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Aims and Scope

The Journal of Physiological Sciences publishes peer reviewed original papers, reviews, short communications, technical notes, and letters to the editor, based on the principles and theories of modern physiology and addressed to the international scientific community. All fields of physiology are covered, encompassing molecular, cellular and systems physiology. The emphasis is on human and vertebrate physiology, but comparative papers are also considered. The process of obtaining results must be ethically sound.

Fields covered:

- Adaptation and environment
- Autonomic nervous function
- Biophysics
- Cell sensors and signaling
- Central nervous system and brain sciences
- Endocrinology and metabolism
- Excitable membranes and neural cell physiology
- Exercise physiology
- Gastrointestinal and kidney physiology
- Heart and circulatory physiology
- Molecular and cellular physiology
- Muscle physiology
- Physiome/systems biology
- Respiration physiology
- Senses

Plenary Lectures

Special Lectures

Memorial Lectures

Plenary Lecture 1

March 28 (Wed) 11:00～12:00 Hall 1

1PL-01AM-1

The role and mechanism of Traditional Chinese Medicine in the prevention and treatment of Parkinson's disease

Wang Xiaomin

Department of Neurobiology, Capital Medical University, Beijing, China

There has been 200 years since the discovery of Parkinson's disease. The current clinical standard of physical therapy is still L-dopa replacement. Traditional Chinese Medicine, including the chinses herbs and acupuncture, showed a significant effect in the treatment of Parkinson's disease. The clinical researches indicated the effectiveness of Chinese herbs plus western drugs vs. western therapy alone. They exhibited significant superiorities in PD symptoms as evidenced by improvements in UPDRS scores. Moreover, Chinese herbs adjuvant therapy was generally safe, well tolerated, and could significantly reduce the side effects caused by conventional medicine. Further study indicated that a variety of Chinese herbs and its monomer substances can protect the dopaminergic neurons in the PD model, the main mechanism including anti-inflammatory, anti-oxidative, anti-apoptosis, neuro-protective and preservation of mitochondrial function. On the other hand, the role of acupuncture in the treatment of Parkinson's disease has been widely recognized at home and abroad. Acupuncture not only has a unique advantage in improving the symptoms of patients with PD, but also can reduce adverse drug reactions, delay the development of disease, improve the quality of life of patients. Basic researches further confirmed that acupuncture can improve the symptoms of a variety of PD animal models, and has a cumulative effect and subsequent effects. The main mechanisms include the protection of dopaminergic neurons, anti-inflammatory and anti-oxidative effects, the regulation of neurotransmitters and neural circuits. More clinical trials with larger numbers of participants are necessary to verify the effectiveness of the Traditional Chinese Medicine, and more investigations are needed to elucidate the target of Chinese herbs and acupuncture. COI:No

Plenary Lecture 2

March 28 (Wed) 14:00～15:00 Hall 1

1PL-01PM-1

Dynamics of Function and Regulation of the Endoplasmic Reticulum

Mori Kazutoshi

Department of Biophysics, Graduate School of Science, Kyoto University, Kyoto, Japan

The endoplasmic reticulum (ER), where newly synthesized secretory and transmembrane proteins are folded and assembled, has the ability to discriminate folded proteins from unfolded proteins and controls the quality of synthesized proteins. Only correctly folded molecules are allowed to move along the secretory pathway, whereas unfolded proteins are retained in the ER.

The ER contains a number of molecular chaperones and folding enzymes (ER chaperones hereafter), which assist productive folding of proteins, and therefore newly synthesized proteins usually gain correct tertiary and quaternary structures quite efficiently. Yet unfolded or misfolded proteins even after assistance of ER chaperones are retrotranslocated back to the cytosol, ubiquitinated and degraded by the proteasome. This disposal system is called ER-associated degradation (ERAD). Thus, the quality of proteins in the ER is ensured by two distinct mechanisms, productive folding and ERAD, which have opposite directions.

Under a variety of conditions collectively termed ER stress, however, unfolded or misfolded proteins accumulate in the ER, which in turn activates ER stress response or Unfolded Protein Response (UPR). The UPR is mediated by transmembrane proteins in the ER, and three ER stress sensors/transducers, namely IRE1, PERK and ATF6, operates ubiquitously in mammals. Thanks to these signaling pathways, translation is generally attenuated to decrease the burden on the folding machinery; transcription of ER chaperones is induced to augment folding capacity; and transcription of components of ERAD machinery is induced to enhance degradation capacity, leading to maintenance of the homeostasis of the ER. If ER stress sustains, cells undergo to apoptosis.

I will talk on the mechanism, evolution, and physiological importance of the UPR. COI:No

Plenary Lecture 3

March 29 (Thu) 15:00～16:00 Hall 1

2PL-01PM-1

Functional diversity of macrophage/monocyte subsets

Akira Shizuo

Laboratory of Host Defense, WPI Immunology Frontier Research Center, Osaka University, Suita, Japan

Macrophages represent a diverse set of phagocytic cells distributed in the whole body. They play a central role in a variety of biological events including host defence against pathogens, tissue remodelling, chronic inflammation, fibrosis and cancer. The heterogeneity of macrophages is recognized as a result of plasticity of these cells to different environmental conditions. However, accumulating evidence indicates the existence of multiple and distinct subsets that exert different biological functions. We previously showed that JMJD3, a HeK27me3 demethylase which is rapidly induced in macrophages in response to LPS is involved in M2 macrophage polarization in helminth infection. We also found that Trib-1 knockout mice show severe lipodystrophy owing to increased lipolysis, accompanied by severe reduction of tissue-resident M2-like macrophages in adipose tissues. Recently we have identified another monocyte subset critical for development of bleomycin-induced lung fibrosis. We named this novel monocyte subset segregated-nucleus containing atypical monocytes(SatM) based on a unique nuclear shape and a hybrid character of monocyte and granulocyte. C/EBPβ deficiency results in a complete lack of SatM. We also identified the progenitor cells of SatM(SMP). Collectively, these results demonstrate that C/EBPβ is a key transcription factor for differentiation of profibrotic SatM from their committed progenitors. COI:No

Special Lecture 1

March 28 (Wed) 17:10～17:55 Hall 1

1SL-01PM-1

Plasticity of cancer cell invasion and metastasis: cell-tissue interplays across scale

Friedl Peter

Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, 2:The University of Texas, MD Anderson Cancer Center, Houston, TX, USA

Cancer cell migration is a plastic and adaptive process generating molecular and physical heterogeneity of migration mechanisms and metastatic routes, including single-cell and collective metastasis. When monitored in vivo using intravital multiphoton microscopy, tissue microniches provide invasion-promoting tracks that enable collective migration along tracks of least resistance. In regions of tissue confinement, invading cancer cells undergo a jamming transition towards collective migration and circulate as both individual cells and multicellular clusters for collective organ colonization. Using multi-targeted interference with integrin adhesion systems, conversion from collective invasion to amoeboid single-cell dissemination followed by increased rates of lung colonization was detected. Similar amoeboid dissemination was induced by hypoxia or stabilization of hypoxia-inducible factor (HIF). The data suggest that metastatic cancer cells can undergo physicochemical reprogramming in response to encountered tissue environments, and thereby balance cell-intrinsic adhesion and mechanocoupling with encountered cues. Dissecting the microenvironmental determinants underlying individual-to-collective plasticity, and vice versa, will enhance to derive combined "antimigration" and cytotoxic therapies and combat metastatic transitions. COI:No

Special Lecture 2

March 28 (Wed) 17:10〜17:55 Hall 2

1SL-02PM-1

Obese-Insulin Resistance and Acute Myocardial Infarction: Roles of Cardiac Mitochondrial Alterations

Chattipakorn Nipon

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Currently, obesity has been considered as an epidemic in which its prevalence is dramatically increased globally. It is well known that a number of serious cardiovascular risk factors already exist in obese subjects including insulin resistance, dyslipidemia, and increased inflammation and oxidative stress. The risk of developing heart disease especially acute myocardial infarction has been shown to be increased in obese subjects. Growing evidence strongly indicates that obese-insulin resistant condition (i.e. a pre-diabetic state) also already impairs left ventricular function. It has been shown that an impaired cardiac mitochondrial function, including increased mitochondrial ROS production, mitochondrial depolarization and mitochondrial swelling, is contributing to impaired left ventricular function under obese-insulin resistant condition. In obese-insulin resistance, the infarct size following the acute cardiac ischemia-reperfusion (I/R) injury is also found to be much larger than those in lean ones. These findings indicate that obesity can markedly aggravate the severity of the impaired cardiac function after cardiac I/R injury. Recently, several new anti-diabetic drugs have been shown to exert cardioprotective effects in addition to their glycemic control. Moreover, device intervention such as the vagal nerve stimulation, has also been shown to be promising in exerting cardioprotection in obese-insulin resistant model with cardiac I/R injury. These benefits have been shown to be due to the improved cardiac mitochondrial function. The increased understanding on the pathophysiological process of obese-insulin resistant condition on the heart will help devising the therapeutic strategies for patients in the future. COI:No

Special Lecture 3

March 28 (Wed) 17:10〜17:55 Hall 3

1SL-03PM-1

A scientific journey from the protein therapy to BNCT (boron neutron capture therapy)

Matsui Hideki

Department of Physiology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

The protein therapy is a newly developed method, which delivers peptides, proteins and biologically active compounds to eukaryotic cells by poly-arginine (three to eleven arginine) protein transduction signal. We are able to control the localization of targeted substances in subcellular compartments, such as nuclei, mitochondria and post synaptic density. It is very convenient, highly efficient and applicable to tissue slices and in vivo whole body. Brain, heart, skeletal muscle, liver, pancreas and lymphocytes are efficient target organs and tissues for the therapy.

By using the protein therapy method, we developed new drug agents, which deliver boron isotope 10B, the fifth element into cancer cells selectively and efficiently. We showed that the drug is very ideal agent for the Boron Neutron Capture Therapy (BNCT). Boron neutron capture therapy (BNCT) is a novel radiation cancer therapy, which selectively destroy malignant cells and spare normal cells. It is a binary method for the treatment of cancer based on the tumor selective delivery of ^{10}B followed by radiation with low energy thermal neutrons. The selective accumulation of ^{10}B in tumors is the key step, which will cause the killing of cancer cells and induce a therapeutic effect. BNCT is recognized as one of the most promising cancer treatment and has been applied to clinical study for the treatment of malignant brain tumors, malignant melanoma, and head and neck cancer.

Okayama University has set up Neutron Therapy Research Center in April 2017 by the support of Ministry of Education, Culture, Sports and Science and Technology- Japan has started to develop a new generation BNCT.

In this presentation, the basic physiological mechanisms of protein therapy and BNCT will be discussed. The future scope of cancer therapy and BNCT will also be discussed. COI:No

Special Lecture 4

March 30 (Fri) 11:35〜12:20 Hall 1

3SL-01AM-1

RARE SUGARS: functional sweeteners to change our life style

Tokuda Masaaki

Department of Cell Physiology, Kagawa University, Faculty of Medicine, Miki, Kagawa, Japan

"Rare sugars" are monosaccharides (minimal functional unit of sugars) that are rarely found in nature, but existing about 50 types. Kagawa University has been successful in producing all of the rare sugars, and has the only research group in the world that prioritized research and development of rare sugars, which has resulted in the ability to produce a high quantity and quality of these precious rare sugars.D-Allulose, one of rare sugars, is a palatable and refreshing sweetener with zero calorie, whose functions include reducing blood sugar levels and lowering lipid accumulation to the body. The former function may be useful for prevention and/or treatment of pre-diabetic and diabetic patients, and the latter for that of atherosclerosis and obesity. It is now under review for the government approval as a functional food for specified health use (FOSHU), while there are many food products containing D-allulose sold on the market such as cakes, bread, and other sweets, soup and beverages in Japan. Japan is the only nation that has legally defined functional foods for health. For those reasons, developments in Japan are often cited as indication of possible developments for Asia, Europe, the United States and the rest of the world.Another rare sugar, D-allose shows another functional benefit to reduce the production of oxygen radicals. The beneficial effect has been shown, for example, to prevent the onset of salt-induced hypertension in rat models. As there are many diseases such as metabolic diseases, cardiovascular diseases, neurodegenerative diseases where oxygen radicals are involved in their pathogenesis, D-allose may be widely used for applicative purposes as a functional food or medicine.Rare sugars are new alternative sweeteners. Utilizing rare sugars effectively in our daily life will be crucial in establishing a "Healthy and Sustainable Society". COI:No

Special Lecture 5

March 30 (Fri) 11:35〜12:20 Hall 2

3SL-02AM-1

Animal movements affected by physical conditions: Introduction of Inverse Problem Approach into Bio-Logging Science

Sato Katsufumi

Atmosphere and Ocean Research Institute, University of Tokyo, Kashiwa, Japan

Gravity acting on an aquatic animal is almost counteracted by buoyancy. As amount of air and lipid in the body fluctuates, total body density deviates around that of surrounding water. Since the first accelerometer was deployed on king penguins in 1996, we have monitored swimming efforts of free-ranging animals in relation to conditions of animals. Diving penguins stopped beating flippers during the final stages of ascent. Propulsive swim speeds of penguins were about 2 m s-1, however, gliding speeds increased after flipper beating stopped. The acceleration during the passive ascent can be attributed to increased buoyancy from the expanding air volume (following Boyle's law) in the body. Estimated air volume inhaled at the surface was positively related with dive depth, which indicates penguins controlled inhaling air volume according to their intended dive depth. On the other hand, Weddell seals were observed to exhale before dives, which means their body density would be affected mainly by the amount of fat. To investigate the effect of body density on stroke effort, accelerometers were deployed on breeding females during lactation. At early lactation, fatter females exhibited only stroke-and-glide swimming. As breeding females consumed its energy stores and their fat layer was depleted, prolonged gliding in descent and continuous stroking in ascent was observed with thinner females. Seals changed swimming gait according to seasonally changing body density. Accelerometers are available for flying animals. Shearwaters and albatrosses mainly relied on soaring, and were passively drifted during resting on the water surface. High-resolution GPS loggers on birds provided zig-zag flight paths and drift movements, which should include information on ocean surface winds and currents. Interspecific comparison indicated that movements of many animals are sensitive to physical conditions. Using this characteristic, we are inversely analyzing animal movements to extract physical conditions of animals and their surrounding environments. COI:No

The Sunao Tawara Memorial Lecture

March 29 (Thu) 10:30〜11:15 Hall 1

2ML-01AM-1

The autonomic regulation of the heart

Ishikawa Yoshihiro

Cardiovascular Research Institute, Yokohama City University School of Medicine, Yokohama, Japan

The autonomic nervous system is the major mechanism of regulating cardiac function. Norepinephrine released from the synaptic terminal binds to the adrenergic receptor on the cardiac cell membrane. This leads to the activation of the stimulatory G proteins, followed by the activation of adenylyl cyclase, a membrane-bound enzyme that produces cAMP from ATP. Cyclic AMP is a major second messenger that activates protein kinase A, which phosphorylates multiple molecules, such as L-type calcium channel or phospholamban, leading to enhanced cardiac inotropism and chronotropism. This was the classic understanding of the molecular mechanisms that regulate cardiac function via the autonomic nervous system. Our laboratory first started the study in this field in the 1990's to identify the molecular diversity of G proteins and its role under both physiological and pathophysiological conditions. We developed a transgenic mouse model that overexpresses G protein in the heart and examined the role of sympathetic activation that occurred exclusively in the heart. This animal model proved that sympathetic activation in the heart is beneficial in the early stage. However, prolonged activation indeed deteriorates cardiac function by inducing cardiac myocyte apoptosis. We also identified specific subtypes of adenylyl cyclase that are dominantly expressed in the heart. Through the generation of multiple gene-manipulated mouse models, we have demonstrated that the specificity of cardiac autonomic regulation is largely dependent upon the enzymatic character of these adenylyl cyclase subtypes. More recent studies have demonstrated that Epac, which is directly activated by cAMP independently from protein kinase A, also plays an important role in regulating cardiac function. Thus, the diversity of molecules involved in cardiac sympathetic regulation in the past 30 years widely opened our understanding of the role of sympathetic nervous system per se under both physiological and pathophysiological conditions. COI:No

The Susumu Hagiwara Memorial Lecture

March 29 (Thu) 11:15〜12:00 Hall 1

2ML-01AM-2

Toward the Mysteries of Sleep

Yanagisawa Masashi

International Institute of Integrative Sleep MedicineWPI-IIIS, University of Tsukuba, Tsukuba, Japan

Although sleep is a ubiquitous behavior in animal species with well-developed central nervous systems, many aspects in the neurobiology of sleep remain mysterious. Our discovery of orexin, a hypothalamic neuropeptide involved in the maintenance of wakefulness, has triggered an intensive research examining the exact role of the orexinergic and other neural pathways in the regulation of sleep/wakefulness. The orexin receptor antagonist suvorexant, which specifically block the endogenous waking system, has recently been approved as a new drug to treat insomnia. Also, since the sleep disorder narcolepsy is caused by orexin deficiency, orexin receptor agonists are expected to provide mechanistic therapy for narcolepsy; they will likely be useful for treating excessive sleepiness due to other etiologies.

Despite the fact that the executive neurocircuitry and neurochemistry for sleep/wake switching has been increasingly revealed in recent years, the mechanism for homeostatic regulation of sleep, as well as the neural substrate for "sleepiness," remains unknown. To crack open this black box, we have initiated a large-scale forward genetic screen of sleep/wake phenotype in mice based on true somnographic (EEG/EMG) measurements. We have so far screened > 8,000 heterozygous ENU-mutagenized founders, and established a number of pedigrees exhibiting heritable and specific sleep/wake abnormalities. By combining linkage analysis and the next-generation whole exome sequencing, we have molecularly identified and verified the causal mutation in several of these pedigrees. Biochemical and neurophysiological analyses of these mutations are underway. Since these dominant mutations cause strong phenotypic traits, we expect that the mutated genes will provide new insights into the elusive pathway regulating sleep/wakefulness. COI:No

Planned Symposia

Planned Symposium 1

China-Japan Joint Symposium (-Towards FAOPS2019-)

From Neuronal Circuits to Behavior

March 28 (Wed) 9:00～10:50 Hall 1

1PS-01AM-1
Reward processing by the dorsal raphe

Luo Minmin

National Institute of Biological Sciences, Beijing and Tsinghua University, Beijing, China

Serotonin neurons in the dorsal raphe nucleus (DRN) provide extensive output to essentially all brain areas and represent the primary source of serotonin in the forebrain. The exact computational role of DRN serotonin neurons remains unclear. Here, I propose that DRN serotonin neurons encode beneficialness, which indicates whether the current state is beneficial to the animal. I will present the following evidences. First, activating serotonin neurons produces reward-like behaviors. Second, these neurons are rapidly activated by natural rewards including sucrose, food, sex, and social interaction. They exhibit the tonic-then-phasic firing pattern during reward anticipatory and consummatory phase. By conditioning the sucrose delivery with a preceding auditory tone, serotonin neurons gradually increase their response during the tone and reach the peak at the point of receiving sucrose. Third, aversive stimuli including quinine and footshock do not activate serotonin neurons, but they inhibit the response to sucrose either during expectation phase or after consumption phase. Fourth, the activity of DRN serotonin neurons are strongly modulated by drugs that produce hedonia effect. Thus, DRN serotonin neurons positively encode a wide range of reward signals during anticipatory and consummatory phases of reward responses. These results lead me to hypothesize that DRN serotonin neurons encode beneficialness (A), which can be calculated by pB-C, where p indicates reward probability, B the predicted reward value, and C the cost. The beneficialness model may explain the extremely diverse behavioral roles of serotonin in modulating different behaviors. COI:No

1PS-01AM-2
Neuronal circuits underlying the regulation of aversive valence in mice.

Watabe M Ayako

Jikei Univ. Sch Med, Chiba, Japan

The amygdala plays a crucial role in the memory formation associated with emotional valence. While the neural mechanisms underlying this associative learning of conditioned stimulus (CS) and an unconditioned stimulus (US) has been intensively studied, the coding and routing of valence-related information of US per se is only partly understood. In particular, both flight and freezing behaviors can be elicited by exposing animals to an environmental threat, but little is known of neural circuits regulating this behavioral switch. To elucidate the neural mechanisms, we focused on the external part of the pontine lateral parabrachial nucleus (PB), which receives nociception-specific inputs and sends direct monosynaptic projections to the central amydala (CeA). We found that optogenetic activation of PB-CeA pathway induced aversive memory when paired with CS, suggesting this pathway may encode aversive valence. We also found that PB-CeA pathway activation triggered impulsive escaping behavior. This prompted us to explore identifying the underlying neuronal circuits. Since a proportion of PB-CeA fibers are known to express calcitonin gene related peptide (CGRP), we used Cre-dependent viruses in genetically engineered mice. The selective activation of CGRP-positive terminals triggered freezing behavior instead of impulsive escaping. These results suggest that the PB-CeC pathway may be part of the central command to trigger defensive behaviors, and might be composed of heterologous population to induce active and passive behaviors. Future study is necessary to dissect out the neural circuits controlling these competing processes. COI:No

1PS-01AM-3
Possible roles of the active zone proteins in neural circuits underlying reward activity in mice

Ohtsuka Toshihisa, Hagiwara Akari

Dept Biochem, Grad Sch Med, University of Yamanashi, Yamanashi, Japan

Neurotransmitter release is a tightly-regulated process by complex interactions of presynaptic active zone proteins. So far, several active zone proteins have been identified and characterized, including CAST, ELKS, Bassoon, Piccolo/Aczonin, RIM1, and Munc13-1. We have reported that CAST regulates basic neurotransmitter release in mouse hippocampal pyramidal cells and controls the size of synaptic ribbon in retinal photoreceptor cells. However, its physiological roles in higher brain functions have been largely unknown. Here we found that the deletion mutant of CAST (CAST KO) showed significantly reduced weaning rate of primiparous female. CAST KO had also less crouching time and increased moving distance during nurture. Moreover, we found that the total amount of drinking water was significantly decreased but preference for sucrose was increased in CAST KO during pregnancy and nurturing. From these results we speculate that exposure of pregnant stress may affect the responsiveness to reward by impaired release regulatory machinery. Our preliminary analyses also revealed that CAST mRNA was expressed in dopaminergic neurons in substantial nigra and ventral tegmental area, consisting of reward related circuits. To uncover its causal relationship between pregnant stress and reward activity by CAST, electrophysiological and mice behavioral analyses are currently under way. And in this symposium I would like to show some additional biochemical data and discuss the contribution of the presynaptic active zone proteins to the reward related circuits. COI:No

1PS-01AM-4
Deciphering Ca^{2+}-dependent signaling pathways underlying cognitive processes

Bito Haruhiko, Kim Ryang, Inoue Masatoshi, Inokuchi Kasumi, Yokoyama Tatsushi, Sakai Kazuki, Miyazawa Yusuke, Ishii Yuichiro, Okamura Michiko, Gammon Nicholas, Kobari Shigetaka, Kondo Yayoi, Sakamoto Masayuki, Fujii Hajime

Dept. Neurochem., Grad. Sch. Med., The Univ. of Tokyo, Tokyo, Japan

Deciphering the intricate and interactive relationship between the information encoded in the genome and the ongoing synaptic activity is critical for understanding the molecular and cellular signaling underlying long-term memory formation and maintenance of long-lasting changes within the brain. We previously identified a novel "inverse" synaptic tagging mechanism in which one of CREB's target gene, Arc, acts as a brake that helps weaken the non-potentiated synapses during the maintenance phase of synaptic plasticity. Arc's rapid induction following strong physiological stimuli is dictated by a potent synaptic activity-responsive element (SARE) present in its enhancer/promoter region, which strikingly harbors a unique cluster of binding sites for CREB, MEF2 and SRF/TCF. We further studied key signaling features underlying SARE activation in vivo during contextual fear memory processing and elucidated the amygdala-specific requirement for CRTC1, a CREB coactivator that translocated from the cytoplasm to the nucleus in a Ca^{2+}-dependent manner, only in the amygdala during fear conditioning. These efforts collectively start to illuminate key molecular and cellular events that are essential in neuronal coding and information processing in the amygdala during fear memory processing. COI:Properly Declared

Planned Symposium 2

Translation of smooth muscle physiology to pathophysiology

March 28 (Wed) 9:00～10:50 Hall 2

1PS-02AM-1
Proof of importance of phospho-CPI-17 at Thr38 in maintenance of normotensive pressure using genetically modified mice

Hori Masatoshi

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CPI-17, an endogenous myosin phosphatase inhibitory protein, is considered a key molecule for Ca2+ sensitization of the contractile apparatus, as well as myosin binding subunit of myosin phosphatase, MYPT1. Here, we have used CRISPR/Cas9 to generate CPI-17-deficient (KO) and Thr38 phospho-resistant mice (TA), then effects of CPI-17 on vascular contractility in vitro and mean blood pressure in vivo were investigated. In isolated thoracic aorta, PDBu induced a sustained contraction of WT mice while no contraction showed from KO or TA mice. High concentration of KCl solution-induced contraction was not different between transgenic and WT mice. In contrast, phenylephrine (PE)-induced contractions in aorta isolated from both mutant strains were significantly smaller than those of WT mice in association with low level of myosin phosphorylation. Phosphorylations of MYPT1 at Thr696 and Thr853 in PE-induced contraction were similar in both mutant strains and WT mice. Taken together, at least in part, PE-induced contraction is regulated by phosphorylation of CPI-17 at Thr38. Finally, the physiological role of CPI-17 in the regulation of blood pressure was investigated using radio telemetry. MBP was significantly decreased in both transgenic mice, even though with a compensatory increase in heart rate. In conclusion, we generated knock-out and constitutively phospho-resistant mouse models of CPI-17 for the first time. Phosphorylation of CPI-17 at Thr38, possibly by protein kinase C, is important to maintain vascular contractility and blood pressure in vivo. COI:No

1PS-02AM-2
Physiology and pathophysiology of thrombin receptor in pulmonary circulation

Hirano Katsuya

Dept Cardiovasc Physiol, Fac Med, Kagawa Univ, Kagawa, Japan

The pulmonary circulation shows some distinct properties from the systemic circulation. Hypoxic pulmonary vasoconstriction is one of the well-known characteristics of the pulmonary circulation. We revealed that the thrombin receptor-mediated vascular effects also differ between pulmonary and systemic circulations. Under physiological conditions, the most frequently reported vascular effect of thrombin in the systemic circulation is an endothelium-dependent vasorelaxation. Many systemic arteries do not show vasoconstrictor response. In contrast, the pulmonary arteries in many species exhibit vasoconstrictor effect in response to thrombin. Pulmonary hypertension characterized by progressive increase in vascular resistance. Increase in vascular resistance is mainly attributable to vasoconstriction, vascular remodeling and thrombosis. Increased coagulability and thrombotic pulmonary arteriopathy are associated with pulmonary hypertension. Collectively, it is hypothesized that the unique property of pulmonary artery regarding the smooth muscle effect of thrombin plays a critical role in the pathogenesis of pulmonary hypertension, by inducing pulmonary vasoconstriction and vascular remodeling. We proved this hypothesis by revealing the ameliorating effects of pharmacological and genetic antagonisms vs. thrombin receptor in experimental pulmonary hypertension. We also found that the expression and vasoconstrictor activity of thrombin receptor are up-regulated in the pulmonary hypertension. As a result, the thrombin receptor antagonism is proposed to be a potential therapeutic strategy for the treatment of pulmonary hypertension. COI:No

1PS-02AM-3
Novel elements that regulate Ca2+ sensitization force in GI smooth muscles

Eto Masumi, Kitazawa Toshio

Dept Mol Physiol & Biophys, Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, USA

Disturbances in the CPI-17-myosin phosphatase pathway that govern SM contraction have been linked to various diseases, such as hypertention, asthma, GI dysmotility and overactive bladder. In addition, reports suggest that MLCK phosphorylation attenuates the CaM binding, although its pathophysiological impacts remain largely unknown. Here, we investigated structure-function relationships of CPI-17 and physiological roles of MLCK phosphorylation in two types of mouse GI SM tissues; stomach fundus (tonic) and ileum (phasic). Addition of the full-length CPI-17 protein to permeabilized fundus strips produced a tonic force. Deletion of either N- or C-terminal tail of CPI-17 dampened the potency of this protein, suggesting specific roles of N- and C-terminal tails in regulating the contraction. In ileum SM, a steady elevation in MLCK phosphorylation occurred in intact strips stimulated with 124mM K+ and carbachol and in the permeabilized strips stimulated with the Ca2+-clamped pCa6.0 solution that produced the phasic force. MLCK phosphorylation in ileum strips was independent of the CaMKKbeta-AMPK pathway, which is reported to phosphorylate MLCK. Addition of ectopic CaM to the permeabilized strips resulted in a tonic contraction in pCa6.0 solution, whereas it did not alter MLCK phosphorylation, suggesting that excess CaM can override a reduction in affinity to CaM. In addition to the canonical Ca2+ sensitization signaling, novel pathways that regulate the myosin phosphatase and MLCK may contribute to defining force development in governing GI motility. COI:No

1PS-02AM-4
Phathophysiology of the common signaling pathway regulating vasospasm and cell migration

Kobayashi Sei

Dept. Mol. Cell. Physiol., Yamaguchi Univ Grad. Sch. Med, Ube, Japan

no abstract COI:No

Planned Symposium 3

Joint Symposium with the Japanese Association of Anatomists

Molecular mechanisms of cell polarity formation

March 28 (Wed) 9:00～10:50 Hall 3

1PS-03AM-1
The role of polarized transport in the cell polarity of mammalian epithelial cells.

Harada Akihiro

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The molecular mechanism of polarized transport such as apical and basolateral transport in epithelial cells still remains elusive compared to basolateral transport. To date, some Rabs have been known to be involved in apical transport. We have previously demonstrated that Rab8a, Rab8b and Rab11 are required for localizing apical markers in the intestine. To know the molecular mechanism in apical transport further, we started searching for binding proteins of Rab8 and Rab11 and identified a novel Rab8-interacting protein, EHBP1L1. EHBP1L1 directly bound GTP-Rab8 and Bin1. EHBP1L1- or Bin1-depleted or dynamin-inhibited small intestinal organoids accumulated apical membrane proteins in lysosomes. Furthermore, in EHBP1L1-deficient mice, small intestinal cells displayed truncated and sparse microvilli, suggesting that EHBP1L1 is required for apical transport. Our data demonstrate that EHBP1L1 links Rab8 and the Bin1-dynamin complex, which generates membrane curvature and excises the vesicle at the ERC for apical transport. COI:No

1PS-03AM-2
Polarized transport in Drosophila photoreceptors

Satoh Kono Akiko

Grad. Sch Integ. Arts and Sci., Hiroshima Univ., Higashi-Hiroshima, Japan

Polarized membrane trafficking is essential for the construction and maintenance of multiple plasma membrane domains in a cell, however, its mechanism is not well elucidated. Highly polarized Drosophila photoreceptor is an excellent model for studying polarized transport. A single cross-section of Drosophila retina contains many photoreceptors with 3 clearly differentiated plasma membrane domains: a rhabdomere, stalk, and basolateral membrane. We have shown before that Rab11 and its effectors, dRip11 and MyoV regulate the polarized transport of Rhodopsin to the rhabdomere (Satoh et al., 2005 Dev; Li et al., 2007 JCB). We recently found Parcas (Pcs), Drosophila homolog of REI1/REI2, recently reported as Rab11GEF in C. elegans (Sakaguchi et al., 2015 Dev Cell), is a Rab11GEF in Drosophila photoreceptors. Pcs strongly co-localizes Rab11, and Pcs deficiency pheno-copies Rab11 loss. We also found that impairment of Crag/Rab10/Ehbp1 results in mislocalization of basolateral protein, Na+K+ATPase to the stalk membrane, and the shortage of basolateral and the elongation of stalk membranes, and the abnormality of Golgi cisternae. The phenotype is similar to, but stronger than the deficiency of AP1/clathrin, which is involved in the basolateral transport, indicating that Crag/Rab10/Ehbp1 is essential for the polarized trafficking to the basolateral membrane. COI:No

1PS-03AM-3
The role of tight junction-associated plaque proteins ZO-1 and ZO-2 in epithelial polarity

Furuse Mikio[1,2], Otani Tetsuhisa[1,2]

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Tight junctions (TJs) play pivotal roles in epithelial barrier function by restricting the passage of solutes through the paracellular pathway. In addition, TJs have been believed to act as a fence that prevents lateral diffusion of membrane proteins and lipids between the apical and basolateral plasma membranes, thereby contributing to the establishment and maintenance of epithelial polarity. Contrary to this model, when cultured epithelial cells lacking TJs were generated by targeted gene disruption of ZO-1 and RNA interference of ZO-2, epithelial polarity was not affected (Umeda et al., Cell 126:741-754, 2006). Here, to clarify whether TJs play any roles in epithelial polarity formation, we completely disrupted both ZO-1 and ZO-2 genes by genome editing in MDCK II cells, which have been widely used in epithelial polarity research. TJ formation was severely impaired in the ZO-1/ZO-2 double knockout cells in agreement with the previous study. Unexpectedly, the polarity of the plasma membrane was disorganized in these cells: membrane proteins normally localized at the basolateral membrane, including Na+-K+-ATPase, E-cadherin and claudins, were also detected in the apical membrane domain. Moreover, the ZO-1/ZO-2 double knockout cells showed aberrant localization of Par3, one of the key components of cell polarity signaling, and failed to form polarized cysts in 3D culture. These results suggest that ZO family proteins are required for TJ formation and play a crucial role in the formation of epithelial polarity in MDCK II cells. COI:No

1PS-03AM-4
Cell polarity regulation by the microtubule crosslinking and stabilization factor, MTCL1

Suzuki Atsushi, Satake Tomoko

Mol Cell Biol Lab, Grad Sch Med Life Sci, Yokohama City Univ, Yokohama, Japan

Recent studies have revealed critical importance of non-centrosomal MTs for cell functions and polarity. In highly differentiated cells such as epithelial cells and neurons, MTs are detached from the centrosome and aligned to form bundles in a cell-type specific manner. Even in less polarized culture cells, MTs are nucleated from not only the centrosome but also the Golgi membrane to connect Golgi stacks laterally. These organizations of non-centrosomal MTs are essential for polarity development of each type of cells. MTCL1 is the MT crosslinking and stabilizing protein, which plays indispensable roles for developing epithelia-specific apicobasal MT bundles and the Golgi-derived MT networks. In this talk, I introduce our recent results on its essential role for establishing neuronal polarity. Mice lacking MTCL1 were born normally but exhibited abnormal motor coordination and degeneration of cerebellar Purkinje (PJ) cells. Knockdown of MTCL1 in PJ cells by in utero gene transfer revealed that loss of MTCL1 disturbs normal development of the AIS (axon initial segment) structures: in severest cases, the cells lost AIS and exhibited lots of dendritic spines on axon, indicating the loss of axonal polarity. Rescue experiments further demonstrated that these defects are caused by disturbed formation of AIS-spanning MT bundle, the restoration of which requires the MT stabilizing activity of MTCL1. I will discuss possible mechanisms by which MTCL1-mediated MT stabilization facilitates the formation of the AIS structure essential for axonal polarity. COI:No

Planned Symposium 4

Joint Symposium with the Japan Society of Acupuncture and Moxibustion

Scientific basis of the oriental medicine: acupuncture mechanism on muscle pain and motor dysfunction

March 28 (Wed) 9:00～10:50 Hall 4

1PS-04AM-1
Indication of acupuncture and moxibustion in musculoskeletal or motor disorders

Kondo Tetsuya

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This year, the society for internal medicine in the United States declared that acupuncture is the first choice for acute, subacute and chronic lumbago. On the other hand, the drug has been regarded as "the fault of the market" with the risk of mortality and addiction. In 1997, National Institute of Health concluded that acute dental pain in addition to nausea and vomiting due to chemotherapy is a good indication of acupuncture judging from a systematic review summarizing 16 reports on controlled trials. A systematic review revealed positive trend toward real acupuncture for recurrent headache against sham acupuncture. These conclusions were followed by British Medical Association aiming at reducing National Health Service costs in 2000. Apart from these, World Health Organization suggested 50 disorders as indications of acupuncture in 1993. These include nine musculoskeletal or motor disorders such as tennis elbow, shoulder stiffness, cervical spondylosis, shoulder periarthritis, rheumatoid arthritis, contusion, ligament rapture, knee osteoarthritis, tension-type headache, and sciatic neuralgia. COI:No

1PS-04AM-2
Muscular and neural mechanisms of muscular pain/hyperalgesia in a rat model of delayed onset muscle soreness (DOMS)

Mizumura Kazue

Dept Phys Ther, Coll Life Health Sci, Chubu Univ, Kasugai, Japan

Muscular pain/hyperalgesia is quite common and a large health problem. Acupuncture and massage are often used for its treatment. To get better treatment it is important to know its generating mechanism. For a model to study its mechanism we have chosen delayed onset muscle soreness (DOMS) that appears after unaccustomed strenuous exercise including lengthening contraction (LC), because in DOMS symptoms that are typical for myofascial pain syndromes are found: the taut band like muscle hardness and referred pain that occurs when the most sensitive spot in the hardness is compressed. We applied LC to the lower hindlimb of rats and found muscular mechanical hyperalgesia of the exercised muscle (DOMS). No invasion of inflammatory cells were found. By pharmacological and biochemical/molecular analyses we found that there are two pathways that lead to DOMS. One is bradykinin B2 receptor to nerve growth factor (NGF) pathway, the other is cyclooxigenase-2 to glial cell-line derived neurotrophic factor (GDNF) pathway. Intramuscular injection of antibodies to these neurotrophic factors reversed mechanical hyperalgesia, demonstrating that they are responsible for DOMS. We also found that these neurotrophic factors are produced by muscle cells/satellite cells in the exercised muscle. Both NGF and GDNF sensitize muscular thin-fiber afferents to mechanical stimuli. This sensitivity change of muscle nociceptors is considered to be the neural basis of mechanical hyperalgesia. In conclusion, muscle itself can produce pain-enhancing substances by its work to cause muscular pain/hyperalgesia. COI:No

1PS-04AM-3
Peripheral and spinal mechanisms of pain in the muscle fascia

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For acupuncturists in oriental medicine, muscle fascia has long been considered not only as a critical pain source but also as an important target for the treatment of patients with myofascial pain. The second skeleton "fascia" is a sheet- or layer-like structure which functionally binds and separates whole body parts, and makes it possible to perform smooth and cooperative muscular work. Except for its supportive and biomechanical properties, however, physiological roles of muscle fascia have never been subjected to intensive exploration in the biomedical sciences. Here we examined peripheral and spinal mechanisms of fascial nociception in rats. Immunohistochemical staining revealed that nociceptive nerve fibers with peptidergic and non-peptidergic axons and terminals were densely distributed in the crural fascia and the thoracolumbar fascia. Peripheral thin-fiber afferents (Aδ- and C-fibers), responding to noxious mechanical, chemical, and thermal stimuli, existed in the fascia. Forty-three % of the C-fibers were polymodal receptors, which have an effector function responsible for a flare reaction of the skin after acupuncture and moxibustion. Nociceptive signals from the fascia was projected mainly to the superficial spinal dorsal horn. Spinal dorsal horn cells receiving input from the thoracolumbar fascia existed, and the cells were sensitized in inflammatory condition. These results show that muscle fascia is not just a supporter surrounding the muscle, but is a nociceptive sensory tissue/organ, and that it may be a target for the treatment of patients with myofascial pain such as stiff neck and low back pain. COI:No

1PS-04AM-4
The analgesic effects and the mechanisms of acupuncture and Kampo medicine

Sunagawa Masataka

Dept. Physiol., Sch. Med., Showa Univ., Tokyo, Japan

Pain is generally treated with drugs such as non-steroidal anti-inflammatory drugs, acetaminophen, tricyclic antidepressants, serotonin noradrenaline reuptake inhibitors, narcotic analgesics, calcium channel $\alpha_2\delta$ ligands, and anti-epileptic drugs. However, in many cases, these drugs do not sufficiently treat the symptoms, and they may lead to the manifestation of undesirable side effects.

Acupuncture has been used to relieve pain. A number of mechanisms have been reported to underlie the analgesic effect of acupuncture, including central mechanisms such as the activation of the descending pain inhibitory system and the endogenous opioid system, gate control theory, diffuse noxious inhibitory control, and the inhibition of glial activation. The release of adenosine has also been reported to be involved as a peripheral mechanism.

Various Kampo medicines are used to treat pain. Among these, we reported the analgesic effect of Yokukansan in a previous meeting. Yokukansan is reported to have various pharmacological effects, including a partial agonistic effect on the serotonin 1A receptor, a downregulating effect on the serotonin 2A receptor, an antagonistic action at the NMDA receptor, an ameliorative effect on glutamate clearance in astrocytes, and an inhibitory effect on glial activation. Kampo medicines are multicomponent; thus, they may have multiple mechanisms of action.

Acupuncture or Kampo therapies should be considered in cases in which general pharmacotherapies are ineffective.

COI: No COI:No

1PS-04AM-5
Effect of acupuncture on vibration-induced finger flexion reflex in humans

Takakura Nobuari

Dept Acupunct, Tokyo Ariake Univ, Tokyo, Japan

A well-known purpose of acupuncture is to ease stiffness or excessive tension within skeletal muscles. Vibration-induced finger flexion reflex (VFR), a somatic motor reflex, is induced by vibratory stimulation to the fingertips. Flexion force of VFR in the forearm finger flexor muscles gradually increases during vibration. The receptors of VFR are reported as skin mechanoreceptors, and this reflex has two reflex circuits: the spinal short loop and supraspinal long loop. VFR is considered a good experimental model to investigate the mechanism of acupuncture in reducing muscle tension because the reflex circuit is relatively well studied in humans. In 1987, VFR was found to be inhibited with acupuncture at the TE5 acupoint, located in the back of the forearm. Based on these findings, we investigated the effect of acupuncture at other acupoints, needle stimulation with different needle manipulations, transcutaneous electrical stimulation, and pressure stimulation on VFR. We found that acupuncture by needle insertion at the acupoints such as LI4 in the upper extremity, diminished flexion force induced by VFR but not for pressure stimulation. This indicates that a noxious component was important in VFR inhibition, and pain-conducting afferent fibers may have an inhibitory connection to relevant spinal motor neurons. The inhibitory effects of acupuncture on VFR may explain why acupuncture can exert effects not only on muscle tension and stiffness in musculoskeletal diseases such as low back pain and neck/shoulder stiffness but also on muscle spasticity in neurological disorders such as stroke. COI:No

Planned Symposium 5

The understanding of molecular mechanisms underlying exercise therapy for the future of neurorehabilitation

March 28 (Wed) 9:00〜10:50 Hall 6

1PS-06AM-1

Exercise regimen that enhances endurance, cognition and motivation

Soya Hideaki

Labo of Exercise Biochemistry, Faculty of Health and Sport Sciences, Univ of Tsukuba, Tsukuba, Japan

Many people are unable to implement exercise regularly, despite its immense benefit to their body and mind.To solve this issue, we proposed a translational study called Brain Fitness, in which we aimed to explore exercise conditions that enhance endurance, cognition and motivation. We developed animal and human exercise models based on lactate threshold level (physiological), and found positive effects of acute mild exercise on regions including the prefrontal cortex (human) and hippocampus (animal); a chronic mild exercise regimen resulted in improved executive function and spatial memory in humans and animals, respectively.Interestingly, such beneficial effects were greater in subjects with higher endurance, which opened the possibility that endurance capacity is an important factor in improving cognitive function through exercise, implying the involvement of cytokines/growth factors acting on brain and brawn simultaneously. Thus, we explored two types of exercise regimens, mild and high-intensity interval, which not only improved endurance, but also enhanced hippocampus-dependent neurogenesis and memory, warranting further mechanistic studies. Interestingly, brain dopaminergic regulation increased with both regimens. Furthermore, a whole-genome hippocampal array study revealed some crucial molecular alterations with both regimens (such as upregulated APOE and IGF2, and downregulated TNF). Therefore, a regimen that is meant to improve cognitive function should also aim to improve endurance and motivation, and should include anti-inflammatory benefits. COI:No

1PS-06AM-2

Anxiety/depressive-like behaviors by mild stress can be improved by treadmill exercise in rats without the change of HPA axis.

Ishida Kazuto, Koike Kohei, Maruyama Akiko, Uenishi Yuki, Wakayama Satomu, Sugiyama Yoshitaka

Dept Phys and Occup Ther, Grad Sch, Nagoya Univ, Nagoya, Japan

Chronic stress is involved in the onset and development of depression. While exercise has stress-buffering effects, the therapeutic significance of exercise is not fully understood. The objectives of this study were, at first, confirm that chronic forced swim stress induces anxiety/depressive-like behaviors after stress exposure, and then investigate whether treadmill exercise alleviates the anxiety/depressive-like behaviors induced by forced swim stress through the alteration of hypothalamic-pituitary-adrenal (HPA) axis response. It has been found that forced swim stress exposure induced abnormal behaviors including forced swim 21 days after stress exposure using forced swim test, open field test and elevated open-platform test. These abnormalities were inhibited by repeated administration of the antidepressant, imipramine, suggesting that these behaviors are anxiety/depressive behaviors. Moreover, treadmill exercise ameliorated depressive behaviors induced by chronic forced swim stress. Interestingly, there was no significant difference of the HPA axis response including serum corticosterone level among groups. These data suggest that this chronic forced swim stress paradigm is mild stress model and the exercise is not affected on the HPA axis. This is the first study that showed that treadmill exercise can rescue anxiety/depressive-like behaviors by not changing the HPA axis in mild stress model. COI:No

1PS-06AM-3

Neural network remodeling induced by rehabilitative training after stroke: Effectiveness and Limitations

Okabe Naohiko, Nakamura Emi, Himi Naoyuki, Miyamoto Osamu

2nd Dept Physiol, Kawasaki Med Sch, Okayama, Japan

Upper limb disability is a common problem in post-stroke patients and task specific training has been regarded as the most reliable therapy. Although descending spinal pathways (corticospinal, rubrospinal and reticulospinal pathways) has been suggested to contribute to functional recovery after stroke, precise mechanism by which task specific training improves motor performance is still elusive. We have demonstrated that skilled forelimb training promotes motor map reorganization through axonal remodeling in the ipsilesional corticospinal pathway and induces task-specific functional improvement in the rat stroke model. However, if both primary and secondary motor cortex was destroyed, any training methods (Skilled forelimb task, rotarod or treadmill) could not improve functional recovery whereas rubrospinal and reticulospinal pathway remained intact. In this severe stroke model, only constraint induced movement therapy which increased the corticospinal projection from the peri-infarct motor cortex promoted functional recovery in the skilled forelimb task. These data indicated that training-induced functional recovery is strongly dependent on the ipsilesional corticospinal projection and it cannot be substituted by other descending spinal pathways, which suggests importance of the strategies to enhance protection or regeneration of corticospinal neurons for effective rehabilitative therapy in severely affected stroke patients. With recent data from our lab, we also discuss molecular mechanism of neural network remodeling. COI:No

1PS-06AM-4

Mechanisms underlying ameliorative and aggravating effects of physical exercise during acute phase after ischemic and traumatic brain injury

Tanaka Junya, Abe Naoki, Taguchi Satoshi, Otoi Takaki

Dept Mol Cell Physiol, Grad Sch Med, Ehime Univ791-0, Ehime, Japan

We examined the ameliorative effects on a rat stroke model of treadmill exercise during acute phase. The model was prepared by transient middle cerebral artery occlusion, and the ischemic lesions were evaluated using magnetic resonance imaging (MRI). The rats were forced to run at 4-6 m/s for 10 min/day for three days. Brain edema was measured on day 5 by MRI, histochemical staining of brain sections and tissue water content determination. Motor function was evaluated by rota-rod test on day 30. Exercise reduced brain edema and ameliorated motor function. The ameliorated effects on brain edema were attributable to reduced expression of aquaporin 4 and Na+/H+ exchangers (NHEs). Elevated plasma corticosterone may have caused the expressional changes. Thus, the physical exercise during acute phase may be favorable for the severe brain injuries. However, adverse effects of early initiation of exercise have been reported. We, therefore, investigated the outcomes of traumatic brain injury (TBI) model rats that were forced to run at 5 m/min for 10 min only once after 1, 2, and 3 days after TBI. Then, we found that larger lost tissue volumes in the injured brains, when the rats were subjected to the forced exercise initiated at the early time points. The aggravation may be mediated by the increased proinflammatory responses. Collectively, physical exercise during the early phase should be done under the careful management; otherwise, it would cause aggravation rather than amelioration. COI:No

Planned Symposium 6

Committee for Promotion of Physiome and Systems Biology

Integrative and theoretical research on the architecture of biological system and its disorder

March 28 (Wed) 15:10〜17:00 Hall 2

1PS-02PM-1

Development of method to predict the toxicity of molecular target drugs

Kariya Yoshiaki, Suzuki Hiroshi

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Molecular target drugs are often associated with the non-predictable and severe adverse events. Such events can be often ascribed to the inhibition of off-target molecules. We analyzed the molecular mechanism to account for the toxicity of sunitinib. In order to identify the off-target molecule(s) of sunitinib, we performed a comparative study between sunitinib and sorafenib. Pharmacokinetic and pharmacodynamic (PK/PD) analysis based on the Kd values of these drugs to tyrosine kinases and drug plasma concentrations suggested that phosphorylase kinase γ-subunit 1 and 2 (PHKG 1/2) are extensively inhibited by sunitinib, but not by sorafenib. It is possible that PHKG 1/2 are the off-target molecules for the toxicity of sunitinib. We performed the pathway analysis and it was found that the inhibition of PHKG 1/2 finally results in the reduction of cellular GSH levels. This hypothesis was confirmed by experiments in mice. Since the administration of α-tocopherol nicotinate results in the recovery of cellular GSH levels, we examined the effect of this antioxidant. The toxicity of sunitinib in mice was markedly reduced by the administration of α-tocopherol nicotinate. Collectively, we could identify the mechanism of development of toxicity of sunitinib based on the PK/PD and pathway analysis/systems-biological analysis, and suggested a method to reduce the toxicity. Furthermore, we would like to also propose a method with which the toxicity of molecular target drugs can be predicted based on the systems-biological method. COI:Properly Declared

1PS-02PM-2

Multi-omics approaches to chronic kidney disease

Uchida Shinichi

Department of Nephrology, Tokyo Medical and Dental University, Tokyo, Japan

Chronic kidney disease (CKD) is a major global health problem. About 13% of the adult population are estimated to have CKD in Japan. The prevalence of end stage kidney disease (ESKD) is also rapidly increasing, with high cost of renal replacement treatment. In Japan, about 40,000 patients were newly introduced to renal replacement therapy in a year, resulting in more than 300,000 patients on dialysis. CKD is also a well known risk factor for cardiovascular mortality and morbidity. Thus, early recognition and treatment of CKD are important to prevent cardiovascular diseases and ESKD. However, there has been no specific drug to treat CKD since we still don't know well about the mechanism how CKD kidney continues to fail irrespective of its primary causes. To identify novel target molecules and mechanisms to develop drugs for CKD, we conducted multi-omics approaches to CKD. The methods used were transcriptomics using microarrays and RNAseq by next generation sequencing, epigenomics, and metabolomics including lipidomics. The CKD model we used was a mouse nephrectomy model in C57BL6 and 129SvJ mice, as CKD-resistant and -prone strains, respectively. Previous QTL analyses and SNP data in both strains were also utilized with these omics data. As results, several candidate molecules which would be important in the progression of CKD were identified, and the validation studies are under way. Utility and problem in the current omics studies and the future directions in the field of system biology will be discussed. COI:No

1PS-02PM-3

Physiological architecture of ion transport system in the epithelial tissue of the inner ear

Hibino Hiroshi[1], Nin Fumiaki[1], Kurachi Yoshihisa[2]

1:Dept Mol Physiol, Sch Med, Niigata Univ, Niigata, Japan, 2:Div Mol Cell Pharm, Dept Pharm Grad Sch Med, Osaka Univ, Osaka, Japan

Cochlea in the mammalian inner ear harbors an extracellular solution characterized by unique $[K^+]$ and potential. This electrochemical environment, which sensitizes sensory hair cells, is likely to be maintained by unidirectional K^+ transport across the double-layered epithelial tissue. Numerous molecular biological and histochemical experiments have identified that different K^+ channels and K^+ transporters are expressed in each membrane domain of the epithelial tissue. Electrophysiological assays have showed that the channels and transporters are active and control intracellular and extracellular $[K^+]$ properties that contribute to the electrochemical environment of the extracellular solution. Nevertheless, it remains uncertain how these ion transport machineries are functionally coupled to drive the unidirectional K^+ transport. To address this issue, we have used *in silico* approach. The model that replicates experimentally identified molecular and physiological characteristics of K^+ flows in the epithelial tissue indicates the architecture of the system for the unidirectional K^+ transport. Furthermore, dysfunction of a deafness-associated channel or transporter interferes the K^+ transport and changes $[K^+]$ properties, possible mechanisms underlying a few types of cochlear disorders. COI:No

1PS-02PM-4

Robustness and vulnerability in cardiac electrophysiological system: from a viewpoint of dynamical system theory

Tsumoto Kunichika[1], Kurata Yasutaka[2], Furutani Kazuharu[1,3], Kurachi Yoshihisa[1]

1:Div Pharma, Grad Sch Med, Osaka Univ, Osaka, Japan, 2:Dept Physiol, Kanazawa Med Univ, Ishikawa, Japan, 3:Dept Physiol, Univ California Davis, Davis, USA

The heart is a huge hierarchical complex system. The contractile function in the heart results from the accurate propagation of action potentials (APs) in cardiomyocytes, i.e., electrical activity in the cellular and tissue system. Therefore, understanding robustness and failure of the electrical activity in the heart will be valuable for integration of functions evoked at each system level within the heart. Cardiac arrhythmia is a certain kind of failure of the electrical activity in the heart and has believed to be evoked by triggered activities following afterdepolarizations. Combining computer simulations in APs with numerical analysis methods based on dynamical system theory, i.e., bifurcation analysis, we investigated stability changes of APs observed in a mathematical ventricular myocyte model. We found that the triggered activity was caused as a result of abrupt changes in the dynamical stability of AP responses in the cardiomyocyte. In this symposium, we will present results from our recent *in silico* studies and would like to discuss the proarrhythmic effects of alteration in repolarization currents from a viewpoint of dynamical system theory. COI:No

1PS-02PM-5

Platform for multilevel systems physiology and its applications

Asai Yoshiyuki[1], Abe Takeshi[2], Kitano Hiroaki[2,3]

1:Dept Sys Bioinf, Grad Sch Med, Yamaguchi Univ, Yamaguchi, Japan, 2:Integrated Open Systems, OIST, Okinawa Japan, 3:SBI, Tokyo, Japan

The importance of systems biological approaches has been well recognized recently in various biological and physiological fields including medicine. Physiologically plausible mathematical models are used as powerful tools to bridge data to the better understanding of the dynamical physiological functions and pathologic conditions. Recently such models are progressively evolving in size and complexity, systematical supports by software is also becoming more and more critical. In this stream, we have developed a modeling platform, PhysioDesigner, with a model descriptive language PHML (Physiological Hierarchy Markup Language) which can cooperate with SBML, a pioneering language for describing models of subcellular biochemical processes. By importing SBML models in a PHML model, it is possible to create an SBML-PHML hybridized model to represent the multilevel structure of physiology. Such languages are computer-readable, which have significant potential to expand the modeling and simulation abilities by cooperating with machine learning and AI technologies. Besides of developing models with mathematical expressions on PhysioDesigner, time series data can be integrated into the models. Simulations of those models can be performed by Flint, a standalone simulator supporting PHML as well as SBML. As an extension of Flint, a cloud-based simulation system Flint K3 is publically available at flintk3.org. In the talk, the software platform will be introduced with some of the simulation studies. COI:No

Planned Symposium 7

Joint Symposium with the Japanese Association of Rehabilitation Medicine

Eating and Exercise changes your Life.

March 28 (Wed) 15:10〜17:00 Hall 3

1PS-03PM-1

Effects of milk product intake during interval walking training on lifestyle-related diseases in older people

Masuki Shizue[1,2], Uchida Koji[1], Morikawa Mayuko[1,2,3], Nose Hiroshi[1,2,3]

1:Dept Sports Med Sci, Shinshu Univ Sch of Med, Matsumoto, Japan, 2:Inst Biomed Sci, Shinshu Univ, Matsumoto, Japan, 3:JTRC, Matsumoto, Japan

[Purpose] We examined whether the milk + carbohydrate (CHO) supplementation during interval walking training (IWT) improved the symptoms of lifestyle-related diseases with enhanced methylation of pro-inflammatory gene. [Methods] Older people (~70 yr) who had performed IWT for >6 mos but with >130 mmHg of systolic pressure and >110 mg/dl of blood glucose used as subjects. After the baseline measurements of peak aerobic capacity (VO_{2peak}) by graded walking test, blood constituents, blood glucose by continuous method (CGM), and carotid arterial compliance (CAC) with Doppler ultrasound (Vivid7) and non-invasive arterial pressure measurement (Finometer), we randomly divided subjects into two groups: MILK and CNT consuming either milk (10 g protein and 9.6 g CHO) + CHO (9 g) or CHO (7 g) alone during 5-month IWT, respectively. After the training, we measured the same variables as before. [Results] In MILK, VO_{2peak} and CAC increased and mean blood glucose decreased with a reduced fluctuation after diet/food intake during the day (all, $P<0.05$), with more methylation of *NFKB2* than in CNT ($P<0.05$); however, none of these occurred in CNT (all, $P>0.06$). [Conclusion] Milk + CHO supplementation during IWT enhanced the improvement of lifestyle-related diseases with enhanced pro-inflammatory gene methylation. COI:No

1PS-03PM-2

Why everyone must exercise: Benefits for the healthy, the specially challenged, and cancer patients

Arakawa Hideki[1], Nakamura Takeshi[1], Tajima Fumihiro[2]

1:Dept of Rehabilitation Medicine, Yokohama City Univ, Yokohama, Japan, 2:Dept of Rehabilitation Medicine, Wakayama Medical Univ, Wakayama, Japan

Exercise is currently advocated for lowering the risk of developing acute and especially chronic diseases. However, the physical activity of daily living is inadequate for maintenance of proper physical fitness. Even healthy individuals need proper exercise. Likewise, the majority of individuals with disabilities complete their rehabilitation programs and return to society; however, physically challenged individuals are more likely to remain in a state of chronic, low-grade inflammation. This condition has been linked to an elevated risk of chronic diseases such as cardiovascular disease, some cancer types, and Type 2 Diabetes. Various exercise and para-sports activities are therefore recommended, especially for wheelchair-bound individuals. The benefits of physical activity to protect against the development and progression of malignant diseases are also well documented and have led to the development of global guidelines on exercise recommendations. Pre-operative exercise in cancer patients is an increasingly common strategy aimed at improving postoperative outcomes, including length of hospital stay, functional capacity and peri-operative complications. It is well known that several neuroendocrine and immunological parameters change after exercise. Exercise also induces changes in the plasma concentrations of various cytokines and activates the entire body; for these reasons, all people should exercise where possible. This session introduces the benefit of exercise and sports. COI:No

1PS-03PM-3

Pannexin-1 in rhino-physiology

Ohbuchi Toyoaki[1], Ueta Yoichi[2], Suzuki Hideaki[1]

1:Dept Otorhinolaryngol-Head and Neck Surgery, Sch Med, Univ Occup Environ Health, 2:Dept Physiol, Sch Med, Univ Occup Environ Health, Kitakyushu, Japan

In airway epithelia, it has been well known that ATP signaling modulates multiple cellular functions such as mucus/ion secretion and mucociliary clearance systems. Pannexin-1 (Panx1) is a second family of gap-junction proteins in vertebrates, and a transmembrane nonselective channel that participate in the release of ATP into the extracellular space. We previously found that the existence of Panx1 in nasal mucosal epithelia in rat and human, and then we hypothesized that various stimuli induce ATP release through the activated Panx1 on the mucosal surface. Panx1 opening is evoked by mechanical stimulation, such as hypotonic stress-induced cell swelling and membrane stretching. In our study, Panx1 blocker inhibited ATP release from human nasal mucosa under hypotonic condition, while other blockers did not, suggesting that the release of ATP from human nasal mucosa may in part be mediated by the Panx1. Current evidence indicates that transient receptor potential (TRP) channel activity involves a relationship between opening of Panx1. We demonstrated that ATP release and ciliary beat frequency (CBF) were significantly potentiated by the heat-sensitive TRPV1 agonist capsaicin, but not by other thermosensitive TRP channels (TRPM8, TRPA1, TRPV1, and TRPV2) agonists in rat. Capsaicin-induced ATP release and CBF increase were significantly inhibited by the Panx1 blockers. Single-cell patch clamping from dissociated rat nasal columnar epithelial cells revealed the TRPV1-Panx1 functional interaction. The Panx1 could be one of the key molecular components in the rhino-physiology. COI:No

1PS-03PM-4

Regulation of swallowing and cough reflexes by sensory stimuli

Ebihara Satoru

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Pneumonia is 3rd leading cause of death in Japan, and the most of patients who died with pneumonia are elderly people. The dysfuvtion of swallowing physiolohy, dysphagia, leads to life-threatening aspiration pneumonia. Since older patients are particularly vulnerable to dysphagia because multiple age-related changes increase the risk of dysphagia, dysphagia is a highly prevalent and growing condition in Japanese people. In order to induce the swallowing reflexes efficiently, sensory inputs to trigger the reflexes are essential. Swallowing reflexes respond to mechanical and chemical stimuli. The combinations of the stimuli is useful to facilitate swallowing refex and should be used in clinical setting. Recent widespread use of food texture and liquid consistency modification as a clinical intervention has created a need to establish clear terminology to describe the target consistencies that are recommended for patients with dysphagia. However, to date, there is no single convention with respect to the terminology used to describe levels of liquid thickening or food texture modification for clinical use, because it is impossible to remove subjectivity in the terminology. Therefore, the levels of liquid thickening or food texture modification should be quantified with an objective device. Here, we are trying to overcome these problems by developing a novel viscometer easily used at caregiving sites. COI:No

Planned Symposium 8

The 9th Annual Meeting and Education Committee of the Physiological Society of Japan

Cooperation between high schools and universities: We can go beyond the fence.

March 28 (Wed) 15:10〜17:00 Hall 10

(No Abstract)

Planned Symposium 9

Joint Symposium with the Japanese Physical Therapy Association

Physiological mechanisms for physical therapy and rehabilitation

March 29 (Thu) 8:30〜10:20 Hall 3

2PS-03AM-1

Cellular mechanisms behind skeletal muscle weakness in rheumatoid arthritis: therapeutic targets for counteracting contractile dysfunction

Yamada Takashi

School of Health Sciences, Sapporo Medical University, Sapporo, Japan

In addition to the primary symptoms arising from inflammatory processes in the joints, muscle weakness is commonly reported by patients with rheumatoid arthritis (RA). A 25-70% reduction in muscular strength has been observed in patients with RA when compared with age-matched healthy controls. The reduction in muscle strength is often larger than what could be explained by the reduction in muscle size in patients with RA, which indicates that intrinsic muscle dysfunction plays an important role in the underlying mechanism of muscle weakness associated with RA. Our previous studies on intact single muscle fibers demonstrate a marked depression in maximal specific force (i.e., force per cross-sectional area) in both fast- and slow-twitch muscle fibers from collagen-induced arthritis (CIA) mice and adjuvant-induced arthritis rats, two widely used animal models for RA. Moreover, these contractile dysfunction was accompanied by a reduction in myofibrillar force production and increased signs of redox modifications in myofibrillar proteins. In this presentation, we highlight the present understanding of RA-associated muscle weakness with special focus on intracellular dysfunction and redox stress, and recent developments of neuromuscular electrical stimulation training as a non-pharmacological intervention. COI:No

2PS-03AM-2

Investigation of basic neurophysiological mechanism for physical therapy by magnetoencephalography

Sugata Hisato

Faculty of Welfare & Health Sci, Oita Univ, Oita, Japan

In the clinical practice of physical therapy, motor imagery and imitation have been widely used as a tool aiding rehabilitation. However, the neural mechanisms of these processes are yet to be fully understood. Gaining a clear understanding of their neural substrates would lead to not only an enhancement of the scientific evidence of physical therapy but also development of new neurorehabilitation practices. Thus, it is important to investigate the basic neurophysiological mechanism for physical therapy using neuroimaging techniques. In this symposium, I would like to introduce the achievements of our magnetoencephalography (MEG) study focused on motor imagery and imitation.Several studies have investigated the relationship between real movement and motor imagery in neural mechanisms. However, the relationship between M1 activity, representing motor information in real movement, and motor imagery has yet to be fully elucidated. We therefore investigated the similarities and differences in M1 activity during real and imagined movements using MEG.Imitation is considered to be a complex process that includes higher-order cognitive and motor functions. This process requires an observation-execution matching system that transforms an observed action into an identical movement. Although recent studies have demonstrated the relationship between the neural substrate of imitation and the mirror neuron system, only a few studies have focused on the mechanisms of imitation from the aspect of neural oscillation. Therefore, we examined oscillatory neural activities associated with imitation. COI:No

2PS-03AM-3

Neural plasticity in motor recovery after brain lesion

Yamamoto Tatsuya[1,2], Murata Yumi[2], Higo Noriyuki[2]

1:Fac Med & Health Sci, Tsukuba Int Univ, Tsuchiura, Japan, 2:Human Informatics Res Inst, AIST, Tsukuba, Japan

Neuromotor systems have the capacity to recover function following local damage. The results of our brain imaging study in macaque monkeys suggested that the ventral premotor cortex (PMv) is involved in functional compensation during the recovery of finger dexterity after lesioning the primary motor cortex (M1), whence the cortico-spinal tract (CST) mainly originates. Here we introduce our data on plastic changes in efferent projections from PMv. We focused on the gene expression of SPP1 (secreted phosphoprotein 1), which is highly abundant in CST neurons in dexterous species (e.g., macaques and humans), and therefore may reflect functional or structural specialization of highly developed CST systems underlying finger dexterity. Better recovery of finger dexterity was observed in monkeys which exhibited higher SPP1-expression in large layer V neurons of PMv after CST lesion. In addition, using an anatomical tracer, we compared the subcortical connections of PMv in the M1-lesioned monkeys with those in the intact animals. Many PMv projections to the subcortical structures such as the deep cerebellar nuclei and red nucleus were observed in lesioned animals, whereas there were few projections to them in intact animals. These results suggest that both qualitative and quantitative changes in PMv projections are involved in functional compensation after lesion of the central nervous system. COI:No

2PS-03AM-4

Central neural mechanisms underlying sympathetic hyperactivity in heart failure

Satoshi Koba

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In heart failure (HF), sympathetic nervous system activity becomes enhanced at rest as well as in response to exercise. Such sympathetic hyperactivity plays a prominent role in disease progression and causes exercise intolerance. Here, central neural mechanisms underlying sympathetic hyperactivity in HF are examined on the basis of experimental data obtained from a rat HF model. Especially, brain regions of which function to regulate sympathetic nervous system becomes abnormal in this disease are discussed as well as causal factors to lead to this dysfunction. Of note, exercise training on the rat model has been shown to normalize central dysfunction, thereby ameliorating sympathetic hyperactivity. In addition, updates on our recent research effort to identify central cardiovascular pathways responsible for abnormal regulation of sympathetic nerve activity in HF are presented. COI:No

Planned Symposium 10

Joint Symposium with the Electrochemical Society of Japan

Opening a new research filed by state-of-the-art electrochemical probes

March 29 (Thu) 8:30〜10:20 Hall 4

2PS-04AM-1

Chemical sensing by using scanning probe microscopy

Takahashi Yasufumi[1,2], Ida Hiroki[3], Zhou Yuanshu[1], Fujii Takuto[4], Sakai Hideki[4], Shiku Hitoshi[5], Fukuma Takeshi[1]

1:Div Elec. Eng. Computer Sci., Kanazawa Univ., Kanazawa, Japan, 2:JST PRESTO, 3:Grad Sch Env, Tohoku Univ., Sendai, Japan., 4:Grad Sch Med Pharm Sci, Univ Toyama, Japan., 5:Grad Sch Eng, Tohoku Univ, Sendai, Japan.

Scanning ion conductance microscopy (SICM) uses a nanopipette for detecting ion current and is an effective tool for non-contact live cell nanoscale topography imaging. The topography image of the sample is effective to set the chemical sensor position around the sample surface for detecting the chemical and eject/collect the chemical from the local region on a single cell. We developed scanning electrochemical microscopy (SECM)-SICM hybrid system to achieve chemical (oxygen, catecholamine, H2O2) and topographical simultaneous imaging. To improve the electrochemical imaging resolution, we have miniaturized the electrode. However, the current response of the nanoscale electrode is too small for detecting the low concentration chemical using conventional current amplifier. To solve the problem, we have developed Pt deposited electrode and FET probe. These probes are effective to detect the uM level of the chemicals.The local cytosol collection is also important technique to reveal the localization of mRNAs at a single cell level. We have developed a hybrid system of SICM and electrochemical syringe to collect the localized mRNA of single living cells. The system uses double-barrel glass nanopipette as a probe. This system successfully detected local differences in Actb mRNA expression levels in single mouse fibroblast cells. High speed SICM is effective to visualize the cell surface dynamic change. COI:No

2PS-04AM-2

Analysis of nanoscale dynamics of the apical membrane morphology in gastric parietal cells using scanning ion conductance microscopy

Fujii Takuto[1], Zhou Yuanshu[2], Shimizu Takahiro[1], Takahashi Yasufumi[2], Sakai Hideki[1]

1:Dept Pharm Physiol, Grad Sch Med Pharm Sci, Univ Toyama, Toyama, Japan, 2:Div Electr Eng & Comput Sci, Kanazawa Univ, Kanazawa, Japan

Gastric parietal cells in the stomach secrete hydrochloric acid (HCl). When the cells are stimulated by secretagogues, intracellular tubulovesicles are fused with each other and connect to the apical membrane. As a result, the morphology of apical interface dynamically changes. So far, morphologies of the cells before and after acid stimulation have been studied using electron microscopy, however, no studies about the apical morphologies and properties in living parietal cells have been done. In this study, we constructed the primary culture of secretagogue-responsive parietal cells from rat gastric mucosa and observed nanoscale structure of the apical surface in the cells using scanning ion-conductance microscopy (SICM). Clear signals of the fluorescence-conjugated H^+,K^+-ATPase antibody could be observed in the living cells without detergent permeabilization, suggesting that the apical membrane rich in H^+,K^+-ATPases is exposed to the extracelluar solution. In SICM analysis, a large microvillus structure formed by apical membranes was visualized in the living parietal cells. After treatment of dibutyryl-cAMP, an acid secretagogue, the apical structures dramatically changed and deep invaginations (μm range) appeared. In summary, we have succeeded to visualize directly the nanoscale dynamics of the apical interface in living parietal cells. COI:No

2PS-04AM-3

Characterization of multicellular spheroids spheroids and embryoid bodies by electrochemical imaging

Shiku Hitoshi

Grad Sch Eng, Tohoku Univ, Sendai, Japan

Bioimaging is a powerful technique to contribute a wide range of research fields including regenerative medicine, environmental science and cell biology. Currently, CCD and CMOS have been used as detectors for fluorescence and chemical luminescence images. Recently, electrochemical imaging device has been a focus of attention because of low cost and miniaturization of the whole microscope apparatus. Here we introduce our recent research topics concerning on the development of electrochemical imaging device and application to the evaluation of multicellular spheroids and embryoid bodies (EBs).

Electrochemical imaging tools allow non-invasive characterization of mammalian embryos and EBs to measure local oxygen concentration and alkaline phosphatase (ALP) activity. In the present study, electrochemical devices have been applied for non-invasive characterization of mouse EBs to measure local oxygen concentration and ALP activity. An addressable electrode array device with interdigitated array (IDA) of 256 to 1,024 points was adopted to visualize ALP activity of mouse EBs. Generally, ALP is accepted as a marker of the pluripotency of the ES cells. We also accomplished non-invasive electrochemical ALP assay in the culture medium at neutral pH condition. Non-invasive respiratory and ALP measurements of the individual mEBs were applicable for selecting the more differentiated cell aggregates, which allowed for further cultivation and successful induction of differentiation.

[1] K Ino et al. Angew Chem Int Ed 51, 6648-6652, 2012; ibid 56, 6818-6822, 2017. COI:No

2PS-04AM-4

A microsensing system for the *in vivo* real-time detection of local kinetics of drug and its physiological relevance.

Ogata Genki[1,2], Asai Kai[3], Sano Yamato[4], Takai Madoka[5], Kusuhara Hiroyuki[4], Einaga Yasuaki[3,6], Hibino Hiroshi[1,2]

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Drugs are effective tools to study physiological phenomena in live animals. The underlying mechanisms will be markedly clarified if local behaviors of systemically administered drugs and their actions *in vivo* could be simultaneously measured. However, conventional methods require considerable samples quantities and have poor sampling rates. Additionally, they cannot address how drug kinetics correlates with target function over time. Here, we developed a system with two different sensors. One is needle-type of boron-doped diamond microsensor with tip diameter ~40 μm, and the other is a glass microelectrode. We first tested an Na^+,K^+,$2Cl^-$-cotransporter blocker, which serves a diuretic but can induce deafness. In the guinea-pig cochlea, changes of bumetanide concentration and the extracellular potential underlying hearing were simultaneously measured in real time. In the rat brain, the kinetics of an antiepileptic drug, which inhibits Na^+ channel, was tracked at the same time as the local field potentials. The actions of other drug type was also monitored *in vivo*. This micrsosensing system will contribute to advances in life science. COI:No

Planned Symposium 8

The Molecular Basis for Plasticity and Dysregulation of the Neural Stem Cells

March 29 (Thu) 8:30〜10:20 Hall 5

2PS-05AM-1

Epigenetic research on the nervous systems and its transition

Nakashima Kinichi

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Neurons, astrocyte and oligodendrocytes are generated from common multipotent neural stem cells (NSCs). In addition to cell-extrinsic cues, proliferation and differentiation of NSCs are regulated by the cell-intrinsic program so called epigenetics. A literature survey in PubMed using "epigenetic" and "brain" as keywords revealed that manuscripts with these keywords started to appear in the late 1970's, however, it seems that epigenetic research in the nervous system was accelerated in the late 1990's. Since then, epigenetic regulations of NSCs have been extensively studied. In the late 2000's, the advent of next generation sequencer pushed forward the epigenetic research in neuroscience field, allowing us to perform comprehensive analyses at the expense of candidate gene approaches. Reprogramming of cell status is exactly the same meaning as reprogramming of the epigenetic status in the cells. By using reprogramming techniques, it has become possible to reprogram somatic cells directory into desired neural cells without passing through iPS cells, enabling us to investigate causes and treatments for diseases much effectively. When we use epigenetic modification enzyme inhibitors, the epigenetic changes are introduced genome widely so that they may induce the expression of undesirable genes, causing adverse effects. However, it has been recently reported that region specific changes of epigenetic modifications can be introduced by using CRISPR/Cas9 systems. In this symposium, I would like to overview such transition of epigenetic research on the nervous systems by introducing our relevant researches. COI:No

2PS-05AM-2

Lineage-restricted dedifferentiation of neurons by transient expression of YAP-TAZ

Fujimura Atsushi

Dept Physiol, Grad Sch Med, Dent, Pharm, Okayama Univ, Okayama, Japan

Neurons were once believed to sustain the differentiated state firmly and avoid the spontaneous dedifferentiation, but recently shown to be dedifferentiated by several signals including Hippo pathway or oncogenic signals. We revealed that the Hippo transducers YAP and TAZ played the pivotal roles in this process. By transient expression of YAP or TAZ, even matured neurons can be dedifferentiated into neural stem cells. We named these cells yNSC after YAP-induced NSC. Interestingly, this process is restricted to the lineage-specific dedifferentiation. Exogenous YAP or TAZ can induce the endogenous YAP and TAZ expression, and confers the self-renewal capacity and differentiation potential to multi-lineages on the matured neurons. These findings suggest that YAP and TAZ alter the cell fate possibly by epigenetic control or maybe by chromatin remodeling. In this session, we will introduce the roles of YAP and TAZ in regulating the cellular plasticity and cell fate. COI:No

2PS-05AM-3

The function of brain turmo stem cell

Michiue Hiroyuki[1,2], Fujimura Atsushi[2], Matsushita Hiroaki[2], Nishiki Tei-ichi[2], Matsui Hideki[2]

1:Neutron Therapy Research Center, Okayama Univ, Okayama, Japan, 2:Dept Physiol, Grad Sch Med, Okayama Univ, Okayama ,Japan

A cancer stem cell (CSC) hypothesis that huge amount of tumor cells are driven by a few CSC is proposed in many malignant tumors. CSC is the top of tumor cell hierarchy that has high tumorigenesis, self-renewal and multipotent. In 1997, CSC was discovered in acute myeloid leukemia (AML) which is characterized as phenotypic, genotypic and clinical heterogeneity. In solid cancer, breast cancer stem cell were first identified in 2003 and GBM, the existence of glioma initiating cell (GIC) was reported in 2004. A membrane glycoprotein CD133, which is a normal neural stem cell (NSC) marker is used to identify GIC. Brain tumor stem cell (BTSC) was identified and maintained in NSC culture conditions. At first, it seemed that the difference of NSC and BTSC was the point of tumorigenesis, because both stem cell could become differentiated into neuron, astrocyte and oligodendrocyte. Recently, BTSC showed tumor specific cell differentiation, for example, neuron, pericyte and endothelial cells for tumor specific growth and survival. In this time, we will show that glioblastoma demonstrates molecular heterogeneity and contains a population of BTSC that contributes to tumor propagation, maintenance, and treatment resistance. COI:No

2PS-05AM-4

Modelling human neurodevelopment and neurological diseases using human pluripotent stem cells

Okano Hideyuki, Imaizumi Kent

Department of Physiology, Keio University School of Medicine, Tokyo, Japan

The mammalian, including human, central nervous system (CNS) contains many diverse neuronal subtypes, and various neurological diseases target specific subtypes. However, the underlying mechanism of neuronal subtype specificity of neurological disease phenotypes remains mostly remains unknown. On the other hand, in our previous study, we developed a culture system to control the regional identity of human pluripotent stem cell (hPSC)-derived neurons along the anteroposterior (A-P) and dorsoventral (D-V) axes (Imaizumi et al., Stem cell Reports, 2015). Here, by modifying this method, we developed a novel method to induce subtypes of human cerebral cortex neurons in a region-specific manner. During mammalian neural development, it is known that the rostrocaudal (R-C) gradient of FGF8 signaling defines this areal identity. In the present study, we examined to recapitulate the cortical R-C patterning in hPSC cultures. We found that expression of the R-C markers of human cortical neurons was appropriately regulated by the gradient of FGF8 signaling activity in vitro, and that their global gene expression patterns resembled those of the corresponding areas of human fetal brains in vivo. Furthermore, this culture system can be used in presumptively modeling upper motor neuron (UMN) phenotype of subtype of familial amyotrophic lateral sclerosis (e.g. ALS-2). Based on these, that our present culture system will contribute to modelling human neurodevelopment and neurological diseases. COI:Properly Declared

Planned Symposium 9

Committee for Young Physiologists

Making ideal research environment -Lab launching and knowledge management-

March 29 (Thu) 8:30〜10:20 Hall 6

2PS-06AM-1

My experiences of difficulties in launching a laboratory

Wada Makoto

Dev Disorders Sect, Dept Brain Rehab, Res Inst of NRCD, Tokorozawa, Japan

For good leadership, intellectual ability, physical ability, persuasion skills, self-management skills, and an unwavering will are required. The needs for ideal principal investigators (PI) are almost the same. To construct rational research plans, intellectual mindset and clarity of thoughts are of course very important. To conduct research projects, physical ability and health are also essential abilities especially in fields of life science. Without being persuasive, getting generous research budgets and human resource of choice are almost impossible. In addition, self-management skills toward proper research ethics prevent temptations to misconducts. Finally, a steadfast motivation and clear vision of one's research goals are very important to stay on track. Those who want to become PIs are required to have such skills to some extents. Unfortunately, almost all persons including me don't have perfect skills for them. Only Julius Caesar, the ancient roman hero might have the right balance of all these abilities. However, Octavianus, who was not as much charismatic as compared to Caesar actually achieved the construction of the Roman Empire by assembling small pieces of powers step by step. The process is helpful for launching laboratory as a PI de fact. When I became a section chief, I had neither any competitive funding nor human resource. With generous assistance from supervisors, I eventually received several grants, human resources and experimental facilities after struggling for several years. I hope sharing my experiences will promote discussions towards making ideal laboratories headed by young researchers. COI:No

2PS-06AM-2

How I enjoy my laboratory life

Murayama Masanori

Lab for Behavioral Neurophysiology, Brain Science Institute RIKEN, Wako, Japan

"All work and no play makes Masa a dull PI" (modified from the movie, *The Shining*). Most researchers live in small spaces; they have inadequate human relations and face severe pressure to secure grants and publish papers, while juggling numerous trivial duties. Therefore, how do they survive such a laboratory life? Although, I do not have an answer to this question, here, I discuss the attitude of believing "Hey! I am so happy in this laboratory!" COI:No

2PS-06AM-3

Knowledge Management for Start-Up Laboratories

Umemoto Katsuhiro

Sch Knowledge Sci, JAIST, Ishikawa, Japan

This short lecture targets mainly at young principal investigators (PIs) or soon-to-be PIs, who are or will soon be managing their own start-up laboratories. The main topic is knowledge management (hereinafter KM), i.e., theory and practice about how knowledge is created, shared, and utilized in such various settings as business organizations, national governments, scientific laboratories, etc.

The new interdiscipline and the management paradigm for the knowledge society in the 21st century was originated by a Japanese scholar, Dr. Ikujiro Nonaka, professor emeritus at Hitotsubashi University and a member of the Japan Academy (Nippon Gakushi-in). He is called "the father of KM" and was ranked as one of top-20-management thinkers by the Wall Street Journal. The KM movement started from his best and long seller book entitled *The Knowledge-Creating Company*, which was co-authored with his former colleague Dr. Hirotaka Takeuchi now professor at the Harvard Business School and published by Oxford University Press in 1995.

Under the main topic, such sub-topics as the following will be discussed: management strategy, process, and leadership for managing scientific laboratories. As for the management strategy, the "emergent strategy" which is flexible against uncertainty, will be explained. As for the management process, the "middle-up-down management," which a mix of top-down and bottom-up managements, will be argued. And as for the management leadership, "phronetic leadership," which is practical and ethical, will be discussed. Finally, an incipient theory of "dynamic duality" will be introduced. COI:No

Planned Symposium 10

Fascination of “D-allulose”; a rare sugar which heals us physically and spiritually

March 29 (Thu) 8:30～10:20 Hall 9

2PS-09AM-1

Naturally Occurring Rare Sugars are Free Radical Scavengers and Can Ameliorate Endoplasmic Reticulum Stress

Mooradian D Arshag[1], Haas J Michael[1], Onstaed-Haas Luisa[1], Tani Yuma[2], Iida Tetsuo[2,3], Tokuda Masaaki[3]

1:Department of Medicine; University of Florida College of Medicine; Jacksonville, FL, USA, 2:Matsutani Chemical Industry, CO., LTD, Hyogo, Japan, 3:Department of Cell Physiology, Faculty of Medicine, Kagawa University, Kagawa, Japan

The effect of naturally occurring rare sugars on free radicals and ER stress was examined in human coronary artery endothelial cells. SO generation was measured using the superoxide-reactive probe 2-methyl-6-(4-methoxyphenyl)-3,7-dihydroimidazo[1,2-A]pyrazin-3-one hydrochloride while phycoerythrin fluorescence based assay was used to monitor scavenging activity of sugars in the presence of hydroxyl or peroxyl radical generators [CuSO4 and azobis (2 amidinopropane) hydrochloride respectively]. ER stress was measured with secreted alkaline phosphatase (SAP) assay and by Western blot analysis of the expression and phosphorylation of key proteins in the unfolded protein response, namely CHOP47, eIF2α and JNK1. D-Glucose (27.5 mM) increased SO generation and decreased SAP secretion indicating ER stress. Treatment of cells with 5.5 or 27.5 mM of D-allulose, D-allose, D-sorbose and D-tagatose reduced SO generation. In a cell free system, all four rare sugars had significantly more SO, hydroxyl and peroxyl radical scavenging activity compared to dextrose. Treatment of cells with rare sugars reduced ER stress. However, unlike other three rare sugars, D-sorbose did not inhibit tunicamycin-induced eIF2α phosphorylation. Conclusion: Naturally occurring rare sugars are free radical scavengers and prevent ER stress in a sugar specific mechanism. COI:Properly Declared

2PS-09AM-2

Rare sugars: natural zero calorie sweeteners in relation to obesity and diabetes to change our life style

Hossain Akram, Yamaguchi Fuminori, Kamitori Kazuyo, Dong Youyi, Tsukamoto Ikuko, Iida Tetsuo, Tokuda Masaaki

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The present world is consequently at a critical point in obesity and diabetic issue. It was mentioned that while physical activity is a key part staving off diabetes, heart disease and dementia, where unhealthy eating with excess sugar and carbohydrate is the key point of being obese. We cannot avoid taking sugar but it is possible to cut down sugar-related energy intake keeping the taste and sweetness of foodstuff unaltered by taking rare sugars D-allulose. We found excellent outcome of D-allulose in controlling body fat and maintaining blood sugar levels. We fed 5% D-allulose to OLETF rats for 60 weeks and body weight, food intake, water intake, blood glucose, serum insulin, body fat were measured. Oral glucose tolerance test was performed. On sacrifice liver, pancreas and other organs were preserved and stained. D-allulose controlled abdominal fat accumulation and thus prevented excess body weight. D-allulose increased insulin resistance through maintenance of fasting, random and OGTT blood sugar and serum insulin levels. Immunostaining of the pancreas showed attenuation of progressive fibrosis with the preservation of insulin producing beta cells. Serum levels of proinflammatory and antiinflammatory cytokines were also controlled well by D-allulose. Rare sugar D-allulose might be a promising strategy for the prevention of obesity and the commencement and prevention of type 2 diabetes. COI:No

2PS-09AM-3

D-Alluose and Lipid Metabolism

Nagata Yasuo

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Fructose has been used as a sweetener and has been reported to be lipogenic and related to the prevalence of obesity worldwide. Therefore, it is important to develop new type of sweetener to substitute fructose. However, D-allulose, one of rare sugars, may be lipogenic since D-allulose is an epimer of fructose. Studies with animals have shown that D-allulose reduces abdominal fat mass. Thus, we investigated the mechanism of lipid-lowering action of D-allulose. In rats fed the 3% D-allulose diet, body weight gain was lower. D-Allulose reduced insulin levels without affecting blood glucose levels. Given that insulin has a regulatory role in glucose and lipid metabolism, the underlying mechanism may be partially through insulin action. Activity of lipogenic enzymes was reduced by D-allulose while that of fatty acid oxidation was enhanced. Feeding D-allulose increased fat oxidation and lowered carbohydrate oxidation, resulting in enhanced 24 h energy expenditure. Anti-obese action has also been reported in diabetic rats and mice.
It is thus likely that suppressive effect of fat accumulation by D-allulose is induced partly through decreased lipogenesis, increased fatty acid oxidation and enhanced 24 h energy expenditure. Unlike fructose, D-allulose is expected to be a new sweetener to maintain and control body weight. COI:No

2PS-09AM-4

Rare sugar D-allulose ameliorates arrhythmic-overeating, obesity and diabetes via GLP-1 and vagal afferents

Iwasaki Yusaku, Yada Toshihiko

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A rare sugar D-Allulose (Allu), zero-calorie sweetener, has been shown to exert anti-obesity and anti-diabetes effects. However, underlying mechanism is unknown. This study examined the effects of Allu on the endocrine and neural systems implicated in obesity and diabetes. Single peroral (po) administration of Allu increased plasma glucagon-like peptide-1 (GLP-1) level, activated vagal afferent neurons, suppressed food intake, and promoted glucose tolerance in normal, diet-induced obese and db/db diabetic mice. Mechanistic analysis revealed increases in insulin secretion and insulin sensitivity, and inhibition of hepatic glucose production. Subchronic Allu administered at the onset of light period (LP), ameliorated LP-specific hyperphagia, visceral obesity and glucose intolerance. These effects were blunted by GLP-1 receptor (GLP-1R) antagonist and in GLP-1R knockout mice, and by vagotomy. These results identify Allu as a novel GLP-1 releaser acting via vagal afferents to restrict feeding and glycemia. When long-termly administered, it counteracts hyperphasia, obesity and glucose intolerance. Allu intake may provide a promising tool for normalizing life-style related functions such as feeding rhythm and for ameliorating obesity and diabetes. COI:Properly Declared

Planned Symposium 11

Novel regulation of Ca^{2+} signaling in physiology

March 29 (Thu) 10:30〜12:20 Hall 2

2PS-02AM-1
Identification of novel targets of S100 proteins and consequent physiological phenomena

Yamaguchi Fuminori[1], Tokumitsu Hiroshi[2], Tokuda Masaaki[1]

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S100 proteins are a large family of EF-hand calcium ion binding proteins and more than 25 members are found in humans. Ca^{2+} stimuli trigger a conformational change and allow interaction with target proteins. S100 proteins regulate many physiological functions such as cell growth, differentiation, and cell cycle regulation by interacting with target proteins including enzymes, receptors, and transcription factors. Recently, we found that S100 proteins bind to some repeat containing proteins such as tetratrico peptide repeat (TPR) proteins. Protein phosphatase 5 is a member of the phosphoprotein phosphatase (PPP) family of serine/threonine phosphatases containing a C-terminal catalytic domain and three N-terminal TPR motifs. The TPR motif consists of a sequence of 34 amino acids, and 3 to 16 copies of the motif are arranged in tandem in the proteins. S100 proteins interact with the TPR domains of PP5 in a Ca^{2+}-dependent manner and significantly activate its phosphatase activity resulting in the regulation of tau and ASK1 dephosphorylation. S100 proteins also regulate TPR-containing FKBP38 immunophilin and U-box E3 ubiquitin ligase CHIP functions. We previously reported that Ca^{2+}/S100 proteins directly associate with other TPR proteins, such as Hsp70/Hsp90-organizing protein (Hop), kinesin light chain, Tom70, FKBP52, and CyP40. Based on the works, we have postulated the existence of an S100-TPR pathway in which S100 proteins are Ca^{2+}-dependent regulators of the novel physiological phenomena. COI:No

2PS-02AM-2
Molecular mechanism of calmodulin-kinase cascade regulated by autophosphorylation and feedback phosphorylation

Tokumitsu Hiroshi

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The enzymatic activity of the Ca^{2+}/calmodulin-dependent protein kinase (CaMK) family member, CaMK kinase (CaMKK) is enhanced by binding with a Ca^{2+}/CaM complex. Accumulated evidence has indicated that CaMKKβ/5'-AMP-activated protein kinase (AMPK) phosphorylation cascade affects various Ca^{2+}-dependent metabolic regulation and cancer growth. Unlike recombinant CaMKKβ that exhibits higher basal activity (autonomous activity), activation of the CaMKKβ/AMPK signaling pathway requires increased intracellular Ca^{2+} concentrations. Here, we demonstrate feedback phosphorylation of CaMKKβ at multiple residues by activated AMPK in addition to autophosphorylation *in vitro*, leading to reduced autonomous, but not Ca^{2+}/CaM-activated, CaMKKβ activity. MS analysis and subsequent site-directed mutagenesis of AMPK phosphorylation sites in CaMKKβ clearly demonstrated that Thr144 phosphorylation by activated AMPK converts CaMKKβ into a Ca^{2+}/CaM-dependent enzyme, consistent with the completely Ca^{2+}/CaM-dependent CaMKK activity of the phospho-mimetic Thr144Glu mutant of CaMKKβ. Furthermore, immunoblot analysis using anti-phospho-Thr144 antibody revealed that the phosphorylation of Thr144 in CaMKKβ was observed in living cells that was further enhanced by exogenous expression of AMPKα. These results indicate that the feedback phosphorylation of CaMKKβ by AMPK regulates the CaMKKβ/AMPK signaling cascade and may be physiologically important for intracellular maintenance of Ca^{2+}-dependent AMPK activation. COI:No

2PS-02AM-3
IRBIT, an integrative regulator of intracellular Ca^{2+} signals and pH environment

Mizutani Akihiro[1], Kawa-ai Katsuhiro[2], Ando Hideaki[2], Yamada Hideomi[3], Seki George[3], Mikoshiba Katsuhiko[2]

1:Dept. Pharmacotherap. Showa Pharmaceutical Univ. Machida, Japan, 2:Lab. for Developmental Neurobiology, BSI, RIKEN, Wako, Japan, 3:Div. of Nephrology and Endocrinology, The Univ. of Tokyo, Tokyo, Japan

IRBIT was first discovered as a binding protein of IP_3 receptors (IP_3Rs). The binding is competitive with IP_3 and does not induce IP_3Rs channel open, indicating that IRBIT functions as a proteinous pseudo-ligand for IP_3Rs. Subsequently, IRBIT was also found to bind to NBCe1, an electrogenic Na^+/HCO_3^- co-transporter, and the binding to NBCe1 drastically enhances the transport activity. Thus, IRBIT is a dual regulator of both intracellular Ca^{2+} signaling and intracellular pH dynamics.Interestingly, both bindings of IRBIT are dependent on its multi-site phosphorylation, which occurs on the characteristic Ser/Thr rich region. Among them, phosphorylations on Ser68, Ser71, Ser74, and Ser77 are essential for binding to the both partners, however, additional Ser/Thr phosphorylations flanking to the essential four Sers are necessary to generate high affinity binding form of IRBIT, and the phosphorylation pattern preferable to each partner is distinct. Considering that Ser/Thr rich region contains putative phosphorylation sites for many classes of protein kinases, including PKA, CaMKII, CaMKIV, AMPK, ATM kinase, CKI, CKII, ERK etc. and that IRBIT phosphorylations indeed take place upon various stimuli, IRBIT may play a role in transducing the intracellular environments into modulation of both intracellular Ca^{2+} signaling and pH dynamics through its "phosphorylation code". COI:No

2PS-02AM-4
Redox dependent regulation of CaM-pedendent enzymes and its physiological significance

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Responses to increased intracellular Ca^{2+} are often mediated by the Ca^{2+}-binding protein calmodulin (CaM). The actions of Ca^{2+}/CaM are mediated by its association with specific target proteins, some of which are known as CaM-binding proteins, which include kinases such as CaM kinases (CaMKs) and a metabolic enzyme of sulfur-containing amino acids as cystathionine γ-lyase (CSE). We have previously reported that cysteine hydropersulfide (CysSSH) was generated by CSE, which in turn may contribute to other CysSSH derivatives of peptides/proteins, involving in their catalytic activities (PNAS, 2014). Recently, we reported a novel signaling function for intracellular CysSSH in inhibiting CaMKIV activity via S-polysulfidation of its Cys198 during the response to endoplasmic reticulum stress (Biochem. J, 2017). Here, we demonstrate that CaMKI, CaMKII, and CSE are candidates of CysSSH derivatives. In vitro incubation of these enzymes either with CysSSH generated by CSE or Na_2S_4, a reactive sulfur donor, resulted in a dose-dependent inactivation of each enzyme activity. Dithiothreitol, a small molecule reducing reagent, restored the each enzyme activity. Interestingly, the enzyme activities of CysSSH-insensitive CSE mutant were higher than that of wild-type, CysSSH has been proposed to auto-inhibit CSE enzyme. On the basis of the above results, we propose that CysSSH regulates CaM-binding proteins via their specific cysteine residues modification. COI:No

Planned Symposium 12

Australia-Japan Joint Symposium (-Towards FAOPS2019-)

Recent advances in physiology/pathophysiology of Ca dynamics and signaling in skeletal, cardiac and smooth muscles

March 29 (Thu) 16:10～18:00 Hall 1

2PS-01PM-1

Divergent effects of disease-linked mutations in RyR2 on Ca^{2+} dynamics in cardiac and non-cardiac cells

Kurebayashi Nagomi

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Type 2 ryanodine receptor (RyR2) is the Ca^{2+} release channel in cardiac muscle. Mutations in RyR2 have been implicated in various arrhythmogenic disorders including catecholaminergic polymorphic ventricular tachycardia (CPVT), idiopathic ventricular fibrillation (IVF), long QT syndrome (LQTS), etc. We aimed to characterize mutant RyR2s carrying CPVT, IVF and LQTS mutations using HEK293 cells and HL-1 cell, a cardiac-derived cell line. Wild type (WT) and mutant RyR2s were expressed in HEK293 cells and spontaneous Ca^{2+} oscillations were monitored using G-GECO1.1 and R-CEPIA1er for cytoplasm and ER Ca^{2+} ($[Ca^{2+}]_{ER}$), respectively. In addition, the Ca^{2+}-induced Ca^{2+} release (CICR) activity was determined with $[^3H]$ryanodine binding assay. CPVT mutants exhibited gain-of-function (GOF) phenotype; they showed more frequent cytoplasmic Ca^{2+} oscillations with reduced $[Ca^{2+}]_{ER}$ and enhanced CICR activity compared to WT. On the other hand, IVF and LQTS mutants showed divergent CICR profiles: some were loss-of-function (LOF) and others were GOF type mutants. The LOF mutants exhibited no Ca^{2+} oscillation and increased $[Ca^{2+}]_{ER}$ in HEK293 cells. In HL-1 cells which have intrinsic WT RyR2, additional expression of exogenous GOF mutants induced frequent Ca^{2+} waves during action potential (AP)-induced Ca^{2+} transients. Most of LOF mutants exert no Ca^{2+} waves and just reduced amplitudes of AP-induced Ca^{2+} transients. Two of LOF mutants, however, evoked frequent Ca^{2+} waves. The characteristics of heterotetramer RyR2 channels composed of WT and mutant monomers remain to be clarified. COI:No

2PS-01PM-2

Linking Ca^{2+} dynamics with morphological aspects to identify smooth muscle pacemaker cells

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1:Dept Cell Physiol, Grad Sch Med Sci, Nagoya City Univ, Nagoya, Japan, 2:Dept Physiol, Monash Univ, Melbourne, Australia

Since the periodical Ca^{2+} release and uptake by sarco-endoplasmic reticulum (the cytosolic Ca^{2+} oscillator) appears to be a ubiquitous mechanism underlying smooth muscle pacemaking, linking intracellular Ca^{2+} dynamics with morphological techniques is fundamental to understand spontaneous activity of smooth muscle. Capillary pericytes expressing NG2-DsRed florescence develop spontaneous Ca^{2+} transients. They employ Ca^{2+}-activated chloride channels to maintain their synchrony and to drive other pericytes in adjacent microvascular units by sending depolarising signals via gap junctions. NG2(+) arteriolar pericytes and NG2(-) venular pericytes also generate synchronous Ca^{2+} transients relying on L-type Ca^{2+} channels. Capillary pericytes may function as pacemaker cells to drive upstream arterioles as well as downstream venules. In the renal pelvis, atypical smooth muscle cells (ATSMCs) exhibit spontaneous Ca^{2+} transients that appear to trigger intercellular Ca^{2+} waves in typical smooth muscle cells (TSMCs), but their specific marker has not yet been identified. Focused ion beam/scanning electron microscopy and subsequent 3D reconstruction of cell structures in the region where ATSMC Ca^{2+} transients are generated revealed the morphology of ATSMCs and their connections with neighbouring cells. The combination with Ca^{2+} imaging, electrophysiology and fluorescent immunohistochemistry, has convincingly recognised ATSMCs as the pacemaker cells that drive their neighbouring TSMC contractions underlying pyeloureteric peristalsis. COI:No

2PS-01PM-3

Ca^{2+}-sensing receptor and PDGF signals in pulmonary arterial hypertension

Yamamura Aya, Sato Motohiko

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Pulmonary arterial hypertension (PAH) is a rare and progressive disease of unknown pathogenesis. Sustained vasoconstriction and vascular remodeling are key pathogenic events that lead to early morbidity and mortality. These events have been linked to cytosolic Ca^{2+} handling in pulmonary arterial smooth muscle cells (PASMCs). We have previously shown that Ca^{2+}-sensing receptor (CaSR) is upregulated in PASMCs from idiopathic pulmonary arterial hypertension (IPAH) patients and contributes to enhanced Ca^{2+} response and augmented cell proliferation. Here, we examined to elucidate the upregulation mechanism of CaSR in IPAH-PASMCs. We focused an enhanced endogenous signal associated with PAH, platelet-derived growth factor (PDGF), in this study. In normal-PASMCs, expression of CaSR was increased by the exposure to PDGF. Expression of PDGFα receptors in IPAH-PASMCs was identical to that in normal-PASMCs, while expression of PDGFβ receptors in IPAH-PASMCs was higher than that in normal-PASMCs. In addition, phosphorylation of PDGFβ receptors by PDGF stimulation in IPAH-PASMCs was long-lasting compared to in normal-PASMCs. Phosphorylation levels of ERK and Akt, which are downstream pathways of PDGF receptors, were also higher in IPAH-PASMCs. Furthermore, in normal-PASMCs, PDGF stimulation caused enhanced cell proliferation and migration. These results suggest that the PDGF signal activates the upregulation mechanism of CaSR in IPAH-PASMCs. In conclusion, the crosslink between CaSR and PDGF signals is a novel pathogenic mechanism contributing to the augmented Ca^{2+} influx and excessive cell proliferation in PAH. COI:No

2PS-01PM-4

Ca^{2+} handling in human skeletal muscle with ryanodine receptor and orai1 variants

Launikonis S Bradley

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Ca^{2+} release through the ryanodine receptor (RyR) is required for the contraction of skeletal muscle. While the muscle is at rest, the RyRs leak Ca^{2+}, which is continuously resequestered by the sarcoplasmic reticulum (SR) Ca^{2+} pump. Variants in skeletal muscle RyRs can cause susceptibility to malignant hyperthermia (MH), a potentially lethal condition that is triggered by volatile anaesthetics to cause uncontrolled Ca^{2+} release leading to excess heat generation via SR Ca^{2+} pump stimulation. However, people who are MH susceptible do not show any muscle pathology in the absence of triggering agents. We wished to examine the effect of RyRs variants on the Ca^{2+} handling at the SR and tubular (t-) system of human muscle. To do this we obtained human muscle from needle biopsies. A Ca^{2+}-sensitive dye was trapped in the t-system of skinned fibres (Cully et al 2017, *Nat Commun*) to provide a novel nano-domain sensor of RyR and t-system Ca^{2+} handling ability. Human MH susceptible fibres were found to have a higher RyR Ca^{2+} leak than non-susceptible fibres, chronically active store-operated Ca^{2+} entry (SOCE) and a greater capacity to extrude Ca^{2+} across the t-system. We also applied our novel techniques to human muscle with Orai1 mutation, the Ca^{2+} channel of SOCE. A depressed steady state of t-system $[Ca^{2+}]$, consistent with constitutively active SOCE was observed. COI:No

Planned Symposium 13

[In association with JSPS Scientific Research (S): Mechanomedicine AMED-CREST/PRIME: Mechanobiology]

Mechanomedicine

March 29 (Thu) 16:10～18:00 Hall 2

2PS-02PM-1

Mechanomedicine

Naruse Keiji

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Introduction of Mechanomedicine COI:Properly Declared

2PS-02PM-2

Mechano-signal pathway regulating tendon and ligament via Mkx

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1:Dept Systems BioMed, TMDU, Tokyo, Japan, 2:CREST, AMED, 3:Dept Molecular Med, The Scripps Research Institute

Despite the unique and important role of tendons/ligaments in musculoskeletal function and disease, research in this area is not as advanced as in most other skeletal tissues. This is partly because critical and specific transcription factors had not been elucidated. In this regard, we identified Mohawk (Mkx) as a specific and critical transcription factor for tendon and ligament development. Mkx is expressed specifically in tendon and ligament cells during embryogenesis and Mkx-deficient mice have hypoplastic tendons throughout the body and deficient type I collagen production in tendon cells. Importantly, we have also found that Mkx expression is maintained in mature tendon/ligament cells in mice and human and demonstrated that MKX expression is clearly decreased in ligaments from patients with OA, correlating deficient expression of important ECM genes such as COL1A1 and TNXB. These findings may reflect a feature of degenerated ACL in OA-affected joints. From another aspect, we showed that MKX expression enhances tendon/ligament differentiation from mesenchymal stem cells, suggesting that MKX could be a tool for tendon/ligament tissue regenerative medicine. Taken together, MKX has been shown to be necessary not only for development but also to maintain the tissue homeostasis and regeneration in adult human tendons and ligaments. COI:No

2PS-02PM-3

Bone homeostasis and Mechanomedicine

Nakashima Tomoki

Dep Cell Signaling, TMDU, Tokyo, Japan

The weight-bearing exercises help to build bones and to maintain them strength. Bone is constantly renewed by the balanced action of osteoblastic bone formation and osteoclastic bone resorption both of which mainly occur at the bone surface. This restructuring process called "bone remodeling" is important not only for normal bone mass and strength, but also for mineral homeostasis. Bone remodeling is stringently regulated by communication between bone component cells such as osteoclasts, osteoblasts and osteocytes. An imbalance of this process is often linked to various bone diseases. During bone remodeling, resorption by osteoclasts precedes bone formation by osteoblasts. Based on the osteocyte location within the bone matrix and the cellular morphology, it is proposed that osteocytes potentially contribute to the regulation of bone remodeling in response to mechanical and endocrine stimuli. These findings provide a scientific basis for future therapeutic approaches to bone diseases. COI:No

2PS-02PM-4

Importance of mechanopathology that sees cancer from the perspective of stroma diversity and stiffness

Enomoto Atsushi

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One of the outstanding features of deadly cancers such as pancreatic cancer is the proliferation of cancer-associated fibroblasts (CAFs) in the stroma. A number of previous studies have shown that the stroma formation leads to stiffening of cancer tissue, which makes cancer cells more aggressive and malignant to easily spread out to adjacent tissues and metastasize to other organs. Another major role of the cancer stroma is the modulation of immune response against cancer cells and tumor angiogenesis. Recent emerging studies, however, have pointed out a possibility that there are diverse populations of CAFs, where some of them have a function to restrain, but not promote, cancer progression. We have recently been interested in the identification of markers or functional proteins that define the characteristics of those diverse CAFs, and found that different CAFs have different functions to regulate the stiffness of the stroma and the deposition of the extracellular matrix, which eventually controls cancer progression and differentiation. In the symposium, we will also discuss the clinical relevance of those findings in the context of pancreatic cancer, which we hope will help us understand the mechanoproperties of cancer and develop new approaches to treat cancer patients. COI:No

2PS-02PM-5

Mechanomedicine of the lung

Ito Satoru

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Pulmonary fibrosis is a pathological feature of dysregulated wound healing after lung inflammation or damage such as interstitial pneumonia and acute respiratory distress syndrome. Fibrotic tissue in the lung is much stiffer than normal lung tissue. Importantly, mechanical cues such as stretch, shear stress and matrix stiffness are considered to be involved in the mechanisms underlying the pathogenesis of pulmonary fibrosis. We have investigated whether mechanical stimuli affect cellular properties of human lung fibroblasts. First, effects of mechanical stress on cellular release of ATP, known as one of danger-associated molecular patterns, were tested. We visualized ATP release during cell stretch in real time by high-sensitivity luciferin-luciferase reaction using a luminescence imaging system while acquiring differential interference contrast cell images with infrared optics (Furuya K, et al., *Medhots* 2014;66:330-44). A single stretch and stretching the plasma membrane by hypotonic solution induced ATP release. In contrast to mechanical stimuli, application of PDGF caused less ATP release from small numbers of the cells. Next, fibroblasts were cultured on different stiffness of polyacrylamide hydrogels ranging from 1 to 50kPa. As substrate stiffness increases, cells spread with stress fiber formation. PDGF-induced cell migration was significantly enhanced by substrate stiffness. Protein expression of α-smooth muscle actin (SMA) was significantly higher on 25kPa substrates than that on 2kPa. Transfection of siRNA for α-SMA inhibited cell migration. Our findings indicate that mechanical cues activate lung fibroblasts, leading to further inflammation and fibrosis of the lung. COI:No

2PS-02PM-6

Urgent problems to be solved in mechanobiology

Sokabe Masahiro

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People have long known that mechanical forces exert profound effects on our body. Space flights cause muscle atrophy and osteoporosis, while weight training leads to reverse results. However, the underlying cellular and molecular mechanisms remain to be solved. The emerging mechanobiology, an interdisciplinary field with a coherent interest on the function of mechanical forces in and for living organisms, is expected to solve this problem. In fact, mechanobiology has revealed that cells can sense not only exogenous/endogenous forces but also stiffness and topography of surrounding microenvironments, utilizing them to regulate fundamental cell functions including proliferation, differentiation and migration. Mechanical forces and cell mechanosensing keep up our healthy body, while unphysiological forces and/or defective mechanosensing cause diverse diseases, such as atherosclerosis, heart failure, muscle atrophy, osteoporosis and carcinogenesis. In the past decade a variety of cell mechanosensor molecules have been identified and their biophysical mechanisms of function have been revealed using cultured cells. However, those in vivo are yet uncovered. Thus the pressing issue is to elucidate molecular and biophysical mechanisms underlying the cell mechanosensing in tissues and organs in situ. This requires cutting edge technologies able to measure and manipulate quantitatively the stress and strain at cell-cell, cell-substrate junctions and organellae in tissues while monitoring intra and inter-cellular mechnaotransduction processes undergoing over short and long terms.
COI:No

Planned Symposium 14

Joint Symposium with the Biophysical Society of Japan

Cutting-edge interdisciplinary physiology for heat production and sensing

March 29 (Thu) 16:10～18:00 Hall 4

2PS-04PM-1

Single-cell analysis of temperature-sensing mechanisms under a photo-thermal conversion microscope

Oyama Kotaro[1,2], Zeeb Vadim[3], Arai Tomomi[2], Itoh Hideki[4,5], Shintani A Seine[6], Suzuki Madoka[1,7], Fukuda Norio[2], Ishiwata Shinichi[4]

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Living cells are constantly exposed to changes in temperature. Recent advances in single-cell thermometry have revealed that living cells can intrinsically increase intracellular temperature by 1° C or higher. In order to elucidate the biological roles of local temperature changes at the single cell level, here we developed a novel microscopic system for the manipulation of local temperatures. Spatiotemporal temperature gradients were generated by an infra-red laser beam focusing on a metal particle, water outside of cells, or cells. At the meeting, we will introduce the unique temperature-sensing mechanisms of various cells revealed by this technique. Likewise, we will discuss our recent findings on the optical control of cellular functions, such as muscle contraction and neurite outgrowth. COI:No

2PS-04PM-2

Analysis of disease mutants of type 1 ryanodine receptor by molecular dynamics simulation and calcum imaging

Yamazawa Toshiko

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In excitable cells, membrane depolarization is translated into intracellular Ca^{2+} signals, and ryanodine receptors (RyRs), located in the sarcoplasmic/endoplasmic reticulum (SR/ER) membrane, are required for intracellular Ca^{2+} release. Malignant hyperthermia (MH) is a disorder of Ca^{2+}-induced Ca^{2+} release (CICR) via the type 1 ryanodine receptor (RyR1) in skeletal muscles. More than 200 mutations have been reported in the RyR1 gene of patients with MH. Most of those mutations have been found in three "hot spots" regions of RyR1. However, few comprehensive study on Ca^{2+} homeostasis associated with structural properties in these mutants to give insight into the mechanism of the pathogenesis have been reported. We investigated properties of RyR1 channels that carry disease-associated mutations at the N-terminal region. HEK293 cells expressing mutant RyR1 channels exhibited alterations in Ca^{2+} homeostasis, i.e., caffeine sensitivity, ER Ca^{2+} contents, resting cytoplasmic Ca^{2+} concentration, mitochondrial Ca^{2+} concentration, and morphological abnormality. molecular dynamics analysis revealed that changes of the electrostatic interactions between the A and B/C domain were strongly correlated with the channel function of the RyR1, suggesting these interactions are primary determinant for MH phenotype. COI:No

2PS-04PM-3

Tumor Bio-imaging Using Thermo-responsive Molecules

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1:Showa Pharm. Univ. Tokyo Japan, 2:QST, Chiba Japan, 3:Kyushu Univ. Fukuoka Japan

Tumor tissues exhibit higher temperatures than healthy tissues. However, the development of a targeted drug delivery system exploiting this phenomenon is challenging because of the minuscule temperature difference and the lack of its molecular implications. However, self-assembling thermo-responsive molecules (UBDs) containing oligoethylene glycol hold significant potential. UBDs exhibit an abrupt self-assembling behavior due to their low critical solution temperature (LCST). They are capable of recognizing the small temperature difference between healthy and tumor tissues, and selectively accumulating within tumor tissues. We prepared three fluorescence-tagged UBDs and confirmed their tumor accumulation by fluorescence imaging. Therefore, we suggest that UBDs selectively accumulate within tumor tissues due to their LCST-dependent self-assembling behavior. COI:No

2PS-04PM-4

Theoretical and experimental approach for mechanisms of temperature compensation in circadian clocks

Kurosawa Gen

Theoretical Biology Lab, RIKEN

Circadian rhythms govern the timing of many physiological events. Mysteriously, the period of the circadian rhythm is stable to temperature although the underlying biochemical reactions usually accelerate with temperature, a paradox that has remained unsolved for more than 60 years. Experiments conducted over the last few decades in insects, mammals, and plants have demonstrated that biological rhythms are governed by cyclical changes in gene expression. However, the topologies of the regulatory network structures for these rhythms differ between species, suggesting that the mechanisms for period stability with temperature are not conserved.
But is it true? We examined the mechanisms for period stability with temperature by combining computational models with distinct network structures and experimental observations from mammalian cell cultures (C6 glioma cells). Unexpectedly, we found that temperature-sensitive amplitude of gene expression, which we call "temperature-amplitude coupling," can stabilize the period with temperature in all models studied, despite differences in structures. Thus, the mechanisms for circadian period stability may be shared across species. Using the cultured cells, we experimentally confirmed the predicted temperature-sensitive amplitude of gene expression, suggesting that temperature-amplitude coupling cause the temperature compensation of the cultured cells (Kurosawa et al. 2017 PLoS Comput Biol).
This study is the collaboration with Atsushi Mochizuki at RIKEN, and Atsuko Fujioka, Satoshi Koinuma, and Yasufumi Shigeyoshi at Kindai. COI:No

2PS-04PM-5

Single-cell thermometry under optical microscope for the physiology of thermogenesis

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1:Inst Protein Res, Osaka Univ, Osaka, Japan, 2:PRESTO, JST, Saitama, Japan

Body temperature in cold environment can be maintained constant by the heat released in, e.g., brown adipocytes that were found thermogenically active in human adults as well as skeletal muscle cells. While the molecular details of temperature sensing in our bodies are being revealed, the heat release that have traditionally been analyzed in the suspension of cells can be now studied in single-cell level due to the development of single-cell thermometry. Instead of measuring oxygen consumption and extracellular acidification rates, these novel methods allow us to consider the heat more directly, possibly paving a way to study thermogenic function in these tissues from the single-cell level for the treatment of obesity and diabetes. In this symposium, I will present the applications of our methods to stimulated skeletal muscle cells and brown adipocytes. I will also briefly overview the current progress and limits in single-cell thermometry. COI:No

Planned Symposium 15

How do we understand the control and function of sleep/arousal?

March 29 (Thu) 16:10～18:00 Hall 5

2PS-05PM-1
A mouse model of sleep disturbance and their phenotype

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Sleep loss is known to induce memory deficits, learning impairment, and anxiety-like behavior. However, the mechanisms underlying the effects of chronic sleep disturbance on mood and behavior remain unknown. In the present study, we developed a chronically sleep-disturbed mouse model by placing mice on a wire net, and investigated their phenotype. Mice reared on a wire net for 3 weeks (sleep-disturbed; SD mice) showed increased wakefulness, decreased amount of rapid-eye movement (REM) and non-REM (NREM) sleep, and attenuated slow-wave activity (SWA) during NREM sleep, compared with control mice. Using this SD model, we evaluated behavioral test battery.SD mice showed an impaired motor learning and increased aggressive behavior, although no differences were found in anxiety-like behavior and special memory/learning ability. The expression of corticotropin-releasing hormone (CRH) mRNA and protein in the hypothalamus and the adrenal gland weight of SD mice were increased, although the plasma concentration of corticosterone was at a similar level to that of control. The contents of serotonin and their metabolite were decreased in brain of SD mice. In addition, mRNA expression of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) that have recently been shown to be associated with aggressive behavior were also altered in brain of SD mice. Our findings may demonstrate a potential new therapeutic approach for common human disorders ranging from insomnia to mood diseases. COI:No

2PS-05PM-2
Mechanisms for inducing fluctuation of autonomic nervous system during REM sleep

Koyama Yoshimasa

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During REM sleep, significant fluctuations in autonomic functions including blood pressure, heart rate, respiration or penile erection can be observed. However, the mechanisms underlying these unique characteristics remain unknown. The cholinergic neurons in the mesopontine tegmental area; laterodorsal/pedunculotegmental nuclei (LDT/PPT), discharging highest during REM sleep or higher during REM sleep and waking, are considered to generate and maintain REM sleep in association with the glutamatergic neurons ventral to the LDT (sub LDT). In addition, some of the cholinergic neurons in the LDT showed phasic firing in close correlation with blood pressure fluctuation during REM sleep, suggesting the involvement of the cholinergic LDT neurons in blood pressure fluctuation during REM sleep. Neuronal firing similar to the cholinergic LDT neurons during REM sleep was observed in the amygdala, which is a center of emotion and causes autonomic fluctuations responding to emotional stimuli during waking. The cholinergic neurons in the LDT project to the amygdala, and injection of nicotinic receptor antagonist (mecamylamine) into the amygdala suppressed the blood pressure increase induced by electrical stimulation to the LDT, suggesting that the cholinergic activation of the amygdale are required for the blood pressure fluctuation during REM sleep. The ascending cholinergic neurons to the amygdala would be basic substrates for inducing fluctuations of autonomic nervous systems during REM sleep. COI:No

2PS-05PM-3
Role of orexin as a link between the limbic system and arousal

Sakurai Takeshi

WPI-IIIS/Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan

Animals shift their sleep-wakefulness state according to their internal state and the external environment by utilizing three major influential elements, i.e., homeostatic, circadian and allostatic factors. Among them, allostatic factors include the nutritional state and external environment, which trigger emotion. For example, stressful and emotionally-salient situations such as encountering predators, adapting to novel situations or expecting a reward require animals to shift their behavior to a vigilant state, along with alteration of their physiological condition through modulation of autonomic and endocrine functions. Studies of efferent and afferent systems of orexin-producing neurons have shown that the orexin neuronal system has close interactions with systems that are involved in the regulations of emotion, energy homeostasis, reward, and arousal. Many studies have suggested that orexin neurons are activated during the behavioural expression of fear or in response to cues associated with danger or reward. These observations suggest that orexin neurons are involved in control of vigilance states in response to outer environment. On the other hand, orexin system affects expression of behavioral response against outer environment via control of monoaminergic neurons. I will discuss functional interplay between the limbic system and arousal system, and involvement of orexin and orexin receptors in these interactions. COI:No

2PS-05PM-4
Mental and physical disorders attributable to desynchronization between social time requirements and an individuals sleep-wake rhythm

Mishima Kazuo

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Circadian rhythm sleep-wake disorder is a family of sleep disorders caused by the inability to entrain sleep/biological rhythms to the 24-h day-night cycle. Using a forced desynchrony protocol, we have revealed that patients with non-24-hour sleep-wake rhythm disorder suffered from both the abnormally long circadian period of the biological clock and decreased photic entrainment. Even in healthy subjects, great individual variations in length of the circadian period have significant effects on sleep timing and morningness/eveningness preferences (chronotypes), causing misalignment between social time and an individual's sleep pattern. Consequently, many people today suffer from internal desynchronization and sleep debt.

The misalignment between social time and an individual's sleep pattern has an enormous effect on mental and physical functions. Our simulation study has shown reduced sleep duration and mood disturbance among evening chronotypes, and sleep debt accumulated over a very short period of time easily evokes comparable depressive mood in normal individuals. Reduced functional connectivity (for mutual suppression) between the medial prefrontal cortex and amygdala, which plays an important role in the processing of emotions, was a neurological basis for depressive mood among individuals with sleep debt that was mild enough to occur in daily life. Furthermore, chronic sleep debt (potential sleep debt) that is often too mild for the individual to perceive it can suppress mental functions, appetite regulation, metabolic processes, and the stress response mechanism.

Planned Symposium 16

Joint Symposium with the Japanese Pharmacological Society

Sensible approaches for sensing channels: From physiology to pharmacology

March 29 (Thu) 16:10〜18:00 Hall 6

2PS-06PM-1

Physiological roles of the Na+ sensing channel, Nax

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1:Nat'l Inst Basic Biol, Okazaki, Japan, 2:SOKENDAI

Na_x is a sodium concentration ($[Na^+]$)-sensitive, and not a voltage-sensitive Na^+ channel. Na_x-knockout mice ingest salt even when they develop hypernatremia by dehydration. In the brain, Na_x channels are preferentially expressed in the glial cells of sensory circumventricular organs (sCVOs), midline structures without the blood-brain barrier. Among sCVOs, the subfornical organ (SFO) is the primary site of $[Na^+]$ sensing for the control of salt-intake behaviors. Dehydration increases $[Na^+]$ in plasma and cerebrospinal fluid. When this occurs, Na_x activation stimulates the release of glial cell lactate, which acts as a gliotransmitter, activating GABAergic inhibitory neurons in the SFO. The SFO neurons that encode salt appetite are angiotensin II receptor type 1a-positive excitatory neurons that project to the ventral part of the bed nucleus of the stria terminalis. Under dehydrated conditions, salt appetite-driving neurons are suppressed through the activation of GABAergic neurons by Na_x signals. In parallel, Na_x are also involved in $[Na^+]$-dependent thirst generation. When dehydrated, Na_x channels in the glial cells of sCVOs are activated, leading to the synthesis of epoxyeicosatrienoic acids (EETs) in Na_x-positive glial cells. EETs released from glial cells are also gliotransmitters that activate neurons with TRPV4 channels in sCVOs, which presumably encode thirst. Other physiological functions of Na_x channels, including neural regeneration, will be discussed. COI:No

2PS-06PM-2 (AP2)

A CALHM1/CALHM3 heteromeric channel mediates purinergic neurotransmission of sweet, bitter, and umami tastes

Taruno Akiyuki[1], Ma Zhongming[2], Ohmoto Makoto[3], Matsumoto Ichiro[3], Tordoff G Michael[3], Foskett Kevin J[2], Maruñaka Yoshinori[1]

1:Dept Mol Cell Physiol, Kyoto Pref Univ Med, Kyoto, Japan, 2:Dept Physiol, Univ Pennsylvania, Philadelphia, PA, USA, 3:Monell Chemical Senses Center, Philadelphia, PA, USA

Sweet, bitter, and umami taste compounds stimulate taste receptors in apical membranes of type II taste cells, leading to action potential-dependent, non-exocytotic release of adenosine triphosphate (ATP), which serves as a neurotransmitter conveying taste information to afferent gustatory nerves. Although we identified calcium homeostasis modulator 1 (CALHM1) as an essential component in the voltage-gated ATP release channel required for purinergic neurotransmission in type II taste cells, the molecular identity of the ATP release channel is not yet fully understood as suggested by the biophysical and pharmacological disparities between CALHM1 gating *in vivo* and *in vitro*. CALHM3 interacts with CALHM1, and the resulting CALHM1/CALHM3 channel exhibits biophysical and pharmacological properties that are identical to those of the ATP release channel in type II taste cells. Both CALHM1 and CALHM3 are expressed exclusively in type II taste cells within taste buds. Knockout of either *Calhm1* or *Calhm3* impairs perception of sweet, bitter, and umami tastes and abolishes ATP release channel current and taste-evoked ATP release in type II taste cells. Thus, the CALHM1/CALHM3 heteroligomer is the voltage-gated ATP release channel required for neurotransmission in type II taste cells. COI:No

2PS-06PM-3

Unveiled cold sensitivity of TRPA1 by the prolyl hydroxylation inhibition-induced sensitization to ROS

Nakagawa Takayuki[1], Kaneko Shuji[2]

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Mammalian TRPA1 plays an important role in pain generation, but its role as a cold nociceptor is still controversial. We previously reported that oxaliplatin, a platinum chemotherapeutic agent, elicits cold hypersensitivity via TRPA1 in mice. Here, we further explored the molecular mechanisms underlying TRPA1cold hypersensitivity. Oxaliplatin or its metabolite analog, dimethyl oxalate (DMO), for 2 h enhanced H_2O_2-evoked TRPA1 activation, suggesting TRPA1 sensitization to reactive oxygen species (ROS). Although hTRPA1 showed little responses to cold, we found that oxaliplatin or DMO pretreatment induced cold-evoked hTRPA1 response, which was mediated through ROS-mediated oxidation. TRPA1 is activated by ROS through oxidative modification of cysteine residues, but also by relief from prolyl hydroxylase (PHD)-mediated hydroxylation of a proline residue (P394). Thus, we determined whether the oxaliplatin-induced hTRPA1 sensitization to cold/ROS is PHD-dependent. We found that oxaliplatin or DMO has an ability to inhibit PHD activity, and the TRPA1 sensitization to cold or ROS was inhibited in a hTRPA1 proline mutant resistant to PHDs (P394A) or by overexpressing PHD2. Furthermore, a PHD inhibitor or P394A mutation mimicked the cold-evoked responses. Finally, we confirmed our in vitro findings could fit an in vivo mechanism of TRPA1 in mice. These results suggest that relief from the PHD-mediated prolyl hydroxylation of TRPA1 enables the channel to sense cold-evoked mitochondria-derived ROS, which endows TRPA1 with indirect cold sensitivity. COI:No

2PS-06PM-4

Critical roles for TRPV2 in the formation and maintenance of intercalated discs in cardiomyocytes

Katanosaka Yuki

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The cardiac muscle is a functional syncytium that composed of terminally differentiated myocytes. The individual cardiomyocytes are mechanically and ionically coupled at intercalated discs. The molecular detail of formation and maintenance of intercalated discs are unclear. We have previously proposed that TRP vanilloid family type 2 channel (TRPV2) serves as a mechanoreceptor at intercalated discs of cardiomyocytes. Here, we show that TRPV2 plays critical roles in the formation and maintenance of intercalated discs, using by temporally-controlled and cardiac-specific TRPV2-deficint mice. The elimination of TRPV2 in the mouse heart resulted in an immediate severe decline in cardiac function accompanied by disorganization of the intercalated discs, which triggered abnormal cell shortening, Ca2+ handling, and myocardial conduction defects. In neonatal cardiomyocytes, stretch induced Ca2+ responses and robust expression of TRPV2 were observed within 12 hours of culture after enzymatic isolation. TRPV2-deficient myocytes showed no Ca2+ response to stretch-stimulation. These myocytes neither formed intercalated discs between neighbouring cells and Ca2+ handling for E-C coupling. In addition, elimination of TRPV2 from juveniles resulted in mild chamber dilation with malformation of intercalated discs and defects in compensated hypertrophic response to hemodynamic stress. These results suggest that TRPV2 is critical for the formation and maintenance of intercalated discs in the hearts. COI:No

2PS-06PM-5

Breathing patterns regulated by mechano-sensing channel Piezo2

Nonomura Keiko[1,2]

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Mechanosensation plays important roles in our life as exemplified by touch sensation and hearing. However, channels responsible for mechanosensation had not been identified for many decades. Our group identified Piezo 1 and 2 as mechanically activated cation channel in 2010. Piezo2 is highly expressed in subsets of sensory neurons. Studies of conditional KO mouse lines and of human patients with genetic mutation revealed that Piezo2 functions as a key mechanotransducer for touch sensation and proprioception. Furthermore, Piezo deficient animal models provide us a unique opportunity to look into roles of mechnaosensation in vivo. We recently found that Piezo2-mediated mechanosensation takes part in the regulation of breathing patterns in both newborn and adult mice. First, newborn mice lacking Piezo2 in whole body or sensory neurons derived from neural crest cells died shortly after birth with breathing defects. In contrast, adult mice lacking Piezo2 in sensory neurons lost firing of vagus sensory nerve induced by inflation of lungs. They also showed increased tidal volume and lost the Hering-Breuer reflex, an apnea induced by lung inflation, which is thought to prevent lung over-inflation. Our data show that mechanosensation mediated by Piezos is involved in multiple physiologically important phenomenon in our body. COI:No

Planned Symposium 20

Taiwan-Japan Joint Symposium (-Towards FAOPS2019-)

Integrated Understanding of Gastrointestinal Physiology - Microbiome, Motility and Membrane Transport

March 30 (Fri) 8:30～10:20 Hall 1

3PS-01AM-1

Function of epithelial K^+ and Cl^- channels in the human colon

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Thromboxane A_2 (TXA_2) is known to be involved in the colonic cancer cell proliferation and inflammatory bowel diseases. Here, regulatory mechanisms of K^+ and Cl^- channels by TXA_2 are reported. We found that expression level of Kv7.1 K^+ channel proteins in the human colorectal cancer tissue is significantly higher than in the accompanying non-tumor mucosa. Kv7.1 was also expressed in human colonic cancer cell lines such as HT29, T84, WiDr and KM12-L4. In the KM12-L4 cells, STA_2, a stable analogue of TXA_2, significantly increased the Kv7.1-derived K^+ currents in parallel with an increased expression of Kv7.1 proteins. A TXA_2 receptor antagonist and a PKA inhibitor blocked these STA_2-induced effects. Interestingly, STA_2-stimulated proliferation of colonic cancer cells was inhibited by a Kv7.1 inhibitor. We also investigated the effect of STA_2 on the CFTR Cl^- current in isolated human colonic mucosa set between Ussing chambers. STA_2 increased the Cl^- current due to Cl^- secretion across the colonic mucosa. The STA_2-induced current was significantly inhibited by a TXA_2 receptor antagonist. The effect of STA_2 was independent of the colonic segment from which the tissue was obtained (from cecum to rectum). The STA_2-induced current was also inhibited by a membrane-permeant cAMP antagonist. STA_2 significantly increased the intracellular cAMP level in a human colonic cell line. Our results suggest that TXA_2-elicited cAMP pathways contribute to the colonic cancer cell proliferation via Kv7.1 K^+ channels and to the colonic Cl^- secretion via CFTR Cl^- channels. COI:No

3PS-01AM-2

Spatial coordination of gut electrical activity is modulated by serotonin signaling and inflammatory factors.

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Gut motility requires spatial coordination of electrical activity. For example, textbooks of physiology describe simultaneous activation of excitatory inhibitory motor neurons for peristaltic movements. Recent studies suggest co-contribution of other cellular members, such as network-forming pacemaker cells, to spatial coordination of electrical activity.

In this presentation, I communicate modulation of micro-coordination of gut electrical activity in animal models. Microelectrode array reinforced by dialysis membranes enabled stable measurements of spatio-temporal electrical activities in many divisions of the gut, including the colon. Under normal condition, pacemaker electrical activity in the ileum was classified into three patters: bumpy, expanding and migrating. Gastric musculatures also displayed migrating electrical activity.

In the ileum application of serotonin accelerated pacemaker activity, and shifted the spatio-temporal pattern of pacemaker activity toward migrating. These changes agree well with the previous observation of propulsive motions in isolated tracts of the ileum.

Inflammation caused by surgical operation and chemical perturbation impaired pacemaker electrical activity with sporadic or complete loss of active area. Anti-inflammatory agents, such as reduced form of glutatione also suppressed pacemaker activity. Addition of serotonin partly restored pacemaker activity, and ondansetron reversed this effect, suggesting that 5-HT_3 receptors play a key role in both initiating and coordinating pacemaker activity. COI:No

3PS-01AM-3

Interplay between gut bacteria and epithelial innate immunity in barrier defects and colon tumorigenesis

Yu C Linda

Graduate Institute of Physiology, National Taiwan University College of Medicine

The gut microbiota and innate immune receptors are involved in the maintenance of epithelial homeostasis and development of colorectal cancers (CRC). Bacterial lipopolysaccharide (LPS) receptor, consisting of three subunits CD14/ TLR4/MD2, is a well-known proinflammatory receptor complex on monocytes that is involved in septic injury. Recent evidence showed upregulation of LPS receptors expression on intestinal mucosa of CRC patients. Our series of studies (Kuo WT et al., Cell Death Diff. 2015; Cancer Res. 2016) demonstrated that LPS regulated epithelial apoptosis and colon carcinogenesis via functional antagonism of CD14 and TLR4. We showed that epithelial CD14-mediated lipid signaling triggered caspase-dependent apoptosis and barrier damage, whereas TLR4 antagonistically promoted cell survival and cancer development. Moreover, eritoran (a molecule similar to lipid A moiety of LPS) acts as a TLR4 inhibitor. Eritoran administration reduced tumor burden in a mouse model of CRC. In vitro cultures of mouse primary tumor spheroids and human cancer cell lines displayed increased cell cycle progression following LPS challenge, and increased cell apoptosis after eritoran treatment. Lastly, E.coli with adherent and invasive characteristics was implicated in tumor development, pointing to an active role of bacteria in carcinogenesis. In sum, dysregulation of the expression pattern of epithelial LPS receptor subunits and enhanced bacterial adherence play critical roles in promotion of colon tumorigenesis. COI:No

3PS-01AM-4

Gut microbiota-derived metabolites shape host physiological homeostasis

Fukuda Shinji[1,2,3,4,5]

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The gut microbiota form a highly complex ecological community together with host intestinal cells. It has been reported that imbalance in the structure of gut microbiota could be a risk factor in human disorders including not merely gut-associated disorders such as inflammatory bowel disease, but also systemic diseases such as metabolic disorders. However, the molecular mechanisms of the host-microbial crosstalk remain obscure. To this end, we firstly established a highly integrated omics-based approach involving genome, transcriptome, and metabolome analyses to elucidate the molecular basis of the function of gut microbiota. Applying this novel method to various mice models, we found that acetate produced from carbohydrate metabolism by probiotic bifidobacteria largely contributes to the protection of mice from enterohaemorrhagic *E. coli* O157:H7 lethal infection through enhancement of gut epithelial barrier function. In addition, we showed that butyrate derived from dietary fiber fermentation by microbial order Clostridiales progresses the induction of regulatory T cell differentiation from naive T cells through epigenetic modification, which suppress colonic inflammation. Furthermore, metabologenomic approach revealed that succinate produced from neonate gut microbiota mediates Clostridiales colonization followed by protection against enteropathogenic infection. Taken together, gut microbiota-derived metabolites are considered to be crucial factors to shape host physiological homeostasis. COI:Properly Declared

Planned Symposium 17

Editorial Board of the Journal of Physiological Sciences

How to get your paper accepted or review other people's papers

March 30 (Fri) 8:30～10:20 Hall 2

3PS-02AM-1

How to get your paper accepted or review other people's papers

Ishikawa Yoshihiro[1], Sato Motohiko[2], Tominaga Makoto[3]

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Getting ones paper accepted is a major interest for researchers, in particular, for young ones. However, it is not always easy to publish a paper in a good peer reviewed journal. For senior researchers, this review process may be familiar since many of them have published numerous papers and also served as referee or editors for journals. For young researchers, there is few opportunities to serve as referee or editor. Accordingly, it is important for young scientists to learn the process of manuscript review during the editorial work of a journal. In the past decade, the number of so called open access journal has been increased dramatically. Such journal contents become available to the public immediately after paper acceptance, and thus more and more scientists can read the papers on that open access journal. Citations may be increased dramatically. In this regard, it is very important to learn the content and character of such open access journals as well. In this symposium, Dr. Makoto TOMINAGA, who serves as editor for Molecular Pain, an open access journal in neuroscience, and Dr. Motohiko SATO, who serves for Scientific Reports, will introduce their journal for young researchers. The Journal of Physiological Sciences, the official English Journal of our Society will be changed to an open access journal in the very near future. We will thus discuss how we should manage this Journal for our Society to contribute toward the progress of physiological research, not only in Asia, but in the world. COI:No

Planned Symposium 18

Joint symposium with the Japanese Society of Pathophysiology

Newly identified circulatory regulation mechanisms and induction of diseases caused by their disruptions.

March 30 (Fri) 8:30～10:20 Hall 3

3PS-03AM-1
Mechanism of Caveolin in Heart Failure

Ichikawa Yasuhiro[1,2,3]

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Background: Caveolae are flask-like invaginations of the plasma membrane that are enriched in cholesterol and caveolins. Three caveolin subtypes (Cav-1, Cav-2, Cav-3) are expressed in heart. Caveolin-3 (Cav-3) is expressed in heart and muscles, and it is a structural protein in cell membranes that is involved in protection of the heart from cardiac stress. However, little is known about its roles in heart failure. Methods and Results: New Zealand rabbits were administered Daunorubicin (Dau) weekly for 9 weeks to induce heart failure. Dau treatment resulted in a reduced left ventricle (LV) ejection fraction. LV samples from rabbits with Dau-induced heart failure showed increased Cav-3 protein expression compared to control tissues, with no change in Cav-1 expression. In addition, electron microscopy images show that Dau treatment increases formation of caveolae. We found similar changes in Cav-3 in failed human heart, suggesting the relevance of our rabbit model. We also used transgenic Cav-3 overexpression (OE) mice and control littermate mice that underwent transverse aortic constriction (TAC). Echocardiography was performed 2 and 4 weeks after surgery. Cav-3 OE mice subjected to TAC had increased survival and reduced cardiac hypertrophy, and cardiac function was maintained compared with control mice. Cav-3 expression in control mice was decreased after TAC treatment compared to OE mice.Conclusion: Cav-3 expression was different in two heart failure models. These results suggest a potential pathophysiologic role for Cav-3 in heart failure. COI:No

3PS-03AM-2
The role of exchanged protein directly activated by cAMP 1 (Epac1) in the development of arrhythmias.

Fujita Takayuki[1], Prajapati Rajesh[1], Nakamura Takashi[1], Cai Wenqian[1], Hidaka Yuko[1], Suita Kenji[1,2], Okumura Satoshi[2], Ishikawa Yoshihiro[1]

1:Cardiovascular Research Institute, Yokohama City University Graduate School of Medicine, 2:Department of Physiology, Tsurumi University of Dental Medicine

β-adrenergic receptor (β-AR) mediated pathway have been reported to play important role in development of clinically significant arrhythmias including atrial fibrillation (AF). β-AR blockade therapy is widely accepted as a useful treatment for arrhythmias. However, their clinical usage has been limited by their critical side effects including suppression of cardiac function. Epac was found recently as one of the down-stream molecules of β-AR. We found that type 5 adenylyl cyclase (AC5) and Epac1 plays an important role especially in some pathogenic processes of β- AR mediated signaling. In both of AC5 knockout mice and Epac1 knockout mice, the duration of AF were shorter than wild type mice. In addition, cardiac AC5 overexpression-induced AF elongation was attenuated by Epac1 deficiency, indicating that Epac1 plays an important role in AC5-mediated AF susceptibility. Consistently, CE3F4, an Epac1 inhibitor, shortened AF in mice. Importantly, the heart rate was not affected by the amount of CE3F4 which is sufficient to exert anti-arrhythmic effect. These findings indicate that Epac1 plays important role in development of AF. Epac1 may be a useful therapeutic target for treatment of arrhythmias. COI:No

3PS-03AM-3
Cardiac myofilament function in transgenic mice overexpressing Epac1 in the heart

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β-Adrenergic receptor (β-AR) stimulation on the heart results in an increase of cardiac output partially through phosphorylation of the myofilament proteins troponin I (TnI) and myosin binding protein C (MyBP-C), the major PKA-mediated phosphorylation. The aim of this study was to investigate the contribution of the exchange protein activated by cAMP 1 (Epac1), a PKA-independent cAMP effector, to the cardiac myofilament response to β-AR stimulation. Ca^{2+} sensitivity of force and ATPase activity as well as tension cost (ATPase activity/force) were significantly increased in cardiac myofilaments from transgenic mice overexpressing Epac1 in the heart (Epac1TG) compared to non-transgenic mice (NTG). In addition, phosphorylation level of myosin regulatory light chain (RLC) was significantly greater in Epac1TG than that in NTG without any changes in the phosphorylation of TnI or MyBP-C. We also observed that pharmacological activation of Epac with 8-CPT-AM, an Epac-specific but not isoform-selective cAMP analogue, increased RLC phosphorylation in perfused hearts isolated from NTG mice, but its increase was blunted by the addition of a phospholipase C (PLC) inhibitor (U73122) or a protein kinase C (PKC) inhibitor (Bisindolylmaleimide I). These results suggest that activation of Epac1 mediates RLC phosphorylation via PLC/PKC signaling pathways, leading to the increase in Ca^{2+} sensitivity of force and ATPase activity as well as tension cost in cardiac myofilaments. COI:No

3PS-03AM-4
Impact of altered uteroplacental circulation on placental function and fetal growth.

Shibata Eiji, Aiko Yukiyo, Aramaki Satoshi, Mori Hiroshi, Hachisuga Toru, Askew David, Inagaki Hirohide, Okabe Keisuke

Dept. of Obstetrics & Gynecology, University of Occupational Environmental Health, School of Medicine, Kitakyushu, Japan

Preeclampsia (PE) and fetal growth restriction (FGR) are both associated with abnormal remodeling of uterine spiral arteries perfusing the placental site. This would be expected to be associated with reduced fetal growth, yet only one third of infants of mothers with preeclampsia are growth restricted. Furthermore mothers with only FGR are lacking maternal symptoms such as hypertension, protein urea, and general edema. The purpose of this study was to assess differences of the microcirculation of uterine spiral arteries, angiogenetic factors, morphology, oxidative stress, inflammatory changes, and nutrient transport function between PE and FGR placenta. The placental perfusion from uterine spiral arteries was analyzed by 3D power Doppler ultrasound with the VOCAL imaging analysis program. Placental morphology was analyzed by image analyzing computer. Amino acid, glucose and fatty acid transporters were analyzed for placental nutrient transport function. Immunohistochemistry was performed using antibodies against oxidative stress markers including heat shock protein, 8-hydroxy deoxy-guanosine, and HNE-modified proteins, and cytokine signals including TNF-alpha and suppressor of cytokine signals 3, and markers of immune cells including CD64 and CD136. We discuss the effect of low placental perfusion in PE and FGR on placental functions such as angiogenesis, morphological changes, and nutrient transport function. COI:No

3PS-03AM-5
Molecular pathophysiological insight into hypertension induced by oral-salt intake and atherosclerosis by commensal microbes and high fat diet.

Ishigami Tomoaki, Chin Lin, Minegishi Shintaro, Kino Tabito, Sasaki Rie, Doi Hiroshi, Arakawa Kentaro, Teranaka Sae, Tamura Kouichi

Yokohama City University Graduate School of Medicine, Department of Medical Science and Cardiorenal Medicine

Both hypertension and atherosclerosis are global health burden for healthy longevity especially among developed countries. Life with daily dietary excess of salt and fat is characteristics for modern developed countries, and definite contributions to cardiovascular diseases and impairment of healthy longevity. Detailed molecular pathophysiological analyses for these diseases should be essential for handling the subjects appropriately. For hypertension, it was suggested that primary molecular abnormalities existed in tubular sodium transports by molecular genetical analyses for human hereditary hypertension. We have analyzed molecular mechanism for sodium sensitivity and hypertension focusing on Nedd4-2 which is ubiquitin ligase for epithelial sodium channels located in aldosterone sensitive distal nephron. Using Nedd4-2 C2 domain knock-out mice, we discovered oral salt sensitive enhancements of sodium reabsorption via ENaC in the distal nephron. The commensal microbes-derived atherosclerosis via the metabolism-dependent mechanisms have received increasing attention. However, whether the effect of the intestinal microbiota on atherosclerosis is mediated by immune mechanisms remain uncertain. Our reports provided evidence for a pathway of immune activation in commensal microbes-derived atherosclerosis, which driven by high fat diet with enhancement of substantial population of splenic B2 cell. COI:No

Planned Symposium 19

Korea-Japan Joint Symposium (-Towards FAOPS2019-)

Exquisite mechanisms for sensing and orchestrating mechanical signals

March 30 (Fri) 14:00〜15:50 Hall 1

3PS-01PM-1

Regulation of cardiac calcium by shear stress signaling

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Lab. Physiol., Coll of Pharm, Chungnam Natl. Univ, Daejeon, Republic of Korea

Cardiac myocytes are subjected to fluid shear stress during the cardiac cycle and hemodynamic disturbance. Atrial myocytes are exposed to high shear stress during blood regurgitation and high intra-atrial pressure. We investigated shear-specific Ca^{2+} signaling and underlying mechanotransduction in single atrial myocytes. Using two-dimensional confocal Ca^{2+} imaging combined with micro-jet apparatus, we found that relatively high shear stress (about 16 dyn/cm^2) induces two types of global Ca^{2+} waves in atrial myocytes: longitudinally propagating slow wave and transversely moving fast wave. The longitudinal Ca^{2+} wave has been found to be triggered by Ca^{2+} release via autocrine activation of P2Y1 purinoceptor-phospholipase C-inositol 1,4,5-trisphosphate (IP3) receptor type 2 signaling. This shear-IP3 signaling, in turn, activated the transient receptor potential melastatin 4, a nonselective cation channel. The generation of shear-induced transverse waves were caused by activations of voltage-dependent Na^+- and Ca^{2+}-channels (action potentials), and were Ca^{2+}-independent. Real-time measurement of ATP release simultaneously with Ca^{2+} wave, and pharmacological and genetic interventions further revealed that shear-induced transverse Ca^{2+} waves are triggered by P2X currents activated by connexin 43-hemichannel-mediated ATP release. Shear-specific mechanotransduction and the subsequent Ca^{2+} waves may be one way for atrial myocytes to measure mechanical stress and alter their Ca^{2+} signaling and excitability. COI:No

3PS-01PM-2

Shear stress-mediated purinergic signaling in vascular endothelial cells

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Vascular endothelial cells (ECs) sense shear stress and transduce blood flow information into functional responses that play important roles in vascular physiology and pathophysiology. However, how ECs perceive shear stress as a signal and transmit it to the cell interior remains unknown. Previously, we found that the shear-stress dependent Ca^{2+} signaling occurs via an ATP-operated channel, P2X4, and that P2X4-mediated shear stress mechanotransduction plays an important role in vascular homeostasis, including in the control of blood pressure, flow-induced vasodilation and blood flow-dependent vascular remodeling. P2X4 activation requires the presence of endogenous ATP released by ECs, but the mechanism of the ATP release remains unknown. To analyze the dynamics of ATP release, we visualized ATP release at the cell surface by using cell-surface attached luciferase. When exposed to shear stress, ECs simultaneously released ATP in both a highly concentrated, localized manner and a diffuse manner. The hot spots of ATP release occurred at caveolin-1-rich regions which are known as cholesterol-rich caveolar domains in the plasma membrane and were blocked by caveolin-1 knockdown with siRNA, indicating involvement of caveolae in the localized ATP release. Immediately after this localized ATP release, an increase in intracellular calcium concentration and subsequent calcium wave occurred at the same site as the localized ATP release, suggesting that the ATP released at caveolae activates nearby P2X4 ion channels, which triggers shear stress calcium signaling in ECs. COI:No

3PS-01PM-3

Role of myocardial subcellular mechano-sensitivity in integrative cardiac function

Iribe Gentaro

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Cardiac mechanics in response to change in preload shows load-independent consistency and simplicity as represented by, for instance, Frank-Starling's law of the heart, time-varying elastance model, etc. However, this simplicity is the result of complex interactions among various load-dependent cellular responses. Mechanical load applied to cardiomyocytes can propagate through the cell via the cytoskeleton to cause mechano-sensitive responses, which are mediated not only by sarcolemmal mechano-sensitive ion channels, but also by organelle. For instance, myocardial stretch activates NADPH oxidase (NOX) 2 to produce reactive oxygen species (ROS), which stimulates ryanodine receptors to increase calcium spark rate, followed by a slow increase in sarcolemmal calcium influx. These responses may facilitate calcium recruitment for contractions against increased preload. When the preload increases, myocardial ATP consumption increases. The required ATP is produced in the process of oxidative phosphorylation in the mitochondrial respiratory chain. Our recent study revealed that myocardial stretch hyperpolarizes mitochondrial membrane potential which is the driving force for ATP synthase. This response might be beneficial for efficient ATP production against increased preload. The mitochondrial hyperpolarization also facilitates electron leak to produce mitochondrial ROS and affect NOX2 ROS production. These load-dependent responses may contribute to maintaining load-independent integrity in cardiac mechano-energetics. COI:No

3PS-01PM-4

Ca^{2+}-activated Cl^- channel TMEM16A in cirrhotic portal hypertension

Yamamura Hisao, Kondo Rubii, Furukawa Nami, Suzuki Yoshiaki, Imaizumi Yuji

Dept Mol Cell Pharmacol, Grad Sch Pharmaceut Sci, Nagoya City Univ, Nagoya, Japan

Portal hypertension is a major complication in patients with chronic liver diseases and cirrhosis, but its pathogenic mechanism remains unclear. Vascular tone of portal vein smooth muscles (PVSMs) is regulated by the activities of several ion channels including Ca^{2+}-activated Cl^- (Cl_{Ca}) channel. TMEM16A is mainly responsible for Cl_{Ca} channel conductance in portal vein smooth muscle cells (PVSMCs). Here, the functional expression of TMEM16A channels in portal hypertension was analyzed using two experimental animal models, bile duct ligation (BDL) mice with cirrhotic portal hypertension and partial portal vein ligation (PPVL) mice with idiopathic non-cirrhotic portal hypertension. Expression analyses revealed that TMEM16A was downregulated in BDL-PVSMCs but not in PPVL-PVSMCs. Whole-cell Cl_{Ca} currents were reduced in BDL-PVSMCs compared to sham- and PPVL-PVSMCs. Sham- and PPVL-PVSMs showed spontaneous contractions which were sensitive to Cl_{Ca} channel inhibitors. On the other hand, BDL-PVSMs represented similar spontaneous contractions, however, the TMEM16A-mediated component was clearly attenuated. This TMEM16A downregulation was mimicked by the exposure to angiotensin II in normal PVSMCs. The angiotensin II-induced downregulation of TMEM16A was reversed by the treatment with angiotensin II receptor blockers. These results suggest that the reduced Cl_{Ca} channel activity due to TMEM16A downregulation causes a lower membrane excitability of PVSMs and results in preventing from development of portal hypertension. COI:No

Planned Symposium 20

Joint symposium with the Japanese Society of Physical Fitness and Sports Medicine

Central cardiovascular regulation supporting emotion and behavior

March 30 (Fri) 14:00〜15:50 Hall 3

3PS-03PM-1

Acute shifts of baroreflex control of sympathetic nerve activity during sleep, exercise and mental stress

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The baroreflex is a negative-feedback control system for maintaining systemic arterial pressure (AP) at a certain constant level. An acute increase in AP induces a reflex decrease in sympathetic nerve activity (SNA), decreasing AP to the basal level. Thus, the relationship between AP and SNA shows an inverse sigmoidal curve. The baroreflex sigmoid function curve has been reported to shift acutely in response to changes in behavioral states. For instance, exercise increases both AP and SNA simultaneously, which can be explained by an acute shift in baroreflex control of SNA upward and to the right. We have reported that mental stress results in an increase in renal SNA, no changes in lumbar SNA and AP, and a decrease in heart rate. This suggests that acute shifts in baroreflex control of SNA can occur in a region-specific manner. In daily activity, including sleep, when awake, exercising, and under stress, each behavioral state generates a state-specific pattern of SNA, determining the AP which meets each behavioral cardiovascular demand. It is therefore likely that acute shifts in baroreflex control of SNA occur in a state-dependent and region-specific manner. In this symposium, we will describe in more detail the mechanisms of acute shifts in baroreflex control of SNA and arterial pressure regulation during sleep, exercise and mental stress. COI:No

3PS-03PM-2

Feedforward control of the cardiovascular system during exercise: insight from animal and human studies

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A feedforward signal descending from higher brain centers (termed central command) plays a role in controlling the cardiovascular system at the initial period of exercise. To identify the physiological role of central command, we have conducted a series of animal and human experiments. Central command increases cardiac sympathetic outflow, which contributes to the initial increases in heart rate and cardiac output. The cardiac responses are further enhanced via centrally-induced activation of the sympathoadrenal system. In addition, centrally-induced muscle vasodilatation occurs at the onset of exercise via the sympathetic cholinergic system in preparation for metabolic demands of skeletal muscles. Concomitantly, central command increases renal sympathetic nerve activity to maintain arterial blood pressure and to redistribute cardiac output to active skeletal muscles. Although evidence regarding central control in some peripheral organs has been accumulated so far, neural circuits responsible for generation of central command, however, remain unknown. The feedforward feature of central command provides us an idea that central command activation occurs sufficiently earlier prior to the exercise onset, taking into a large time lag of cardiovascular effector organs. Recently, we found that activation of the prefrontal cortex occurs 5 s prior to the onset of voluntary exercise. In the symposium, we would like to discuss about feedforward control of the cardiovascular system and possible central sites responsible for generation of central command. COI:No

3PS-03PM-3

Does motivation affect blood pressure? Cardiovascular regulation during reward-oriented behaviors in rats

Yamanaka Ko, Waki Hidefumi

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Our behaviors are strongly affected by motivation. For example, an action associated with a desirable outcome (reward) is performed more quickly and accurately than an action associated with an undesirable outcome (no reward). Given that it is necessary to quickly supply blood flow to muscles to promote such motivational behaviors, motivation may also have effects on autonomic cardiovascular regulation. However, little is known about the effects of motivation on the autonomic cardiovascular responses underlying motivational behaviors and their neuronal circuit mechanisms. To approach this issue, we recorded arterial pressure (AP) using telemetry in head-fixed rats performing a lever-exercise task. During the behavioral task, AP phasically increased in response to lever movement. AP was lower in the error trials in which motivation was considered to be low than in the correct trials. Furthermore, even in the correct trials that showed similar lever trace patterns, baseline AP progressively decreased through a day-long session, because of increasing satiation level and fatigue. These results suggest that cardiovascular activity may reflect animals' motivational state. Recently, we are trying to manipulate the activity of neurons in the hypothalamus and other limbic regions in the brain during the behavioral task. We would like to introduce these ongoing studies in the symposium. COI:No

3PS-03PM-4

Central regulation of the cardiovascular reaction during temporal and repeated social defeat stresses

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It is known that neurons in the dorsomedial area (DMH) and the perifornical area (PeF) in the hypothalamus play an essential role on the autonomic cardiovascular response evoked by psychological stress. A social defeat stress (SDS) that mimics an interpersonal issue at workplace or school is a kind of psychological stress, but it is unclear how neurons in the hypothalamus participate in the cardiovascular response during the SDS. In addition, orexin neurons are strictly localized within the DMH and PeF, and may be involved in the cardiovascular response during the SDS. In this presentation, we reveal the cardiovascular response, distributions of the c-Fos expression and role of the orexin neurons during the temporal (one day) and repeated (2 weeks) SDSs in conscious Wistar rats. Blood pressure (BP), heart rate (HR) significantly increased during both SDSs. However, repeated SDS did not alter the baseline BP and HR. The number of c-Fos expressed neurons in the DMH and PeF increased after both SDSs, but the number of orexin neurons in the DMH and PeF did not change compared to non-stress control animals. In the repeated SDS animal, the ratio of c-Fos expressions in the orexin neurons of the DMH increased compared to that of the temporal SDS group. These results suggest that neurons in the DMH play a crucial role in the cardiovascular response evoked by the SDS and the orexin neurons in the DMH are particularly involved in the stress-induced response. COI:No

3PS-03PM-5

Central neural circuitries underlying sympathetic adjustments to exercise

Koba Satoshi

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Sympathetic nervous system activity increases during exercise. Motor command signal emanating from the rostral brain, called central command, stimulates the brainstem, thereby producing parallel activation of somatomotor and sympathetic neurons. Central command activation has been shown to stimulate multiple central cardiovascular regions, including the rostral ventrolateral medulla (RVLM). Nevertheless, information is not available regarding neural pathways from *central command origin* to *sympathetic nervous system*. Our recent research efforts have been focused on exploring central neural circuitries underlying sympathetic adjustments to exercise. Our experimental techniques include anterograde/retrograde neural tracings combined with immunohictochemistry and *in vivo* observation of sympathetic nerve activity combined with optogenetics. The experiments have allowed us to find several neuronal pathways that possibly play a role in exercise-elicited sympathoexcitation. Updates on our findings are discussed. COI:No

Planned Symposium 21

Joint symposium with the Japanese Society for Behavioral Neuroendocrinology

Neurophysiology underlying intra- or inter-species communication

March 30 (Fri) 14:00〜15:50 Hall 4

3PS-04PM-1

Individual recognition and the 'face inversion effect' in medaka fish

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Individual recognition is essential for maintaining various social interactions in a group, and face recognition is one of the most specialised cognitive abilities in IR. We used both a mating preference system and an electric shock conditioning experiment to test IR ability in medaka, and found that signals near the face are important. Medaka required more time to discriminate vertically inverted faces, but not horizontally shifted faces or non-face objects. The ability may be comparable to the classic "face inversion effect" in humans and some other mammals. Extra patterns added to the face also did not influence the IR. These findings suggest the possibility that the process of face recognition may differ from that of other objects. The complex form of recognition may promote specific processing adaptations, although the mechanisms and neurological bases can be different from mammals in medaka. Such ability is important for shaping animal societies. COI:No

3PS-04PM-2

Neuronal mechanisms of temporal processing for electric communication in mormyrid weakly electricfish

Kohashi Tsunehiko

Neurosci. Inst., Div. Biol. Sci., Grad. Sch. Sci., Nagoya Univ, Nagoya, Japan

Precise temporal interval between successive communication signals conveys critical information regarding such as phenomes and punctuations in human speech, call types in animal communication and syllables in bird songs. Central neurons that respond selectively to temporal intervals of sensory input have been identified in wide range of species from mammals to insects. Neuronal mechanisms that create the interval selectivity, however, have been technically difficult to reveal, largely due to the lack of in vitro preparations in which cellular mechanisms are readily accessible while interval selectivity is reproducible as in vivo. Electrosensory neurons of weakly electric mormyrid fish offer a unique opportunity to overcome this problem. Pulse-type mormyrid fish communicate with other fish by varying the interpulse intervals (IPIs) between electric organ discharge (EOD) that they generate in the tail. The timing of each EOD from a neighboring fish is encoded by peripheral electroreceptors into precisely timed spikes and is conveyed to the midbrain posterior exterolateral nucleus (ELp), where there are neurons exhibit selective response to particular IPIs. Recently, we developed an in vitro whole-brain preparation of mormyrid Brienomyrus brachyistius in which local ELp circuitry remain intact and the interval selectivity of ELp neurons can be reproduced by direct stimulation of the afferents. This preparation have allowed us to reveal synaptic mechanisms and membrane excitability to shape the interval tuning of ELp neurons. COI:No

3PS-04PM-3

Behavioral, physiological, neurological and genetic bases of tameness in mice

Koide Tsuyoshi

MGRL, Nat Inst Genet, Mishima, Japan

Tameness is a behavioral characteristic with two potential components: reluctance to avoid humans (passive tameness) and motivation to approach humans (active tameness). To quantify these two different components of tameness separately in mice, we established three behavioral tests: the 'active tame', 'passive tame', and 'stay-on-hand' tests. We previously analyzed with the tame tests for two groups of mouse inbred strains: domesticated strains (laboratory strains) and wild strains. We found that most of the domesticated strains showed significantly greater passive tameness than wild strains, whereas there was no significant difference in active tameness. The results suggested that domesticated strains were predominantly selected for passive tameness over the course of their domestication but no attempts were made to select for active tameness in mice. Therefore, we tried to elucidate genetic and neural mechanism underlying active tameness by conducting selective breeding using genetically diverse wild-derived heterogeneous stock (WHS) made from eight wild inbred strains. As a result, the selected groups exhibit significantly higher active tameness than control groups, and following genetic study revealed that at least two closely linked loci on chromosome 11 associate with increased active tameness. Further behavioral studies revealed that selected groups exhibit higher social interaction behavior comparing the control groups. Our current attempts to analyze c-Fos positive neurons as well as RNA transcriptome will provide information for neural and molecular bases of active tameness in mice. COI:No

3PS-04PM-4

Toward the elucidation of the mechanism of affiliative relationship among interspecific species

Okabe Shota, Takayanagi Yuki, Yoshida Masahide, Onaka Tatsushi

Dept Physiol, Jichi Medi Univ, Tochigi, Japan

Social animals develop affiliative relationship with not only intraspecific but also interspecific individuals. While the oxytocin system is known to have an important role in regulating social interaction of intraspecific partners, the role of oxytocin neurons for interspecific affiliative relationship remains unclear. In addition, whether gentle stroking contributes to establishment of heterospecific affiliative relationship is unknown. The aim of this study was to investigate the role of gentle stroking and oxytocin neurons in forming affiliative relationships between humans and animals.
Virgin female rats (LEW/CrlCrlj) received stroking stimuli by hands of experimenter for 4 weeks between 3 and 7 weeks of age (S3-7 group), for 4 weeks between 7 and 11 weeks of age (S7-11 group), or for 8 weeks between 3 and 11 weeks of age (S3-11 group). Control rats did not receive stroking stimuli (N3-11 group). Rats of S3-7 and S3-11 groups emitted more frequently 50-kHz ultrasonic vocalizations, an index of positive emotion, during stroking as compare to the N3-11 group. In addition, S3-7 and S3-11 groups showed following behavior toward the hand of experimenter. Stroking induced expression of Fos protein in oxytocin neurons of the hypothalamic paraventricular nucleus of S3-7 and S3-11 groups. These findings suggest that stroking activates oxytocin neurons, which may contribute to forming an affiliative relationship. COI:No

3PS-04PM-5

Mutual gaze induces reciprocal human-dog communication via endocrine alterations

Nagasawa Miho[1], Ogawa Misato[1], En Shiori[1], Onaka Tatsushi[2], Sakuma Yasuo[3], Mogi Kazutaka[1], Kikusui Takefumi[1]

1:Azabu Univ, Sagamihara, Japan, 2:Jichi Medical Univ, 3:Univ of Tokyo Health Sciences

Animals have developed empathy-related neural and behavioral systems, which is clearly observed in mother-infant relationship, such as bonding. Neurochemically, Oxytocin plays a pivotal role in forming mother-infant bonding in both sides, and there is a positive loop of attachment-parenting behavior via the oxytocin system in the infant-mother dyad. Humans strengthen emotional bonds as we gaze into each other's eyes; therefore, we tested whether humans and dogs establish the emotional bonding by using eye-gazing as an attachment behavior in dogs and wolves. We discovered that such gaze-mediated bonding also exists between dogs and their owners, and even between Japanese dogs which are included in the genetically closest cluster to wolves. Mutual gazing increased oxytocin levels in both dogs and their owners, but on the other hand, wolves did not show much mutual gazing. Administration of oxytocin increased gazing in dogs, and this behavioral change was transferred to their owners; increase of oxytocin levels in the owners. Japanese dogs use eye-gazing towards their owners similar to European breeds; however, there is a disparity between the dog sexes when it comes to the owners' oxytocin secretion. Japanese dogs also showed different attachment behaviors from both European breeds and wolves, and they likely use additional strategies to substitute gaze when forming the human-dog bond. COI:No

Symposia

Symposium 1

New Prospects of Peptide Hormone Research -Development of novel hormone search technology and diversity of hormone function-

March 28 (Wed) 9:00～10:50 Hall 5

1S-05AM-1
Identification of the novel bioactive peptides using model animal

Ida Takanori

Peptide, Frontier, Miyazaki Univ, miyazaki, Japan

In both vertebrates and invertebrates, there are many orphan G protein-coupled receptors (GPCRs), for which ligands have not yet been identified. Identification of their endogenous ligands is very important for understanding the function and regulation of such GPCRs. Indeed, That has enhanced our understanding of many physiological processes including feeding behavior, sleep-awake system, stress reaction, immunological system and reproduction. Here, we identified five Drosophila endogenous ligands, CCHamide-1, CCHamide-2, dRYamide-1, dRYamide-2 and trissin of the Drosophila orphan GPCRs and LURY-1 of the C.elegans orphan GPCRs using functional assays with the reverse pharmacological technique. And, in various invertebrates, we identified orthologous peptides to Drosophila peptides. Some of these peptides modulate feeding behavior and metabolism. These results suggest that identification of novel invertebrates bioactive peptide might facilitate the elucidation of various physiological function and have a useful possibility for the ligand searching of mammal orphan GPCRs. COI:No

1S-05AM-2
Big angiotensin-25 (Bang-25): a novel glycosylated angiotensin-related peptide isolated from human urine

Nagata Sayaka, Kitamura Kazuo

Univ of Miyazaki, Miyazaki, Japan

The renin-angiotensin system (RAS) plays key roles in the regulation of blood pressure and body fluid balance. In 2006, we isolated and characterized proangiotensin-12 (proang-12), a component of the tissue RAS in rat small intestine. Similar angiotensin-related peptides had never been reported in humans. In a search for bioactive peptides using an antibody against the N-terminal portion of Ang II, we identified and characterized a novel angiotensin-related peptide from human urine as a major molecular form. We named the peptide Big angiotensin-25 (Bang-25) because it is composed of 25 amino acids and is N-glycosylated on its 14th amino acid (Asn) and has a cysteine linked to its 18th amino acid (Cys). We then assessed the distribution of Bang-25 in tissues by immunostaining with an antiserum raised against the C-terminal portion of the peptide. We found that Bang-25 was abundantly expressed in a number of human tissues, including kidney, heart, adrenal gland, pancreas and placenta. In the kidney, Bang-25 is localized predominantly to podocytes. Then, compared with Aogen, Bang-25 is rapidly cleaved by chymase to Ang II, but is resistant to cleavage by renin. In the present study, we have isolated and identified a novel Aogen-derived peptide, Bang-25. The identification of Bang-25 suggests the existence of a RAS processing cascade different from the renin-catalyzed cleavage of Aogen to Ang I, and provides a potential target for assessing tissue RAS status and for the development of new therapeutic approaches to related diseases. COI:No

1S-05AM-3
Neuromedin U precursor-related peptide: a newly identified neuropeptide with prolactin-releasing activity

Mori Kenji, Miyazato Mikiya, Kangawa Kenji

Dept Biochem, Natl Cerebral & Cardiovascular Ctr Res Inst, Suita, Japan

Neuromedin U (NMU) is a neuropeptide which is widely distributed in the gut and central nervous system. The precursor for NMU contains multiple cleavage sites for proteolytic processing. This suggests that the precursor might generate additional peptides. Here, we identified such a processing product by purification, and named this peptide neuromedin U precursor-related peptide (NURP). Immunoaffinity chromatography of rat brain extracts demonstrated that NURP was present as two mature peptides of 33 and 36 residues. NURP immunoreactivity was detected in the pituitary, small intestine, and brain of rats by radioimmunoassay, with the most intense reactivity in the pituitary. Intracerebroventricular administration of NURP to both male and female rats induced a robust increase in the plasma concentrations of prolactin and failed to alter the plasma concentrations of other anterior pituitary hormones. In contrast, NURP was unable to stimulate prolactin release from dispersed anterior pituitary cells harvested from male rats. Pretreatment of rats with bromocriptine, a dopamine receptor agonist, blocked the prolactin-releasing activity of NURP. In rats pretreated with the antagonist sulpiride, intracerebroventricular administration of NURP did not increase plasma prolactin concentrations more than administration of saline. These data suggest that NURP induces prolactin release by acting indirectly on the lactotrophs of the pituitary; dopamine from the hypothalamus, which inhibits prolactin release, may be involved in NURP activity. COI:No

1S-05AM-4
Diversity of ghrelin function and adaptation to starvation

Sato Takahiro, Kojima Masayasu

Inst Life Sci, Kurume Univ, Fukuoka, Japan

When faced with starvation, we must survive while achieving two contradictory goals of exploring food and preserving energy in the body. Therefore, our body has a mechanism to adapt to the emergency of starvation, and one of the candidate substances controlling this mechanism is ghrelin. Ghrelin of gastrointestinal hormone is mainly secreted from the stomach, and physiologically, the serum ghrelin concentration is highest under fasting conditions. On the other hand, the ghrelin receptor is widely distributed not only in the digestive tract but also in the brain, the pituitary gland, and the pancreas. Therefore, ghrelin shows various physiological functions such as promotion of growth hormone secretion, hyperphagia, fat accumulation and the like. In addition to these known functions, we have shown that ghrelin is essential for maintaining body temperature during fasting. In this presentation, we will introduce the mechanism of ghrelin regulating body temperature, and consider how ghrelin's various functions are coordinately regulated in order to achieve the object of starvation adaptation. In addition to searching for new hormones, further functional analysis will be carried out on known hormones and it is expected to lead to an understanding of starvation adaptation based on hormonal diversity as shown in this presentation, that is, maintenance of homeostasis. COI:No

Symposium 2

A new insight of the synaptic formation from molecular level to circuit level.

March 28 (Wed) 9:00～10:50 Hall 7

1S-07AM-1

Microglial contribution of neuronal circuit formation during development

Miyamoto Akiko[1], Wake Hiroaki[1], Ishikawa Ayako[2,5], Murakoshi Hideji[3], Eto Kei[4,5], Yoshimura Yumiko[2,5], Nabekura Junichi[4,5]

1:Div System Neurosci, Dept Physiol and Cell Biol, Grad Sch Med, Kobe Univ, Hyogo, 2:Div Visual Info Proc, Natl Inst Physiol Sci, Aichi, 3:Supportive Center for Brain Res, Natl Inst Physiol Sci, Aichi, 4:Div Homeostatic Develop, Natl Inst Physiol Sci, Aichi, 5:Dept Physiol, Sch Life Sci, SOKENDAI, Hayama

Microglia, which are the immune cells in the central nervous systems, are one of the glial cells. It has been revealed that microglia also have some actions for synaptic connection during physiological condition. For example, microglia selectively contact onto synapses in intact brain and are also involved in circuit refinement via synapse elimination at ischemic penumbra region and developmental period. Recently, we found that microglia induce filopodia, which are precursor of spine, during cortical development using in vivo two photon imaging technique. Injection of microglia activation inhibitor decreased cortical spines in density. Microglia specific ablation also decrease spine density and miniature EPSC frequency. Finally, we investigate whether microglia induced filopodia formation affect to cortical function. To reveal that we investigated the synaptic connection between cortical layers by uncaging photostimulation method. Usually, strength of excitatory synaptic connections from layer 4 to layer 2/3 pyramidal cells are prominent. However, these are reduced in microglia ablated mice. Taken together, this finding suggests that microglia contributed to neuronal circuit maturation not only via synapse elimination but also via synapse formation. COI:No

1S-07AM-2

A septin-dependent synaptic regulation required for spatial discrimination

Ageta-Ishihara Natsumi, Kinoshita Makoto

Dept Mol Biol, Grad Sch Sci, Nagoya Univ, Nagoya, Japan

A novel spatial context is recognized by cell assemblies through comparison and differentiation between a firing pattern encoding the current unfamiliar surroundings and those of familiar ones. In theory, separation among similar but not identical firing patterns relies on decorrelation via high-threshold synapses. A representative case is those formed between the perforant path (pp) from the entorhinal cortex and granule cell (GC) dendrites in the hippocampal dentate gyrus (DG). However, the pp-GC synapse is poorly characterized at molecular/ultrastructural level. Here we show that mice that lack a pan-neuronal subunit of the septin cytoskeleton pass hippocampus-dependent tasks. Intriguingly, however, they consistently underperform in specific tasks that require discrimination among distinct spatial contexts. AAV vector-mediated, DG neuron-selective supplementation of the subunit restores the performance in NAPR, while the local depletion recapitulates the defects in wild-type mice. Fine morphometry with serial section TEM and 3D reconstruction of asymmetric synapses in three major hippocampal subregions identifies a specific postsynaptic anomaly that is most severe in the pp-GC synapse. Depletion of the subunit from primary cultured DG neurons recapitulates the morphological anomaly *in vitro*. These and other findings collectively indicate that a partial loss of septin function from pp-GC synapse is primarily responsible for the attenuated spatial pattern separation. Molecular mechanisms underlying the neuron-selectivity and the unique mode of synaptic regulation are under investigation. COI:No

1S-07AM-3

Neuronal activity-dependent millisecond Ca^{2+} dynamics activate multiple proteins for synaptic vesicle control

Mochida Sumiko

Dept Physiol, Tokyo Med Univ, Tokyo, Japan

For reliable transmission at chemical synapses, neurotransmitters must be released dynamically in response to neuronal activity in the form of action potentials. Stable synaptic transmission is dependent on the efficacy of transmitter release and the rate of resupplying synaptic vesicles to their release sites. Accurate regulation is conferred by proteins sensing Ca^{2+} entering through voltage-gated Ca^{2+} channels opened by an action potential. Presynaptic Ca^{2+} concentration changes are dynamic functions in space and time, with wide fluctuations associated with different rates of neuronal activity. Thus, regulation of transmitter release includes reactions involving multiple Ca^{2+}-dependent proteins, each operating over a specific time window. Classically, studies of presynaptic proteins function favored large invertebrate presynaptic terminals. I have established a useful mammalian synapse model based on sympathetic neurons in culture. In this symposium I summarize the use of this model synapse to study the roles of presynaptic proteins in neuronal activity for the control of transmitter release efficacy and synaptic vesicle recycling. COI:No

1S-07AM-4

The roles of visual experience in the maturation of secondary visual cortex

Yoshimura Yumiko

Nat Inst Physiol Sci, Okazaki, Japan

Visual cortical functions are refined by visual experience during postnatal development. We previously reported that visual deprivation during development did not reduce the strength of visual responses in rat V1, although it decreased orientation/direction selectivity slightly. V1 neurons send their output signals to the secondary visual cortex (V2), suggesting the susceptibility of V2 neurons to visual deprivation. We investigated this possibility in the lateromedial area (LM), one subarea of V2, using 4-week-old rats raised normally or with binocular deprivation beginning just before eye opening. We conducted unit recordings from LM neurons under anesthesia. Binocular deprivation severely decreased the strength of visual responses in LM neurons, although it did not affect orientation/direction selectivity. Flavoprotein fluorescence imaging of cortical areas, including V1 and LM, showed that the ratio of LM to V1 responses in deprived rats was about half of that in normal rats, indicating that visual responses in LM were commonly weakened by deprivation.To explore the change in neural circuits underlying the decreased visual responsiveness in LM, we morphologically analyzed projections from V1 to LM. In normal rats, the axons of V1 neurons labeled by GFP targeted heavily layer 4 of LM. Binocular deprivation substantially decreased these axons, suggesting that visual experience seems necessary for the establishment of projections from V1 to LM. Therefore, visual experience likely contributes to the maturation of visual responsiveness in V2 neurons through the establishment of long-range projections from V1 to V2. COI:No

1S-07AM-5

The C1q complement family proteins and glutamate receptors: bridge over the synaptic cleft

Matsuda Keiko, Yuzaki Michisuke

Dept. Physiol, Keio Univ Sch Med, Tokyo,Japan

C1q is identified as the initial protein of the classical complement pathway in the innate immune system. A number of C1q-related family proteins sharing globular domain of C1q have been shown to be expressed in CNS. We have demonstrated the crucial function of C1q family members as a new class of synapse organizer, together with their receptors across over synaptic cleft. Cbln1 released from parallel fiber plays an indispensable role in the formation and function of parallel fiber-Purkinje cell synapses. This synapse formation depends on direct binding of Cbln1 to the amino-terminal domains (ATD) of glutamate receptor GluD2 in Purkinje cells and simultaneous binding to presynaptic neurexin. Another C1q family member, C1ql2 and C1ql3 is expressed in dentate gyrus granule cells and released from mossy fiber (MF) terminals at MF-CA3 synapse in hippocampus. In analogy to Cbln1-GluD2 binding, C1ql2 and C1ql3 bind to ATD region of kainate-type of glutamate receptors (KARs) and recruit functional postsynaptic KAR complexes at MF-CA3 synapses. Moreover, C1ql subfamily members directly bind to NRX3 containing sequences encoded by exon25b insertion at splice site 5. Recently, the important role of C1ql subfamily members in synapse formation has been revealed in various brain regions. For example, C1ql1 expressed in inferior olivary nucleus regulate the formation and maintenance of climbing fiber - Purkinje cell synapses in the cerebellum. In this talk, I would like to discuss the function of C1q family members and their receptors in certain type of synapse organization. COI:No

Symposium 3

Protective mechanisms against hypoxia: from molecules to whole body

March 28 (Wed) 9:00～10:50 Hall 8

1S-08AM-1

Hypoxia detection mechanism in adrenal medullary cells

Harada Keita, Inoue Masumi

Dept Cell and Systems Physiol, UOEH, Fukuoka, Japan

We and other have reported that rat adrenal medullary (AM) cells lose hypoxia sensitivity one week after birth, whereas guinea-pig AM cells maintain it until adulthood. Therefore, exploring what is different between AM cells in adult rats and guinea pigs would provide insight into the mechanism for hypoxia detection. Guinea-pig AM cells conspicuously secreted catecholamine (CA) in response to CN^- or CCCP (a complex IV inhibitor and a protonophore, respectively), but rat cells showed a little. The time course of depolarization of the mitochondrial membrane potential ($\Delta\Psi_m$) in guinea-pig AM cells in response to CN^-, chemical hypoxia, was slower than that in rat AM cells, and this difference was abolished by oligomycin, an F_1F_0-ATPase inhibitor. The extent of CCCP-induced decrease in cellular ATP in guinea-pig AM cells was larger than that in rat AM cells. The relative expression level of F_1F_0-ATPase inhibitor factor in guinea-pig adrenal medullae was smaller compared with that in rat adrenal medullae, and the opposite was true for F_1F_0-ATPase α subunit. To conclude, guinea-pig AM cells secrete more CA in response to chemical hypoxia than rat AM cells and this higher susceptibility in the former is accounted for by large extent of reverse operation of F_1F_0-ATPase with the consequent decrease in ATP. These results support the notion that the large extent of reversed operation of F_1F_0-ATPase due to insufficient expression of the inhibitor factor may play the major role for hypoxia detection in AM cells. COI:No

1S-08AM-2

Carotid body chemosensing: adaptation and pathology

Pokorski Mieczyslaw

Dept Nursing & Specialist Care, Opole Med Sch, Opole, Poland

Oxygen is fundamental for the cell metabolism in all aerobic organisms. Both deficit and surplus in tissue oxygenation cause morbidity and can induce long-term sequelae. To meet the objective of being beneficial for health, O_2 should be maintained within a narrow range. Thus, O_2 must be strategically sensed and its supply rapidly controlled mostly via respiration. The adaptive capability of the respiratory system relies on feedback mechanisms initiated by carotid body (CB) O_2-sensitive glomus cells. The CB role in chronic hypoxia, and its intermittent hypoxia variety, is inextricably linked to hypoxia inducible factors, master regulators of the adaptive processes. However, the sensor of acute hypoxia is still elusive. This sensor is liable to respond to changes in O_2 content along the hypoxia-hyperoxia continuum, rather than to any single O_2 level, and would translate the response to ventilatory changes. Recently, a research interest focuses on novel mechanisms of hypoxia-sensing. Odorant receptors, engaged in sensory signaling in olfaction, are ectopically expressed in CB. These receptors mediate hyperventilation by sensing lactate instantaneously produced when O_2 level drops. Other candidate proteins such as galanin, neuroglobin, or YKL-40 recently unraveled in CB play an interactive role in the adaptive plasticity of O_2 sensing, carotid body aging, and the suppression of hypoxic ventilatory reactivity in neurodegenerative and metabolic pathologies. Different intertwined mechanisms shape the acute ventilatory response to hypoxia, but none can yet be considered the ultimate hypoxia sensor. The enigma of O_2 sensing in the tissue goes on. COI:No

1S-08AM-3

Central mechanism of hypoxic respiratory regulation

Okada Yasumasa[1], Yazawa Itaru[2], Takeda Kotaro[1,3], Okazaki Shuntaro[1,4], Uchiyama Makoto[5], Fukushi Isato[1], Yokota Shigefumi[6], Mori Yasuo[5], Mieczyslaw Pokorski[7], Onimaru Hiroshi[1,8]

1:Lab Electrophysiol, Clin Res Ctr, Murayama Med Ctr, Tokyo, 2:Pharm Pharmaceut Sci, Hoshi Univ, Tokyo, 3:Fac Rehab, Sch Health Sci, Fujita Health Univ, Toyoake, 4:Fac Human Sci, Waseda Univ, Tokorozawa, 5:Dept Syn Chem Bio Chem, Grad Sch Eng, Kyoto Univ, Kyoto, 6:Dept Anat Morphol Neurosci, Shimane Univ, Izumo, 7:Opole Med Sch, Opole, Poland, 8:Dept Physiol, Showa Univ, Tokyo

It has been described that hypoxia is sensed solely by peripheral chemoreceptors in respiratory control. Indeed, hypoxia does not increase but suppresses ventilation in peripheral chemodenervated anesthetized animals. However, hypoxia augments ventilation in unanesthetized awake animals even after peripheral chemodenervation, indicating the existence of the central hypoxia-sensitive mechanism. To investigate the central mechanism of hypoxic respiratory regulation, we used in vitro brainstem-spinal cord preparations isolated from newborn rats or mice. The preparation generates spontaneous respiratory output but lacks peripheral tissue. Thus, it is ideal in the study of purely central mechanism of hypoxic respiratory responses. When the superfusate gas composition is switched from control (95% O_2, 5% CO_2) to hypoxic (95% N_2, 5% CO_2), respiratory frequency increases transiently followed by a decrease in respiratory frequency. Based on reports on intrinsic hypoxic sensitivity of astrocytes, we propose that not only a subset of neurons but astrocytes in the hypothalamus and medulla play roles in respiratory hypoxic sensing. Further studies are necessary to identify hypoxic sensing molecules in the sensor cells. COI:No

1S-08AM-4

Effects of Hypoxia on Circulatory Function

Pearson James[1,2], Tsuchimochi Hirotsugu[1], Schwenke Daryl[3], Sonobe Takashi[1], Jin Huiling[1], Shirai Mikiyasu[4], Ogo Takeshi[4]

1:Dept Cardiac Physiology, National Cerebral and Cardiovascular Center, Osaka, Japan, 2:Dept Physiology, Monash University, Melbourne, Australia, 3:Dept Physiology, Otago University, Dunedin, New Zealand, 4:Dept Advanced Med Res Pulmonary Hypertension, NCVC, Osaka, Japan

Acute exposure to hypoxia evokes a hyperemic response through vasodilation in both cerebral and coronary arterial vessels, but vasoconstriction in the pulmonary arteries. The latter response facilitates the redistribution of blood flow to better aerated lung regions when hypoxia occurs regionally. However, hypoxic pulmonary vasoconstriction (HPV) is maladaptive when hypoxia globally affects the lungs. Chronic exposure to continuous or intermittent hypoxia (IH) results in endothelial dysfunction. Multiple neurohormonal factors, inflammation and exposure to ROS cause vascular remodeling and impairment of blood flow control in both the heart and lungs. Increased muscularization of small and intermediate size vessels occurs in both coronary and pulmonary circulations. Chronic IH exposure reduced the ability to increase distal coronary perfusion through conducted dilation due to impairment in endothelium derived hyperpolarization factor production. On the other hand, sympathoadrenal activation following chronic IH exposure greatly attenuates HPV through increased β-adrenoceptor mediated dilation and iNOS signaling. It is now becoming clear that conditions such as insulin resistance, which also involve oxidative stress, when combined with exposure to chronic hypoxia or chronic IH, exacerbate cardiopulmonary endothelial dysfunction and vascular remodeling. COI:No

Symposium 4

Space mouse experiments using a new rodent experimental platform “MARS”

March 28 (Wed) 9:00～10:50 Hall 9

1S-09AM-1

Development of new experimental platform 'MARS' - Multiple Artificial-gravity Research System - to elucidate the impacts of micro/partial gravity on mice

Shiba Dai, Suzuki Tomoko, Simomura Michihiko, Yumoto Akane, Senkoji Teruhiro, Mizuno Hiroyasu, Shirakawa Masaki

JEM Utilization Center, JAXA

The Japan Aerospace Exploration Agency project focused on elucidating the impacts of partial gravity (partial *g*) and microgravity (μg) on mice using newly developed mouse habitat cage units (HCU) that can be installed in the Centrifuge-equipped Biological Experiment Facility in the International Space Station. Here, we present an overview of this space mouse project by JAXA and the development of the new hardware. We also present the results of the first mission with onboard 1 g control. The JAXA rodent experimental platform 'MARS' - Multiple Artificial-gravity Research System - presents a novel opportunity to elucidate the specific effects of μg on mice and also enhances our ability to conduct partial *g* experiments in space for future human space exploration. COI:No

1S-09AM-2

The molecular mechanism of muscle atrophy in space

Okada Risa[1], Tsubouchi Hirona[1,2], Shimbo Miki[1], Hamada Michito[1], Muratani Masafumi[3,4], Shiba Dai[5], Shirakawa Masaki[5], Kudo Takashi[1], Takahashi Satoru[1]

1:Dept. Ana. Emb., Fac. Med., Univ. Tsukuba, 2:Mas. Pro. Med. Sci., Grad. Sch. Com. Hum. Sci., Univ. Tsukuba, 3:Dept. Genome Biol., Fac. Med., Univ. Tsukuba, 4:TMRC, Fac. Med., Univ. Tsukuba, 5:JEM Utili. Cent., JAXA

Skeletal muscle is one of the most robustly affected organs under different gravitational conditions and it is known that space environment causes muscle weakness, however, the molecular mechanism of muscle atrophy in space is largely unknown. In the "Mouse Epigenetics" project, 12 mice were housed under microgravity (MG) or artificial earth-gravity (AG) in "Kibo", the Japanese Experimental Module onboard the International Space Station, for 35 days, and the weight of both soleus and gastrocnemius muscles were significantly decreased in MG compared with AG and ground control (GC) mice. (*Shiba D et al., 2017, Sci. Rep.*)

To reveal the molecular mechanism of muscle atrophy during spaceflight, we conducted transcriptome analysis of these soleus muscles to measure the gene expression patterns using RNA sequencing, resulting in significantly up- or down-regulated genes in MG mice compared with AG mice. To examine the relations of these genes and muscle atrophy, we introduced some of them into C2C12 myotubes using recombinant adenovirus vectors and identified several genes as candidates that cause a reduction in myotube diameter.

These results suggest that candidate genes that we found through the comprehensive analysis and *in vitro* screening may have roles in microgravity-induced muscle atrophy. COI:No

1S-09AM-3

Osteoporosis in spaceflight and osteoclasts

Shinohara Masahiro

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Bone is a key biological organ which characterizes the class of vertebrates, and has a critical role as a component of the skeletal-locomotor system. Bone is also known to be a calcium reservoir regulated by the endocrine system and an immunological organ that harbors and mobilizes hematopoietic stem cells. Thus, maintenance of the bone tissue homeostasis is very important not only for performing daily activities but also keeping healthy physical conditions. Mechanical loading on the bone is the most critical factor for maintaining normal bone homeostasis. Under mechanical unloading conditions such as spaceflight and long-bed rest, bone mass is dramatically decreased due to enhanced osteoclastic bone destruction, which leads to osteoporosis, a bone disease with a high risk of bone fracture. Here, mechanisms of bone loss induced by the mechanical unloading are summarized and discussed by focusing on the bone-degrading osteoclasts and their differentiation factor RANKL. The elucidation of molecular mechanisms will not only help pathological understandings but also development of a novel therapeutic strategy for disuse osteoporosis. COI:No

1S-09AM-4

Plastic alteration of the vestibular system induced by gravitational change including microgravity and hypergravity

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The vestibular system especially otolith organs and semicircular canals is the sensory system to detect the linear acceleration and angular velocity. Traditionally, the vestibular system is known to participate in eye movement and body balance, however recent studies have clarified the other physiological function through the vestibular system using gravitational change; muscles and bone, food intake, circadian rhythm, body temperature and cardiovascular system. The vestibular system is known to be highly plastic; i.e., the sensitivity of the vestibular system is altered if subjects are maintained in a different gravitational environment. This plasticity is considered to be one of the reasons for post-space flight deconditioning. Accordingly, examining the detail of its mechanism is important to develop post-space flight deconditioning treatment. For this purpose, we examined changing of the gene expression in the peripheral and central part of the vestibular system in mice after exposure to microgravity or hypergravity, and we found that plastic alteration was occurred even at the peripheral vestibular organ including saccule and utricle. Furthermore, cardiovascular response induced by central photostimulation in the vestibular system was altered in rats after exposure to hypergravity. These results indicate that plastic alteration in the peripheral vestibular organ might affect the downstream of the vestibular pathway. COI:No

Symposium 5

The generality and the specificity of signal processing circuit in the retina

March 28 (Wed) 9:00～10:50 Hall 10

1S-10AM-1

Determination of regions required for plasma membrane expression in metabotropic glutamate receptor type 6

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Metabotropic glutamate receptor type 6 (mGluR6) in retinal ON-bipolar cells (BCs) and ionotropic AMPA/KA glutamate receptors in OFF-BCs play critical roles in the parallel processing of visual information. These glutamate receptors are specifically localized at the dendritic tips of respective BCs. Although the molecular determinants of ionotropic glutamate receptors for the polarized localization have been extensively described, the molecular mechanisms how mGluR6 is targeted and expressed at the dendritic tips of ON-BCs have been poorly understood. In order to identify the amino acids in mGluR6 that act as targeting signals for plasma membrane expression, we here generated various C-terminally deleted mGluR6 and examined their expression using heterologous expression systems in cultured hippocampal neurons and HEK293 cells. We showed that the mutated mGluR6 lacking the last 14 amino acids was expressed at the plasma membrane, while the mutant lacking the last 15 amino acids was not transported to the plasma membrane. We also generated a series of mGluR6 mutants, in which the C-terminal intracellular domain (CTD) was N-terminally deleted. We showed that the mutated mGluR6 missing the entire CTD was not transported to the plasma membrane, while the mutant lacking all amino acid except the last C-terminal residue of CTD was targeted to the plasma membrane. These results suggest that the cell surface targeting of mGluR6 may involve multiple amino acids within the last 15 residues of the CTD. COI:No

1S-10AM-2

Physiological and immunohistchemical analyses of histamine receptors in retinal neurons.

Miyachi Ei-ichi[1], Ohkuma Mahito[1], Horio Kayo[1], Imada Hideki[2]

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The expression of histamine receptor has been reported in amacrine cells of mouse and rat retinae. In order to confirm that histamine modulates the membrane potential in mouse amacrine cells, we measured voltage-gated currents using whole-cell configuration of patch-clamp technique. Under voltage-clamp conditions, the amplitude of voltage-gated outward currents was enhanced by the application of 100 μM histamine in 65% of amacrine cells. Histamine also increased the amplitudes of voltage-gated inward currents in amacrine cells. When antagonists of the histamine H1, H2, or H3 receptors were applied to histamine-sensitive amacrine cells, all three types of these inhibitors reduced the effect of histamine. Fura-2 based calcium-imaging technique and immunohistochemical analyses were also used in the gerbil. A bath application of 100 μM histamine or histamine agonists increased the intracellular calcium concentration in some retinal ganglion cells. Next, we examined the localizations of H1, H2 and H3 receptors in the gerbil retinae from 1 to 350 postnatal days. We found that H1, H2 and H3 receptors expressed respectively on retinal ganglion cells. H1 receptor expresses through the retinal maturation. On the other hand, the expressions of H2 and H3 receptors became maximum from 14 to 21 postnatal days. These results suggest that histamine may be one of the important neurotransmitters and/or neuromodulators in the visual information processings of the mammalian retina. COI:No

1S-10AM-3

Molecular mechanisms underlying the establishment of spatially asymmetric inhibitory connectivity in retinal direction selective circuits

Yonehara Keisuke

DANDRITE, Aarhus University, Aarhus, Denmark

Spatially asymmetric neuronal connectivity is a key organizing principle that characterizes neuronal systems. Well-known examples are orientation-selective cells in the visual cortex and direction-selective (DS) ganglion cells in the retina. The main aim of my research is to elucidate the key molecular mechanisms underlying functional development of neural circuits by focusing on this organizing principle. I recently identified congenital nystagmus gene Frmd7 as a key regulator in the establishment of spatially asymmetric inhibitory connectivity from inhibitory starburst amacrine cells to DS cells in the retina. However, it remains unknown what kind of molecular signaling the Frmd7 is involved in. To investigate the role of multi-gene functions in the establishment of asymmetric connectivity from starburst cells to DS cells, I have created a catalogue of developmental cell type transcriptome for inhibitory amacrine or excitatory ganglion cell types that are involved in retinal direction selectivity. We have been performing functional screening for mutant mouse lines of the identified genes by assessing gaze-stabilizing reflex, followed by micro-electrode spike recordings from isolated retinas. Here I would like to discuss the result of our functional screenings and possible signaling cascades in which Frmd7 may be involved. Our insights will provide causal link between gene function, asymmetric circuit development, motion computation and eye movement control. COI:No

1S-10AM-4

How the cone photoreceptor synapse encodes the visual scene

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In daylight, the cone outer segment converts the light input from a visual scene into a time-varying electrical signal. In order for a visual percept to occur, this signal must be re-encoded as a rate of vesicle release at the cone synapse, which is among the most structurally complex synapses in the CNS. The spatiotemporal glutamate gradient in the cone synaptic cleft is sampled by the dendritic contacts of more than 12 different bipolar cell types. These types start the process of breaking down the visual scene into parallel components for subsequent processing in the retina and higher visual centers. The functional dichotomy between bipolar cell types that depolarize at light-on (On cone bipolar cells) and light-off (Off cone bipolar cells) is well understood. Instead, we focus on how the cone signal is differently transformed as it flows to the 5 types of Off (ionotropic glutamate receptor-expressing) bipolar cells. I show that the individual Off bipolar types have different thresholds for responding to cone transmitter release and encode the visual signal over different light decrement ranges. Differences in bipolar cell response threshold result from type-specific distances between cone ribbon vesicle release sites and the sites of bipolar cell cone contacts, the precise number of cone contacts each bipolar cell type makes, the glutamate affinity of different receptors expressed by each Off bipolar cell type, and the localization of glutamate transporters on the cone terminal surface. Our findings suggest that parallel processing at the cone to bipolar cell synapse is more elaborate and systematic than currently thought. COI:No

1S-10AM-5

Lrit1, a leucine-rich immunoglobulin-like transmembrane protein, is required for the synapse formation between photoreceptor cells and bipolar cells in the retinal circuit

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Precise neuronal connection mediated by synapse formation is crucial for correct development and function of the mammalian brain and retina. However, the molecules involved in this process and their functional mechanisms are still poorly understood. Here, we found that Lrit1 and Lrit2, two leucine-rich membrane proteins, are expressed in the retinal photoreceptor and bipolar cells, and that Lrit1 plays an essential role for the synaptic connection between photoreceptor cells and bipolar cells. Lrit1-deficient retinas exhibited an irregular distribution and aberrant morphology of cone photoreceptor pedicles. In addition, Lrit1 deficient mice displayed a prolongation of signal transmission in ERG analysis. Coimmunoprecipitation experiments in cultured cells demonstrated that Lrit1 interacts with a glutamate receptor, mGluR6, which localizes to the bipolar postsynaptic terminals and is essential for the ON bipolar functions. Our results suggest that Lrit1 is required for the photoreceptor-ON bipolar synapse formation via interacting with mGluR6 in bipolar postsynaptic terminals. COI:No

Symposium 6

Molecular/Cellular Physiology and Pathology of the Collective cell migration

March 28 (Wed) 15:10〜17:00 Hall 1

1S-01PM-1
Molecular mechanism underlying directional collective migration of endothelial cells in angiogenesis

Fukuhara Shigetomo[1], Wakayama Yuki[2], Fujiwara Masakazu[1], Sonoi Rie[1], Mochizuki Naoki[2]

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Angiogenesis is a complex morphogenetic process by which new blood vessels form from pre-existing vasculature, and regulates various physiological and pathophysiological processes such as embryogenesis, wound healing, cancer and diabetic retinopathy. In angiogenesis, endothelial tip cells located at the vascular front and the trailing stalk cells undergo directional collective cell migration to elongate new blood vessels. However, the underlying mechanisms of their directional collective cell migration during angiogenesis remain still largely unknown. Here, we addressed this question by performing fluorescence-based bio-imaging analyses using zebrafish as a model animal. By live-imaging endothelial cell morphology, movement and front-rear polarity, we showed that endothelial tip and stalk cells actively moved forward by establishing the front-rear polarity in angiogenesis. We also found that endothelial tip cells established the front-rear polarity by contacting with the stalk cells, thereby moving forward. In addition, our in vivo live-imaging analyses together with in vitro wound healing assay suggested that endothelial stalk cells established the front-rear polarity by sensing the pulling force by the cells in front and by pushing force by the cells in back. In this symposium, we discuss how mechanical interaction between the endothelial cells regulates their directional collective cell migration in angiogenesis. COI:No

1S-01PM-2
Intercellular propagating wave of ERK activation and its role in collective cell migration.

Aoki Kazuhiro

Div Quantitative Biol, Nat Inst Basic Biol, NINS

Cell migration is a fundamental process in many physiological and pathological contexts. In general, there are two modes of cell migration: single cell migration and collective cell migration. The collective cell migration refers to a movement of cell groups with physical and functional cell-cell connections, and involves an inherent process of embryonic development, wound healing and cancer invasion. The biophysical framework of collective cell migration has been extensively investigated in recent years; however, it remains elusive how chemical inputs from neighboring cells are integrated to coordinate the collective movement. Here, we provide evidence that propagation waves of extracellular signal-related kinase (ERK) mitogen-activated protein (MAP) kinase activation determine the direction of the collective cell migration. A wound healing assay of Mardin-Darby canine kidney epithelial (MDCK) cells revealed two distinct types of ERK activation wave, a tidal wave from the wound and a self-organized spontaneous wave in regions distant from the wound. In both cases, MDCK cells collectively migrated against the direction of the ERK activation wave. The inhibition of ERK activation propagation suppressed collective cell migration. ERK activation wave spatiotemporally controlled actomyosin contraction and cell density. Furthermore, optogenetic ERK activation wave reproduced the collective cell migration. These data provide new mechanistic insight into how cells sense the direction of collective cell migration. COI:No

1S-01PM-3
Collective migration and 3D morphogenesis of epithelial cells induced by cellular contractile forces on a viscoelastic substrate

Haga Hisashi

Faculty of Advanced Life Science, Hokkaido University

Collective migration and three-dimensional morphogenesis of epithelial cells plays pivotal roles in many biological events as it is observed in embryogenesis, wound healing, and cancer metastasis. Epithelial cells adhere to a substrate and form sheet structures. Mechanical properties of the extracellular substrate such as viscoelasticity and geometrical constraints are understood as factors that affect cell behaviors. We found that epithelial cells (MDCK cells) cultured on a soft collagen gel exhibit collective movement, whereas the cells moved randomly on a stiff glass substrate. We also found the emergence of leader cells at the leading edges of colonies of collectively migrating cells. Lumen formation occurred when an epithelial sheet on a collagen gel was overlaid with another collagen gel. Immediately after the collagen gel overlay, an epithelial sheet folded from the periphery, migrated collectively, and finally formed a luminal structure. Moreover, MDCK cells formed a 3D structure on a viscous substrate such as Matrigel. The structures appear as a tulip hat. We revealed that the 3D tulip hat-like morphology changed in a substrate viscosity-dependent manner. In addition, the cellular contractile forces generated in the edge of the cell sheets were required for the tulip hat-like morphogenesis. Our studies indicate that the substrate viscoelasticity and the cellular contractile force are involved in collective cell movement and morphological changes observed during 3D morphogenesis. COI:No

1S-01PM-4
Possible participation of the intra-cell collective pH distribution in the maintenance of direction of the metastatic tumor cell collective migration

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A growing body of evidence demonstrates the consequence of the collective cell migration not only physiologically but also pathophysiologically, and elucidation of the molecular mechanism maintaining the direction of migration on cell collectives can be an one of the up-to-date issue. At least two cell types, classified into leading/leader cell (LC) and following/follower cell, construct cell collectives. A limited part of LC may sense and/or originate the direction of migration accompanied by high motility, however, there are still many enigmas including what can be the origin of the high motility. The elevated expression of sodium ion/proton exchanger 1 (NHE1) exhibited a correlation with activities of *in vitro* invasiveness as well as metastasis in the mouse model of a head and neck squomous cell carcinoma lymph node metastasis through enhanced ability for controlling migration direction. NHE1 excretes proton by using the influx of sodium ion as the driving force, hence alkalinize intracellular pH (pHi). Determination of pHi distribution among cell collectives indicated lower pHi in LCs than FCs, and knockdown as well as forced expression experiments suggested low activity of the NHE1 in LCs. We would like to discuss as to how contributions to the motility of, and also participation in the migration polarity of cell collectives are possible for the lower pHi in LCs. COI:No

Symposium 7

Neural circuit mechanisms of odor-guided emotional and motivated behaviors: from innate to learned behaviors

March 28 (Wed) 15:10〜17:00 Hall 4

1S-04PM-1
Neural circuit for acquiring distinct odor-guided motivated behaviors in mice

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Odors elicit various motivated behaviors such as the attraction to food odor and the aversion from predator odor. Some odor-guided motivated behaviors are innately acquired and others are learned through experience. We are interested in the learning mechanism of how animals associate particular odors with attractive and aversive behaviors, and examined the olfactory cortical regions which are activated in relation to the odor-guided behavioral learning. We found that the olfactory tubercle (OT), a part of the olfactory cortex and also a part of the ventral striatum receiving massive dopaminergic input from midbrain, has functional domains whose activation is correlated to the acquisition of odor-guided attractive or aversive behavior. While neutral odor does not elicit significant activation in the OT, after the association of the same odor with food reward, the mice became attracted to the odor and the anteromedial domain of the OT was activated. In contrast, after the association of the same odor with electrical foot shock, the mice became aversive to the odor and the lateral domain of the OT was activated. We further examined the development of the OT in neonatal mice, and found that the lateral domain well developed just after birth and that the anteromedial domain was immature at birth and then developed to maturity within 3 weeks after birth. This developmental time course raises the possibility that functional domains in the OT are also important for the acquisition of odor-guided behaviors at early neonatal periods. COI:No

1S-04PM-2
Odor-induced analgesia and anxiolytic effects

Kashiwadani Hideki

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Several odorous compounds act on the living animals and could modulate the physiological responses pharmacologically. However, it is not known exactly whether the "odor" (in other words, "the sense of smell") has the function or not. To address the question, we examined the analgesic effects of odor exposure and found that the odor of linalool, one of the terpenoids, had the significant analgesic effects in mice. Furthermore, central olfactory system and hypothalamic orexin neurons are essential for the effects. In addition to the analgesic effects, we also found that the linalool odor also induced anxiolygic effects without impairing spontaneous motor activity. COI:No

1S-04PM-3
Sensory representations and integrational processing for innate versus learned olfactory behaviors and beyond

Kobayakawa Ko, Matsuo Tomohiko, Kobayakawa Reiko

Inst Biomed Sci, Kansai Medical Univ, Osaka, Japan

Behaviors are regulated by innate and learned mechanisms. Neuronal mechanisms to integrate both information to induce appropriate behaviors for survival are largely unknown. We have previously shown that odor-induced innate and learned behaviors are regulated by separate pathway in the olfactory epithelium. Recently, we showed that both information are antagonistically integrated by the Htr2A-positive cells in the central amygdala (CeA-Htr2A+), to control hierarchical relationships in which innate behaviors are prioritized over learned behaviors. From these results, we propose an olfactory behavior model, namely separate olfactory sensory inputs and antagonistic integration for reregulating behavioral outputs. In addition to these conceptual advances, we have also developed several powerful tools for research of olfactory behaviors, including potent innate fear inducers termed thiazoline-related fear odors (tFOs), whole brain activity mapping techniques, and deep brain imaging apparatus in freely moving animals and procedures of multidimensional behavioral analyses for olfactory emotional behaviors. By combining these research tools, we are studying mechanisms how receptor genes code for neural information of various innate behaviors, which may lead to an understanding of how brain code information that is tightly linked to behavioral output. We are also studying mechanisms how different kinds of information are integrated to regulate behaviors. COI:No

1S-04PM-4
Neural Circuit Mechanisms Underlying Olfactory Emotional Behaviors in Zebrafish

Yoshihara Yoshihiro

RIKEN Brain Sci Inst, Wako, Japan

Many olfactory cues pervade the aquatic environment of fish and induce various behaviors crucial for their survival and species preservation, such as searching foods, escaping from danger, finding potential mates, and memorizing physiologically important contexts. In this symposium, I will summarize recent advances in our knowledge on functional architecture of the olfactory neural circuitry mediating three distinct types of emotional behaviors in zebrafish. Feeding cues and sex pheromones attract fish by evoking different types of positive emotion, whereas danger signals elicit negative emotion to induce escape response that is important for risk avoidance even without visible presence of predators. We recently identified olfactory receptors and neural circuits that mediate those distinct types of olfactory emotional behaviors. In this talk, I will focus on our recent progress about the identification of two alarm pheromones and the elucidation of coincidence-detection neural-circuit mechanism underlying the robust escape response in zebrafish. COI:No

Symposium 8

Recent Progress in the Estrogenic Regulation of Social Behavior and Sexual Differentiation

March 28 (Wed) 15:10〜17:00 Hall 5

1S-05PM-1
Formation and function of female-biased sexually dimorphic cell group in male-biased sexually dimorphic nucleus

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We recently found a novel sexually dimorphic cell group in the mouse brain. This cell group locates a ventral part of the principal nucleus of the bed nucleus of the stria terminalis (BNSTp). The BNSTp is a male-biased sexually dimorphic nucleus, but the ventral part of the BNSTp (BNSTpv) shows female-biased sex differences in volume and neuron number. Postnatal testicular testosterone and pubertal ovarian hormones are critical for sexually dimorphic formation of the BNSTpv. The BNSTpv of male mice was feminized by neonatal orchidectomy, while the BNSTpv of female mice was defeminized by treatment with testosterone during the postnatal period. Sex differences of the BNSTpv emerged before puberty and became prominent in adulthood. Ovarian hormones during puberty affect the BNSTpv to increase volume and sustain larger number of neurons. For understanding of the physiological functions of the BNSTpv, we measured neuronal activity with c-Fos in the BNSTpv of female mice during sexual and maternal behaviors. The number of c-Fos cells in the female BNSTpv did not change during sexual behavior. On the other hand, the number of c-Fos cells was increased by exposure to pups in virgin females exhibiting lower maternal behavior performance, but not in primiparous mothers exhibiting higher performance. The BNSTpv of female mice may contain a neuronal cell population involved in the inhibition of maternal behavior and/or promotion of avoidance response to pups. Additionally, we found an area homologous with the mouse BNSTpv in the marmoset brain, suggesting the BNSTpv is not specific for mice. COI:No

1S-05PM-2
The effects of sexual hormone on the synaptic input into the medial preoptic area.

Amano Taiju[1,2], Ito Kazuki[1], Sato Keiichiro[1], Sakurai Takeshi[3], Maejima Takashi[4], Minami Masabumi[1], Kuroda Kumi[2]

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Virgin male mice tend to be aggressive toward pups. It is known that removal of testis, called castration, has effects to decrease the aggressive behaviors and to promote parental behaviors. However, the mechanisms of sexual hormone to modulate the behavioral pattern has not been well elucidated. In this study, we focused on the medial preoptic area (cMPOA) which is one of the important brain areas for the parental behaviors. We performed whole cell patch clamp recordings from cMPOA of the adult castrated C57BL/6J mice. The amplitude of inhibitory postsynaptic potentials in the cMPOA, induced by electrical stimulations (eIPSP) of stria terminalis were significantly decreased in castrated mice compared with sham operated mice. Administration of testosterone propionate or estradiol to castrated mice, but not dihydrotestosterone, restored the amplitude of eIPSP in the cMPOA. In the behavioral tests, optogenetic inhibition of the GABAergic synaptic inputs from medial amygdala to cMPOA resulted in the decreased the aggressive behaviors toward pups. These results suggest that testosterone propionate and estradiol may increase the aggressive behaviors toward pups via increasinge the inhibition in the cMPOA. COI:Properly Declared

1S-05PM-3
Non-genomic signalling by estrogen drives social behaviour in rodents

Vasudevan Nandini

School of Biological Sciences, University of Reading, WhiteKnights Campus

The steroid hormone estrogen is essential for the display of sexually dimorphic social behaviours such as sex and aggression in the mouse. What are the molecular mechanisms that estrogens use to drive these behaviours? Estrogens regulate transcription slowly by binding to classical, intracellular nuclear receptors such as the estrogen receptor α (ERα) and ERβ but also signal rapidly via membrane estrogen receptors (mERs) that activate kinases and calcium flux. Some candidate mERs include the former orphan GPCR, GPER1 or GPR30 as well as ERα or ERβ which may also localize to the plasma membrane. Work in our laboratory demonstrated that a third pathway that we called coupled or integrated signalling exists where rapid non-genomic signaling by 17β-E potentiates transcription via the phosphorylation of the ERα. This coupled pathway is probably important for behaviours that are activated over long-time scales such as the lordosis behaviour in female rodents. We demonstrate that GPER1 activation is sufficient for lordosis behaviour and regulates spinogenesis in hypothalamic nuclei that are relevant to lordosis behaviour. In the male mouse, we show that GPER1 activation can rapidly decrease anxiety and regulate the level of PSD-95, a dendritic spine marker. We also show that estrogen can rapidly drive aggression, over time scales that are commensurate with non-genomic action. The possible mechanisms that underlie this phenomenon will be discussed. These data suggest that non-genomic signalling by estrogens may predominate in the male rodent. COI:No

1S-05PM-4
Mechanisms of estrogen action on neural networks of social behavior

Ogawa Sonoko

Lab. Behav. Neuroendocrine, Uni.Tsukuba, Tsukuba, Japan

We have been studying brain mechanisms of social behavior by focusing on the role of two types of estrogen receptors, ERα and ERβ. Previous studies have revealed that they are differentially involved in the regulation of sex-typical expression of social behavior by gonadal steroids such as testosterone (after being aromatized) and estradiol. In a series of studies using adeno-associated viral vector mediated RNA interference methods, we determined brain site(s) and time in development responsible for estrogen action via ERα and ERβ in the expression of sexual, aggressive and parental behavior (Sano et al., EJN, 2012; Sano et al., PNAS, 2016; Nakata et al., eNeuro, 2016). For instance, in adult male mice, sexual behavior requires ERα in the ventromedial nucleus of the hypothalamus (VMN) and medial preoptic area (MPOA), whereas ERα in the VMN, but not in the MPOA, is necessary for aggressive behavior. Although it is not necessary at the time of testing, ERα activation in the medial amygdala (MA) during pubertal period is crucial for male mice to fully express male-type social behavior in adulthood. In postpartum females, pup-directed caring behavior was reduced by a lack of ERα in the MPOA, but the levels of aggression toward male intruder mice were increased by ERβ knockdown in the MPOA. In this talk, we will overview these findings and our more recent studies with the use of fiber photometry for measurement of neuronal activity of ERα expressing cells by focusing on neural network of social behavior. (Supported by JSPS KAKENHI 15H05724) COI:No

Symposium 9

Frontiers in membrane transport research: approaches toward understanding regulatory mechanisms by small molecule ligands

March 28 (Wed) 15:10～17:00 Hall 6

1S-06PM-1
Probing conformational change in inward rectifier K^+ (Kir) channels by small molecules

Inanobe Atsushi, Kurachi Yoshihisa

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Tetrameric and pseudo-tetrameric cation channels possess an ion conduction pathway at the center of "pseudo-"4-fold symmetry axis. The pathway at the membrane plane has physical constraints, the selectivity filter and bundle crossing, which are believed to behave as the gates. The constraints sandwich a dilated area so called the central cavity where the majority of therapeutic agents targeting the channels is accommodated. The mode of action shares with the cause of life-threatening drug-induced QT prolongation. So far, the association of drugs with the central cavity has drawn attention. On the other hand, the binding of Kir channels with peptide toxin at external vestibule of the pore or chloroquine at the cytoplasmic pore evoke the current blockage. Therefore, the interaction of compounds at the ion conduction pathway causes the channel inhibition by perturbing conformational transition and/or by acting as the physical constraint for ion permeation. Previously we reported that bacteriostatic agent, proflavine, is a pore blocker of Kir3.2. However, we also found that the drug inhibits Kir1.1 by the association of at least three compounds to the extracellular region. These results present the allosteric modulation of Kir1.1 by proflavine and the multiple modes of proflavine recognition by Kir channels. But it also suggests the concerted movement of the corresponding region. Dynamic change in conformation around this area has not been estimated from previous studies. Therefore, allosteric modulators potentially prove useful for studying functionally important conformational changes of ion channels. COI:No

1S-06PM-2
Molecular determinants of human cardiac Kv1.5 channel block by small molecule ligands, SKF-96365 and efonidipine

Ueda Rika, Ding Wei-guang, Matsuura Hiroshi

Dept Physiol, Shiga Univ Med Sci, Otsu, Shiga, Japan

The voltage-gated Kv1.5 channels conduct the ultra-rapid delayed rectifier K^+ current (I_{Kur}) and play an important role in repolarization of the human atrial action potential. The present study investigated the molecular determinants of the small molecule ligands binding to the human Kv1.5 (hKv1.5) channel, using site-directed mutagenesis combined with whole-cell patch-clamp technique, as well as computer docking simulations. hKv1.5 channel was blocked by SKF-96365, which is a transient receptor potential canonical (TRPC) channel blocker, with half-maximal inhibitory concentration (IC_{50}) of 2.5 μM and efonidipine, which is an L-type and T-type calcium channel blocker, with IC_{50} of 0.84 μM. The channel block time constant was lower in 10 μM SKF-96365 of 1.7 ms than 3 μM efonidipine of 80.8 ms. hKv1.5 channel block by SKF-96365 for each of the pore domain mutants T479A, T480A, R487V, I502A, V505A, I508A, L510A, V512A and V516A was significantly different from the corresponding WT control. The docking simulation predicted that SKF-96365 interacted with Met478, The479, The480, Val505, Ile508, Ala509, Val512, Pro513 and Val516 in hKv1.5 channels. Although It was also predicted that efonidipine interacted with these amino acid residues, SKF-96365 and efonidipine had different inhibition effects. These results suggest that the binding sites in the Kv1.5 channel have important roles in channel blocking and the ligand structures affect the inhibition effects. COI:No

1S-06PM-3
X-ray Crystallographic Structures of Na,K-ATPase in Complex with Cardiotonic Steroids

Ogawa Haruo[1], Motoyama Kanna[1], Cornelius Flemming[2], Vilsen Bente[2], Toyoshima Chikashi[1]

1:IMCB, The Univ. of Tokyo, Japan, 2:Dept. of Biomedicine, Aarhus Univ., Denmark

Na,K-ATPase is one of the most important members of the P-type ATPases, which establishes gradients for sodium and potassium ions across the membrane. These ion gradients are used in many fundamental processes, notably, excitation of nerve cells. Na,K-ATPase is also known as a target molecule for cardiotonic steroids (CTS), including digoxin prescribed for congestive heart failure and supraventricular arrhythmias for three centuries. However, due to high affinity to Na,K-ATPase and poor specificity to organs, their clinical usage is limited. To overcome these problems, modification of sugar, lactone ring or steroid moieties in CTS may be necessary. Thus, high resolution structures of Na,K-ATPase in complex with CTSs have been awaited. Here, we report our recent crystal structures in complex with variety of CTSs. These structures provide detailed information on the interaction of Na,K-ATPase and CTS and may allow us to design better drugs for treating heart failure, arrhythmias and cancer. COI:No

1S-06PM-4
Molecular basis for Ca^{2+} binding of ryanodine receptor for channel activation and its regulation by caffeine

Murayama Takashi

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Ryanodine receptor (RyR) is a Ca^{2+} release channel in the sarcoplasmic reticulum of skeletal and cardiac muscles and indispensable to muscle contraction. Although the RyR channel is activated by Ca^{2+}, the actual mechanism of Ca^{2+} binding remains largely unknown. Here, we report molecular basis for Ca^{2+} binding of RyR for channel activation and its regulation by caffeine. RyR2 carrying mutations in the putative Ca^{2+} and caffeine-binding sites were functionally analyzed. The results were interpreted by recent near-atomic resolution RyR1 structures at various ligand states. We demonstrated that a hydrophobic interaction between tryptophan and isoleucine in the caffeine-binding site makes the Ca^{2+} binding pocket larger and less favorable for Ca^{2+}. Caffeine alters orientation of the tryptophan to break the interaction, making the pocket smaller and more favorable for Ca^{2+}. The tryptophan then forms another hydrophobic interaction with a phenylalanine that locates opposite side of the tryptophan, which also contributes to Ca^{2+} sensitizing effect of caffeine. Thus, the tryptophan residue in the caffeine-binding site switches two hydrophobic interactions to regulate the Ca^{2+} sensitivity. We identified three CPVT mutations that break the hydrophobic interaction to hypersensitize the channel to Ca^{2+}. Our results elucidated the initial step of activation of the RyR channel by Ca^{2+} and explain how mutations cause disease states at molecular level. COI:No

1S-06PM-5
Visualization and inhibition of a cancer specific amino acid transporter, LAT1

Nagamori Shushi

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As growing cancers have strong metabolic demands, nutrient transporters are constitutively activated to facilitate the nutrient uptake for robust the cell growth. Among these transporters, L-type amino acid transporter 1 (LAT1) transports a lot of essential amino acids such as leucine. While LAT1 is found in limited normal tissues, most of cancer cell lines express LAT1. By means of comprehensive proteomics combined with uptake assays, we have evaluated the role of LAT1 in cancer cells. LAT1 was the main transporter particularly responsible for the uptake of leucine which is not only a protein building block but also works as a signaling molecule and stimulates mTORC signaling for tumor growth. Thus, LAT1 is most likely important for the growth of cancer cells and has been considered as an attractive target for both cancer diagnose and treatment. Based on our research of LAT1 transport function, we have been developing two series of compounds. One of them is amino acid derivatives as PET probes which are selectively transported by LAT1, and another is non-amino acid small compounds as anti-cancer drugs which inhibit LAT1 function in non-competitive manner and suppresses tumor growth. The LAT1 non-competitive inhibitors showed low toxicity but strong anti-cancer effects on various type of cancer cells in animal models. In addition, the newly developing LAT1 specific PET probes might be able to use for the companion diagnosis with the LAT1 targeted anti-cancer drugs. Small compounds allow a cancer specific amino acid transporter LAT1 to be a very promising target for both cancer diagnosis and therapy. COI:No

Symposium 10

Phyletic memory embedded in the olfactory and visual systems activates emotion

March 28 (Wed) 15:10〜17:00 Hall 7

1S-07PM-1
Distinctive neuronal responses to snake images in the macaque subcortical visual pathway -a fear circuit embedded during primate evolution?-

Nishimaru Hiroshi, Dihn Ha, Le Van Quan, Matsumoto Jumpei, Takamura Yusaku, Ono Taketoshi, Nishijo Hisao

Syst. Emotional Sci., Univ. of Toyama, Toyama, Japan

In the animal kingdom, the ability to detect and avoid danger is crucial for survival. For primates, it has been proposed that snakes are the first of the modern predators to appear and they were the main selective pressure that favored primates which developed their visual system to rapidly detect snakes (Isbell, 2009). Behavioral studies show that both human and nonhuman primates can visually detect snakes more quickly than other visual stimuli. The subcortical visual pathway including the superior colliculus (SC) and pulvinar, bypasses the striate cortex and is involved in subconscious visual perception. It is connected to the amygdala which is the center for fear. To unravel the neuronal substrate for rapid snake detection in monkeys, we recorded neuronal responses in this pathway during a delayed nonmatching-to-sample task, in which the they were required to discriminate various visual stimuli including snakes. Both SC and pulvinar neurons responded faster and stronger to snakes compared to other visual stimuli. Furthermore, neurons in the medial prefrontal cortex (mPFC) which receives robust inputs from this pathway, also responded rapidly and strongly to snake images. Neuronal responses to each visual stimulus in the pulvinar and mPFC were unaffected by low-pass filtering of the images and were significantly correlated. These results suggest that the subcortical visual pathway send coarse and rapid information on snakes to the cortical system in a bottom-up process. COI:No

1S-07PM-3
Pheromonal communication in humans

Shinohara Kazuyuki

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Data on humans have generated the greatest controversy regarding the existence of pheromonal communication. Recently, chemosensory communications are now recognized to be crucial for mediating a variety of social behaviors, which form the basis for ontogenetic and phylogenetic survival. In the present paper, evidence on chemosensory communication in humans, with special reference to reproduction and survival, will be presented. In a broader perspective, pheromones can be classified as primers, releasers, signalers, and modulators. There is good evidence to support the presence of all of them in humans. Examples include affects on the menstrual synchrony (primer effects); breast crawl of new born baby (releaser effects), olfactory recognition of newborn by its mother (signaler effects); fear induced by alert pheromone released by other persons who feel fear (modulator effects). COI:No

1S-07PM-4
Human psychophysiological responses to odors: danger, food and conspecifics

Okamoto Masako[1], Touhara Kazushige[1,2]

1:Grad Sch Agric Life Sci, Univ, Tokyo, Japan, 2:ERATO Touhara Chemosensory Signal Project

It has been frequently shown that pleasantness is the primary dimension of olfactory perception. From an ecological perspective, odor serves as a cue for things to avoid, such as toxic substances or microbial hazard, and things to approach such as food, conspecifics of the opposite sex or kin. Hedonics of the odors is probably associated with those ecological meanings. In the case of non-human animals, some olfactory cues are likely to be innate. Mice avoid some predator odors, even if they have never been encountered to the predator. In humans, experience and learning inevitably play a major role in associating meaning to odors. However, there still may be unlearned, innate aspects. In this presentation, we will talk about psychological and physiological responses of humans to odors, especially focusing on body odors and malodors. Based on this, we will discuss the ecological meaning of odors for humans. COI:No

1S-07PM-5
Odor-induced autobiographical memories clustered in the first decade of life

Masaoka Yuri

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A feeling of going back in time is experienced more strongly for odor-evoked autobiographical memories than for those elicited by verbal or visual cues. Unlike other autobiographical memory, odor memory (AM-odor) elicits strong emotions accompanied by a feeling of reality for specific memory in individuals, and specifically clustered in the first decade. We measured magnetic resonance imaging (fMRI) and respiratory activity to investigate the link between respiratory pattern and neural activity during emotional response of AM-odor. Deep and slow breathing associated with increased emotional arousal, pleasantness and familiarity toward AM-odor were observed. Brain activation of AM-odor was identified in the posterior orbitofrontal cortex (OFC) as well as the olfactory limbic areas. Psychophysiological interaction analysis showed that the increase posterior OFC activity was paralleled with activations of the parahippocampus and precuneus. AM-odor involved wide areas of activations, and showed more emotionally intense and positive mood than memories cued by other sensory modalities. Pleasant memory consolidation will be discussed in a view of olfaction coexisted with slow and deep breathing pattern. COI:No

Symposium 11

Physiology of circadian rhythm disorder as understood from basic principles

March 28 (Wed) 15:10〜17:00 Hall 8

1S-08PM-1

Physiological dissociation between internal- and external- rhythms.

Nakamura Wataru

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Circadian rhythmicity is a fundamental feature of biological organization. It has been well established that the suprachiasmatic nucleus (SCN) of the hypothalamus is the predominant pacemaker governing circadian rhythms in mammals. The endogenous circadian clock in the SCN is normally synchronized to the 24-h changes in the light-dark cycle. The clock consists of multiple autonomous cellular oscillators distributed throughout the SCN. Without the inter-cellular coupling for synchronizing individual rhythms, the SCN is unable to produce a stable, holistic rhythm. Several studies, using ex vivo image analysis, indicate that there are orderly arrangement among SCN oscillators, with phase varying in a topologically specific manner. What is a situation where the intercellular coupling is diminished? We focused on the effect of aging on circadian output, and found that the amplitudes of day-night differences in neural activity were significantly reduced in aged mice. Furthermore, I will present that an age-related decline of reproductive functions can be improved by coordinating environmental LD cycles in middle aged female mice. These results suggest that age-related changes in early onset estrous cycle irregularities and resultant infertility are strongly dependent on biological clock functioning. Desynchronization between the environment and the biological clock (i.e., circadian timing shift) has a major impact on female reproductive functions. Furthermore, normal reproductive functioning can be rescued by manipulation of environmental timing signals. COI:No

1S-08PM-2

Effects of the isolation of suprachiasmatic nucleus on circadian system

Nakamura Takahiro[1], Mizuta Shuto[1], Nakamura Wataru[2]

1:Lab Animal Physiol, Sch Agri, Meiji Univ., Kawasaki, Japan, 2:Dept Oral Chrono-Physiol, Grad Sch Biomed Sci, Nagasaki Univ, Nagasaki, Japan

The central circadian clock, located in the suprachiasmatic nucleus (SCN) of the hypothalamus, generates daily oscillations responsible for organising the timing of most behaviors and physiological events in mammals. Neural multiple unit activity rhythms and locomotor activity rhythms disappear after SCN isolation (SCNi), which severs all nerves originating from the SCN (Inouye and Kawamura, 1979; Stephan et al., 1977). Conversely, it has been reported that the diffusible signal from SCN transplant within the semipermeable polymeric capsule regulates circadian behavioral rhythms (Silver et al., 1996). Here, we assessed the importance of neural outputs in the circadian system. A micro-knife was inserted into the brain and rotated to create the SCN island in adult male C57BL/6J mice. Post-surgery, wheel-running activities were recorded under light-dark cycle, constant darkness, and 6-h advance or delay shifts. The activity levels of circadian behavior in SCNi mice were significantly lower than those in sham-operated mice. Both groups showed free-running rhythms in constant darkness; however, no difference was detected in the free-running period. SCNi mice showed fluctuating onsets in light-dark cycle and rapid phase shifts in 6-h phase advance. These results suggest that the neural signal from the SCN plays an important role in the amplitude and photic entrainment of circadian behavioral rhythm. COI:No

1S-08PM-3

Effects of hypoxia on the daily locomotor activities of mice

Masubuchi Satoru[1], Yano Takako[1], Komatsu Kouji[1], Nakamura Wataru[2], Ota Akinobu[3], Karnan Sivasundaram[3], Takeuchi Kosei[4], Hosokawa Yoshitaka[3], Todo Takeshi[5], Umezawa Kazuo[6], Shiomi Toshiaki[7]

1:Dept Physiol, Aichi Med Univ, Nagakute, Japan, 2:Physiol, Grad Sch Biomed Sci, Nagasaki Univ, Nagasaki, Japan, 3:Dept Biochem, Aichi Med Univ, Nagakute, Japan, 4:Dept Biol, Aichi Med Univ, Nagakute, Japan, 5:Dept Radiat biol and Med Genet, Grad Sch Med, Osaka Univ, Suita, Japan, 6:Dept Mol Target Med, Aichi Med Univ, Nagakute, Japan, 7:Dept Sleep Med, Aichi Med Univ, Nagakute, Japan

Acute mountain sickness (AMS) occurs when moving to a low oxygen environment. AMS often develops or worsens overnight. Hypoxia has a stronger effect on arterial oxygen saturation at nighttime in humans. We examined circadian effects to hypoxia on mouse locomotor activities. Under 12h: 12h Light-Dark condition (LD), Daytime (9h from light on) hypoxia (8%) exposure strongly affected following 24h activities of ICR mice. Daytime hypoxia reduced the activity in the following active phase (AP) and increased the activity in the activity ending phase (AEP). Because several clock gene expressions in forebrain had changed by Daytime hypoxia, we tested molecular clock-deficient, Cry1 & Cry2 double knockout mice (CryDKO). Under LD, activity of CryDKO increases in early Dark phase and this peak respond similarly with wild type mice (activity reduction in AP and increase in AEP). However, this response had disappeared by disappearance of daily activity peak under constant darkness. From these results, we propose the daily activity rising system (DARS). DARS is an activity driver that responds to hypoxia systematically and is regulated differently from the molecular clock system. COI:No

1S-08PM-4

Pathophysiological insights of circadian rhythm disorder: A report of mouse-cohort study

Yagita Kazuhiro

Dept Physiol and Systems Bioscience, Kyoto Pref. Univ. Med

Circadian clocks regulate the daily fluctuations of essential biological processes from the molecular to organismal levels to predict and adapt to the cyclic environment of our rotating planet. Cell-autonomous circadian clocks exist in both the SCN and peripheral cells throughout the body, suggesting that circadian clocks may function as an interface connecting cyclic environmental changes and cellular physiology.We performed a prospective study observing the effects of different types of scheduled light-dark shift conditions in wild-type mice, we reared mice for 630 days under two chronic jet lag conditions with distinct programs of light and darkness: an 8-h phase delay every 7 days (Delay) or an 8-h phase advance every 4 days (Advance). Delay represents a mild condition in which mice are entrained, whereas Advance represents a condition of circadian rhythm disorder in which mice are not entrained. Our results showed that the 26.5% (9/34) mice reared under the Advance shift condition died or were sacrificed by humane endpoint throughout the experiment, whereas the first death under Delay condition occurred on day 611. In Advance mice of shorter lifespan, which showed splenomegaly and increased myeloid cells in bone marrow, which indicates that chronic inflammation existed in those mice. These findings strongly suggest that the prospective intervention study using mice provides important information to understand the pathophysiology of circadian rhythm disorder. COI:No

Symposium 12

Development of new compounds as a research tool to investigate physiological events

March 28 (Wed) 15:10〜17:00 Hall 9

1S-09PM-1

Development of a novel magnetic organic compound

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We previously reported a novel magnetic organic compound, *N,N'-bissalicylideneethylenediamine iron* [Fe(Salen)], as an anti-cancer agent with intrinsic magnetic property. In addition to anticancer effect, its magnetic property has the following outstanding functions: 1) It can be attracted by a magnet. 2) It can be visualized by magnetic resonance imaging (MRI). 3) It *per se* generates heat, *i.e.* hyperthermia when exposed to alternating magnetic field (AMF). In the course of our study, we identified the magnetic chemical key structure of Fe(salen) generating the magnetic property using Super Photon ring-8 GeV (SPring-8) (RIKEN). This result showed that the unique angle configuration of Fe-O-Fe (146.359°) in the crystal structure of Fe(Salen) contributes to the generating original magnetism. Based on the proven magnetic performance of Fe(Salen), we primarily hypothesized that the Fe(Salen) molecule can endow each commercially available anti-cancer drug, Paclitaxel (PTX) or Methotrexate (MTX) , with intrinsic magnetic property by chemically tethering the drug counterpart. Therefore, we have designed specific covalent linkage of the Fe(Salen), to commercial available drugs, such as PTX or MTX. Fe(Salen) serves as a magnetically-responsive scaffold, generating the magnetic property in addition to the original cytotoxic property of PTX or MTX. In conclusion, this covalent coupling technique can be an effective method for improving the therapeutic index of clinically available drugs. COI:No

1S-09PM-2

Synthesis of bioactive compounds connected with functional molecules

Hoshino Yujiro

Grad Sch Environ Infrom Sci, Yokohama National Univ, Yokohama, Japan

Drug Delivery System (DDS), which can control the administration of several drugs, makes rapid progress and has gained more attention owing to enhanced oral bio-availability enabling reduction in dose and protection of drugs from the hostile environment in gut. It was recently reported that μ-oxo-N,N'-bis(salicylidene)ethylenediamine iron [Fe(Salen)]2O, a magnetic metal complex, has inherently anti-tumor activity and generates heat in an alternating magnetic field (AMF). It opens the door to a possibility of the design and synthesis of magnetic molecules using organic synthetic techniques. Therefore, we investigate the synthetic methods of [Fe(Salen)]2O complexes having functionalized molecules for their use in DDS. In this presentation, we will report the design and synthesis of magnetic molecules having fluorescent agent for easily tracing it in tissues. Introduction of fluorescein derivatives into salen ligand was achieved by linking together through formation of amide bonds or cycloaddition reaction. COI:No

1S-09PM-3

COA-Cl as a novel adenosine-like compound with pro-angiogenic activity

Igarashi Junsuke

Grad Sch Health Sci, Morinomiya University of Medical Sciences, Osaka, Japan

COA-Cl is a synthetic compound with a structure related to adenosine. COA-Cl exerts strong angiogenic activity in multiple experimental settings. Here I explore the mechanisms by which COA-Cl promotes angiogenic signaling events, using cultured human umbilical vein endothelial cells (HUVEC) as a model. When HUVEC were treated with COA-Cl, a G-protein coupled receptor $S1P_1$ was activated to induce phosphorylation of the MAP kinases ERK1/2, without stimulating the VEGF receptor. COA-Cl promoted tube forming activity of HUVEC in the presence of co-cultured normal human dermal fibroblasts (NHDF). Inhibition of $S1P_1$ as well as that of ERK1/2 attenuated tube formation responses of HUVEC to COA-Cl. Thus, COA-Cl directly modulates the endothelial $S1P_1$ receptor. We then explored the effects of COA-Cl on adjacent non-endothelial cell types. COA-Cl led to significant increases in VEGF secretion into culture media from NHDF as well as from mouse C2C12 skeletal myotubes, in association with the induction of their VEGF mRNA. In NHDF, COA-Cl elevated cAMP content, leading to the phosphorylation of a transcription factor CREB at Ser133, a PKA site. Phosphorylation and activation of the CREB elevated promoter activity of the VEGF gene, modulated by a transcriptional co-activator PGC-1α. PGC-1α is regulated by the cAMP/PKA pathway in several cell types including skeletal muscle and fat tissue. Collectively, I propose that COA-Cl promotes two distinct signaling cascades: the $S1P_1$ receptor/ERK1/2 pathway within vascular endothelial cells, and the cAMP/CREB/ PGC-1α/VEGF pathway within surrounding non-endothelial cells. COI:No

1S-09PM-4

Screen of small molecules targeting activator of G-protein signaling

Sato Motohiko[1], Takahashi Rie[1], Yamamura Aya[1], Hayashi Hisaki[1], Umezawa Kazuo[2], Osada Hiroyuki[3]

1:Dept Physiol, Aichi Med Univ, Nagekute,Japan, 2:Dept Molecular Target Medicine, Aichi Medical University, 3:Chemical Biology Research Group, RIKEN Center for Sustainable Resource Science, Wako, Japan

We previously identified Activator of G-protein Signaling 8 (AGS8) from the myocardium of rats subjected to repetitive ischemia. AGS8 regulated hypoxia-induced apoptosis of cardiomyocytes and angiogenic events of vascular endothelial cells. Association of AGS8 to Gβγ subunit was a key step for AGS8-mediated events, and a peptide, which disrupted the AGS8-Gβγ interaction, blocked those events. To examine a potential of AGS8 as a therapeutic target in boarder applications, we explored small molecules that inhibited AGS8-Gβγ interaction. Utilizing yeast-based system, in which growth of cells was linked to activation of G-protein signaling by AGS8, we screened compounds of the RIKEN NPDepo library. Of 459 compounds screened, we found one compound prevented AGS8-mediated growth of yeast, but it did not influence growth by the other AGS. This compound effectively inhibited formation of AGS8-Gβγ complex in mammalian cell without cytotoxicity. Then, an effect of this compound was examined in human umbilical vein endothelial cell (HUVEC), in which AGS8 was involved in activation of vascular endothelial growth factor receptor (VEGFR) and tube formation. As observed when AGS8 was suppressed in cells, the compound attenuated phosphorylation of VEGFR-2 and down stream molecules, and inhibited tube formation of HUVECs. These data indicate an importance of AGS8-Gβγ complex and a potential of this compound for therapeutic application. COI:No

1S-09PM-5

TRPA1 channel agonists protect against intestinal inflammation and fibrosis

Kurahara Hai Lin[1], Hiraishi Keizo[1], Hu Yaopeng[1], Doi Mayumi[1,2], Koga Kaori[2], Onitsuka Miki[2], Inoue Ryuji[1]

1:Dept Physiol, Sch Med, Fukuoka Univ, 2:Dept. Pathol., Sch. Med., Fukuoka Univ.

BACKGROUND AND AIMS: The TRPA1 channel is highly expressed in the intestinal myofibroblasts, but its contribution to gut physiology/pathophysiology is unclear. Here, we evaluated the function of myofibroblast TRPA1 channels in intestinal remodeling. METHODS: InMyoFib cells stimulated by TGF-β1 to induce in vitro fibrosis. Trpa1 knockout mice were generated by CRISPR/Cas9 system. A murine chronic colitis model was established by weekly intrarectal TNBS administration. Crohns disease (CD) patients sample were used for pathological staining and quantitative analyses.RESULTS: In TNBS chronic colitis model mice, the extents of inflammation and fibrotic changes were more prominent in TRPA1-/- knockout than in wild-type mice. One-week enema administration of TRPA1 agonists suppressed fibrotic lesions in wild-type mice, but not in TRPA1 knockout mice. TRPA1 channel agonists found by screening from 103 biological food library counteracted TGF-β1-induced expression of HSP47, type 1 collagen, and α-smooth muscle actin, and reduced Smad-2 phosphorylation and myocardin expression. In stenotic intestinal regions of CD patients, TRPA1 expression was significantly increased. TRPA1/HSP47 double-positive cells accumulated in the stenotic intestinal regions of both CD patients and TNBS-treated mice. CONCLUSION: TRPA1 may protect against intestinal inflammation and fibrosis and thus may be a novel therapeutic target for highly incurable inflammatory/fibrotic disorders. COI:No

Symposium 13

[JST PRESTO "Creation of Innovative Technology for Medical Applications Based on the Global Analyses and Regulation of Disease-Related Metabolites"]

Frontier Study of Disease-Oriented Metabolomics by Young Researchers

March 29 (Thu) 8:30〜10:20 Hall 1

2S-01AM-1
Sulfur metabolism as a new therapeutic target for the treatment of heart failure

Nishida Motohiro[1]

1:Div Cardiocirc Signal, Okazaki Inst Integr BiosciNat Inst Physiol Sci, Natl Inst Nat Sci, Okaaki, Japan, 2:Grad Sch Pharm Sci, Kyushu Univ, Fukuoka, Japan, 3:SOKENDAI, Dept Physiol Sci

Covalent modification of GTP-binding protein (G protein) by endogenous electrophiles, such as 8-nitro-guanoshine 3', 5'-monophosphate (8-nitro-cGMP), promotes myocardial early senescence during the transition of mouse heart from compensative hypertrophy to heart failure. Exogenous treatment with NaHS improved heart failure by eliminating 8-nitro-cGMP accumulation. However, using a novel techniques to measure intracellular reactive sulfur species, we found that NaHS per se hardly eliminates electrophiles in vitro, and formation of more nucleophilic sulfur species, such as Cys persulfide/polysulfide in proteins, predominatly eliminates endogenous electrophilies in the heart. We also revealed that hypoxic stress or exogenous treatment with an electrophile, such as methylmercury (MeHg), induces mitochondrial hyper-fission via electrophilic modification of rodent dynamin-related protein 1 (Drp1). Polysulfide detection assay revealed that endogenous Drp1 forms Cys persulfide in rat cardiomyocytes, and persulfide level is dramatically reduced by MeHg exposure. Treatment with NaHS for 24 hrs completely suppresses MeHg-induced modification, activation of Drp1, mitochondrial hyper-fission, and mechanical stress-induced cardiac injury. These results strongly suggest that S-polythiolation of Drp1 underlies suppression of eletrophile-mediated cardiac vulnerability to hemodynamic overload, and offers new therapeutic possibilities. COI:No

2S-01AM-2
Functional characterization of FTSJ1, a X-linked mental retardation-related gene

Wei Fan-Yan

Dept Mol Physiol, Kumamoto Univ. Kumamoto, Japan

Genetic mutations in X chromosome-linked genes have been associated with mental retardation (XLMR). Recently, linkage analyses performed in Belgian, Chinese and Japanese families have identified Ftsj1 gene as a novel candidate gene. Ftsj1 shares homology with a bacterial 23S rRNA methyltransferase FTSJ. However, the molecular function of Ftsj1 and its pathological relevance in mental retardation have remained unknown. Using Ftsj1 knockout mice, we demonstrate that Ftsj1 methylates cytosolic transfer RNAs (tRNAs) at position 32 and 34. While the FTSJ1 KO mouse developed normally, we observed a decreased protein synthesis level in hippocampus of FTSJ1 KO mice using puromycin-mediated in vivo pulse-labeling technique. Especially, there was a marked decreased of synaptic proteins including glutamate receptors and signaling molecules. The decreased protein synthesis level resulted in the electrophysiological and morphological abnormalities in hippocampal neurons of FTSJ1 KO mice. There results suggest that the accumulation of hypomodified tRNAs disturbs neuronal protein synthesis, which ultimately contributes to the development of mental retardation in Ftsj1-deficient mice and human. COI:No

2S-01AM-3
Systemic response as a causal/exacerbating factor for psychiatric disorders

Hayashi-Takagi Akiko

Lab of Medical Neurosci, Gunma Univ, Gunma, Japan

Major depression and type 2 diabetes mellitus are common chronic illnesses. Moreover, each disorder can be among the most disabling chronic disorders, and when they occur comorbidly, they are even more detrimental bidirectionally. However, molecular and cellular mechanisms underlying these bidirectional exacerbating factors of these comorbidity remain elusive. A potential clue to understand the psychiatric disorders can be systemic response, in which maladaptive neural circuits are often modulated metabolic dysregulation and impaired immune response. In order to fundamentally understanding of psychiatric disorders and its comorbid disorders within the multi-organ context, we here developed a longitudinal and multiaxial testing, which consists of in vivo 2-photon synapse imaging, assessment for daily activity, circadian rhythm, a level of stress hormone such as catecholamines, cortisol (corticosterone in mice model), and 23 kinds of cytokines. With use of this longitudinal and multiaxial assessment, we compared the model mice of depression, diabetes mellitus (DM), and these comorbidity. Interestingly, comorbid mice (DM and depression comorbidity), compared with control, depression-, and DM-model mice, exhibited more exacerbating depressive-like phenotypes such as decrease in diary activity and food intake, disturbed circadian rhythm, increase in corticosterone level. In this symposium, we will present the detailed data for behaviours, imaging, and comprehensive analyses of cytokines, and discuss about the mechanism of molecular mechanisms of systemic response as a causal/exacerbating factor for psychiatric disorders COI:No

2S-01AM-4
Aging-related alterations in the murine intestinal environment induce an obese-type gut dysbiosis

Fukuda Shinji[1,2,3,4,5]

1:Inst. Adv. Biosci., Keio Univ., Yamagata, Japan, 2:JST PRESTO, Saitama, Japan, 3:KISTEC-KAST, Kanagawa, Japan, 4:TMRC, Univ. Tsukuba, Ibaraki, Japan, 5:Metabologenomics, Inc., Yamagata, Japan

Aging is a progressive decline of cellular functions that ultimately affects whole body homeostasis. Alterations in the gut microbiota associated with aging have been reported, however the molecular basis of the relationships between host aging and gut microbiota remains obscure. By using longitudinal microbiome and metabolome characterization, we show that the aging-related alterations in the intestinal environment lead to gut dysbiosis with a potential to induce obesity in mice. In middle-age mice, we observed more than a 2-fold increase in fecal carbohydrates derived from dietary polysaccharides and a significant reduction of gut microbial diversity resembling the microbiota characteristic of obese mice. Consistently, fecal microbiota transplantation from middle-age specific pathogen-free (SPF) mice into young germ-free mice resulted in increased weight gain and impaired glucose tolerance. Our findings provide new insights into the relationships between host aging and gut dysbiosis, and may contribute to development of a possible solution to aging-related obesity. COI:Properly Declared

2S-01AM-5
Mapping of de novo monoamine synthesis reveals anxiety behavior related nucleus with high serotonin turnover in mouse brain

Sugiura Yuki, Eiji Sugiyama

Dept Biochem, Sch Med, Keio Univ, Tokyo,Japan

Neurons regulating cognitive circuits use monoamines as key neurotransmitters and/or modulators. Such monoaminergic neurons form nuclei projecting their axons precisely into targeted brain regions, where the local concentrations of monoamines were tightly regulated. So far, imaging techniques to visualize protein and mRNA localizations have been utilized to uncover anatomical wiring of the monoaminergic circuits. On the other hand, although that synaptic concentrations of monoamines play critical roles for cognitive functions, however, whole brain monoamine concentration maps are not available due to technical limitation. Here, we generated serotonin (5-HT), dopamine (DA) and norepinephrine (NE) distribution atlas of the mouse brain in semi-quantitative manner by imaging mass spectrometry. We found paraventricular nucleus of the thalamus (PVT) and substantia nigra pars reticulata as novel 5-HT rich nuclei. Moreover, by taking IMS advantage enabling simultaneous mapping of three monoamines, part of those nuclei co-contain DA or NE, might be crosstalk nuclei connecting 5-HT and catecholamine circuits. Among these, we suggest decreased 5-HT concentration in PVT as well as dorsal raphe nucleus and median raphe nuclei could induce anxiety behavior occurred in an acute tryptophan depletion model, in which drop of serum Trp followed by reduction of whole brain 5-HT level are observed. Our data suggest acute reductions of serum Trp resulted in 5-HT concentration in specific nuclei with high 5-HT turnover, subsequently resulted in abnormal behavior. COI:No

Symposium 14

Unexpectedly Responded.
- Curious Reflex in System Physiology-

March 29 (Thu) 8:30〜10:20 Hall 2

2S-02AM-1
Relationship between vision and postural control while viewing motion movies

Sugiura Akihiro

Dept of Radiol Technol, Gifu Univ of Med Sci, Gifu Japan

Our study group has shown interested in the relationship between vision and postural control because to reveal detail of this relationship leads to overcoming the visually induced motion sickness. Several new findings in this relationship were obtained from our late experimental study. In this presentation, I explain the aim and detail of following experimental studies. First is to investigate the effect of stereoscopic viewing and degree of awareness of motion sickness on postural control. In this study, the movie, which showed several balls randomly positioned and making sinusoidal reciprocating movement, was projected on a white wall 2 m in front of the subjects through a two-dimensional (2-D)/3-D convertible projector. To measure body sway during movie viewing, the subjects stood statically erect on a Wii Balance Board. In conclusion, we found that posture changed according to the motion in the movie and that the longer the viewing time, the higher the synchronization accuracy. These tendencies depended on the level of awareness of motion sickness or the 3-D movie viewed. Second is to verify the effect of unpredictable components on that. The subjects watched 8 sinusoidal moving movies (direction setting: 2, added unpredictable component settings: 4) each. The results revealed the following: First, a viewing depth-direction predictable motion movie had high phase synchronization acuity. In contrast, the unpredictable motion component considerably affected the viewing side-direction motion movie. Second, the relationship between the amount of added unpredictable motion components and the synchronization acuity was poor. COI:No

2S-02AM-2
Application of physiological control systems analysis on the vestibular-postural control system

Fujiki Nobuhiro

Dept Ergonomics, UOEH, Kitakyusyu, Japan

The vestibular-posture control system could be defined as a system that keeps the posture constant, that is, it controls the center of gravity of the body constant against to the changes in the linear and/or rotational acceleration. Although there are several ways to evaluate the balance function such as stabilometer, it seems not so much sensitive to detect the risk of falls in elderly. We considered that if we apply physiological control systems analysis on the vestibular-postural control system, we may obtain more sensitive and accurate method to evaluate the function of the human subject. We used galvanic vestibular stimulation as a mimic of the natural acceleration change and recorded the sway of the center of gravity as the output of the system. We analyzed the transient and the frequency response of the output against to the step and sinusoidal electrical stimulation change of the input, respectively. I will report the experimental set-up, procedures for the data acquisition and analysis. COI:No

2S-02AM-3
Estimation of dynamic characteristics of baroreflex-mediated vagal heart rate control

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The arterial baroreflex system controls the sympathetic and vagal nervous systems. Although dynamic characteristics of the sympathetic baroreflex have been well identified, dynamic characteristics of the vagal baroreflex are poorly understood partly because of the difficulty in measuring baroreflex-related vagal nerve activity (VNA). We examined the dynamic heart rate (HR) response to an isolated carotid sinus pressure (CSP) input before and after vagotomy in anesthetized rats. The dynamic HR response after vagotomy was used to estimate the transfer function from CSP to HR via the sympathetic system. We assumed that the transfer function from CSP to VNA might show dynamic characteristics similar to the sympathetic baroreflex neural arc (a derivative filter with high-cut properties), excepting the sign of the signal transduction. We also assumed that the transfer function from VNA to HR might show dynamic characteristics similar to those estimated using electrical vagal nerve stimulation. The vagal transfer function from CSP to HR was constructed from the product of the transfer function from CSP to VNA and that from VNA to HR. This was further combined with the sympathetic transfer function from CSP to HR to obtain an overall transfer function model from CSP to HR. The developed model was able to describe a peculiar phase change observed in the transfer function from CSP to HR before vagotomy. The present results may provide a clue to understand the baroreflex-mediated VNA regulation. COI:No

2S-02AM-4
What is a role of the vestibular mediated autonomic reflex?

Morita Hironobu, Abe Chikara

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Although the vestibular system plays a significant role in controlling the arterial pressure (AP) response to gravitational changes, the physiological significance of this response remains unclear. Hypergravity causes orthostatic fluid shift from the intrathoracic compartment to the legs; this could result in reduced venous return and cardiac output, followed by decreased AP. Thus, the vestibular system-mediated pressor response may counteract the hypergravity-induced hypotension. In this regard, the vestibular system acts as a regulator of AP in order to prevent hypotension. On the other hand, microgravity causes a headward fluid shift that could result in increased venous return and cardiac output, followed by increased AP. Thus, if the vestibular system has a physiological significance in the control of AP during gravitational changes, it should induce a depressor response under microgravity conditions. Conversely, however, it induces a pressor response; i.e., the vestibular system induces a pressor response irrespective of the direction of the changes in gravity and the AP. These results suggest that the vestibular system-mediated pressor response is a type of stress response but not a purposeful response. This pressor response is, however, effective in preventing hypotension under hypergravity conditions and on postural change from recumbency to upright standing. COI:No

2S-02AM-5
Reflex arc of teeth-clenching pressor response in rats.

Nishida Yasuhiro[1], Shoji Ichiro[2], Maruyama Satoshi[3], Ishiwata Ryo[1], Hruma Megumi[1]

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Electrical stimulation on the masseter muscles in anesthetized rats evokes teeth clench to generate pressor responses and slight tachycardia, named "teeth-clenching pressor response". We examined the mechanisms on the teeth-clenching pressor response. Intravenous dantrolene, a ryanodine receptor antagonist, blocked muscle contraction, resulted in abolishing the pressor response and tachycardia, indicating that the muscle afferents or the produced pressure on the molar regions induces the responses. Conversely, sinoaortic denervation didn't alternate the pressor response, indicating that the pressor response may not be a simple peripheral response. We will show the research results examined on the sensor mechanisms, such as sensor characteristics, afferent nerves, efferent limbs in the teeth-clenching pressor response, and the characteristics of the reflex. Finally, we will show why slight the tachycardia is at the teeth-clenching pressor response. COI:No

Symposium 15

Cellular and molecular mechanisms of epileptogenesis

March 29 (Thu) 8:30～10:20 Hall 7

2S-07AM-1
Microglia disrupt synapse E/I balance in epileptogenesis

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Fever (typically greater than 38.5° C)-induced febrile seizures are the most common type of seizures in early childhood. Prolonged febrile seizures could afterwards initiate the development of epilepsy, i.e., epileptogenesis; however, the cellular and molecular mechanisms that link febrile seizures and epilepsy remain unclear. Here we report that microglia, the brain-resident immune cells, disrupt the excitatory versus inhibitory balance (E/I balance) of synapses in the dentate neural circuits. Microglia detected the increase in brain temperature during experimental febrile seizures through activation of transient receptor potential vanilloid 4 (TRPV4), a member of the thermosensitive TRP channel family. The TRPV4-mediated Ca^{2+}-influx led microglia to preferentially engulf inhibitory synapses, resulting in a decrease of the density of inhibitory synapses in the dentate gyrus. Finally, minocycline, an inhibitor of microglial activation, decreased the delayed seizure severity after febrile seizures. Thus, our study provides a novel mechanism by which the brain hyperthermia impairs the synapse E/I balance via activation of microglia in epileptogenesis. COI:No

2S-07AM-2
Neuronal inhibition and seizure suppression by a metabolic enzyme, lactate dehydrogenase

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Epilepsy is characterized by hyperexcitation of electrical activities in the brain, and therefore antiepileptic drugs have been designed to act on molecules that regulate the electrical activities (for example, ion channels and synaptic receptors). However, these antiepileptic drugs are not effective for one-third of epileptic patients. Thus, new antiepileptic drugs are required for the drug-resistant epilepsy. In the present study, we studied the neuronal action of the ketogenic diet, which is known as a diet treatment effective for the drug-resistant epilepsy. Using slice patch-clamp techniques, we found that neurons were hyperpolarized by a metabolic switch mimicking the ketogenic diet. This hyperpolarization was mediated by lactate dehydrogenase (LDH), a metabolic enzyme on astrocyte-neuron lactate shuttle. Inhibition of LDH enzyme not only hyperpolarized neurons *in vitro*, but also suppressed seizures in a mouse model of epilepsy *in vivo*. We then explored clinically-used antiepileptic drugs acting on the LDH enzyme, and found that LDH was moderately inhibited by an antiepileptic drug stiripentol. By modifying the chemical structure of stiripentol, we finally identified a compound that inhibits LDH and suppress seizures *in vivo* more potently. In conclusion, new drug for the drug-resistant epilepsy can be developed by targeting LDH enzyme and modifying the chemical structure of stiripentol. COI:No

2S-07AM-3
Persistent barrage firing in cortical interneurons: an endogenous mechanism for suppressing seizures?

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Neural circuits are typically maintained in a state of dynamic equilibrium by balanced synaptic excitation and inhibition. However, brain regions that are particularly susceptible to epilepsy may have evolved additional specialized mechanisms for inhibiting over-excitation. Here we identify one such possible mechanism in the cerebral cortex and hippocampus of mice. Recently it was reported that some types of GABAergic interneurons can slowly integrate excitatory inputs until eventually they fire persistently in the absence of the original stimulus. This property, called persistent firing or retroaxonal barrage firing, is of unknown physiological importance. We show that two common types of interneurons in cortical regions, neurogliaform cells and fast-spiking cells, are unique in exhibiting barrage firing in acute slices and in vivo. In slices, barrage firing could reliably be triggered by trains of excitatory synaptic input, as well as by exposure to proconvulsant bath solutions. Using pair recordings in slices, we confirmed that barrage firing neurogliaform cells can produce synaptic inhibition of nearby pyramidal neurons, and that this inhibition outlasts the original excitation. The ubiquity of neurogliaform and fast-spiking cells, together with their ability to fire persistently following excessive excitation, suggests that these interneurons may function as cortical sentinels, imposing an activity-dependent brake on undesirable neuronal hyperexcitability. COI:No

2S-07AM-4
Axonal burst and epileptogenesis

Kamiya Haruyuki

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Epilepsy is one of the common neuronal disorders caused by hyperexcitability of cortical neuronal networks, and epileptic discharges often spread over in time and space. Towards understanding the cellular changes underlying epileptogenesis, we have been studied using the subcellular electrophysiological recordings from single axon terminals of the hippocampal mossy fibers which compose of *en passant* structure with multiple boutons. We specifically aimed to test the possibility of generation of ectopic burst from distal axon, away from the physiological spike initiation sites at proximal axon or at axon initial segment. It was reported that the density of sodium conductance was highest at the proximal axon of mossy fiber, though significantly high levels were detected throughout the rest of the axon. This implies that the excitability of distal axon is much higher than other cellular regions such as somatodendritic compartment. Consistent with this notion, mild elevation of potassium concentration around the distal axon reliably evoked ectopic bursts of the mossy fiber terminals in mouse hippocampal slices. Numerical simulation using a realistic model of hippocampal mossy fiber, based on both anatomical and electrophysiological data, also supported that change in excitability of distal axon induced burst discharges originated from ectopic foci. All these findings suggest that distal axon may trigger axonal bursts upon change in local microenvironment, and initiate the first step for epileptogenesis. COI:No

Symposium 16

Approaches to autonomic dysfunction in cardiovascular diseases: From molecular to clinical aspects

March 29 (Thu) 8:30～10:20 Hall 8

2S-08AM-1

A Pathogenic Mutation in the Acetylcholine-activated Potassium Channel Provides a Therapeutic Target in Familial Sinus Nodal and Atrial Bradyarrhythmias

Yamazaki Satoru[1], Yamada Noriaki[2], Asano Yoshihiro[2], Kitakaze Masafumi[1], Takashima Seiji[3]

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Normal heart rate and rhythm are essential for proper heart function. Because disorders of impulse generation and/or propagation in the cardiac conduction system culminate in life-threatening symptomatic bradyarrhythmias, the ion channels responsible for these phenomena represent promising therapeutic targets. Here, we describe a family affected with hereditary sinus node dysfunction and atrial bradyarrhythmias. Next-generation sequencing analysis and positional cloning revealed a novel nonsynonymous mutation (Kir3.1-N83H) in KCNJ3, which encodes the inwardly rectifying potassium channel Kir3.1, a subunit of the acetylcholine-activated potassium channel (IKACh channel). The mutation caused a gain of IKACh channel function by increasing the basal current, even in the absence of G protein-coupled receptor stimulation. In zebrafish harbouring the mutation, the specific IKACh channel blocker NIP-151 repressed the current increase and improved all bradyarrhythmia phenotypes. Thus, we conclude that the mutation (Kir3.1-N83H) in KCNJ3 is one of the causes of bradyarrhythmias, and propose that a specific IKACh channel blocker could be used to treat bradyarrhythmias resulting from gain-of-function mutations in KCNJ3. COI:No

2S-08AM-2

Description of drug properties based on effects on arterial baroreceptor reflex

Yamamoto Hiromi[1], Kawada Toru[2], Sugimachi Masaru[2], Miyazaki Shunichi[1]

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The arterial baroreflex system is one of major negative feedback systems for stabilizing arterial pressure (AP). To understand arterial baroreflex-mediated AP regulation, a framework of open-loop systems analysis has been used. In this analysis, the arterial baroreflex system may be divided into two principal subsystems: a neural arc from pressure input to efferent sympathetic nerve activity (SNA), and a peripheral arc from SNA to AP.
Beta blockers have been commonly used as a treatment for cardiovascular diseases such as hypertension. We compared the effects of intravenous metoprolol (ML) and carvedilol (CL) on baroreflex-mediated sympathetic circulatory regulation in vagotomized and anesthetized rats. Both ML and CL did not significantly affect the SNA response but abolished the HR response to stepwise changes in carotid sinus pressure (CSP). Although ML maintained the range of AP response to CSP, CL significantly narrowed the range of AP response to CSP. ML reduced aortic flow (AoF) without affecting stroke volume (SV) or the relationship of peripheral vascular resistance (PVR) versus SNA. CL decreased AoF and SV, and attenuated the PVR response to SNA.
In conclusion, both ML and CL eliminated baroreflex-mediated sympathetic HR response, which is attributable to the beta-blocking effect. In addition, CL significantly reduced PVR, manifesting a relatively strong alpha-blocking action. The open-loop systems analysis is useful to investigate the effects of a given drug on baroreflex-mediated sympathetic circulatory regulation. COI:No

2S-08AM-3

Role of sympathetic nerve activity in the pathogenesis of hypertension

Yoshimoto Misa, Miki Kenju

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Excess activation of sympathetic nerve activity has been believed to be a main cause of hypertension. Excess activation of sympathetic nerve activity results in increases in total vascular resistance, heart rate, and cardiac contractility. This causes an increase in systemic arterial pressure, renin secretion, and sodium reabsorption, resulting in hypertension. However, how and when sympathetic nerve activity increases and causes hypertension remains unclear. We have studied the role of sympathetic nerve activity as a cause of hypertension using conscious rats. In this symposium we will introduce our recent findings using Dahl salt-sensitive (DS) rats and spontaneously hypertensive stroke prone rats (SHRSP). In DS rats, 8% high sodium diets were loaded over 14 days. In DS rats, a high salt loading increased arterial pressure from 104 mmHg to 141 mmHg, while renal and lumbar sympathetic nerve activity remained unchanged during the initiation of hypertension over 14 days. In SHRSPs, systemic arterial pressure was 140 mmHg at 9 weeks of age and it increased with aging to 170 mmHg at 13 weeks old. During the development of hypertension in SHRSPs, renal and lumbar sympathetic nerve activity did not increase. Taken together, these results using two hypertensive models showed that the initiation and development of hypertension were not associated with an increase in sympathetic nerve activity. It is therefore concluded that excess activation of sympathetic nerve activity may not be a primary cause of the hypertension in DS and SHRSP models. COI:No

2S-08AM-4

The novel method to identify the arterial barorefelex function from continuous arterial pressure waveform with power spectral analysi

Saku Keita

Center for Disruptive Cardiovascular Medicine, Kyushu University, Fukuoka, Japan

Background: The importance of arterial baroreflex function in cardiovascular disease is widely known, thus the practical methods to identify the transfer function (H) is required in clinical. H approximates a 1st-order low pass filter with the fixed corner frequency of 0.05 Hz in rats. In theory, a feedback system with H attenuates variability (PD) to PD/(1+H). We hypothesized that the baroreflex attenuates the power spectrum density (PSD) of arterial pressure (AP) in its operating frequency (< 0.1Hz) as long as PD remains unchanged irrespective of baroreflex function, thereby H can be identified from PSD. Methods: We used Wister-Kyoto rats. We created graded baroreflex dysfunction by sinoaortic denervation (SAD) (sham: no SAD, n=9, partial: denervation only in right side, n=8 and total: bilateral denervation, n=6). One week after SAD, we calculated PSD from continuous AP recording (12hours). We took the ratio of PSD at 2 frequencies (0.01 Hz/0.1 Hz), normalized the value by PSD of SAD and estimated the baroreflex gain. At the end of each experiment, we isolated baroreceptors and measured the open-loop baroreflex gain. Results: The lability (standard deviation) of AP increased in partial SAD and SAD groups (sham: 7.96±0.94, partial SAD: 9.06±1.37, SAD: 18.5±5.04 mmHg, $p < 0.001$). Estimated baroreflex gain linearly correlated with open-loop baroreflex gain (y=2.05x-0.02, R2=0.83). Conclusion: Power spectrum anaysis of AP time series allows us to identify broreflex function. K.S. works in a department endowed by OMRON Healthcare. COI:Properly Declared

2S-08AM-5

Half-minute cardiovascular regulation is impaired in patients with heart failure with preserved ejection fraction

Fuke Soichiro, Mizobuchi Asako, Tsushima Ryu, Tanaka Masamichi, Yumoto Akihisa, Saito Hironori, Sato Tetsuya

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[Background] Detrended fluctuation analysis (DFA) of RR interval is a method for determining the autocorrelation function of cardiovascular regulation. Although the short-term exponent is reported to be decreased in patients with heart failure, the differences in exponents between heart failure with preserved ejection fraction (HFpEF) and heart failure with reduced ejection fraction (HFrEF) remains unknown.[Methods] Twenty patients diagnosed with HFpEF, defined as brain natriuretic peptide (BNP) > 100 pg/ml and EF > 50% without significant valvular diseases, 20 patients diagnosed with HFrEF, defined as BNP > 100 pg/ml and EF < 50%, and 20 control subjects were enrolled. After RR intervals of 24-h Holter electrocardiography were resampled at 1 Hz, a DFA was performed. The short-term ($\alpha 1$, 4 to 10 s) and long-term scaling exponents ($\alpha 2$, 11 to 3600 s) were compared among the three groups. The $\alpha\beta$ filter was applied to calculate the instant magnitude of the exponent.[Results] In DFA, $\alpha 1$ was significantly decreased in the HFpEF ($P < 0.05$ vs. control) and HFrEF ($P < 0.05$ vs. control) groups. After the application of an $\alpha\beta$ filter, the short-term cardiovascular regulation within 20 s was disturbed in the HFpEF group, and short-term (within 30 s) and midterm (around 180 s) regulation was disturbed in the HFrEF group.[Conclusion] The short-term cardiovascular regulation was disturbed in HFpEF patients, while short- and mid-term cardiovascular regulation was disturbed in patients with HFrEF. COI:No

Symposium 17

[Co-sponsored by Grant-in-Aid for Scientific Research on Innovative Areas "Thermal Biology"]

Novel mechanisms for thermosensory processing: perception, emotion and behavior

March 29 (Thu) 16:10〜18:00 Hall 3

2S-03PM-1
Transient receptor potential melastatin 2 (TRPM2), a thermo-sensitive metabolic sensor

Kashio Makiko

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Living organisms are continually surrounded by changes in ambient temperature. Hence, we evolved the ability to sense temperature to lead autonomic/behavioral adaptive responses. Thermo-sensitive transient receptor potential (TRP) channels, so-called thermo-TRPs, constitute important temperature sensors in diverse species. Thermo-TRPs are expressed not only in sensory neurons to detect environmental temperature change but also in deep organs which are not exposed to drastic temperature changes exceeding daily fluctuation of body temperature, suggesting functional regulation of their activity and the roles at body temperature.
TRPM2 is broadly expressed in various tissues and cells such as the brain, pancreas, spleen, kidney and immunocytes which are continually influenced by core body temperature. TRPM2 function is regulated by intracellular Ca^{2+} ($[Ca^{2+}]_i$), endogenous agonists (e.g. ADPR and cADPR) and redox signal reflecting signal inputs to the cells. Interestingly, temperature threshold of TRPM2 is dramatically reduced by redox signal, enabling its activation at body temperature to cause $[Ca^{2+}]_i$-increase. Moreover, TRPM2 roles in body temperature regulation have also been suggested. TRPM2 has been shown to be expressed in warmth-sensitive neurons in hypothalamus and peripheral sensory/sympathetic neurons, and involved in autonomic hypothermic response and thermoregulatory behavior, respectively.
These results strongly suggest TRPM2 implications in body temperature-regulated processes and its relation with metabolism. TRPM2 roles as a body temperature sensor integrating systemic and cellular metabolic states will be discussed. COI:No

2S-03PM-2
Different thermosensory pathways mediate perception and thermoregulatory behavior

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Thermoregulatory behavior (e.g., cold-seeking behavior in a hot environment) is utilized to choose an optimal thermal environment. Although many thermoregulatory behaviors are driven by environmental thermosensory signaling from skin thermoreceptors to the brain, the thermosensory neural pathways have been unknown. Here we ablated the rat spinothalamocortical thermosensory pathway by bilaterally lesioning the thalamus. The functional ablation of this pathway was confirmed with elimination of skin temperature-dependent electroencephalographic responses recorded from the primary somatosensory cortex. In a two-floor thermal plate preference test, control rats displayed cold- and heat-avoidance thermoregulatory behaviors. Of note, the thalamic-lesioned rats also exhibited intact thermoregulatory behaviors, indicating that the spinothalamocortical pathway is not involved in behavioral thermoregulation. We next examined the involvement of the lateral parabrachial nucleus (LPB), which mediates thermosensory signaling for autonomous thermoregulation. Inhibition of neurons in the LPB with bilateral nanoinjections of muscimol eliminated the cold- and heat-avoidance behaviors. These results indicate that an LPB-mediated afferent pathway, but not the spinothalamocortical pathway, transmits the cutaneous thermosensory signals required for behavioral thermoregulation. This finding may contribute to understanding of the central mechanisms for the generation of thermal comfort and discomfort underlying thermoregulatory behavior. COI:No

2S-03PM-3
Coding of cutaneous temperature in excitatory and inhibitory neurons of the primary somatosensory cortex

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Thermal sensation is critical for adapting to new environments. Cutaneous thermal information is transmitted to cortical regions, such as the primary somatosensory cortex (S1), via the spinal cord and the thalamus. The S1 has roles in processing of sensory information, such as touch, pain, and temperature. Electrical stimulations of the S1 cause thermal sensation in human and lesion of this area causes loss of thermal sensation. Thus, the S1 has critical roles in thermal sensation. In the S1, excitatory neurons transmit sensory information to other brain regions, and inhibitory neurons regulate excitatory neuronal activities, and the balance between excitation and inhibition is critical for sensory processing. However, little is known about how these neurons process cutaneous temperature with single cell resolution and how warm and cold sensation are processed in S1 neurons. To elucidate this, we observed excitatory and inhibitory neuronal activities of layer 2/3 in the S1 in response to thermal stimulations using two-photon microscopy. Thermal stimulation evoked calcium responses in S1 excitatory and inhibitory neurons. In addition, some neurons respond to cold or warming stimulation and the majority of excitatory and inhibitory neurons activated by both cold and warming. These results provide a comprehensive explanation of coding of cutaneous temperature in the excitatory and inhibitory neurons in the S1. COI:No

2S-03PM-4
Brain regions involved in thermal perception: where we sense, feel, and evaluate thermal stimulus?

Nagashima Kei[1], Aizawa Yuka[1], Harada Tokiko[2], Nakata Hiroki[3], Sadato Norihiro[2]

1:Fac Human Sci, Waseda Univ, Tokorozawa, Japan, 2:Psychopysiol, NIPS, Okazaki, Japan, 3:Human Life Env, Nara Women's Univ, Nara , Japan

The conscious perception about thermal stimuli is divided to two categories: thermal sensation and pleasantness/unpleasantness. The aim of the present study was to identify brain regions involved in the thermal perception. Sixteen subjects had thermal stimulus of either 41.5° C or 18.0° C at the forearm. during whole-body stimulus of 47.0° C, 32.0° C, or 17.0° C. The local stimuli were delivered on the right forearm with the Peltier device, and the whole-body stimuli were conducted with a water-perfusion suit. The local stimulation was conducted five times with a 30-s interval. The brain activation was assessed by functional magnetic resonance imaging, and the subjects reported rating of thermal perception following. There were no differences in the discriminative component among the three whole body temperatures, although the hedonic component was different. Significant activation of medial prefrontal cortex extending to anterior cingulate cortex, bilateral insula and the right inferior parietal lobe were observed, which overlapped with across the 6 experimental conditions. Regions including insula were related to thermal sensation evaluated, whereas medial prefrontal cortex was activated while the thermal pleasantness was estimated. Although the brain activation was not specific to hot and cold stimuli, the insula and medial prefrontal cortex may be responsible for evaluating thermal perception. COI:No

Symposium 18

Advances in iPS cell technology for elucidating tissue development and organogenesis.

March 29 (Thu) 16:10〜18:00 Hall 7

2S-07PM-1
Patient specific iPS cell technology reveals abnormal activation of TGFβ signaling as a pathogenesis of left ventricular non-compaction cardiomyopathy

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Left ventricular non-compaction (LVNC) is the third most prevalent cardiomyopathy in children and has been hypothesized a developmental defect of the embryonic myocardium as the pathogeneisis. Here, we showed that patient-specific induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) generated from patients carrying cardiac transcription factor, TBX20, mutation with LVNC recapitulated the pathological phenotype at the single-cell level and was associated with perturbed transforming growth factor beta (TGFβ) signaling. We demonstrated that LVNC iPSC-CMs had decreased proliferative capacity due to abnormal activation of TGFβ signaling. TBX20 regulated expressions of TGFβ signaling modifiers including the known genetic cause of LVNC, PRDM16, and genome editing of PRDM16 also causes proliferation defect in iPSC-CMs. Inhibition of TGFβ signaling and genome editing of the TBX20 mutation were sufficient to reverse the disease phenotype. Our study demonstrates that LVNC iPSC-CMs are a useful tool for the exploration of novel mechanisms underlying poorly understood cardiomyopathies including LVNC. COI:No

2S-07PM-2
Fabrication of scaffold-free human vascular graft by periodic hydrostatic pressurization

Yokoyama Utako[1], Saito Junichi[1], Ito Hiroaki[2], Tadokoro Tomomi[3], Taniguchi Hideki[3], Ishikawa Yoshihiro[1]

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Biological tissue-engineered blood vessels are desired for patients with arterial obstructive disease. We previously demonstrated that periodic hydrostatic pressure (PHP) induced fibronectin fibrillogenesis and enabled to fabricate implantable vascular grafts consisting of rat smooth muscle cells (SMCs). In the present study, we investigated human-derived cells as potential sources of biological grafts. First, we used human umbilical arterial SMCs (hUASMCs) and fabricated ten layered cell sheets under the same PHP culture condition as rat SMCs, in which cell seeding and PHP (110-180 kPa, 0.002 Hz) for 24 h were repeated. The multi-layered graft (2.0 mm by 1.5 mm) was successfully sutured at the aorta of nude rat in which the same size of aortic tissue was resected (n=4). Two months after implantation, echocardiography confirmed patency, and all patch grafts were endothelialized. Next, we investigated the effect of PHP in induced pluripotent stem cells (iPS cells), and found that higher pressure with faster cycle of PHP (110-550 kPa, 0.005Hz) significantly induced fibronectin mRNA expression (1.5-fold vs. atmospheric pressure, n=4, p=0.004). The above-mentioned PHP enabled to fabricate eight layered cell sheet, although tensile strength of the iPS cell sheets were lower than that of hUASMC sheet. These data suggest that hUASMCs and iPS cells are potential source of human vascular graft. COI:No

2S-07PM-3
Generation of the vascularized liver organoids by a fusion of iPSC liver buds

Tadokoro Tomomi, Matsuno Tatsuya, Yamazato Tasuku, Taniguchi Hideki

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Cell culture technology including 3D organoid culture is advanced rapidly in the field of regenerative medicine. Previously, we generated vascularized organoids named liver buds, which contain human induced pluripotent stem cells (iPSCs) derived hepatic progenitors, human mesenchymal stem cells (MSCs), and human umbilical vein endothelial cells. Cell mixtures on matrigel are self-condensed and self-organized, and vascular networks are formed in the liver buds. As its self-condensation is necessary to generate vascularized liver bud, size and shape control is limited. In this study, we developed a novel method to generate the vascularized liver organoid by a fusion of small liver buds so that vascularized liver organoids can be designed in any size and shape. As small liver buds were fused, vascular networks in a fused liver organoid were formed in 3 days. Moreover, branching of vascular networks in the fused liver organoid was controlled by the amount of MSCs. When fused liver organoids were transplanted on the surface of brain, 30.8% of transplants were engrafted and vascular networks were connected to the host vasculature while small liver buds cannot be engrafted. Finally, fused liver buds showed the upregulation of genes related to liver functions compared to small liver buds. Taken together, it is demonstrated that fused liver organoid forms functional vascular networks and promotes liver functions. This technology will be applicable to other types of organoids and contribute to the tissue reconstruction. COI:No

2S-07PM-4
Induction of functional lung epithelial cells from human pluripotent stem cells

Gotoh Shimpei[1]

1:Dept Repir Med, Grad Sch Med, Kyoto Univ, Japan, 2:Dept Drug Disc Lung Dis, Grad Sch Med, Kyoto Univ, Japan

The lung is a vital organ for gas exchange and involved in defense to pathogens. It consists of complicated branching structure covered with various epithelial cells. Multi-ciliated cells and mucus-secreting cells work for eliminating pathogen by muco-ciliary clearance in airway region of the lung. Alveolar type II cells secrete pulmonary surfactant and differentiate into alveolar type I cells, thereby contribute to gas exchange and immunological defense. For lung disease modeling, animal models have long been used by combining drug-induced lung injury modeling and reverse genetics. However, due to the difference of genetic backgrounds, there has always been a limitation to understand the results of mouse models to human diseases. Emerging technologies such as human iPS cells, CRISPR-CAS9 system and "organoid" culture, have enabled us to conceive the concept of human disease modeling in a dish. Human iPS cells have been expected as a source of human derived lung cells, because primary cells are difficult to obtain from the lung for technical and ethical reasons. Then, we have worked on developing the methods to differentiate iPS cells into lung cells. Accumulating data involving our studies have shown that stepwise differentiation strategy recapitulating the developmental stages proved to be an effective approach for generating lung cells. We have recently established the methods of generating functional hiPSC-derived airway and alveolar epithelial cells, respectively. These methods would be beneficial for future drug discovery and regenerative medicine. COI:Properly Declared

Symposium 19

New insights into the neural dysfunction due to impaired glucose metabolism

March 29 (Thu) 16:10〜18:00 Hall 8

2S-08PM-1
How much does the variety of carbohydrates in a diet control brain development and mental health?

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1:Neural Dev, Tokyo Met Inst of Med Sci, Tokyo, Japan, 2:Schizo Res, Tokyo Met Inst of Med Sci, Tokyo, Japan

As you can see from the popularity of low-carb diet in these days around the world, it has been known among the people with scientific proof, that the excess carbohydrate in your meals has a bad effect for the etiology and progression of obesity and diabetes. However, the evidence of effects for brain function is scarce; what's more during developing period. Digested nutrients in the intestine are absorbed into the blood stream to convey throughout the body including the brain. Since neurons acquire the nutrients indirectly from the blood vessel through glial cells, small wander that changing blood component affects the neurons and glial cells function. Carbohydrates are mainly classified as simple and complex carbohydrates. We now focus on how much different effect on the brain function does each carbohydrate have. We aim at not only the analysis of changing behaviors depending on the variety of carbohydrates, but also identification of neural cells and molecules causing the changing behaviors. It's a fact that the less carbohydrates you take, the thinner you are. Some carbohydrates being bad for going on a diet are not also bad for brain function, we consider. We'd like to propose the more safety or superior carbohydrates for brain function based on the scientific proof. COI:No

2S-08PM-2
Pyruvate starvation induces rapid neuronal and Schwann cell death under high glucose conditions

Yako Hideji, Sango Kazunori

Diabetic Neuropathy Project, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan

Pyruvate is produced from glucose through the glycolysis and plays an essential role in energy metabolism. In addition, exogenously applied pyruvate has been shown to act as a potent anti-oxidant and anti-inflammatory molecule under pathological conditions, such as diabetic retinopathy and nephropathy. However, it remains unclear if exogenous pyruvate has beneficial effects on neurons and Schwann cells under hyperglycemia, thereby being a potential therapeutic agent against diabetic peripheral neuropathy.

We observed rapid and massive cell death of primary cultured rat dorsal root ganglion neurons and immortalized mouse Schwann cells IMS32 under exposure to high glucose and pyruvate-deficient conditions. Further analyses using IMS32 cells resulted in the following findings; 1) the cell death was induced by pyruvate starvation with high glucose, but not with galactose or mannitol, 2) pyruvate deficiency *per se* rapidly increased the metabolites of the polyol pathway, such as sorbitol and fructose, 3) pyruvate starvation with high glucose markedly suppressed the mitochondrial respiration and ATP production, and 4) the cell death under pyruvate deficiency with high glucose was completely prevented by the treatment of some TCA cycle metabolites (e.g. 2-oxoglutaric acid). These findings suggest that exogenous pyruvate plays a major role in the maintenance of the glycolysis-TCA cycle pathway under high glucose conditions, and the rapid cell death resulting from its starvation may be, at least partly, attributed to the attenuation of mitochondrial ATP production. COI:No

2S-08PM-3
Effects of blood glucose fluctuation and hypoglycemia on diabetic neuropathy

Kato Ayako, Tatsumi Yasuaki, Kato Koichi

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It is suggested that postprandial hyperglycemia and hypoglycemia due to diabetes treatment could be involved in the development of diabetic complications. As for diabetic neuropathy, neuropathy associated with impaired glucose tolerance (IGT) has been reported. There is evidence for the involvement of reactive oxygen species (ROS) in the pathogenesis of diabetic neuropathy. It has been previously shown that hyperglycemia enhanced oxidative stress and caused peripheral nerve dysfunction. However, the effects of hypoglycemia and blood glucose fluctuation on diabetic neuropathy remain unclear. Our data demonstrated that not only constant high glucose, but also glucose fluctuation and hypoglycemia enhanced oxidative stress and induced cell death in immortalized adult mouse Schwann (IMS32) cells and that polyol pathway hyperactivity and ER stress might be involved in the increased oxidative stress and cell death caused by glucose fluctuation and hypoglycemia. These findings suggest that hypoglycemia and blood glucose fluctuation might cause nerve dysfunction in diabetes, leading to the onset and progression of diabetic neuropathy. COI:Properly Declared

2S-08PM-4
TREM2: A Novel Link between Diabetes and Cognitive Impairment

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Dementia is expanding worldwide, in conjunction with the increase of diabetes and obesity. As dementia is one of the major causes of disability and dependence, it is urgently needed to develop predictive markers and effective treatments for dementia. Diabetes has been reported to be a risk factor of dementia, and the possible mechanisms underlying diabetes-related dementia have been implicated in hyperglycemia, inflammation, and insulin resistance; however, its mechanistic details remain to be elucidated. Triggering receptor expressed on myeloid cells 2 (TREM2) is a cell surface protein exclusively expressed on myeloid lineage cells such as monocytes and microglia. Although genome-wide association studies suggested the pathological implication of TREM2 in dementia, the pathophysiological significance of TREM2 in diabetes and obesity remains unclear. We previously reported the unfavorable pro-inflammatory (M1)/anti-inflammatory (M2) balance of monocytes in obese diabetic patients. We further found that in high-fat diet-fed mice, TREM2 was more up-regulated on M1 macrophages as compared to M2 macrophages. In non-obese diabetic patients, we found that serum TREM2 levels were correlated with diabetes-related risk factors of dementia, including hyperglycemia and aggravation of inflammation. Notably, the elevation in serum TREM2 levels was associated with the risk of cognitive impairment. Here, we summarize recent advances on the pathophysiological significance of TREM2 in cognitive impairment associated with diabetes. COI:No

Symposium 20

Recent Progress of Renal Tubular Transporters

March 29 (Thu) 16:10～18:00 Hall 9

2S-09PM-1
Functional Characterization of Monocarboxylate Transporter 9 as a Multi-Functional Organic Solute Transporter (MFOST)

Anzai Naohiko[1,2], Jutabha Promsuk[2], Ouchi Motoshi[2], Furihata Tommy[1]

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An orphan transporter member of the 16th solute carrier family (SLC16), named monocarboxylate transporter 9 (MCT9), has amino acid sequence only 30-35% identity to its family members and distinguishes from MCT1 to 4. MCT9 has been suggested to be involved in the onset of hyperuricemia or gout by genome-wide association studies (GWAS). Since experimental data about MCT9 function has not been reported yet except carnitine efflux, we tried to clarify its physiological roles. The quantitative RT-PCR (qRT-PCR) against human tissue cDNAs revealed that the mRNA expression of MCT9 is quite higher in kidney than other tissues. In situ hybridization showed its high expression in renal proximal convoluted tubule and collecting duct. *Xenopus* oocyte expressing MCT9 demonstrated the $[^{14}C]$urate transport in Na^+-, Cl^- and voltage-independent manner and the $[^{14}C]\beta$-hydroxybutyrate transport in Na^+-and Cl^- dependent manner. MCT9-mediated β-hydroxybutyrate transport was inhibited by several organic anionic drugs while MCT9-mediated urate transport was not. Thus, since MCT9 mediates the transport of carnitine, urate as well as β-hydroxybutyrate differently, we have to call this clone Multi-Functional Organic Solute Transporter (MFOST). COI:No

2S-09PM-2
New aspects of pH regulation through bicarbonate transporter: from kidney to multiple organ.

Yamazaki Osamu[1], Sohma Yoshiro[2,3], Seki George[4], Hayashi Matsuhiko[1]

1:Apheresis and Dialysis center/General Medicine, Med, Keio Univ, Tokyo, Japan., 2:Dept.Pharm, Med, Keio Univ, Tokyo, Japan., 3:Dept. Pharm, Pharm, Intl Univ of Health and Welfare, Tochigi, Japan., 4:Yaizu City Hospital

Na^+-HCO_3^- cotransporter NBCe1 encoded by SLC4A4 plays essential roles in the regulation of intracellular/extracellular pH. Homozygous mutations in the NBCe1 cause proximal renal tubular acidosis (pRTA) associated with extrarenal manifestations such as ocular abnormalities and migraine. We analyzed nonsynonymous single nucleotide polymorphisms (SNPs), and K558R variant had a significantly reduced transport activity. (Pflugers Arch 461:245, 2011) We also demonstrated that the NBCe1 Leu522 plays an important role in the structure and trafficking of NBCe1. The trafficking defect may underlie the dominant negative effect, leading to migrane. (Pflugers Arch 465:1281, 2013) These and other results suggest that the defective NBCe1 activity in astrocytes can cause migraine potentially through dysregulation of synaptic pH.We also demonstrated a cellular chloride sensing mechanism that plays an important role in the regulation of NBCe1B, leading to the maintenance of cellular ion homeostasis and epithelial fluid and electrolyte secretion. (PNAS 112:E329, 2015)IRBIT, 1,4,5-trisphosphate receptors binding protein binds NBCe1B and has an important role of regulating pH through NBCe1B at many organs. Our findings revealed pH regulating system in human multiple organs, not only kidney or epithelial grand, but also synaptic system in neuron, ocular system and gastrointestinal tract. COI:No

2S-09PM-3
Controlling phosphate in the body including kidney and other organ

Segawa Hiroko, Kaneko Ichiro, Miyamoto Ken-ichi

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Inorganic phosphate (Pi) is an essential physiologic compound for several biologic functions, including intracellular signal transduction, energy exchange, production and function of cell membranes, and also the composition of hydroxyapatite in the bone and teeth. Deviations from normal serum Pi concentrations cause severe clinical disorders. Even slight chronic elevations have been associated with increased rates of death due to cardiovascular complications that are common among patients with chronic kidney disease. On the other hand, prolonged hypophosphatemia, caused by, e.g., malabsorption, renal Pi losses, or inherited disorders, results in symptoms such as osteomalacia, hypercalciuria, and bone demineralization. The extracellular concentration of Pi depends to a large extent on mechanisms that control renal excretion of Pi. Renal handling of Pi is controlled by complex regulatory networks that involve several organs and several endocrine factors. Dietary Pi, parathyroid hormone, 1,25-dihydroxyvitamin D3, and fibroblast growth factor 23 are major regulators of Pi homeostasis. Two families of sodium-dependent Pi transporters, type II (SLC34A1-A3) and type III (SLC20A1-A2), are responsible for the inward transport of extracellular Pi. Recently, SLC53A1 was added to the Pi transporter families. SLC53A1/Xpr1 is originally identified a xenotropic and polytropic retrovirus receptor and it exports intracellular Pi to outside. This symposium will give an overview for controlling of Pi homeostasis and the Pi transporters in the body including kidney and other organ. COI:No

2S-09PM-4
Two cystine transporters

Nagamori Shushi

Lab Biomol Dyn, Nara Med Univ, Kashihara, Japan

Cystine, one of the oldest amino acids, was found from a kidney stone by Wollaston in 1810. Cystinuria is an inherited disorder caused by the defect of amino acid transport systems for cystine in the apical membrane of renal proximal tubules. The failure of cystine reabsorption results in recurrent nephrolithiasis which leads to severe renal dysfunctions. It has been known that a heterodimeric amino acid transporter called $b^{0,+}$ AT/SLC7A9 and rBAT/SLC3A1 is responsible for cystine transport and mutations in either SLC7A9 or SLC3A1 genes result in cystinuria. Thus, the mutations of these two subunits have been studied extensively. However, although molecular identification of mammalian transporters seems to be virtually settled, some long-postulated transporters remain to be uncovered. The second cystine transporter contributed to renal reabsorption of cystine was the one of them. Its genetic defect has been proposed to be responsible for a type of cystinuria distinct from that caused by the mutations of $b^{0,+}$ AT and rBAT. Recently, we have found AGT1/SLC7A13 as the second cystine transporter with proposed characteristics and provided a possible clue to the genetics of new type of cystinuria.I will present the latest researches about one of the mutations in $b^{0,+}$ AT/SLC7A9 and the characterization of AGT1/SLC7A13. COI:No

Symposium 21

Conjunctive experimental and theoretical approaches promote elucidation of the functioning mechanisms of non-classical voltage-gated ion channels

March 30 (Fri) 8:30～10:20 Hall 4

3S-04AM-1

Integrative approach with electrophysiological and theoretical methods reveals an unusual voltage sensing mechanism in PKD2L1 channel

Numata Tomohiro, Inoue Ryuji

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Numerical model-based simulations provide important insights into ion channel gating when experimental limitations exist. Here, a novel strategy combining numerical simulations with patch clamp experiments was used to investigate the role of net positive charges in the putative transmembrane segment 4 (S4) for atypical, positively-shifted voltage-dependence of polycystic kidney disease 2-like 1 (PKD2L1) channel. Charge-neutralising mutations of K452Q, K455Q and K461Q in S4 reduced gating charges, positively shifted the Boltzmann-type activation curve and altered the time-courses of activation/deactivation of PKD2L1, indicating that this region constitutes part of a voltage sensor. Numerical models of wild-type (WT) and mutant PKD2L1-mediated currents were reconstructed from their voltage-dependent gating parameters. However, this modelling required an additional scaling factor to adjust the maximal conductance (G_{max}), which depends on the polarity of the membrane potential. Subsequent single-channel conductance (γ) measurements revealed that voltage-dependence of G_{max} in WT can be explained by the additional voltage-dependence of γ, which is greatly changed in PKD2L1 mutants. Finally, a possible structural basis for the observed voltage-dependence of PKD2L1 was discussed by homology modelling based on the atomic structures of PKD2 and NaVAb. COI:No

3S-04AM-2

Multi-hierarchical analysis of TRPM4 arrhythmogenicity by experimental and numerical approaches

Hu Yaopeng[1], Shen Yanghua[2], Kurahara Lin[1], Hiraishi Keizo[1], Ichikawa Jun[1], Numata Tomohiro[1], Okamura Yasushi[3], Zhu Xin[2], Inoue Ryuji[1]

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TRPM4 channel is a Ca^{2+}-activated monovalent cation channel abundantly expressed in the heart. Voltage change profoundly affects TRPM4 channel gating in concert with physiological modulators such as intracellular Ca^{2+} and PIP_2. In this study, we investigated this implications in cardiac excitability by electrophysiological measurements and multi-hierarchical numerical simulations.Voltage-dependent activation of TRPM4 channel re-evaluated by ionomycin-permeabilized cell-attached recording. Detailed gating analysis yielded the voltage-dependent rate constants described as the complex functions of Ca^{2+}. Numerical simulation using these constants explained well why TRPM4 channel activation turns arrhythmogenic under remodeling conditions. However, whole-atrium 3D-simulation showed that spatially homogenous TRPM4 upregulation may be anti-arrhythmic by earlier termination of reentry. Quantitative analysis with patch clamping and FRET-based PIP_2 measurement revealed that decreased PIP_2 level greatly suppresses TRPM4 activity by positively shifting its voltage-dependence of gating. Numerical simulations incorporating these changes into cardiac AP model suggested that PIP_2 depletion may counteract arrhythmic changes resulting from TRPM4 upregulation in remodeled cardiomyocytes. In addition, this protective mechanism appears compromised in an arrhythmogenic TRPM4 mutant E7K. COI:No

3S-04AM-3

Approaches to structural dynamics of the voltage-gated H^+ channel gating.

Yuichiro Fujiwara

Dept Physiol, Grad Sch Med, Osaka Univ, Suita, Japan

The voltage-gated H^+ channel (Hv) is a H^+-permeable four-transmembrane domain protein that corresponds to the voltage-sensor domain of other voltage-activated ion channels and phosphatases. The functional unit of Hv is a homo dimer assembled by the cytoplasmic coiled-coil domain. Although atomic structures of Hv have been solved, the structural mechanism how Hv senses the membrane potential, how Hv opens the gate and how the dimerization controls the gating have been unknown. I have tried to dissect the structural rearrangement of the transmembrane region associated with the channel gating. In this symposium, I will introduce multiple approaches to the structural dynamics of Hv that revealed that the structural uniqueness of Hv deeply involved in the gating process. COI:No

3S-04AM-4

Structural basis for the modulation of voltage sensor movement by auxiliary subunits in voltage-gated KCNQ1 channels

Nakajo Koichi

Dept Physiol, Osaka Med Coll, Takatsuki, Japan

The classical voltage-gated potassium channels such as Kv1 (Shaker) have a tetrameric structure with four voltage sensing domains (VSDs). Each VSD senses depolarization, moves upward (up-state) and contributes to eventual opening of the pore domain. KCNQ1 channel is a shaker-type voltage-gated potassium channel, and has a similar tetrameric structure, which has been confirmed by a recent cryo-EM structure. KCNQ1 channels are expressed in various tissues including heart, intestine and inner ear. KCNQ1 channels compose ion channel complex with different subtypes of auxiliary KCNE proteins depending on where they are expressed; for example, KCNQ1 forms ion channel complex with KCNE1 and underlies the slow delayed rectifier potassium current in heart, while KCNQ1 and KCNE3 form constitutively active potassium current in epithelial cells, assisting chloride ion transport by recycling potassium ions. Apparently, KCNE subunits dramatically affect and change KCNQ1 channel gating. Recent findings suggest KCNE proteins modulate KCNQ1 channels by directly interacting with the VSDs. To elucidate how KCNE3 interacts with KCNQ1 channel and makes it constitutively active, we took advantage of zebrafish KCNE3, which cannot make KCNQ1 channels constitutively active, and successfully identified a latter half of the transmembrane domain of KCNE3 is required for the constitutive activity of the KCNQ1-KCNE3 complex. With the recent docking model of the KCNQ1-KCNE3 complex in mind, we propose the interaction between the latter half of KCNE3 and the S1 segment of KCNQ1 stabilizes the up-state of the VSDs. COI:No

3S-04AM-5

Voltage-activated K^+ channels increase the concentration of ER Ca^{2+} store

Murata Yoshimichi, Kazama Itsuro, Maruyama Yoshio

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Ion channels working at plasma membrane are assembled in endoplasmic reticulum (ER), then they are transported to their destination via Golgi apparatus. But it is unclear that whether they are conferred function and physiological role(s) in the intracellular membrane.

We observed large conductance Ca^{2+}-activated K^+ current in nuclear envelopes (peri-nuclear ER membrane) of pancreatic acinar cells and HEK293 cells using the patch-clamp technique. Consequently we tried to elucidate role(s) of this channel in ER membrane. We evaluated alteration in free Ca^{2+} content in the ER expressing MaxiK channel and indicated that MaxiK-expressing HEK293 cells have larger Ca^{2+} content in ER than wild type (WT) HEK293 cells.

We tried to reveal the mechanisms for the elevation of Ca^{2+} concentration in the ER by MaxiK channel. Which property of MaxiK is important for this phenomenon? We measured G-CEPIAer fluorescence intensity in HEK293 cells expressing K^+ channels having various properties; intracellular Ca^{2+} sensitivity, inward rectification or voltage dependence. HEK293 cells expressing SK1, IK1 or Kir2.1 channels did not show the elevation of Ca^{2+} concentration in the ER. On the other hand, Kv1.2-expressing cells had larger Ca^{2+} content in the ER than WT HEK293 cells. Furthermore, HEK293 cells expressing mutant MaxiK channels, much or less sensitive to $[Ca^{2+}]_i$ showed the elevated ER Ca^{2+} level as the cells expressing WT MaxiK channels. These results indicate that voltage sensitivity of the channels could be important to this phenomenon. COI:No

Symposium 22

Front line of the pathological analysis of neuropsychiatric disorders

March 30 (Fri) 8:30～10:20 Hall 5

3S-05AM-1

Trends technology in pathological analysis of neuropsychiatric diseases

Matsushita Masayuki

Dept Mol&Cell Physio, Grad Sch Med, Univ Ryukyus, Japan

Life sciences has made remarkable progress due to technological innovation such as next generation sequence technology, regenerative medicine technology, gene editing technology and so on. In this symposium, each symposist reports the pathological analysis and treatment development of neuropsychiatric disorders by applying these techniques. As an introduction of this session, I briefly introduce success examples on diseases that we will take up in this symposium such as schizophrenia and mood disorder, applying the latest technology. COI:No

3S-05AM-2

Impact of omega-3 fatty acids on prevention and treatment of depression

Matsuoka Yutaka

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Depression is a common mental condition and account for a large burden of disability. Pharmacologically focused approaches clearly do not meet clinical needs, while clinicians and researchers are facing the huge challenge of developing effective treatments despite of the advance of neurosciences. As detailed in our Consensus Statements, nutritional medicine should now be considered as a mainstream elements of psychiatric practice (Sarris et al. Lancet Psychiatry 2015; Sarris et al. World Psychiatry, 2015). Omega-3 polyunsaturated fatty acids (PUFAs) also known as n-3 fatty acids or fish oil are essential nutrients and must be obtained by dietary sources. Omega-3 PUFAs have a range of neurobiological activities in modulation of neurotransmitters, anti-inflammation, anti-oxidation and neuroplasticity, which could contribute to the psychotropic effects. Evidence from epidemiological and clinical studies have revealed that omega-3 PUFAs play an important role in the prevention and treatment of depression. According to biological specificity and safety consideration, omega-3 PUFAs is a potential treatment option for pregnant women, adolescents, and physically vulnerable subjects. This presentation describes my recent work of fish intake on preventing depression (Matsuoka et al, Transl Psychiatry, 2017) and extends the notion that nutrition in psychiatry is a modifiable environmental factor and calls for more researches on prospective clinical studies to justify the application of omega-3 PUFAs not only in daily clinical practice but also in self-management. COI:No

3S-05AM-3

Cell biological studies of familial Parkinson's disease using iPS cells

Bono Keiko[1,2], Okano Hirotaka[1]

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Parkinson's disease (PD) is a common neurodegenerative disorder and the number of patients is increasing with the aging of the population. Mutations of the retromer component Vacuolar protein sorting-35 (VPS35) is linked to autosomal dominant forms of familial Parkinson's disease, PARK17. VPS35 is essential for endosome-to-Golgi retrieval of membrane proteins. However, the physiological and pathological roles of Vps35 in neuronal functions are poorly understood. Here we demonstrate that the PD-associated VPS35 mutation caused dysfunction of retromer in neurons differentiated from PARK17 patient's iPS cells. We have analyzed three independent iPS cell lines from controls and from PD patients heterozygous for the VPS35 mutation, respectively. After differentiation into dopaminergic neurons, we observed the movement of the retromer and early endosomes in neurons using fluorescent live imaging. It was observed that the retromer moved in cytoplasm together with early endosomes, and the movement of early endosome was slower in the disease group compared with healthy control. These results focusing on retromer function may have a potential to be a breakthrough for development of therapeutic drugs targeting retromer in Parkinson's disease and Alzheimer's disease as well, which are the two major neurodegenerative diseases. COI:No

3S-05AM-4

Dissociation of CS-US association in fear memory by manipulating the activity of parietal association cortex

Suzuki Akinobu[1,2], Ushijima Sakurako[1,2], Murayama Emi[1,2], Ohkawa Noriaki[1,2], Matsuo Mina[3], Nishizono Hirofumi[2,3], Inokuchi Kaoru[1,2]

1:Dept Biochem, Grad Sch Med Pharm Sci, Univ of Toyama, Toyama, Japan, 2:CREST, Japan Science and Technology Agency, 3:Div of Animal Exp Lab, Life Sci Res Cen, Univ of Toyama, Toyama, Japan

In the usual contextual fear conditioning, the animals have two types of learning [(1) learning the context (CS) and, (2) learning the association between the context (CS) and the shock (US)]. Because these types of learning co-occur during conditioning, it is difficult to identify brain regions required for CS-US association. In this study, using modified CPFE paradigm, in which the mice received paired or unpaired presentations of the CS and US during conditioning, we performed Arc CatFISH method to detect brain regions required for the CS-US association and found that parietal association cortex (PtA) responded to CS-US signals.To test whether PtA regulates CS-US association at behavioral level, we established new behavioral paradigms to measure CS, US, and CS-US independently. By employing lentivirus harboring 3GTRE-ArchT-EYFP and cfos-tTA transgenic mice, we found that PtA regulates CS-US association.We next asked whether manipulating the PtA activity erase CS-US associate memory that has been once formed. We labeled with ArchT-EYFP a subset of PtA neurons that responded during reactivation. Fifteen minutes optical silencing immediately after CS exposure 24h after reactivation suppressed fear memory when mice were tested 24h later without optical silencing, indicating that manipulating the PtA activity erase CS-US associative memory. COI:No

3S-05AM-5

An approach from familial psychiatric patients in Ryukyu Islands: Disease modeling based on induced pluripotent stem cell and high-impact variant

Takamatsu G[1], Kaname T[3], Okano J H[4], Matsushita M[1]

1:Dept Mol Cel Physiol, Grad Sch Med, Univ Ryukyus, Okinawa, Japan, 2:Dept Neuropsych, Grad Sch Med, Univ Ryukyus, 3:Dept Gen Med, Nat Cent Chi Heal Dev, Tokyo, Japan, 4:Div Reg Med, Jikei Univ Sch Med, Tokyo, Japan

Bipolar disorder and schizophrenia are common and complex psychiatric diseases characterized by high genetic heterogeneity. Therefore, it would be a rational approach to expand pathological findings in high-risk pedigrees (i.e. genetically recognized subtype with high penetrance variants) to general cases. Patient-derived induced pluripotent stem cells (iPSCs) with high-impact variants could be useful disease cell models of psychiatric diseases. However, while successful for simply inherited traits such as Alzheimer's disease with pathogenic APP mutations, finding the subtype with high penetrance variants remains a challenge in bipolar disorder and schizophrenia. Although DISC1 mutation or 22q11.2 deletion in schizophrenia have been established, high-risk pedigree-based study has not reached its potential. Here, to develop novel iPSC disease models of bipolar disorder and schizophrenia, we searched for families with a high prevalence in Ryukyu Islands and performed genome sequencing and iPSCs generation. We found (1) a large family with multiple bipolar disorder and (2) a family with an early-onset schizophrenia monozygotic twin from cousin marriage in an isolated island. We present the relation between genomic segments shared in the family and neuronal phenotype. Our works are expected to establish novel cellular and molecular models of bipolar disorder and schizophrenia. COI:No

Symposium 23

Zinc homeostasis-dependent regulation of cellular functions and physiological events

March 30 (Fri) 8:30～10:20 Hall 6

3S-06AM-1
Requirement of zinc transporter ZIP10 for epidermis and hair follicle development

Fukada Toshiyuki

Tokushima Bunri University, Faculty of Pharmaceutical Sciences, Molecular and Cellular Physiology

Skin tissues, in particular the epidermis and hair formation, are severely affected by zinc deficiency. However, the zinc-mediated mechanisms that maintain the cells that form the epidermis and hair have not been established. Here we report that the zinc transporter ZIP10 is highly expressed in the outer root sheath of hair follicles and plays critical roles in epidermis and hair follicle development. We found that ZIP10 marked epidermal progenitor cell subsets and that ablating *Zip10* caused significant epidermal hypoplasia accompanied by down-regulation of the transactivation of p63, a master regulator of epidermal progenitor cell proliferation and differentiation. Both ZIP10 and p63 are significantly increased during epidermal development, in which ZIP10-mediated zinc influx promotes p63 transactivation. Collectively, these results indicate that ZIP10 plays important roles in epidermal development via, at least in part, the ZIP10-zinc-p63 signaling axis, thereby highlighting the physiological significance of zinc regulation in the maintenance of skin epidermis and hair follicles. COI:No

3S-06AM-2
MG53 interaction with Zn for wound healing and regenerative medicine application

Ma Jianjie

Dept Surgery, Davis Heart Lung Res Inst, Ohio St Univ, Columbus, OH, USA

Zinc is an essential trace element that participates in a wide range of biological functions, including wound healing. Although Zn deficiency has been linked to compromised wound healing and tissue repair in human diseases, the molecular mechanisms underlying Zn-mediated tissue repair remain unknown. Our previous studies established that MG53, a TRIM (tripartite motif) family protein, is an essential component of the cell membrane repair, and Zn binding to MG53 is indispensable to assembly of the cell membrane repair process . Mutagenesis studies showed that both RING and B-box motifs of MG53 constitute Zn-binding domains that contribute to MG53-mediated membrane repair. We also demonstrated that the recombinant human MG53 (rhMG53) protein can facilitate wound healing with scar mitigation. Thus, targeting the synergy between Zn and rhMG53 represents a potential effective means for tissue repair and regenerative medicine. COI:No

3S-06AM-3
Modulation of the activity of thermo-sensitive TRP channels by zinc ion

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Zinc is one of the most abundant metals in organisms and the only metal used in all enzyme classes such as DNA polymerase and alcohol dehydrogenase. In addition, many reports have shown that zinc modulates a variety of ion channels, including thermo-sensitive transient receptor potential (TRP) channels. We reported that extracellular zinc inhibits TRPM5 activation. In whole-cell patch-clamp recordings, extracellular application of $ZnCl_2$ inhibited TRPM5 currents with intracellular 500 nM free Ca^{2+}, and the inhibition was dose-dependent (IC50 = 4.3 μM at -80 mV). Extracellular application of $ZnCl_2$ also inhibited temperature-dependent TRPM5 activation. Furthermore, we determined the amino acid residues required for inhibition of the Ca^{2+}-evoked TRPM5 currents by $ZnCl_2$. In addition, we reported the possibility that zinc could modulate the TRPA1 activation by alcohol. Thus, zinc modulates the activity of thermo-sensitive TRP channels under physiological conditions and its inhibition could be involved in the various physiological functions. COI:No

3S-06AM-4
Physiology and biochemistry of zinc-requiring ectoenzymes

Kambe Taiho

Grad Sch Biostudies, Kyoto Univ, Kyoto, Japan

The essential trace element zinc is widely required in cellular functions. Thus, zinc homeostasis is tightly controlled within narrow boundaries, in which zinc transport proteins play indispensable roles. Moreover, zinc transporters also enable a variety of zinc-dependent proteins and enzymes to play roles in numerous and varied biological reactions. Recent studies start to reveal the relationships between the functional activations of these proteins/enzymes and roles of transporters. Zinc-requiring ectoenzymes have received much attention because they are involved in important physiological functions and thus regarded as potential therapeutic targets in the treatment of diseases. Zinc-requiring ectoenzymes become active by being metallated with zinc at their active site during the secretory process. We have shown that ZNT5-ZNT6 heterodimers and ZNT7 homodimers supply zinc into the early secretory pathway, and activate a number of ectoenzymes such as alkaline phosphatases, matrix metallopeoteinase 9, and carbonic anhydrase IX (CAIX). However, CAIX can be metallated with zinc mediated by ZNT4 homodimers, suggesting that metallation processes by zinc would be dependent on a structural property including coordination motif of each ectoenzyme. Here, I will present our data about activation mechanisms of zinc-requiring ectoenzymes, focusing on their structural properties. I will also summarize and discuss zinc homeostasis-dependent regulation of cellular functions and physiological events. COI:No

Symposium 24

Structure and Function of Inter-areal Circuits in Cerebral Cortex

March 30 (Fri) 8:30～10:20 Hall 7

3S-07AM-1

Ipsilateral and contralateral corticocortical projection-dependent subcircuits in layer 2/3 of rat frontal cortex

Ueta Yoshifumi[1], Kawaguchi Yasuo[2,3]

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Layer 2/3 (L2/3) pyramidal cells in the neocortex project primarily to other cortical areas, with output providing three major classes of innervation: feedforward projections to higher cortical areas, feedback projections to lower cortical areas, and commissural connections innervating the contralateral cortex. In the frontal cortex, the projection subtypes and connectional preferences of pyramidal cells have been well investigated in layer 5, but little is known about the organization of L2/3 projections. Here, in the rat secondary motor cortex (M2), we compared the physiology, morphology, and synaptic connectivity patterns across the three corticocortical projection subtypes in L2/3. In L2, we identified two segregated projection subtypes, commissural cells innervating contralateral M2 (cM2p, contralateral M2-projecting) and neurons providing feedforward projections to the perirhinal cortex (PRC). On the other hand, in L3, cM2p cells overlapped with neurons providing feedback projections to the primary motor cortex. L2 cM2p and ipsilateral PRC-projecting (iPRCp) cells were distinct in their electrophysiological and morphological characteristics, and made unidirectional, cM2p to iPRCp, synaptic connections. These results suggest that, in the frontal cortex, L2/3 is vertically organized into distinct subcircuits providing feedforward, feedback, and commissural connectivity with other cortical areas. COI:No

3S-07AM-2

Axon branches for inter-areal connections of layer 2/3 neurons grow more rapidly than branches for intra-areal connections in the mouse neocortex

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Direct connections between different cortical areas have been shown to be important for sensorimotor integration. Developmental processes of these connections, however, have not been fully understood. By using a subtype-specific promoter and our new sparse labeling method combined with tissue clearing of flat-mounted cortices, we visualized a population of layer 2/3 (L2/3) neurons in the mouse primary somatosensory area (S1) that had axons projecting to a distant ipsilateral target as well as to the contralateral hemisphere. We found that after the main axonal shaft crossed the midline, collateral branches projecting to the ipsilateral hemisphere emerged around postnatal day 3 at the level of layer 5. Only a few among these collateral branches reached the distant target areas, such as the primary motor area (M1) and the secondary somatosensory area (S2), and these far-reaching branches grew at a higher rate than those projecting to the local targets. This observation raises the possibility that a small number of branches might be selected at a very early stage, if not at the timing of the budding, to target an area distant from the original area of the soma. COI:No

3S-07AM-3

Dynamics of thalamocortical activities during motor execution and learning

Tanaka H Yasuyo[1], Tanaka Yasuhiro[1], Kondo Masashi[1], Terada Shin-Ichiro[1], Kawaguchi Yasuo[2], Matsuzaki Masanori[1]

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Animals can learn varieties of action repertoire for smooth and efficient goal-oriented behaviors. Within the primary motor cortex, the representation of well-learned movement is stabilized through motor learning (Masamizu *et al.*, 2014; Peters*et al.*, 2014). The thalamus functions as a hub to transmit signals from the basal ganglia and cerebellum, which also play pivotal roles in motor learning, to the neocortex. However, the temporal dynamics of thalamocortical axonal activity during motor execution and learning remain largely unknown. Here, we conducted two-photon calcium imaging of thalamocortical axons in the mouse motor cortex during a self-initiated lever-pull task. We demonstrate that thalamocortical activities evolve their specific representation for movements through the learning and employ various temporal dynamics depending on their projecting layers. Furthermore, lesion of either the dorsal striatum or deep cerebellar nuclei impaired motor learning. Our results suggest that layer-specific thalamocortical signals for execution of skilled movement evolve for motor memory, and this requires the basal ganglia and cerebellum. COI:No

3S-07AM-4

A cortico-cortical mechanism underlying perceptual memory consolidation during sleep

Murayama Masanori

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Sleep has fundamental roles for consolidation of an animal's motor and sensory learning experiences. During sleep, bottom-up inputs from sensory organs to the brain are largely silenced and inactive. However during the non-rapid eye movement (NREM) phase synchronous oscillations ranging from 0.5 to 4 Hz (slow wave activity) occur across cortical regions. These observations have led to the hypothesis that interregional transfer of internal information during NREM sleep has a significant role in memory consolidation. Recently, we identified a cortical top-down circuit that underlies somatosensory perception in the mouse hindpaw. However, the role of top-down cortical inputs during sleep in memory, particularly in the consolidation mechanism, has yet to be examined. We developed a novel perceptual learning task that requires sleep for memory consolidation and examined the role of top down input during sleep. During NREM sleep between the learning and retrieval periods, the optogenetic inhibition of an anatomically identified cortical top-down input from M2 to S1, but not vice versa, resulted in the suppression of functional communication causality from M2 to S1, the absence of reactivated S1 neurons, and behavioral deficits in texture memory consolidation. In NREM sleep and sleep-deprived states, closed-loop asynchronous or synchronous M2-S1 co-activation, respectively, reduced or prolonged memory retention. Top-down cortical information flow in NREM sleep is thus required for perceptual memory consolidation. COI:Properly Declared

3S-07AM-5

Anatomy and physiology of layer 2/3 projection neurons in mouse barrel cortex

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Interareal signaling between distinct cortical regions is crucially involved in perceptual and cognitive functions of our brain. However, the organizing principle of cortical interareal connections is still not established. We have investigated how layer 2/3 pyramidal neurons in primary somatosensory barrel cortex (S1) in mice anatomically connect to other cortical regions and how these neurons generate functionally tuned outputs toward their targets. Selective labeling of S1 layer 2/3 neurons revealed that their major projection targets are secondary somatosensory cortex (S2) and primary motor cortex (M1) of the ipsilateral hemisphere. Single-cell electroporation and subsequent 3-D reconstruction suggested that S2-projecting (S2p) and M1-projecting (M1p) S1 neurons are mostly dedicated projection neurons without forming other major projection targets. In vivo whole-cell recordings from awake behaving mice revealed that S2p and M1p neurons had distinct intrinsic membrane properties and exhibited markedly different membrane potential dynamics during behavior. Interestingly, associating a whisker stimulus with a reward caused signals conveyed by S2p and M1p neurons to be further tuned, dynamically changing large-scale signal flows in sensorimotor cortex. Thus, our study offers insights into the cellular and synaptic organization of cortical interareal connectivity, providing the evidence for differential regulation of membrane potential dynamics in S1 layer 2/3 neurons having different major projection targets. COI:No

Symposium 25

Illumination of Synapse Physiology -Frontier researches of synaptic functions-

March 30 (Fri) 8:30～10:20 Hall 8

3S-08AM-1

Development of innovative wide-view mapping of synaptic ensemble in the psychiatric model

Hayashi-Takagi Akiko

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The dysregulation of dendritic spines is thought to be involved in a variety of psychiatric disorders, but the links between spines and disorders have been largely correlational because of lacks of a technique for manipulating individual spine. To overcome this problem, we developed a synaptic optoprobe, AS-PaRac1, which is unique not only because it can specifically label the recently potentiated spine, but can also selectively induce shrinkage in just those spines containing AS-PaRac1. This indicates AS-PaRac1 specifically visualizes the synaptic plasticity, which can be erased by blue light. We here aim at the next-generation of AS-PaRac1. First, we developed the improved AS probe (AS-ver3) for more effective shrinkage property, which is essential for future application of this probe for bigger brain such as marmoset. Secondly, by simultaneous three colour imaging of an activity-dependent expression of the presynaptic marker Vamp2-mTurquoise2, the postsynaptic neuron markers tdTomato, together with AS-mVenus as a potentiated synaptic marker, we aim to visualize the potentiated neuronal circuits. To identify the ideal probe with a proper temporal regulation, the selection of promoter and protein degradation sequences were extensively compared in the mice, which were induced with the corresponding probes into the visual cortex, were dark reared for 2 d, being followed by short light stimulation. With the development and application of these tools, studies on synaptic ensemble will pioneer new discoveries, eventually leading to a comprehensive understanding of how the brain works. COI:No

3S-08AM-2

Optical manipulation of monoamin signal for elucidating synaptic basis of reward and punishment learning

Yagishita Sho

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Reward or punishment transiently activates or suppresses dopamine (DA) activity to drive conditioned learnings. Specifically, the phasic release of dopamine reinforces the preceding sensorimotor events while the transient decrease in the dopamine causes the opposite effect. In contrast to strong evidences for the role of dopamine in behavioral learning, the cellular and synaptic mechanisms processing the dopamine signals remains elusive. Using optical techniques to manipulate dopamine and glutamate signals in mouse brain slices, we have explored synaptic bases for reinforcement learning at the level of single dendritic spine. First, we found that reward-related phasic activation of dopamine had a critical time window (0.3 - 2 s) for the enhancement of spine structural plasticity, a structural basis for long-term potentiation, of D1-spiny projection neurons (SPNs) in the nucleus accumbens (NAc). Next, we examined whether spines detect optogenetically mimicked DA dips, subsecond decrease in DA concentration in response to punishment, for the structural plasticity of D2-SPN. We found that DA dip as short as 0.4 s enhanced spine enlargement. We also revealed that these plasticity mechanism is relevant to reward or punishment learning by optogenetic stimulation of synaptic inputs to NAc. Collectively, these results clarified the synaptic mechanisms underlying reinforcement learning. COI:No

3S-08AM-3

Roles of synaptic plasticity at recurrent circuit in the dentate gyrus of the hippocampus

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Hilar mossy cells (MCs) of the dentate gyrus receive inputs from granule cells (GCs) and project their glutamatergic axons back to ipsilateral and contralateral GCs along the longitudinal axis of the hippocampus, thereby establishing an excitatory recurrent circuit. In addition, MCs make synapses with hilar GABAergic interneurons, generating feed-forward inhibition onto GCs. While MCs have been implicated in both memory formation and epileptogenesis, little is known about dynamic changes in synaptic strength at monosynaptic and disynaptic pathways from MC to GCs. Here, we investigated whether MC-GC synapses can undergo long-term plasticity. To this end, we performed whole-cell patch clamp recordings from GCs in acute hippocampal slices of young-adult rats. We found that repetitive activation of MC axons with brief bursts induced presynaptic form of long-term potentiation (LTP) of MC-GC EPSCs (MC-GC LTP). Remarkably, MC-GC LTP requires postsynaptic brain-derived neurotrophic factor (BDNF)/TrkB and presynaptic cAMP/PKA signaling. By using optogenetic methods, we found that this LTP is selectively expressed at MC-GC synapses, but not at the disynaptic inhibitory circuit. Taken together, our findings indicate that MC-GC LTP modulates output of GCs by changing excitation/inhibition balance and may contribute to dentate gyrus-dependent form of learning and epilepsy. COI:No

3S-08AM-4

Regulation of surface dynamics of AMPA receptors and cognitive processes by Arc

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1:Med Innov. Ctr, Grad Sch Med, Kyoto Univ, Kyoto, Japan, 2:Dept Neurochem, Grad Sch Med, Univ Tokyo, Tokyo, Japan, 3:Ctr Dis Biol Intgr Med, Grad Sch Med, Univ Tokyo, Tokyo, Japan

The neuronal immediate early gene Arc plays critical roles in regulation of AMPA receptor (AMPA-R) trafficking and in memory processes. However, the exact AMPA-R dynamics associated with Arc-mediated mechanisms remains unknown. To address this issue, we here recorded subunit-specific AMPA-R lateral diffusion during structural plasticity in wildtype (WT) and Arc-null (Arc-KO) hippocampal neurons. We found that long-term potentiation (LTP) of surface GluA1 levels in spines with volume expansion was undistinguishable between WT and Arc-KO neurons. In contrast, surface GluA1 gradually decreased in non-expanding spines of WT neurons during the late phase of structural plasticity, and this effect was abolished in Arc-KO neurons. Interestingly, LTP of surface GluA2 levels showed a significant increase in Arc-KO neurons in expanded spines immediately after structural plasticity, while no effect was observed on non-expanded spines. Thus, disruption of Arc affected distinct of AMPA-R pools during early and late phases of LTP in potentiated and non-potentiated spines, without perturbation of plasticity induction and expression. These findings strikingly correlated with the normal acquisition yet dysfunctional behavioral refinement in Arc-KO mice in target-switching tasks. Thus, our findings suggest a novel synaptic mechanism by which Arc regulates cognitive refinement processes while preserving synaptic plasticity and learning. COI:Properly Declared

Symposium 26

Neural mechanisms of decision-making revealed by physiology, genetics, and animal psychology

March 30 (Fri) 8:30～10:20 Hall 9

3S-09AM-1

Role of the nigrostriatal dopamine system in response inhibition

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Dopamine (DA) neurons in the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) have been shown to encode "reward prediction error " and implicated in reinforcement learning. It has become increasingly clear, however, that a subgroup of DA neurons in the SNc encodes a signal related to "salience" rather than reward prediction error. Here we show that DA neurons encoding salience regulate a cognitive ability, called "response inhibition", to inhibit planned or ongoing motor actions that would lead to unwanted outcomes. We recorded single-unit activity from DA neurons in the SNc and VTA and neurons in the caudate nucleus, while monkeys performed the saccadic countermanding task. After the monkey gazed a fixation point, the point disappeared and a saccadic target was presented. In 70% of the trials, the monkey was required to make a saccade to the target. In the remaining 30%, the fixation point reappeared as a "stop signal" after the onset of the saccadic target. The monkey was required to cancel a planned saccade. We found that DA neurons in the SNc, but not in the VTA, exhibited a significant excitation to the stop signal. This excitatory response decreased when the monkey failed to cancel a planned saccade. We also found that caudate neurons, which receive DA projections mainly from the SNc, exhibited a significant excitation to the stop signal as well. Furthermore, injecting haloperidol, D2 antagonist, into the caudate nucleus impaired the performance of canceling a planned saccade. Our findings suggest that the nigrostriatal DA pathway transmits a signal that suppresses a planned saccade in the response inhibition paradigm. COI:No

3S-09AM-2

Representation of reward seeking and punishment avoiding signals in single neurons in primate caudate

Nakamura Kae, Yasuda Masaharu, Ueda Yasumasa

Dept Physiol, Kansai Medical University, Osaka,Japan

While the decision making is often based on the expectation of rewards, aversive information and its interaction with reward system may also influences the process. However, the neuronal mechanisms that mediate decision making through analyses of both appetitive and aversive information is poorly understood. Using an eye movement task in which monkeys chose one of cues associated with appetitive, neutral, and aversive outcomes, we revealed that both appetitive and aversive cues may influence monkeys' choice behavior and emotional state measured by task behavior and autonomic responses. We further found that neurons in the caudate, an input channel of the basal ganglia, encode appetitive and aversive information, or both. Inconsistency to the activity for appetitive and aversive Pavlovian conditioning indicated that the activity did reflect emotional context such as motivation or fear per se. The differential activity instead developed as the monkeys made optimal decision making. Thus, beyond its established role in mediating actions aimed at rewards, different population of caudate neurons are involved in choice behavior in different emotional context. COI:No

3S-09AM-3

Physiological roles of striatal projection neurons revealed by optogenetics

Sano Hiromi[1,2], Nambu Atsushi[1,2]

1:Div System Neurophysiol, NIPS, Okazaki, Japan, 2:Dept Physiol Sci, SOKENDAI, Okazaki, Japan

The basal ganglia (BG) play important roles in voluntary movements, motor learning and decision making. The striatum is one of the input nuclei of the BG, and modulated by glutamatergic inputs from the cerebral cortex and by dopaminergic inputs from the midbrain. The striatum is composed of two types of projection neurons, i.e., striatonigral direct pathway and striatopallidal indirect pathway neurons. Striatonigral neurons send afferents to the substantia nigra pars reticulata (SNr) or the internal segment of the globus pallidus (GPi), while striatopallidal neurons project to the external segment of the globus pallidus (GPe). In the classical model of the BG, striatonigral neurons suppress the activity of the output nuclei of the BG, i.e. the SNr/GPi, and disinhibit thalamic and cortical neurons, while striatopallidal neurons increase the activity of the SNr/GPi by the sequential connection through the GPe and subthalamic nucleus. To elucidate the mechanisms of the BG functions, it is essential to investigate information processing along the BG circuit. We have applied optogenetics and electrophysiological recordings to examine the effects of selective activation of striatonigral and/or striatopallidal neurons to clarify the information flow through each pathway. COI:No

3S-09AM-4

The causal role of the orbitofrontal cortex in updating stimulus-outcome relationships

Ogawa Masaaki

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In classical appetitive conditioning, a neutral stimulus is repeatedly followed by an outcome (i.e. reward or no reward). The subject is then able to predict the outcome during the presentation of the stimulus (i.e. conditioned stimulus: CS). Previous studies have shown that neurons in the orbitofrontal cortex (OFC) develop firing in response to both CS and outcomes, and that OFC is necessary to use acquired stimulus-outcome relationships to facilitate subsequent adaptive behaviors. However, how temporally and context-specific OFC activity in response to CS or outcomes could contribute to such behaviors has remained elusive.

To address these questions, mice were trained to learn CS1-reward and CS2-no reward associations in a blocked manner, and the stimulus-outcome relationships were then reversed. We found that optogenetic inhibition of OFC specifically in the face of omission of expected reward following CS1 impaired extinction of conditioned response (CR) during the presentation of CS1. Intriguingly, this OFC inhibition also impaired subsequent CS2-reward association, despite the fact that OFC was not inhibited during the latter association. Furthermore, this impairment was not observed when a new CS3 instead of CS2 was introduced. Finally, the effect of OFC inhibition following CS1 on the CS2-reward association was layer-specific. These results pinpoint the causal role of the orbitofrontal cortex in updating stimulus-outcome relationships. COI:No

Symposium 27

Role of mucosa in regulating smooth muscle contractility

March 30 (Fri) 14:00～15:50 Hall 2

3S-02PM-1
Mucosal and muscularis immune responses-motility linkage in digestive tract

Hori Masatoshi

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Normal gastrointestinal motility is essential for maintaining healthy gastrointestinal luminal environment, especially healthy intestinal microflora. That is, periodic germ excluding by defecation is indispensable for healthy intestinal microflora construction. Turbulence of the intestinal microflora injuries the barrier function of the gastrointestinal mucosal epithelium and develops mucosal inflammation by bacterial invasion into the mucosa. For example, intestinal obstruction alters composition of intestinal microflora resulted in induction of mucosal inflammation to induce gastrointestinal motility disorder. Mucosal Th1 or Th2 signaling induces hypo- or hyper- gastrointestinal motility, respectively, but the molecular mechanisms are not still unified well. On the other hand, in recent years, it was found that the signal from normal intestinal flora propagated submucosally via mucosal epithelial cells, resulting in the activation of muscularis resident macrophages. The macrophages produced Bone Morphogenetic Protein2 (BMP2) to stimulate enteric neuron which in turn inhibited gastrointestinal motility. This is the first report to prove that intestinal bacteria in the gastrointestinal lumen indirectly modulated gastrointestinal motility. Thus, mucosal and muscularis immune responses have positive and negative influences on gastrointestinal motility, but only a small part of its molecular mechanism has been elucidated. In this symposium, I would like to think about mucosal immunity - muscularis immunity - gastrointestinal motility coupling in summary. COI:No

3S-02PM-2
The Urinary Bladder: modulation of detrusor smooth muscle contractility by the mucosa

Fry Henry Christopher[1], Kanai John Anthony[1]

School of Physiology, Pharmacology & Neuroscience, University of Bristol, UK, 2:Department of Pharmacology & Chemical Biology, 3:University of Pittsburgh, 4:Pittsburgh, Pennsylvania, 5:USA

The bladder wall is composed of detrusor smooth muscle, overlain on the inner surface by a mucosa that itself consists of urothelium, to face the bladder lumen, and a suburothelium containing sensory nerves, muscle elements, blood vessels and interstitial cells. The mucosa has several functions: to provide a tight epithelial interface between urine and underlying tissues; a sensory function to transduce external stresses and directly influence detrusor contractile function. When exposed to external stresses the urothelium releases neuromodulators including acetylcholine (ACh), ATP, prostaglandins and nitric oxide. ACh and ATP are released from urothelium by different but interacting routes that lead ultimately to purinergic activation of afferent nerves. Several questions arise: how are ACh and ATP release mediated from urothelium; can their release explain the mode of action of some drugs used to treat bladder pathologies; what is the route whereby released ATP activates sensory nerves? The presence of mucosa overlaying detrusor smooth muscle itself greatly increases spontaneous contractile activity of the bladder wall. Questions arising are: does the mucosa itself have intrinsic contractile activity; does the mucosa exert an action on detrusor by diffusion of chemical mediators or by direct cell-to-cell interaction; what is the role of the mucosa in mediating much-enhanced spontaneous contractions associated with some bladder pathologies? COI:No

3S-02PM-3
Novel therapeutic strategies targeting the epithelium and airway contractility in asthma

Bourke E Jane

Biomedicine Discovery Institute, Monash University, Melbourne, Australia

Damage to the airway epithelium in asthma may not only compromise its crucial barrier function, but also increase secretion of inflammatory cytokines and mucous, and reduce capacity to produce relaxing factors that oppose airway contraction. Anti-inflammatory glucocorticoids do not target airway remodeling, while bronchodilator efficacy of β_2-adrenoceptor agonists is limited in severe disease. Novel drugs that target epithelial repair and function and improve dilator responsiveness offer promise to overcome these limitations of current therapy.

The peptide hormone, relaxin, is a Relaxin Family Peptide Receptor 1 (RXFP1) receptor agonist with unique combined abilities in the lung. In mouse models of allergic airways disease, chronic relaxin treatment reversed established epithelial thickening, airway fibrosis and hyperresponsiveness. Relaxin also directly elicited partly epithelium-dependent bronchodilation in rat, guinea pig and human airways. Tested in combination, relaxin enhanced responsiveness to both glucocorticoids and β_2-adrenoceptor agonists.

Free fatty acid receptors 1 and 4 (FFAR1, FFAR4) are expressed in airway epithelium and smooth muscle. ω-3 fatty acids are endogenous agonists for FFAR4 shown to accelerate recovery from epithelial injury. Both FFAR1- and FFAR4-selective synthetic agonists caused airway relaxation that was maintained even with β_2-adrenoceptor desensitisation.

The mechanisms underlying these promising preclinical findings remain to be fully elucidated to support the development of relaxin and FFAR agonists as novel therapeutics for asthma and other lung diseases. COI:No

3S-02PM-4
Spontaneous activity in mucosa of seminal vesicles originates from epithelial basal cells

Takeya Mitsue[1], Hashitani Hikaru[2], Hayashi Tokumasa[3], Nakamura Kei-ichiro[4], Takano Makoto[1]

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In seminal vesicles (SVs) of the guinea pig, the mucosa plays a crucial role in generating spontaneous SV smooth muscle contractions. We have reported that SV mucosal cells with a morphology resembling holly leaves, generate asynchronous spontaneous Ca^{2+} transients that are abolished by the blockade of sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA). Here we further explored the morphological and functional properties of the spontaneously active cells in situ. Tetrodotoxin-insensitive electrical slow waves were irregularly generated in the basal layer of the mucosa. Fluorescent dye injected into the cells from an impaled microelectrode visualised a concentric spread of the dye to the neighboring cells that had a similar morphology to that of the mucosal cells firing spontaneous Ca^{2+} transients. Slow waves were abolished by the blockade of SERCA or Ca^{2+}-activated Cl^- channels (CaCCs) as well as by reducing extracellular Cl^-, suggesting that they result from the opening of CaCCs triggered by spontaneous Ca^{2+} release from ER. Immunohistochemical study revealed that the spontaneously active cells were pancytokeratin-positive basal cells distributed just beneath the secretory columnar epithelium. Thus, it is likely that the epithelial basal cells generate spontaneous activity to drive the spontaneous contractions in the SV smooth muscle. COI:No

Symposium 28

Molecular guideposts to direct "healthy time"; Chrono-medicine reveals homeostatic and adaptive protection pathways

March 30 (Fri) 14:00～15:50 Hall 5

3S-05PM-1
Molecular basis of chronotherapy in cancer treatments

Ikeda Masaaki[1], Kumagai Megumi[1,2], Nakajima Yoshihiro[3], Sasaki Yasutsuna[4], Fujita Ken-ichi[4]

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Chronotherapy is a promising approach for treating a variety of diseases. As the onset of disease can occur in a temporally restricted time period during the day, selecting the appropriate treatment time might optimize the therapy for the disease. Recently, several targeted drugs for cancer chemotherapy have been developed that can be administered orally. This efficient drug administration method allows greater flexibility in selecting the optimal time of day for treatment. To investigate the molecular mechanism of chronotherapy during anticancer drug treatment, we established *Bmal1* knockout cells utilizing the CRISPR/Cas9 system and compared the antiproliferation rates in normal or *Bmal1* gene knockout cells in the presence or absence of anticancer drugs. The multikinase inhibitors regorafenib and sorafenib significantly inhibited the proliferation of *Bmal1* knockout cells. Cells synchronized to the 24-h rhythm by dexamethasone were more sensitive to gefitinib than unsynchronized cells. These findings suggest that the sensitivity of cells to molecularly targeted anticancer drugs is regulated by the circadian system. COI:No

3S-05PM-2
Intracellular signaling to integrate synchronous functioning of circadian adaptation system

Tamaru Teruya[1], Kawamura Genki[2], Yoshitane Hikari[3], Fukada Yoshitaka[3], Ozawa Takeaki[2], Takamatsu Ken[1]

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Healthy time is sustained with circadian adaptation system (CAS), in which daily genome-wide physiological rhythms and adaptive pathways are orchestrated by circadian clocks through the core feedback loop with clock genes (Bmal1, Clock, Cry1/2, Per1/2) and their regulatory loops including protein regulatory events. CAS is composed with the multi-cellular circadian oscillators, which can be synchronized by environmental cues including cell stresses, such as heat, reactive oxygen species and UV, involving in pathogenesis. Desynchony of multi-cellular physiologies possibly relates to the cause of certain diseases. Therefore dysfunction/ desynchrony of CAS exacerbates various health problem and diseases. Among potential molecular basis to integrate synchronous functioning of CAS, we here focuses on: 1) Interplays between molecular clocks and adaptive protection pathways, which is coordinated via heat-shock response and anti-oncogene pathways to evoke protection systems during cell stress-triggered clock resetting. 2) What is the common intracellular signaling to synchronize clocks at cell-to-cell level? It still remains elusive about common molecular code to synchronize multi-cellular circadian clocks by various external cues. As the highly potential candidate, we have identified a structural basis in BMAL1 protein, tentatively called S (synchrony) region. COI:No

3S-05PM-3
SIK3-mediated signaling pathway involved in circadian rhythm, sleep, and metabolism

Hayasaka Naoto[1,3], Hirano Arisa[2], Miyoshi Yuka[3], Tokuda T Isao[4], Yoshitane Hikari[2], Matsuda Junichiro[5], Fukada Yoshitaka[2]

1:Dept Neurosci II, Res Inst Environ MedRIEM, Nagoya Univ, Nagoya, Japan, 2:Dept of Biol Sci, Sch Sci, Univ Tokyo,Tokyo, Japan, 3:Dept Anat and Neurobiol, Kindai Univ Sch Med, Osaka-Sayama, Japan, 4:Dept Mech Engineer, Ritsumeikan Univ, Shiga, Japan, 5:Lab Animal Model Human Diseases, NIBIOHN

Salt-inducible kinase 3 (SIK3) plays a crucial role in various aspects of metabolism as well as in sleep regulation and skeletal development. In the course of investigating metabolic defects in the Sik3-deficient mice (Sik3-/-), we observed that circadian rhythmicity of the metabolisms was phase-delayed. Sik3-/- mice also exhibited other circadian abnormalities, including lengthening of the period, impaired entrainment to the light-dark cycle, phase variation in locomotor activities, and aberrant physiological rhythms. Ex vivo suprachiasmatic nucleus slices from Sik3-/- mice exhibited destabilized and desynchronized molecular rhythms among individual neurons. In cultured cells, Sik3-knockdown/knockout resulted in abnormal bioluminescence rhythms. Expression levels of PER2, a clock protein, were elevated in Sik3-KD/KO cells but down-regulated in Sik3-overexpressing cells, which could be attributed to a phosphorylation-dependent decrease in PER2 protein stability. This was further confirmed by observation of PER2 accumulation in the Sik3-/- fibroblasts and liver. Collectively, the results indicate that SIK3 plays key roles in circadian rhythms by facilitating phosphorylation-dependent destabilization of PER2. COI:No

3S-05PM-4
The regulation of cardiac mitochondrial function by the circadian clock

Kohsaka Akira

Dept Physiol, Wakayama Med Univ, Wakayama, Japan

The heart continuously requires oxidative energy, bioenergy primarily produced by mitochondria, across the daily 24 hr light/dark cycle. Recent studies have revealed that mitochondria are highly dynamic and the dynamic processes are under the control of the circadian clock system. We have previously reported that the heart expresses functional molecular clocks which not only orchestrate daily rhythms of cardiac physiology but also regulate cardiac energy homeostasis in mice. The heart tissue without *Bmal1*, a core circadian clock gene, exhibits severe defects in cardiac energy metabolism, including glucose and lipid transports, fatty acid oxidation, and mitochondrial biogenesis. Importantly, mice deficient in cardiac *Bmal1* function develop severe heart failure with age, which results in early mortality. These findings indicate that the molecular clock in the heart plays an important role in the production of mitochondrial bioenergy, and thus maintains cardiac function. Here, I will introduce recent findings on mitochondrial dynamics and bioenergetics that are linked to the function of the circadian clock system. COI:No

3S-05PM-5
Circadian regulation of allergy

Nakamura Yuki, Nakao Atsuhito

Dept Immunol, Sch Med, Univ Yamanashi, Yamanashi, Japan

Symptoms and laboratory parameters of allergic diseases exhibit prominent 24-hour variations. For instance, in most allergic rhinitis patients, symptoms worsen overnight or early in the morning. Accordingly, there are benefits to nighttime dosing of anti-allergy medications in such patients. Although the circadian pathophysiology of allergic diseases is well documented, the biological basis of this phenomenon remains poorly understood. We have shown that the circadian clock plays a key role in temporal regulation of allergic reaction, and may therefore underlie the circadian pathophysiology of allergic diseases. In this symposium, I will introduce our findings that highlight the emerging role of the circadian clock as a potent regulator of allergic reactions. Given the strong influence of circadian rhythms on allergic diseases, we believe that research on how the time of day impacts allergic reaction which we may call chronoallergology will provide new insight into previously unknown aspects of the biology of allergies. Such knowledge should facilitate novel strategies for prevention and treatment of these diseases. COI:No

Symposium 29

Oxidative stress and disease

March 30 (Fri) 14:00〜15:50 Hall 6

3S-06PM-1
Modulation of signaling mechanisms in the heart by thioredoxin 1

Sadoshima Junichi

New Jersey Medical School, Newark, USA

Myocardial ischemia/reperfusion and heart failure are the major cardiac conditions in which an imbalance between oxidative stress and anti-oxidant mechanisms is observed. The myocardium has endogenous reducing mechanisms, including the thioredoxin (Trx) and glutathione systems that act to scavenge reactive oxygen species (ROS) and reduce oxidized proteins. The Trx system consists of Trx, Trx reductase (TrxR), and an electron donor, NADPH, where Trx is maintained in a reduced state in the presence of TrxR and NADPH. Trx1, a major isoform of Trx, is abundantly expressed in the heart and exerts its oxidoreductase activity through conserved Cys32 and Cys35, reducing oxidized proteins through thiol disulfide exchange reactions. In this lecture, molecular targets of Trx1 in the heart, namely class II histone deacetylase, AMP activated protein kinase, and mechanistic target of rapamycin, will be introduced. I will then discuss how Trx1 regulates the functions of its targets, thereby affecting the extent of myocardial injury caused by myocardial ischemia/reperfusion and the progression of heart failure. COI:No

3S-06PM-2
Roles of Nox4 in neural stem/precursor cell proliferation and neurogenesis in the hippocampus

Ago Tetsuro

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Reactive oxygen species regulate the growth of neural stem/precursor cells and participate in hippocampus-associated learning and memory. However, the origin of these regulatory reactive oxygen species in neural stem/precursor cells is not fully understood. We found that Nox4, a reactive oxygen species-producing NADPH oxidase, is expressed in primary cultured neural stem/precursor cells and in the adult mouse brain. Nox4 inhibitors attenuated the bFGF-induced proliferation of cultured neural stem/precursor cells in a dose-dependent manner. Furthermore, lentivirus-mediated Nox4 overexpression increased the production of H_2O_2, the phosphorylation of Akt, and the proliferation of cultured neural stem/precursor cells. The Nox4-mediated increase in proliferation was blocked by Nox4 inhibitors. Nox4 did not significantly affect the potential of cultured neural stem/precursor cells to differentiate into neurons or astrocytes. Although the pathological and functional changes in the hippocampus induced by the neurotoxin trimethyltin were not significantly different between wild-type and $Nox4^{-/-}$ mice, the proliferation of neural stem/precursor cells and neurogenesis in the subgranular zone of the dentate gyrus were significantly impaired in $Nox4^{-/-}$ animals. Interestingly, recovery from the trimethyltin-induced impairment in recognition memory was significantly attenuated in $Nox4^{-/-}$ mice. Collectively, our findings suggest that Nox4 participates in neural stem/precursor cell proliferation and neurogenesis in the hippocampus following injury, thereby helping to restore memory function. COI:No

3S-06PM-3
Redox control of cardiac mitochondrial quality by reactive cysteine persulfides

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Defective mitochondrial dynamics is increasingly recognized as a contributing factor in mortality and morbidity after myocardial infarction. We here found that the dynamin-related GTPase Drp1 mediated the induction of myocardial early senescence in mouse hearts after myocardial infarction (MI). We found that post-translational (electrophilic) modification of Drp1 at the C-terminal cysteine residue is essential for Drp1 polymerization and activation induced by hypoxia/reoxygenation. This Cys residue was found to form Cys persulfide or polysulfide as an inactive form, and depletion of sulfur or suppression of polysulfuration of Drp1 resulted in increase in its GTPase activity. We also identified a small compound that specifically inhibits Drp1-dependent mitochondrial fission without suppressing basal Drp1 GTPase activity. Proteomic profiles using flag-tagged Drp1 revealed that hypoxia treatment significantly increased the interaction of flag-Drp1 with actin-based cytoskeletal proteins. The interaction of Drp1 with cytoskeleton led to Drp1 assembly at fission sites, and the small molecule suppressed Drp1-dependent mitochondrial fission and heart failure after MI. These results suggest that the mitochondria-cytoskeleton interaction through Drp1 is essential for hypoxia-induced mitochondrial fission and myocardial senescence after MI. COI:No

3S-06PM-4
Sulfur respiration: novel mitochondrial energy metabolism in humans

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Reactive persulfides such as cysteine hydropersulfide (CysSSH) are found physiologically and prevalently in prokaryotes, eukaryotes, and mammals (human) in vivo. The chemical properties and abundance of these species suggest a pivotal role for reactive persulfides in cell-regulatory processes. Researchers proposed that CysSSH and related species can behave as potent antioxidants and cellular protectants, and may function as redox signaling intermediates. Here, we discovered moonlighting (dual) functions of cysteinyl-tRNA synthetases (CARSs), serve as the principal cysteine persulfide synthase (CPERS) in vivo. Targeted disruption of a gene encoding mitochondrial CARS (CARS2) in mice and human cells revealed that persulfides derived from CARS2 are critically involved in the mitochondrial energy metabolism. Therefore, the long time known SULFUR RESPIRATION in anaerobic microorganisms is now identified in mammals and humans for the first time. Further investigating CARS-dependent persulfide production may thus clarify aberrant redox signaling in oxidative stress and energy metabolism in mammals.

Reference:
Akaike, T., et al., Cysteinyl-tRNA synthetase governs cysteine polysulfidation and mitochondrial bioenergetics. Nature Commun. 8, 1177 (2017). COI:No

Symposium 30

New developments in evaluating myocardial function

March 30 (Fri) 14:00〜15:50 Hall 7

3S-07PM-1

Development of proarrhythmia risk prediction method using human induced pluripotent stem cell-derived cardiomyocytes

Yamazaki Daiju, Kanda Yasunari

Division of Pharmacology, National Institute of Health Sciences

Torsade de pointes (TdP) associated with QT prolongation is one of the causes of drug withdrawal from the market. Since QT prolongation is a surrogate marker of TdP, an in vitro hERG assay has been carried out according to the ICH S7B guideline. Whereas it has prevented withdrawals of drugs by TdP, drug-induced proarrhythmia has still been a major concern in the safety assessment. Thus, it is expected to establish prediction method for drug-induced proarrhythimia using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs).Here, we developed electrophysiological evaluation method for proarrhythmia risk prediction using hiPSC-CMs. Based on the microelectrode array data of compounds with different TdP risk from hiPSC-CMs, we provided relative TdP risk score to each drug depending on the extent of field potential duration change or early afterdepolarization occurrence. Furthermore, we calculated the margins between free concentration of each drug in medium and free effective plasma concentrations. Using the relative TdP score and margins, we succeeded in classifying drugs into three risk categories. This categorization system demonstrated high concordance with torsadogenic information obtained by the CredibleMed database.In this symposium, we will present our proarrhythmia risk prediction method using hiPSC-CMs and discuss its usefulness and limitation. COI:No

3S-07PM-2

Assessment of the cardiac contractile toxicity by pattern-cultured hiPSC-CMs

Naito Atsuhiko

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Cardiac toxicity is the leading cause of attrition during preclinical and clinical drug development. Human iPSC-derived cardiomyocytes (hiPSCMs) have been tested for their use in safety pharmacology and are shown to be somewhat useful for evaluating pro-arrhythmic potential of the drugs. In contrast, the use of hiPSCMs for evaluating the toxicity against cardiac contraction/relaxation has still been limited, because hiPSCMs are known to be immature and do not reproduce the critical contractile characters of the cardiomyocytes in the adult human heart tissue. In the present study, we developed a novel culture system with patterned culture of hiPSCMs in a 96 well format. In contrast to hiPSCMs cultured in monolayer sheet, contraction/relaxation velocity and the movement of patterned hiPSCMs showed positive correlation between the contraction frequency. Patterned hiPSCMs also exhibited distinct, mechanism of action-based contractile responses against various inotropic molecules, suggesting its usefulness for assessing the effect of drugs against cardiac contraction/relaxation. We tested the acute-to-chronic effect of several anticancer drugs using patterned hiPSCMs and found the molecule-specific, not class-specific, time-dependent effect of anticancer drugs on cardiomyocyte contraction/relaxation. Our results indicate that patterned hiPSCMs would be useful for evaluating the toxicity, and efficacy as well, of drugs on cardiac contraction and/or relaxation during drug development. COI:No

3S-07PM-3

Development of a multidisciplinary approach to maturate electrophysiological properties of hiPS-cardiomyocytes

Kurokawa Junko[1], Ashihara Takashi[2], Furutani Kazuharu[3], Nagamori Shushi[4], Kanda Yasunari[5]

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Human iPS cell-derived cardiomyocyte (hiPS-CM) is conceptually promising as an unlimited source of human cardiomyocytes for pre-clinical safety assessment of cardiac function. However, the fetal phenotype of hiPS-CM is a major obstacle for accurate evaluation of toxicological concentrations. In order to develop a technique for evaluating arrhythmogenicity of drugs in electrophysiologically-matured hiPSC-CMs, we transduced a ventricular-specific gene KCNJ2 into the cells, which alters fetal spontaneous action potentials (AP) to ventricular-like quiescent AP. *In silico* mathematical models taught us necessity of KCNJ2 expression to stop automaticity, which is consistent with experimental results. We would like to discuss on multidisciplinary approaches to increase predictability of drug-induced arrhythmogenic risks in this presentation. (Supported by AMED17mk0104027h0203) COI:No

3S-07PM-4

Novel computational evaluation method for stability of action potential prolongation based on repolarization reserve

Murakami Shingo, Tomida Taichiro, Mikami Yoshinori, Ito Masanori, Adachi-Akahane Satomi

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Repolarization reserve is a physiological concept that normal repolarization in cardiac myocytes is determined by the multiple and redundant mechanisms. In this symposium, we will present our proposal to employ this frequently-used but vaguely-defined concept for improving the inaccuracy of the hERG test for drug-induced arrhythmia prediction. We redefined repolarization reserve by using a human ventricular myocyte model and quantified the activated repolarization reserve by measuring the current injected during action potential (AP) clamp simulation with AP waveforms of various durations (APDs). The relationship between APD and activated repolarization reserve was named as the repolarization reserve curve. The curve quantitatively showed that the repolarization reserve is activated by the prolongation of APD. The model analysis also showed that the inactivation of voltage-dependent L-type Ca^{2+} channel current as well as the activation of I_{Ks} contribute to the repolarization reserve. We then examined if the repolarization reserve curve predicts APD prolongation under various conditions. The quantified repolarization reserve curves accounted for arrhythmic risks in the presence of various drugs by utilizing empirical proarrhythmic factors, Triangulation, Reverse use-dependence, transmural Dispersion (TRIaD). Thus, our novel method for quantifying the repolarization reserve will be useful for the prediction of drug-induced arrhythmia. COI:No

Symposium 31

Network-level Mechanisms in the Master Circadian Clock

March 30 (Fri) 14:00～15:50 Hall 8

3S-08PM-1

Critical roles of AVP neurons in the central circadian clock of the SCN

Mieda Michihiro

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As the central pacemaker in mammals, the circadian clock in the suprachiasmatic nucleus (SCN) of the hypothalamus is a heterogeneous structure consisting of multiple types of GABAergic neurons. Although individual cells have a cellular clock driven by autoregulatory transcriptional/translational feedback loops of clock genes, interneuronal communication among SCN neurons is likely essential for the SCN to generate a highly robust, coherent circadian rhythm. However, mechanisms underlying the SCN neuronal network remain unclear. We have been focusing on the roles of arginine vasopressin (AVP)-producing neurons, which are located in the SCN shell. Disruption of cellular clocks specifically in AVP neurons by deleting *Bmal1* resulted in attenuation of circadian behavior rhythm and lengthening of free-running period due to reduced coupling of SCN neurons. In addition, manipulating the cellular circadian period of AVP neurons by specific deletion or overexpression of *CK1δ* altered the behavioral circadian period accordingly. Furthermore, lack of GABA release from AVP neurons due to the specific deletion of *Vgat* drastically impaired coupling of SCN neurons and reduced the amplitude of circadian behavior rhythm. Thus, AVP neurons of the SCN may be an essential component for the generation of circadian rhythm and the determination of circadian period. COI:No

3S-08PM-2

Ultradian Calcium Rhythms in the Paraventricular Hypothalamic Nucleus

Enoki Ryosuke[1], Wu Yu-er[2], Oda Yoshiaki[3], Huang Zhi-Li[2], Honma Ken-ichi[3], Honma Sato[3]

1:Hokkaido Univ, Grad Sch Med, Bioimaging, Sapporo, Japan, 2:Dep Pharm, Sch Basic Med Sci, Fudan Univ, Shanghai, China, 3:Hokkaido Univ, Res & Educ Cent Brain Sci, Sapporo, Japan

The hypothalamic suprachiasmatic nucleus (SCN), the master circadian clock in mammals, sends major projections to the paraventricular nucleus of the hypothalamus (PVN). The PVN is known to play critical roles in regulating endocrine and autonotmic functions. It is composed of a group of neurons including neurosecretory cells that synthesize posterior pituitary hormone and those regulate anterior pituitary functions. The PVN and adjuscent areas are also known as a hub region where circadian information from the SCN is relayed to the other brain areas to control physiology and homeostasis in the body. Despite this importance, the network-level dynamics in the PVN is unknown. Here we monitored the Ca^{2+} dynamics in a large population of neurons in the cultured hypothalamic slices containing both SCN and PVN. We found the ultradian Ca^{2+} rhythms in the PVN, which are synchronous in the entire neuronal network. Frequency of the rhythms is regulated in accordance with the phase of SCN circadian Ca^{2+} rhythms. The synchronous ultradian Ca^{2+} rhythms were disrupted by tetrodotoxin (TTX) and blocked by the AMPA/NMDA receptor blockers NBQX/APV. In contrast, the Ca^{2+} level was enhanced by the $GABA_A$ receptor blocker Gabazine. These results indicate that PVN generates ultradian rhythms and that SCN circadian information is transformed to the ultradian Ca^{2+} rhythms via glutamate and GABA signaling. COI:No

3S-08PM-3

In vivo monitoring of clock gene expression rhythms in the brain of freely moving mice

Ono Daisuke[1], Honma Ken-ichi[2], Honma Sato[2]

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In mammals, the suprachiasmatic nucleus (SCN) in the hypothalamus plays a critical role in the expression of circadian rhythms. In the SCN, circadian rhythms are generated by a transcription/translation negative feedback loop involving several clock genes and their protein products. This molecular oscillation in the SCN is thought to transmit the output signals for expressing circadian rhythms in cellular functions such as cytosolic calcium and spontaneous firing.Photonic bioimaging technique is a powerful tool for measurement of biological functions in living cells. Especially bioluminescence is a useful reporter for long-term recording because of its low toxicity and high quantitativeness, which enabled us to obtain characteristics of circadian property from cell to tissue level. It is important to measure gene expression in specific brain areas which control animal behavior from conscious animals, because sleep and wakefulness can only be assessed in living animals. In this research, we established in vivo measurement of clock gene expression in the SCN using an optical fiber in freely moving mice. COI:No

3S-08PM-4

Intercellular coupling between pacemaker clock neurons in *Drosophila melanogaster*

Yoshii Taishi

Graduate School of Natural Science and Technology, Okayama University, Japan

The fruit fly *Drosophila melanogaster* has been an excellent model for studying neurogenetics of animal behaviors. One of the most successful examples is the field of chronobiology, where the Drosophila researches made a significant contribution by the discovery of the *period* gene, the first clock gene. The *period* gene is expressed in about 150 neurons in the fly brain, a very little number of neurons composing a pacemaker neuron network compared to that of mammals. Among them, a cluster of four small neurons located in the lateral brain, so-called s-LNv, has been proposed as master pacemaker neurons, which express a neuropeptide, Pigment-dispersing factor (PDF) in Drosophila. The s-LNv neurons send signals to other pacemaker neurons via PDF/PDF-receptor. We recently found that a cluster of two pacemaker neurons located in the dorsal brain, so-called DN1a, express a neuropeptide, CCHamide1 (CCHa1) and this signal goes to the s-LNv neurons through the CCHa1 receptor. Thus, s-LNv and DN1a are reciprocally coupled via the neuropeptidergic circuit. Interestingly, the Drosophila CCHa1 receptor is a homolog of the mammalian gastrin-releasing peptide receptor, which is involved in intercellular coupling in mammalian clocks, suggesting a well conserved clock networks across a wide variety of animal species. COI:No

3S-08PM-5

Mathematical modeling of dual coupling mechanisms in the suprachiasmatic nucleus

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The suprachiasmatic nucleus (SCN) is a hierarchical multioscillator system, in which neurons are coupled through vasoactive intestinal polypeptide (VIP), arginine vasopressin (AVP), neurotransmitter GABA, gap junctions, and others. Recent study (Ono et al., 2016) showed that the VIP and AVP are both essential for synchronized cellular activities in the neonatal SCN of CRY double-deficient mice ($Cry1,2^{-/-}$), while attenuation of both signals leads to desynchronized aperiodic activities in the adult SCN. Coculture with the neonatal wild-type SCN revealed that the cells remained aperiodic when both signals were present. The circadian rhythmicity was however restored when either of the two signals was blocked, implying competing effect of the two signals. We present here a mathematical model for the network of SCN neurons coupled by two interaction functions corresponding to VIP and AVP signals. The competing effect was modeled as a phase shift between their input signals. We show that three factors, i.e., oscillator strength, strength of coupling, and timing of the dual couplings, are essential for reproducing the experimental observation of the network dynamics in the SCN. COI:No

Symposium 32

The relationship between arterial stiffening and cerebral, cardiovascular or cognitive function: effect of exercise

March 30 (Fri) 14:00〜15:50 Hall 9

3S-09PM-1
Effects of shear stress during exercise on the vascular endothelial function

Iwamoto Erika

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Vascular endothelial dysfunction is a well-established an early cardiovascular risk marker. Moreover, endothelial dysfunction is associated with cerebrovascular events such as chronic stroke and vascular dementia. Exercise-induced increases in shear stress release the vasoactive substance from the endothelium and have beneficial effects on vascular function. In fact, exercise training has been shown to improve endothelial function in cardiovascular patients. In addition, exercise training increases resting cerebral blood flow in ischemic lesions and improve cognitive function via endothelium-dependent mechanisms. These results suggest that endothelial function is a key factor to understand the vascular adaptation to exercise. Endothelial function in peripheral conduit arteries (e.g., brachial, femoral, popliteal arteries) is commonly assessed by a noninvasive method, ischemia-induced flow-mediated dilation (FMD). Interestingly, recent studies measured shear-mediated dilation using hypercapnia to assess the endothelial function in the carotid artery. Analogous to the FMD in the peripheral conduit arteries, the decreased carotid shear-mediated dilation could serve as a potentially useful measure of cerebrovascular endothelial function. In this symposium, my presentation would like to focus on the change in blood flow and shear stress during exercise and how these changes affect vascular endothelial function. Additionally, I will discuss our ongoing carotid study. COI:No

3S-09PM-2
Relationship between cerebrovascular arteriosclerosis and cognitive impairment

Hirasawa Ai, Shibata Shigeki

kyorin University, Tokyo, Japan

The number of dementia patients is expected to increase in Japan as the population ages. Alzheimer's disease (AD) and vascular dementia (VaD) are common subtypes of dementia in the elderly. Recently, the development of arteriosclerosis is shown to be a potential risk factor not only for VaD, but also for AD. For example, our previous study proved a significant association between arterial stiffness measured by brachial-ankle pulse wave velocity and cognitive impairment in the elderly with dementia. However, it remains unknown how cerebrovascular arteriosclerosis would affect onset of AD and VaD. Transcranial Doppler (TCD) ultrasound is a non-invasive measurement for cerebral hemodynamic and cerebrovascular function to identify its arteriosclerosis. In our recent study, we addressed the relationship between cerebral hemodynamics measured by TCD ultrasound and cognitive function in AD and VaD. Moreover, we found alterations in the cerebral hemodynamics and pathophysiology of AD and VaD such as hippocampus hypoperfusion and atrophy. In this symposium, I would like to focus on our recent findings regarding the relationship between cerebrovascular function and dementia in the elderly. COI:No

3S-09PM-3
Aortic-cerebral Hemodynamic Transmission: Windkessel function and Exercise Training

Sugawara Jun

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High cerebral pressure and flow fluctuations could be a risk for future cerebrovascular disease. To determine whether acute systemic vasoconstriction affects the dynamic pulsatile hemodynamic transmission from the aorta to the brain, we characterized the dynamic relationship between the changes in aortic pressure (AoP; estimated from the radial arterial pressure waveforms) and the cerebral blood flow velocity (CBFV) at the middle cerebral artery (via a transcranial Doppler) during a mild lower body negative pressure (LBNP) stimulation using frequency-domain analysis. The normalized transfer function gain from pulsatile AoP to pulsatile CBFV at 0.07-0.20 Hz is significantly augmented with -20 and -30 mmHg of LBNP, and that such a response is associated with the increase in systemic vascular resistance but not with the end-tidal CO_2 response. These results suggest that systemic vasoconstriction deteriorates the central arterial dampening effect on pulsatile hemodynamics (e.g., Windkessel function) toward the brain, particularly in slow oscillations (e.g., 0.07-0.20 Hz). In this session, we will introduce the following investigation to examine the influence of endurance exercise training on the central arterial dampening effect on pulsatile hemodynamics toward the brain. COI:No

3S-09PM-4
Effects of different mode of exercise training on arterial stiffness and endothelium-derived relaxing factor

Iemitsu Motoyuki

Fac Sport Health Sci, Ritsumeikan Univ, Shiga, Japan

Increased arterial stiffness with advancing age is a risk factor for cardiovascular disease. Many studies have showed beneficial effect of aerobic exercise training on arterial stiffness in the elderly. Additionally, habitual high intensity resistance exercise increases arterial stiffness. Our previous study demonstrated that active physical activity (>about 200kcal/day) leads to be lower risk of arterial stiffness in middle-aged and older adults. Moreover, stretching exercise and high-intensity interval exercise reduce arterial stiffness. Thus, the effects of exercise training on arterial stiffness are differed with different exercise mode. However, a molecular mechanism of effects of different exercise mode on arterial stiffness remains unclear. Nitric oxide (NO), which is produced from L-arginine by endothelial NO synthase (eNOS) in endothelial cells leads to vasodilation. Our recent studies showed that aortic NO-derived vasodilation in response to aerobic exercise training participates in the mechanism underlying the reduction in arterial stiffness in middle-aged and older adults. Furthermore, we examined the effect of different mode of exercise training on eNOS signaling pathway. In this symposium, my presentation would like to focus on the effect of different mode of exercise training on arterial stiffness and endothelium-derived relaxing factor. COI:No

3S-09PM-5
Exercise-induced oxidative stress is associated with impaired dynamic cerebral blood regulation.

Ogoh Shigehiko

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Many previous clinical studies suggest that cardiovascular disease increases a risk of onset of dementia via an impairment in cerebral blood flow regulation. Thus, it is reasonable expectation that there is a strongly relationship between cerebral blood flow regulation and cognitive function. In other words, an adequate cerebral blood regulation should be necessary to maintain normal brain function. However, our previous studies demonstrated that onset of heavy exercise improved cognitive function despite an attenuated cerebral blood flow regulation. This paradox between these findings remains unknown. On the other hand, free radicals may be involved in the metabolic regulation of dynamic cerebral blood flow regulation. As exercise is an established stimulus for oxidative nitrosative stress, especially during the transition from moderate to severe intensity, it is reasonable to suggest that the impaired dynamic cerebral autoregulation may prove the consequence of increased free radical formation. Our previous study examined whether dynamic cerebral autoregulation and blood brain barrier function would become compromised as a result of exercise-induced oxidative nitrosative stress. In this symposium, my presentation would like to focus on the effect of exercise-induced free radical on cerebral blood flow regulation. COI:No

Award Presentations

AP1－AP2	19th Promotion Award of the Physiological Society of Japan for Young Scientists
AP3－AP5	8th Hiroshi and Aya Irisawa Memorial Promotion Award for Young Physiologists
AP6	8th Aya Irisawa Memorial Promotion Award for Excellence by Women Physiologists
AP7	8th Hiroshi and Aya Irisawa Memorial Award for Excellent Papers in The Journal of Physiological Sciences
AP8	8th Hiroshi and Aya Irisawa Memorial Award for Excellent Papers on Research in Circulation in The Journal of Physiological Sciences

AP1 (2P-017)

Measuring dynamics of releasable synaptic vesicles and their plastic changes at hippocampal mossy fiber boutons.

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Neurons use chemical synaptic transmission to communicate with each other. In response to the arrival of an action potential at the presynaptic active zone, synaptic vesicles undergo exocytosis and discharge their cargo contents, neurotransmitters. Extensive electrophysiological studies have revealed detailed kinetics of exocytosis itself, but little is known about the kinetics of the preceding steps, such as tethering, docking and priming at mammalian CNS synapses. We managed to visualize single synaptic vesicles near the plasma membrane, and examined their exocytosis and the kinetics upstream at the hippocampal mossy fiber boutons (hMFBs). We employed total internal reflection fluorescence (TIRF) microscopy to directly visualize dynamics of single synaptic vesicles adjacent to the plasma membrane at high spatial resolution. In addition, we have combined high temporal resolution measurements of presynaptic capacitance and EPSCs to measure the kinetics of exocytosis. Readily releasable vesicles mostly consisted of already-tethered vesicles in the TIRF field. Vesicle replenishment had fast and slow phases, and TIRF imaging suggests that the fast phase depends on vesicle priming from already-tethered vesicles. Application of cAMP, a molecule crucial for long-term potentiation (LTP), mainly increases the vesicular release probability rather than the number of readily-releasable vesicles or their replenishment rate, likely by changing the coupling between Ca^{2+} channels and synaptic vesicles. COI:No

AP2 (2PS-06PM-2)

A CALHM1/CALHM3 heteromeric channel mediates purinergic neurotransmission of sweet, bitter, and umami tastes

Taruno Akiyuki[1], Ma Zhongming[2], Ohmoto Makoto[3], Matsumoto Ichiro[3], Tordoff Michael[3], Foskett J[2], Marunaka Yoshinori[1]

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Sweet, bitter, and umami taste compounds stimulate taste receptors in apical membranes of type II taste cells, leading to action potential-dependent, non-exocytotic release of adenosine triphosphate (ATP), which serves as a neurotransmitter conveying taste information to afferent gustatory nerves. Although we identified calcium homeostasis modulator 1 (CALHM1) as an essential component in the voltage-gated ATP release channel required for purinergic neurotransmission in type II taste cells, the molecular identity of the ATP release channel is not yet fully understood as suggested by the biophysical and pharmacological disparities between CALHM1 gating *in vivo* and *in vitro*. CALHM3 interacts with CALHM1, and the resulting CALHM1/CALHM3 channel exhibits biophysical and pharmacological properties that are identical to those of the ATP release channel in type II taste cells. Both CALHM1 and CALHM3 are expressed exclusively in type II taste cells within taste buds. Knockout of either *Calhm1* or *Calhm3* impairs perception of sweet, bitter, and umami tastes and abolishes ATP release channel current and taste-evoked ATP release in type II taste cells. Thus, the CALHM1/CALHM3 heteroligomer is the voltage-gated ATP release channel required for neurotransmission in type II taste cells. COI:No

AP3 (2P-058)

Novel regulation mechanisms of the GIRK channel activity by small molecules

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G-protein-gated inwardly rectifying K^+ (GIRK) channels control various physiological functions. For example, GIRK1/2 heterotetramers in the brain regulate neuronal excitability; GIRK1/4 heterotetramers in the heart regulate heart rate. We previously identified a novel GIRK activator, ivermectin (IVM), and more recently two novel GIRK inhibitors, astemizole (AST) and terfenadine (TER). We reported that IVM directly activates GIRK current in a PIP_2-dependent, $G_{\beta\gamma}$-independent manner. Here we present novel findings of the regulation mechanisms of GIRK channels by these small molecules. (1) By using *Xenopus* oocytes expressing M2 muscarinic receptors with GIRK channels, we observed that IVM potentiates the ACh-induced current, suggesting that IVM not only acts as an activator but also an allosteric modulator of GIRK channels. (2) By examining the effects of AST and TER in the oocytes expressing different GIRK subunits, we observed that AST- and TER-mediated inhibitions are GIRK1 dependent. (3) Mutation of a GIRK1-specific amino acid residue located in the pore helix close to the selective filter, Phe137, to Ser abolished the inhibition of GIRK current by AST and TER, suggesting that the Phe137 in GIRK1 may play important roles in the channel gating. Taken together, the present data shows the effects of the novel activator and inhibitors on GIRK channels and the structural determinants for the regulations. The results provided us with a clue toward the identification of the novel gating mechanisms of GIRK channels by small molecules. COI:No

AP4 (1O-05AM-1)

An alternative permeation mechanism through the K^+ channel

Sumikama Takashi, Oiki Shigetoshi

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For the mechanism of ion permeation, there is a "dogma", so-called "knock-on" mechanism, in which the ions in the selectivity filter (SF) of the channel are electrically repelled out by an incoming ion. The idea behind the knock-on mechanism is the implicit assumption that the selectively permeable K^+ should have high affinity to the SF over impermeable Na^+. Consequently, the tightly bound K^+ ions need to overcome the energy barrier for the permeation, and an incoming ion has been attributed to be a source of external force to expel the bound ions, such as through collisions. However, the knock-on mechanism cannot account for the experimental data of the water-ion flux coupling ratio ($CR_{w\text{-}i}$). At high K^+, the $CR_{w\text{-}i}$ value was one, indicating up to three K^+ ions occupy the SF transiently, by which the downstream K^+ was repelled. On the other hand, at lower K^+, the $CR_{w\text{-}i}$ was more than one, and the SF contains only one K^+ that cannot be repelled in the absence of coming ion. Here, we investigated the permeation mechanism through the KcsA channel at the atomic level using the molecular dynamics simulation. In our simulation, the concentration-dependent values of $CR_{w\text{-}i}$ were reproduced. The ion and water trajectories revealed that K^+ ions in the SF flowed out towards the extracellular space well before a coming ion has entered the SF. The result indicates that the implicit assumption that K^+ should be tightly bound to SF is not valid, rather, the loose binding of K^+ in the SF allows rapid permeation. COI:No

AP5 (3O-07AM-2)

Angiotensin II increases Ca^{2+} transients by activating $Ca_V1.2$ Ca^{2+} channels through casein kinase 2 in immature cardiomyocytes

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Angiotensin II (AngII) plays important roles in cardiovascular regulation in perinatal mammalians. Here we found that AngII significantly increased twitch Ca^{2+} transients by robustly activating L-type $Ca_V1.2$ Ca^{2+} channels ($Ca_V1.2$) in mouse neonatal ventricular cardiomyocytes (NVCM) but not adult ventricular cardiomyocytes. This response to AngII was mediated by AT_1 receptors and β-arrestin2. To elucidate possible involvement of protein kinases in this system, we examined the effects of array of kinase inhibitors in NVCM, revealing that AngII activated $Ca_V1.2$ channels through Src-family tyrosine kinases (SFK) and casein kinase 2 (CK2). Overexpression of CK2α' β but not c-Src directly activated recombinant $Ca_V1.2$ channels composed of C-terminally truncated α_{1c}, the distal C-terminus of α_{1c}, β_{2c}, and $\alpha_2\delta_1$ subunits, by phosphorylating threonine 1704 located at the interface between the proximal and the distal C-termini of $Ca_V1.2$ α_{1c} subunits. A cyclin-dependent kinase inhibitor, $p27^{Kip1}$ (p27), inhibited CK2α' β, and AngII removed this inhibitory effect through phosphorylating tyrosine 88 of p27 via SFK in cardiomyocytes. Coimmunoprecipitation revealed that $Ca_V1.2$ channels, CK2α' β, and p27 formed a macromolecular complex. These results indicate that stimulation of AT_1 receptors by AngII activates $Ca_V1.2$ channels sequentially through β-arrestin2, SFK, p27, and CK2α' β in immature but not adult cardiomyocytes, thereby likely exerting a positive inotropic effect in the immature heart. COI:No

AP-6

Neurotransmitter release efficacy controlled by neuronal activity-dependent millisecond Ca^{2+} dynamics

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For reliable transmission at chemical synapses, neurotransmitters must be released dynamically in response to neuronal activity in the form of action potentials. Stable synaptic transmission is dependent on the efficacy of transmitter release and the rate of resupplying synaptic vesicles to their release sites. Accurate regulation is conferred by proteins sensing Ca^{2+} entering through voltage-gated Ca^{2+} channels opened by an action potential. Presynaptic Ca^{2+} concentration changes are dynamic functions in space and time, with wide fluctuations associated with different rates of neuronal activity. Thus, regulation of transmitter release includes reactions involving multiple Ca^{2+}-dependent proteins, each operating over a specific time window. Classically, studies of presynaptic proteins function favored large invertebrate presynaptic terminals. I have established a useful mammalian synapse model based on sympathetic neurons in culture. In this symposium I summarize the use of this model synapse to study the roles of presynaptic proteins in neuronal activity for the control of transmitter release efficacy and synaptic vesicle recycling. COI:No

AP-7

Nicotine inhibits activation of microglial proton currents via interactions with α7 acetylcholine receptors

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Alpha 7 subunits of nicotinic acetylcholine receptors (nAChRs) are expressed in microglia and are involved in the suppression of neuroinflammation. Over the past decade, many reports show beneficial effects of nicotine, though little is known about the mechanism. Here we show that nicotine inhibits lipopolysaccharide (LPS)-induced proton (H^+) currents and morphological change by using primary cultured microglia. The H^+ channel currents were measured by whole-cell patch clamp method under voltage-clamp condition. Increased H^+ current in activated microglia was attenuated by blocking NADPH oxidase. The inhibitory effect of nicotine was due to the activation of $\alpha 7$ nAChR, not a direct action on the H^+ channels, because the effects of nicotine was cancelled by $\alpha 7$ nAChR antagonists. Neurotoxic effect of LPS-activated microglia due to infammatory cytokines was also attenuated by pre-treatment of microglia with nicotine. These results suggest that $\alpha 7$ nAChRs in microglia may be a therapeutic target in neuroinflammatory diseases. COI:No

AP-8

Interactions between β-adrenergic vasodilation and cervical sympathetic nerves are mediated by α_2-adrenoceptors in the rat masseter muscle

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Sympathetic activity is important for the regulation of the hemodynamics of jaw muscles. Disturbances in intramuscular blood flow evoked by the modulation of the sympathetic vasomotor response may be related to muscle dysfunctions. Sympathoexcitation can increase or decrease blood flow in the masseter muscle (MBF). These conflicting effects may be attributed to interactions between the neural and humoral mechanisms of MBF regulation, because sympathoexcitation induces the activation of both the sympathoadrenal system and the cervical sympathetic nerves. In this study, we examined the interactions between the neural and humoral mechanisms of MBF regulation and investigated the factors mediating these interactions in urethane-anesthetized rats. Stimulation of the adrenal nerve (AN) projecting into the adrenal medulla increased MBF, and this increase was mediated by β-adrenoceptors. Sectioning of the superior cervical sympathetic trunk (CST) significantly inhibited MBF increase induced by AN stimulation during high activity in the CST, but not during low activity. Furthermore, AN stimulation with clonidine after CST sectioning induced a significant increase in MBF; however, phenylephrine had no observable effects. Pretreatment with yohimbine or propranolol significantly inhibited the increase in the MBF. These findings suggest an interaction between β-adrenergic vasodilation evoked by circulating adrenaline and the cervical sympathetic nerves, which is mediated by the α_2-adrenoceptors in the masseter muscle. COI:No

Oral Presentations
Day 1

March 28 (Wed), 11:00 – 12:00

1O-03AM-1－1O-03AM-5	Skeletal muscle and bone
1O-04AM-1－1O-04AM-5	Circulatory physiology 1
1O-05AM-1－1O-05AM-5	Channel・Transporter 1
1O-06AM-1－1O-06AM-5	Cell physiology 1
1O-07AM-1－1O-07AM-5	Cell physiology 2
1O-10AM-1－1O-10AM-5	Neuroscience1

Oral Session 1

Skeletal muscle and bone

March 28 (Wed) 11:00～12:00 Hall 3

1O-03AM-1

Roles of irisin in interactions between muscle and bone during mechanical unloading in mice.

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Mechanical unloading, such as long term bed rest and space flight, induces disuse muscle atrophy and osteoporosis. However, its precise mechanisms are not fully understood. The interactions between muscle and bone have been noted. We therefore examined roles of the humoral factors linking muscle to bone during mechanical unloading in male C57BL6/J mice with hindlimb unloading (HU) and sciatic neurectomy (SNX). HU was achieved by tail suspension. Sciatic nerve was bilaterally excised. The mice were subjected to analysis of muscle and bone mass using quantitative computed tomography and the mRNA levels in muscle and bone 3 and 4 weeks after HU and SNX surgery, respectively. HU and SNX decreased muscle mass surrounding the tibia and trabecular bone mineral density (BMD) in the tibia. As for humoral factors linking muscle to bone, HU and SNX declined fibronectin type III domain-containing 5 (FNDC5), precursor of irisin, mRNA levels in the soleus and gastrocnemius muscle. FNDC5 mRNA levels in the soleus muscle were positively correlated with trabecular BMD in the tibia of control and HU mice. Irisin blunted osteoclast formation from mouse bone marrow cells. Bone morphogenetic protein (BMP) and phosphatidylinositol 3-kinase (PI3K) signaling inhibitors blunted FNDC mRNA levels enhanced by fluid shear stress in mouse myoblastic C2C12 cells. In conclusion, the present study first showed that mechanical unloading reduces irisin expression in the skeletal muscle of mice presumably through BMP and PI3K pathways. COI:No

1O-03AM-2

Transmembrane domain-deletion mutant of junctophilin 1 reduced contractile force of mouse skeletal muscle through inhibiting junctional membrane-targeting of L-type calcium channels

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In skeletal myocytes, $Ca_V1.1$ L-type calcium channels (LTCC) form a complex with ryanodine receptors at junctional membrane (JM) where the sarcolemma is closely apposed to sarcoplasmic reticulum (SR) membranes. Junctophilins (JP) are known to stabilize the JM complex by bridging the sarcolemma and SR. We previously showed that the physical interaction of $Ca_V1.1$ with JP is important for the proper localization and function of LTCC in cultured myotubes. In this study, we investigated the effect of overexpression of JP1 mutant lacking a transmembrane domain (JP1ΔTM) on excitation-contraction coupling (ECC). JP1ΔTM was not specifically clustered to JM but diffusely localized over the entire plasma membrane, and inhibited proper JM-targeting of $Ca_V1.1$ in cultured myotubes. This result indicated that expression of the mutant exerted a dominant-negative effect on endogenous JP1. To examine the in vivo effect of JP1ΔTM expression, we applied an adeno-associated virus mediated gene delivery system. In adult mouse muscles, JP1ΔTM expression impaired JM-targeting of LTCC without disrupting JM morphology. Action potential elicited Ca^{2+} transient was substantially reduced by JP1ΔTM expression without affecting SR Ca^{2+} content. Moreover, contractile force of the JP1ΔTM-expressing muscle was dramatically reduced compared to the control. Taken together, JPs recruit LTCCs to JM through physical interaction and ensure robust ECC in skeletal muscle. COI:No COI:No

1O-03AM-3

Antinociceptive effect of hyaluronic acid sodium on ankle osteoarthritis model

Jimbo Syunsuke[1,2], Terashima Yoshinori[1,2], Takebayashi Tsuneo[3], Ichise Nobutoshi[2], Sato Tatsuya[2], Teramoto Atsushi[1], Yamashita Toshihiko[1], Tohse Noritsugu[2]

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We developed a new animal model of ankle osteoarthritis (OA) to research the mechanisms of pain in OA, and examined the effect of hyaluronic acid (HA) on pain in ankle OA. We used 45 rats. These rats were divided into three groups (OA rats, HA rats, Sham rats).OA rats were induced by injecting monoiodoacetate (MIA) into the tibiotarsal joint of the right hind paw on two consecutive days. Sham rats were induced by saline on two consecutive days. OA and Sham rats were injected saline on 7, 14, 21 days after injection of MIA/Saline. HA rats were injected HA on 7, 14, 21 days after injection of MIA. The pain related behavior was assessed by mechanical and thermal withdrawal responses on the day before MIA/saline injection, and 7, 14, 21, 28 days after injection. We also measured the walking stride length. We observed histopathological changes of each ankle joint using Osteoarthritis Research Society International score on 28 days. In behavior evaluation, suppression of allodynia and decrease in stride were seen after injection of HA. In histological evaluation, cartilage degeneration was suppressed by injection of HA. HA may suppress the excitability of intraarticular pain receptors, then peripheral sensitization may be suppressed, and central sensitization does not occur. HA improved pain behavior and stride length of ankle OA. HA also suppressed the MIA induced cartilage degeneration histologically. COI:No

1O-03AM-4

Skeletal muscle fiber type shifting for hibernation in a mammalian hibernator, Syrian hamster

Yamaguchi Yoshifumi[1,2], Fujimoto Takayuki[1], Sato Yuya[1], Ando Risa[1], Chayama Yuichi[1], Anegawa Daisuke[1], Taii Hiroki[1], Shigenobu Shuji[3], Miura Masayuki[1]

1:Dept Genet, Grad Sch Pharma Sci, Univ Tokyo, Tokyo, Japan, 2:HIB, ILTS, Hokkaido Univ, Sapporo, Japan, 3:NIBB, Okazaki, Japan

Hibernation is an adaptive strategy to survive during cold period with little or no food by dramatic suppression of metabolisms and extensive utilization of stored fat as a fuel. During hibernation, animals exhibit low body temperature and go into immobility. Such immobility induces severe muscle disuse atrophy (MDA) in non-hibernators including mice, rat, and human, whereas mammalian hibernators have been reported to be less sensitive to MDA during hibernation. A mammalian hibernator, Syrian hamster (Mesocricetus auratus), can be induced to hibernate in a laboratory condition and thus an ideal model animal to study hibernation. Using RNA-seq analysis and immunohistochemistry we found that Syrian hamsters reduced fast-twitch muscle fibers in back muscle and leg muscles under several months of cold exposure, the pre-hibernation period. This fiber type shifting was accompanied with elevated expression of genes involved in muscle atrophy in hibernation period compared to non-hibernation period, suggesting that MDA occurred predominantly in fast-twitch muscle fibers. Interestingly, expression of myostatin, a myokine to inhibit myogenesis and muscle hypertrophy, also decreased in most skeletal muscles during hibernation period. Thus, Syrian hamsters undergo dynamic remodeling of skeletal muscles from fast-type to slow-type fibers as an adaptation to fat dominant nutrient state and immobility. COI:No

1O-03AM-5

Effect of l-caldesmon on osteoclastogenesis in RANKL-induced RAW264.7 cells

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This study intended to investigate the function of non-muscle caldesmon (l-CaD) in osteoclastogenesis by using a cell model of RANKL-induced differentiation of RAW264.7 murine macrophages to osteoclasts (OCs). Upon RANKL treatment, RAW264.7 cells undergo cell-cell fusion into multinucleate and TRAP-positive large OCs with a concomitant increase of l-CaD expression. Using gain- and loss-of-function in OC precursor cells followed by RANKL induction, we showed that the expression of l-CaD in response to RANKL activation is an important event for osteoclastogenesis. To determine the effect of l-CaD phosphorylation in osteoclastogenesis, three decoy peptides of l-CaD were used with, respectively, Ser-to-Ala mutations at the Erk- and Pak1-mediated phosphorylation sites, and Ser-to-Asp mutation at the Erk-mediated phosphorylation sites. Both the former two peptides competed with the C-terminal segment of l-CaD for F-actin binding and accelerated formation of podosome in RANKL-induced OCs, while the third peptide did not significantly affect the F-actin binding of l-CaD and decreased the podosome formation in OCs. With the experiments using dephosphorylated and phosphorylated l-CaD mutants, we further showed that dephosphorylated l-CaD mutant facilitated RANKL-induced TRAP activity with an increased cell fusion index, whereas phosphorylated l-CaD decreased the TRAP activity and cell fusion.Clearly, both the level of l-CaD expression and the extent of l-CaD phosphorylation play an important role in RANKL-induced osteoclast differentiation. COI:No

Oral Session 2

Circulatory physiology 1

March 28 (Wed) 11:00～12:00 Hall 4

1O-04AM-1

Transcripts associated with glycolysis are increased in rat embryonic heart just after the initiation of the heartbeat.

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Backgrounds: We previously reported that the heartbeat in the rat embryo begins at embryonic day 10.0 (E10.0). Although glycolysis plays a central role in ATP production in fetal heart, it remains unclear how the heart responds to increased ATP demand just after the initiation of the heartbeat. **Methods and Results:** The embryo from a pregnant Wister rat at E10.0 was removed and divided into two groups by the heart without (pre-) or with (post-) heartbeat. Total RNA was extracted from the heart and microarray analysis was conducted. A functional group of transcripts showing a significant increase in post-heartbeat group compared with pre-heartbeat group was searched by pathway analysis. The pathway of glycolysis ranked 2nd (23.8% of matched entities), next to the best matched pathway of striated muscle contraction. Gene expression of Hk1, Pfkp, Pklr, and Pkm, which are rate-limiting enzymes in glycolysis, were higher in post-heartbeat group by 1.57, 1.55, 1.43, and 1.24 folds than those in pre-heartbeat group, respectively. Although there were no changes in glucose transporters between two groups, gene expression levels of Gygm and Pgm1, which are associated with glycogenolysis, were significantly increased in post-heartbeat group. **Conclusions:** The findings suggest that glycolytic flux is increased just after the initiation of the heartbeat in rat embryonic heart. Enhanced glycogenolysis may contribute to increased substrate supply. COI:No

1O-04AM-2

Electrophysiological characterization of the field potential duration in human induced pluripotent stem cell-derived cardiomyocytes sheets

Izumi-Nakaseko Hiroko[1], Kanda Yasunari[2], Nakamura Yuji[1], Hagiwara-Nagasawa Mihoko[1], Wada Takeshi[1], Ando Kentaro[1], Naito T Atsuhiko[1], Sekino Yuko[1,3], Sugiyama Atsushi[1]

1:Dept Pharmacol, Fclt Med, Toho Univ, Tokyo, Japan, 2:Div Pharmacol, NIHS, Kanagawa, Japan, 3:Lab Chem Pharmacol, Grad Sch Pharmaceut Sci, Univ Tokyo, Tokyo, Japan

Although the field potential duration (FPD) in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) recorded with microelectrode array systems has been corrected by the clinically used formulae of Fridericia and/or Bazett in many studies, electrophysiological rationale for the use of these formulae has not been established. First, we analyzed the effect of beating rate on FPD in the hiPSC-CMs sheets with electrical stimuli and a HCN channel inhibitor zatebradine. Next, we examined the relationship between the electrophysiological properties and the expression levels of ion channel genes in the cell sheets. Zatebradine slowed the spontaneous beating rate which allowed to analyze FPD changes at various pacing cycle lengths. Non-linear equation for correcting FPD in the cell sheet; $FPDc = FPD/RR^{0.22}$ with RR given in second was obtained, which may make it feasible to assess net repolarization delay by various chemical compounds with a chronotropic action. Rate-dependent change in the repolarization period was smaller in the cell sheets than that reported on the human hearts, which can be partly explained by lower gene expression level of hKCNJ2 and hKCNE1. COI:No

1O-04AM-3

Biological Pacemaker Created by HCN4-overexpressing Human Induced Pluripotent Stem Cell-derived Cardiomyocytes

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1:DeptCardiovascular Med, Okayama Univ Grad Sch, 2:Dept Chromic Kidney Dis Cardiovasc Dis, Okayama Univ Grad Sch, Okayama, Japan

Background: Biological pacemaker is expected to solve the persisting problems of a mechanical pacemaker. Enhancement of the funny current (I_f) flowing through hyperpolarization-activated cyclic nucleotide-gated (HCN) channels and attenuation of the inward rectifier K^+ current (I_{K1}) flowing through inward rectifier potassium (Kir) channels are essential for generation of a biological pacemaker. Recently, we reported a biological pacemaker created by HCN4-overexpressing mouse embryonic stem cell-derived cardiomyocytes (mESC-CMs) that originally show poor I_{K1} currents. Next, we generated HCN4-overexpressing human induced pluripotent stem cells (hiPSC), and we induced cardiomyocytes that originally show poor I_{K1} currents.
Methods and Results: We generated HCN4-overexpressing hiPSC-derived cardiomyocytes (hiPSC-CMs). Human *Hcn4* gene was inserted into the AASV1 locus, a safe harbor locus for inserting transgenes, by TALENs. HCN4-overexpressing hiPSC-CMs expressed an approximately 11-times higher level of the *Hcn4* gene than did non-overexpressing hiPSC-CMs. HCN4-overexpressing hiPSC-CMs showed significantly larger I_f and more rapid beating than did non-overexpressing hiPSC-CMs. The beating rate of HCN4-overexpressing hiPSC-CMs responded to ivabradine, an I_f inhibitor, and to isoproterenol, a beta-adrenergic receptor agonist.
Conclusions: We established HCN4-overexpresssing hiPSC-CMs that show rapid spontaneous beating and responses to ivabradine and isoproterenol. The results indicated that these cells could be applied to a biological pacemaker. COI:No

1O-04AM-4

Effect of dimensional factor of cell culture on cardiac differentiation of human induced pluripotent stem cells

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Cardiac regenerative medicine using induced pluripotent stem cells (iPS) with various culture methods is studied and developed for practical realization. We focused on the fact that the difference in dimensionality of cell culture influences the morphology of cells and hypothesized the dimensional factor influences the myocardial differentiation induction efficiency of iPS cells. Here, we co-cultured human umbilical vein endothelial cells (HUVEC), human gingival fibroblast cells (HGF), and iPS cells three dimensionally (3D) and two dimensionally (2D) using collagen and Matrigel. Then we measured cell activity by MTT assay. In addition, we performed motion analysis of beating cells to assess successful cardiac differentiation by phase-contrast microscopy. Cellular viabilities, derived from absorbance measured by MTT assay, were 0.258 ± 0.041, 0.130 ± 0.015, 0.462 ± 0.024, and 0.488 ± 0.103 for 3D-Matrigel, 3D-collagen, 2D Matrigel, and 2D collagen, respectively. Dimensional factor significantly affected the cellular viability ($P<0.0001$, two-way analysis of variance). In addition, cells in 2D culture showed contraction while those in 3D culture did not. From these results, it was suggested that dimensionality of cell culture affects cardiac differentiation of iPS cells. COI:No

1O-04AM-5

Downregulation of mitochondrial PDP1 is required for the early stage differentiation of embryonic stem cell to cardiac myocytes

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Mitochondria are crucial for maintaining the properties of embryonic stem cells (ESCs) and for regulating their subsequent differentiation into diverse cell lineages, including cardiomyocytes. However, mitochondrial regulators that manage the rate of differentiation or cell fate have been rarely identified. This study aimed to determine the potential mitochondrial factor that controls the differentiation of ESCs into cardiac myocytes. We induced cardiomyocyte differentiation from mouse ESCs (mESCs) and performed microarray assays to assess mRNA expression changes at differentiation day 8 (D8) compared with undifferentiated mESCs (D0). Among the differentially expressed genes, Pdp1 expression was significantly decreased (27-fold) on D8 compared to D0, which was accompanied by suppressed mitochondrial indices, including adenosine triphosphate (ATP) levels, membrane potential, ROS, and mitochondrial Ca2+. Notably, Pdp1 overexpression significantly enhanced the mitochondrial indices and pyruvate dehydrogenase activity and reduced the expression of cardiac differentiation marker mRNA and the cardiac differentiation rate compared to a mock control. In confirmation of this, a knockdown of the Pdp1 gene promoted the expression of cardiac differentiation marker mRNA and the cardiac differentiation rate. In conclusion, our results suggest that mitochondrial PDP1 is a potential regulator that controls cardiac differentiation at an early differentiation stage in ESCs. COI:No

Oral Session 3

Channel・Transporter 1

March 28 (Wed) 11:00〜12:00 Hall 5

1O-05AM-1 (AP4)

An alternative permeation mechanism through the K^+ channel

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For the mechanism of ion permeation, there is a "dogma", so-called "knock-on" mechanism, in which the ions in the selectivity filter (SF) of the channel are electrically repelled out by an incoming ion. The idea behind the knock-on mechanism is the implicit assumption that the selectively permeable K^+ should have high affinity to the SF over impermeable Na^+. Consequently, the tightly bound K^+ ions need to overcome the energy barrier for the permeation, and an incoming ion has been attributed to be a source of external force to expel the bound ions, such as through collisions. However, the knock-on mechanism cannot account for the experimental data of the water-ion flux coupling ratio ($CR_{w\text{-}i}$). At high K^+, the $CR_{w\text{-}i}$ value was one, indicating up to three K^+ ions occupy the SF transiently, by which the downstream K^+ was repelled. On the other hand, at lower K^+, the $CR_{w\text{-}i}$ was more than one, and the SF contains only one K^+ that cannot be repelled in the absence of coming ion. Here, we investigated the permeation mechanism through the KcsA channel at the atomic level using the molecular dynamics simulation. In our simulation, the concentration-dependent values of $CR_{w\text{-}i}$ were reproduced. The ion and water trajectories revealed that K^+ ions in the SF flowed out towards the extracellular space well before a coming ion has entered the SF. The result indicates that the implicit assumption that K^+ should be tightly bound to SF is not valid, rather, the loose binding of K^+ in the SF allows rapid permeation. COI:No

1O-05AM-2

Contraction rhythm homeostasis on warmed cardiomyocytes

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Myocardium is contracted according to calcium concentration. On the other hand, myocardium is also under the control of contraction independent of calcium concentration. For example, there is a contractile force change dependent on sarcomere length called the length-tension relationship, and a contraction rhythm under constant calcium concentration called SPOC (spontaneous oscillatory contraction). That is, myocardium is controlled both calcium concentration-dependent and independent. How does the myocardium compatibilize these properties? We recently discovered that using immature cardiomyocytes from neonatal rats, myocardial cells cause contractile oscillation at temperatures of 38 to 42 ° C. We named this contractile oscillation as HSOs (Hyperthermal Sarcomeric Oscillations). Interestingly, spontaneous beats and HSOs occur simultaneously when warming cardiomyocytes playing spontaneous beats (with changes in calcium concentration). HSOs occur even when the calcium concentration is constant. Therefore, this phenomenon is a very valuable observation object to investigate how myocardium is controlled not only depending on calcium concentration but also independently. As a result of detailed examination, HSOs showed that the oscillation amplitude was changed but the oscillation cycle was kept constant, while sarcomere, which also performed HSOs, changed the average length according to calcium concentration. The compatibility of maintaining this state and responsiveness is considered to be a type of homeostasis. COI:No

1O-05AM-3

Measurement of membrane potential change in phagosome of phagocytes

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1:Integrative Physiol, Grad Sch Med, Osaka Univ, Suita, Japan, 2:Dept Material Sci, JAIST, Nomi, Japan

Phagocytes are immune cells that eliminate pathogens and apoptotic cells by engulfing them directly. After engulfing pathogens, phagocytes form phagosome, a specific compartment separated from plasma membrane, and digest them in it. We have shown that voltage-gated proton channel Hv1/VSOP is important for the regulation of membrane potential in the course of ROS production and degranulation, both of which are essential for pathogen killing. This channel is localized not only on plasma membrane, but also on phagosome and intracellular vesicles, but it is still unclear when and to what extent proton channels are activated on phagosome and intracellular vesicles, because actual values of their membrane potential remain unclear. A technique for measuring accurately their membrane potential is not yet established. To overcome this problem, we developed the method of visualization of membrane potential inside cells. Merm2 developed in our laboratory is Fluorescence resonance energy transfer (FRET)-based protein that responds to membrane potential. We found that Merm2 was exclusively localized on plasma membrane of macrophage/monocyte cell line RAW264.7 and moved to phagosome after phagocytosis of IgG-coated beads. Thus, we could monitor the change of membrane potential in plasma membrane and phagosomes during the phagocytosis. We are now trying to determine actual value of both membrane potentials and which molecules are responsible for keeping and modulating membrane potential in phagosome of RAW cells. COI: No. COI:No

1O-05AM-4

TRPM7 FUNCTIONS AS A KINASE AND AN ION CHANNEL IN ENAMEL DEVELOPMENT

Shin Masashi[1], Ogata Kayoko[1,2], Tsumuraya Tomoyuki[3], Oka Kyoko[2], Okamoto Fujio[1], Kajiya Hiroshi[1], Katagiri Chiaki[3], Ozaki Masao[2], Matsushita Masayuki[3], Okabe Koji[1]

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Purpose: Transient receptor potential M7 (TRPM7) is a bi-functional protein containing a kinase domain and an ion channel. TRPM7 is highly expressed in ameloblasts during tooth development. We compared TRPM7 K1646R (KR, kinase dead) mice, which channel function is intact, and TRPM7 epithelium specific conditional knock out (cKO) mice to elucidate the two functions in enamel development. Materials & Methods: TRPM7 KR and cKO were characterized by patch clamp, histological analyses, micro-CT, Scanning Electron Microscope (SEM) and immunoprecipitation assay. Results: There was no difference in the amplitudes of the Mg2+-inhibited cation (MIC) current in ameloblasts from wild-type (WT) and TRPM7 KR. In contrast, the amplitudes of the MIC current in TRPM7 cKO cells was significantly smaller than that in WT. TRPM7 KR mice had minor enamel defects by micro-CT and SEM. Interestingly TRPM7 cKO mice had more severe enamel malformations than TRPM7 KR. In ameloblasts from TRPM7 KR mice, phosphorylation of intracellular molecules including Smad 1/5/9, p38 and cAMP response element binding protein (CREB) was inhibited. An immunoprecipitation assay showed that CREB bound to TRPM7. Conclusion: These findings suggest that TRPM7 kinase domain has the functions to phosphorylate CREB, Smad1/5/9 and p38 during enamel development. Additionally, TRPM7 channel function is also important for the enamel formation. COI:No

1O-05AM-5

Activation of theTRPM7 channel raises basal $[Mg^{2+}]_i$ only in the absence of extracellular Na^+

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The TRPM7 channel is permeable to various divalent cations, including Mg^{2+} and Ca^{2+}. It has been reported that naltriben, a δ opioid receptor antagonist, activates the TRPM7 channel and induces Ca^{2+} influx through the channel (Schafer et al., 2014). To examine whether TRPM7 is a physiological Mg^{2+} channel in mammalian cells, we studied the effect of naltriben on the Mg^{2+} homeostasis in rat ventricular myocytes. Cytoplasmic free Mg^{2+} concentration ($[Mg^{2+}]_i$) of acutely-isolated single cells was estimated with a fluorescent Mg^{2+} indicator mag-fura-2. After $[Mg^{2+}]_i$ was lowered by soaking the cells with a high-K^+ and Mg^{2+}-Ca^{2+}-free solution, $[Mg^{2+}]_i$ was recovered by extracellular perfusion with the Ca^{2+}-free Tyrode's solution (1 mM Mg^{2+}). The initial rate of increase in $[Mg^{2+}]_i$ was analyzed as the Mg^{2+} influx rate (0.22 ± 0.03 μM/s). The Mg^{2+} influx rate was increased by naltriben (2-50 μM) in a concentration-dependent manner with half maximal effective concentration (EC_{50}) of 24 μM and the maximum rate of 0.71 μM/s. This EC_{50} value is similar to that reported for activation of recombinant TRPM7 overexpressed in HEK293 cells. Naltriben (50 μM) caused little change in basal $[Mg^{2+}]_i$ (~1 mM) in the Ca^{2+}-free Tyrode's solution, but significantly raised $[Mg^{2+}]_i$ to 1.31 ± 0.03 mM in 90 min after removal of extracellular Na^+, which was restored to the basal level by re-addition of extracellular Na^+. The results suggest a significant contribution of the TRPM7 channel to physiological Mg^{2+} homeostasis in heart cells. COI:No

Oral Session 4

Cell physiology 1

March 28 (Wed) 11:00～12:00 Hall 6

1O-06AM-1

Astroglial glutamate dysregulation in the locus coeruleus impairs endogenous analgesia in rats with chronic pain

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Patients with chronic pain show reduced endogenous analgesia induced by a conditioned noxious stimulus. Here we tested in rats after L5-L6 spinal nerve ligation (SNL) whether chronic neuropathic pain impairs descending noradrenergic inhibition from the locus coeruleus (LC). A subdermal injection of capsaicin was used to examine noxious stimulation-induced analgesia (NSIA), evoked LC glutamate and spinal noradrenaline release, and evoked LC neuronal activity in normal and SNL rats. We also examined the role of presynaptic metabotropic glutamate receptors (mGluRs) or the astroglial glutamate transporter-1 (GLT-1). SNL increased basal extracellular glutamate concentration in the LC and basal spinal cord noradrenaline release which was associated with an increased basal LC neuronal activity and a down-regulation of GLT-1 in the LC 6 weeks after nerve injury. SNL reduced NSIA and capsaicin-evoked release of glutamate in the LC and noradrenaline in the spinal cord. Capsaicin-evoked LC neuronal activation was masked in SNL rats. In normal rats, selective knock-down of GLT-1 in the LC mimicked these effects of SNL. Removing auto-inhibition of glutamatergic terminals by mGluR blockade or increasing GLT-1 expression by histone deacetylase inhibition restored NSIA in SNL rats. These results suggest that glutamate dysregulation in the LC consequent to down-regulation of GLT-1 contributes to LC dysfunction and impaired pain-evoked endogenous analgesia in chronic pain. COI:No

1O-06AM-2

Mechanical control of oligodendrocyte morphogenesis and maturation by mechanosensors

Shimizu Takeshi[1,2,3], Osanai Yasuyuki[2,3], Tanaka F Kenji[4], Abe Manabu[5], Natsume Rie[5], Sakimura Kenji[5], Hida Hideki[1], Ikenaka Kazuhiro[2,3]

1:Grad School of Med Sci, Nagoya City Univ, Nagoya, Japan 2:Natl Inst Physiol SciNIPS, Okazaki, Japan 3:SOKENDAIThe Graduate University for Advanced Studies, Hayama, Kanagawa, Japan, 4:School of Medicine, Keio Univ, Tokyo, Japan 5:Brain Res Inst, Niigata Univ, Niigata, Japan

Oligodendrocytes (OLs) are myelinating cells in the central nervous system (CNS). Recent studies have shown that mechanical environment influences property and function of various cell types. Externally and internally applied mechanical stimulation can be transduced to intracellular biochemical signals through focal adhesion-related mechanosensors activating intracellular mechanotransducers. However, molecular mechanisms underlying mechanical control of OLs remain unknown. We found that knocking-down of mechanosensors, such as p130Cas and YAP, in OLs affected their morphology and reduced the interactions between OLs and dorsal root ganglion (DRG) neurons in a co-culture system. When OL precursor cells seeded on a silicon chamber were mechanically extended by stretch machine, or when shear stress was applied to differentiating OLs, the mechanical stress modulated OL morphology via the mechanosensor activities. Newly produced knock-in mice for the mechanosensors using the previously reported FAST system revealed that OL-specific overexpression of the mechanosensors in vivo decreased the number of mature OLs in the CNS white matter. These results suggest that the mechanosensors control OL morphogenesis and the number of mature OLs. Mechanical control of myelination is a new concept and might provide us with a novel target for the treatment of demyelinating diseases. COI:No

1O-06AM-3

Identification of a female-biased sexually dimorphic region in a male-biased sexually dimorphic nucleus of mice and roles of neonatal testicular testosterone in sexually dimorphic formation

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The principal nucleus of the bed nucleus of the stria terminalis (BNSTp) is a male-biased sexually dimorphic nucleus. Calbindin is abundantly expressed in the BNSTp and therefore used as a marker for identification of the BNSTp. However, the BNSTp identified by Nissl staining is different from the BNSTp detected with calbindin (thereafter CALB-BNSTp). To identify the subregion does not express calbindin in the BNSTp, we performed *in situ* hybridization for p21 protein (Cdc42/Rac)-activated kinase 3 (Pak3), another marker for the BNSTp, and immunostaining for calbindin. We found that the ventral part of the BNSTp (thereafter BNSTpv) expressed Pak3 but not calbindin. The BNSTpv showed a female-biased sex difference in volume and neuron number. Next, we examined the effects of neonatal testicular testosterone on the sexual differentiation of the BNSTpv. The volume and neuron number of the BNSTpv were significantly increased in males by orchiectomy at postnatal day (PD) 1 and significantly decreased in females by treatment with testosterone or dihydrotestosterone at PD1 or estradiol at PD1-5. These findings suggest that the BNSTpv showing female-biased sex differences is a part of the BNSTp showing male-biased sex differences. Neonatal testicular testosterone may act to decrease the volume and neuron number of the BNSTpv via binding directly to the androgen receptor and indirectly to the estrogen receptor after aromatization. COI:No

1O-06AM-4

Neurotrophic effect of microglia on spinal cord neurons is enhanced by ectokinase.

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Since regeneration of injured spinal cord is hardly perturbed by the barrier such as formation of fibrotic scar tissue and decline in growth factor secretion, it is hypothesized that the elimination of these barriers could promote regeneration from chronic spinal cord injury (SCI). This hypothesis is supported by our preliminary experiments showing the decline of fibrous scar, microglial activation and functional recovery in SCI model mice after the intrathecal injection of p38 MAP kinase (p38) protein as a ectokinase. In this study, we determine whether activated microglia by p38 ectokinase could directly promote the survival of spinal cord neuron. We obtained cultured microglia and neural progenitor cells (NPC) from adult mice spinal cord, and the differentiated spinal cord neurons were induced by the incubation of NPC with low-nutrient medium. Neuronal cell death was induced by low-glucose medium, and was determined by MTT assay and FDA/PI assay. We found that microglial conditioned medium prepared protects cell death effectively. In addition, this protective activity was significantly enhanced by incubation of microglia with p38 ectokinase. These results suggest that activated microglia stimulated by p38 ectokinase could secrete the regeneration-promoting factor for spinal cord neuron. COI:No

1O-06AM-5

Electrophysiological characterization of the vasopressin neuron from mouse embryonic stem cell

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Recently, hypothalamic tissues have successfully induced from both murine and human embryonic stem cell (ESC). Variety of peptidergic neurons including vasopressin, NPY, and melanocortin and also astrocytes are found in this murine ESC-derived hypothalamic tissue culture (ES-Hypo) system. However, these neurons were identified immuno-histologically, and those functions are poorly understood. On an aim to explore the physiological nature of these peptidergic neurons from mESC, we have engineered the vasopressin::GFP cell line ($AVP^{GFP/+}$) from mESC using TALEN system. After the hypothalamic induction, $AVP^{GFP/+}$ cells can be identified by fluorescence microscope and the expression of AVP was confirmed with immunocytochemistry. Some of the $AVP^{GFP/+}$ cells showed action potentials, which are abolished by TTX. Glutamate (50 μM) depolarized membrane potential and induced action potentials. GABA (60 μM) also depolarized and induced action potentials in short duration. The provocative effect of GABA is reported in immature vasopressin neurons from neonatal murine brain. So far, these data from $AVP^{GFP/+}$ cells show no discrepancies with native vasopressin neurons. It is supposed this system would contribute to elucidate the detailed mechanism of vasopressin release. (COI:No) COI:No

Oral Session 5

Cell physiology 2

March 28 (Wed) 11:00～12:00 Hall 7

1O-07AM-1

Mechanical stress modulates the homeostasis of periodontal ligament

Fujita Ayano[1], Morimatsu Masatoshi[1], Nishiyama Masayoshi[2], Takashiba Shogo[1], Naruse Keiji[1]

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Periodontal ligament (PDL), which connects the teeth to the alveolar bone, is always exposed to mechanical stress such as occlusal force. Mechanical stress modifies periodontitis. However, it was difficult to obtain the quantitative data related to mechanical stress effect on PDL in vivo. Here we study the effects of stretch and pressure on PDL cells. First, we cultured PDL cells under uniaxial cyclic stretch for 16 hours. After 4 hours, PDL cells and actin fibers were aligned in the vertical direction to the stretch axis. These results suggested that mechanical stress regulate the orientation of PDL cells to support the teeth. Second, we used high hydrostatic pressure microscope to observe PDL cells under the high pressure conditions in real time. As a result, high hydrostatic pressure (over 20 MPa) contracted PDL cells, but did not change the actin bundle structure. The length of minor axis of pressurized cells decreased to about 60 % in few minutes. After the release of pressure, contracted cells restarted spreading on the surface. Our data suggested that excessive occlusal force induces the collapse of PDL and occlusal force at biological range modulates homeostasis of PDL. COI:No

1O-07AM-2

The involvement of membrane rafts in the signal transduction of abnormal vascular smooth muscle contraction mediated by SPC/Fyn/ROK pathway: Part II

Kishi Hiroko, Kajima Miki, Kajiya Katsuko, Zhang Ying, Morita Tomoka, Lyu Bochao, Zhang Min, Kobayashi Sei

Dept Mol Cell Physiol, Yamaguchi Univ Grad Sch Med, Ube, Japan

Rho-kinase (ROK)-mediated Ca^{2+}-sensitization of vascular smooth muscle (VSM) plays a critical role for abnormal VSM contractions such as vasospasm. Previously we identified sphingosylphosphorylcholine (SPC) and Fyn tyrosine kinase, as a novel signaling molecule to induce the ROK-mediated Ca^{2+}-sensitization of VSM contractions. We also found that SPC induced the translocation of Fyn and ROK from cytosol to plasma membrane, where they colocalized with caveolin-1, suggesting the translocation to membrane rafts, by immunofluorescent staining and confocal microscopy. However, as we reported in the previous meeting, we observed that SPC (30 μM, 5 min) induced the translocation of Fyn, but not of ROK to membrane rafts using Na_2CO_3 extraction followed by sucrose density gradient fractionation. Since SPC induced the activation of ROK markedly after 15 min, we extended the stimulation by SPC up to 15 min and analyzed the translocation of Fyn and ROK in the present study. The results showed SPC (30 μM, 15 min) increased the distribution of Fyn to low density (raft) fractions, which suggested persisting translocation of Fyn to membrane rafts. However, ROK was detected in only high density (non-raft) fractions. Surface plasmon resonance studies showed that recombinant Fyn bound to raft model membrane. Those findings suggested that SPC induced the translocation of Fyn to membrane rafts and the translocation of ROK to non-raft membranes in the proximity of membrane rafts. COI:No

1O-07AM-3

Fyn-mediated tyrosine phosphorylation of a novel signaling molecule, paxillin controls stress fiber formation and migration of cancer cells

Yokobayashi Shiori[1], Ying Zhang[1], Okamoto Takahumi[1], Morita Tomoka[1], Kishi Hiroko[1], Yamamoto Takeshi[2], Kobayashi Sei[1]

1:Dept Mol Cell Physiol, Grad Sch Med,Yamaguchi Univ, Ube, Japan, 2:Dept Clin Lab Sci, Yamaguchi Univ, Ube, Japan

Previously we identified Fyn tyrosine kinase as an upstream molecule for the Rho-kinase-mediated stress fiber formation and cell migration. However, the molecular mechanism between the two kinases has been unknown. Here, we identified paxillin (Px) as a novel downstream molecule of the activated Fyn by combined use of pulldown assay and MALDI-TOF mass spectrometry. Furthermore, we demonstrated that the constitutively active Fyn (ca-Fyn) bound directly the N-terminus of Px (Px-N) and that both shRNA-mediated knockdown of Px and overexpression of Px-N inhibited the migration and the invasion of highly metastatic breast cancer cells, suggesting that the direct binding between ca-Fyn and Px-N may play a critical role in the cancer cell migration and invasion. In addition, overexpression of ca-Fyn, but not dominant negative Fyn (dn-Fyn), in the cancer cells induced phosphorylation at several tyrosine residues in the Px-N. The point-mutation of tyrosine among these sites abolished stress fiber formation and migration of the cancer cells. Taken together, these results strongly indicate that Fyn-mediated phosphorylation of tyrosine residues at Px-N regulates stress fiber formation and migration of the breast cancer cells. COI:No

1O-07AM-4

Class II phosphoinositide 3-kinase isoforms PI3K-C2αand PI3K-C2β play essential roles in pinocytosis in vascular endothelial cells

Aung Thuzar Khin[1], Yoshioka Kazuaki[1], Aki Sho[1], Pham Quynh Hoa[1], Sarker Kabir Azadul[1], Islam Shahidul[1], Ishimaru Kazuhiro[1], Takuwa Noriko[1,2], Takuwa Yoh[1]

1:Department of Physiology, Kanazwa University School of Medicine, Ishikawa, Japan, 2:Department of Health and Medical Sciences, Ishikawa Prefectural Nursing University, Ishikawa, Japan

The class II phosphoinositide 3-kinase (PI3K) enzymes comprise three isoforms, PI3K-C2α, PI3K-C2β and PI3K-C2γ. Our previous studies showed that PI3K-C2α is involved in receptor endocytosis. However, isoform-specific functions of class II PI3Ks and detailed mechanisms of class II PI3K actions still remain to be explored. Among three class II isoforms, both PI3K-C2α and PI3K-C2β are expressed in endothelial cells (ECs). In this study, we investigated roles of PI3K-C2α and PI3K-C2β in pinocytosis by which ECs uptake large amounts of fluids and solutes to transport them between the plasma and interstitial fluid compartments. In human umbilical vein ECs (HUVECs), PI3K-C2α and PI3K-C2β were distributed in several different cell organelles including early endosomes, late endosomes, recycling endosomes and lysosomes with their partial colocalization. Pinocytosis in HUVECs, which was determined by uptake of FITC-dextran added to the culture medium with confocal microscopic observations, was a clathrin- and dynamin-dependent process. Knockdown of either PI3K-C2α or PI3K-C2β significantly reduced pinocytosis. However, double knockdown of both isoforms showed no additive inhibitory effect. These observations indicate that PI3K-C2α and PI3K-C2β play non-redundant roles in pinocytosis. COI:No

1O-07AM-5

Indispensable role of α and β isoforms of class II phosphoinositide 3-kinases (PI3K) in the uterine smooth muscle contraction during labor

Sarker Kabir Azadul[1], Sho Aki[1], Yoshioka Kazuaki[1], Kuno Koji[2], Okamoto Yasuo[1], Aung Thuzar Khin[1], Pham Quynh Hoa[1], Islam Shahidul1, Takuwa Noriko[1], Takuwa Yoh[1,2]

1:Department of Physiology, Kanazawa University School of Medicine, 2:Cancer Research Institute, Kanazawa University, Kanazawa Japan

The PI3Ks comprise three classes and regulate diverse cellular processes. We previously demonstrated that class II PI3K α isoform (PI3K-C2α) expressed in endothelial cells plays crucial roles in developmental and pathological angiogenesis. Functional role of another class II PI3K member, PI3K-C2β, is poorly understood. While PI3K-C2α KO mice show embryonic lethality, PI3K-C2β KO mice are apparently normal. In this study, we generated smooth muscle (SM)-specific double KO mice of PI3K-C2α and PI3K-C2β (SM-DKO mice) by mating the floxed (PI3K-C2$\alpha^{f/f}$ and PI3K-C2$\beta^{f/f}$) mice with SM22α-Cre mice. While we were studying the phenotypes of DKO mice, we found that pregnant SMC-DKO mothers, but not single KO mothers of either PI3K-C2α or PI3K-C2β, delivered smaller numbers of pups compared with control mice. The numbers of fetuses in the uteri at the term pregnancy did not differ between DKO and control mice. Neither the gross and microscopic structures of the uteri during non-pregnant and pregnant stages or the expression of various contractile proteins in uterine smooth muscle did not differ between DKO and control mice. Isolated uterine smooth muscle from SM-DKO mice showed reduced spontaneous and oxytocin-induced contraction compared with control mice. These results suggest that both PI3K-C2α and PI3K-C2β play important roles in the uterine smooth muscle contraction during labor. COI:No

Oral Session 6

Neuroscience 1

March 28 (Wed) 11:00〜12:00 Hall 10

1O-10AM-1

Breathing-dependent cognitive decline during memory retrieval

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Recent research suggests that cognitive performance can be altered by the inspiratory (I) activity generated from the brainstem. Previous human studies, however, yielded inconsistent results on the memory performance during the I phase. Rather, we hypothesized that cognitive performance is regulated by the timing when the retrieval process crosses a certain point in the respiratory cycle. To determine the respective roles of the respiratory phases in performance, we employed healthy subjects performing a delayed matching-to-sample task with visual information. Because this effect can be ascertained by task difficulty, we set different degrees of difficulty using Stable and Phased sessions, which have constant and variable intervals between cue exposures in the task, respectively. Here, we demonstrate a novel phenomenon, in which subjects avoided final retrieval (a motor response) of the task during the expiratory (E)-to-I phase (EI) transition, and then, once the retrieval process passed through the EI transition, the accuracy was decreased. This breathing-dependent decline was revealed with a task of higher difficulty during retrieval (Phased session). This is the first demonstration that certain breathing phases cause cognitive decline. We propose that this fluctuation is short-term freeze of cognition in the respiratory cycle. Our results suggest that cancellation of breathing-dependent cognitive decline might be crucial for the maintenance and credibility of successful performance in daily life and sports. COI:No

1O-10AM-2

Chloride ion channels play an important role in the cerebral vasomotion underlying the infra-slow oscillation of EEG.

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Dept Physiol, Tohoku Univ, School of Medicine, Sendai, Japan

Distinct rhythmic oscillations of the brain activity are recognized at various frequency ranges. Oscillations at much slower ranges (about 0.1Hz) are collectively known as infra-slow oscillation, whose origin and nature have not been well characterized. In our previous optical imaging study, we found that the reflected green light (530nm) from the exposed rat cortex was oscillating in its intensity at that infra-slow range, and the spatial pattern of the oscillated signal was propagated like a stable wave over the cortex. Here, we show that the oscillation in the optical signal reflects the vasomotion, a rhythmical oscillation of the cerebral arteries in their diameters, and a slow electrical potential change could be detected near the oscillating vessels. We also found that neural activity in a hypothalamic nuclei was synchronized with the rhythmical signal oscillation. Experimental administration of a neurotransmitter, known to be secreted from the hypothalamic nuclei, could abolish the vasomotion. Changing the chloride ion concentration around the blood vessels or administrating chloride channel blockers (niflumic acid and its derivative) directly on the vessels showed a similar inhibitory effect. These results support an idea that rhythmical release of the transmitter may lead to opening of chloride channels, which in turn, triggers the constriction/dilation cycle of the cerebral arteries underlying the vasomotion, thus, the infra-slow oscillation of the cortical electrical potential. COI:No

1O-10AM-3

Noncanonical noradrenergic neuromodulation mediated by dopamine receptor

Kobayashi Katsunori, Suzuki Hidenori

Dept Pharmacol, Nippon Med Sch

The central monoaminergic system has been implicated in the pathophysiology of psychiatric disorders. Despite decades of investigation, however, the detailed pathophysiological mechanisms still remain unknown. We have shown that dopamine D_1-like receptor signaling is greatly enhanced in the hippocampal dentate gyrus and CA3 in several lines of mouse models of psychiatric disorders. Here we show that this D_1-like receptor signaling can be activated by noradrenaline. Electrophysiological recordings were made using acute hippocampal slices prepared from adult mice. Bath-applied noradrenaline potentiated synaptic transmission at the mossy fiber-CA3 excitatory synapse. This noradrenaline-induced potentiation was largely resistant to blockers of adrenaline receptors and significantly reduced by dopamine D_1-like receptor antagonists. It has been shown that intense neuronal activation by electroconvulsive stimulation strongly augments D_1-like receptor signaling at the mossy fiber synapse. Consistently, the noradrenaline-induced synaptic potentiation was also strongly enhanced by electroconvulsive stimulation. These results indicate that noradrenaline has a noncanonical modulatory effect on the hippocampal synapse that is mediated by dopamine D_1-like receptor. Our finding raises a possibility that the essential pathophysiology of psychiatric disorders is excess crosstalk between noradrenergic and dopaminergic system via such a noncanonical pathway. COI:No

1O-10AM-4

Effect of optogenetically stimulation of dopaminergic fibers on exploration behavior and learning and memory in kindled mice

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1:Dept. Physiology, Faculty of Medicine, Tarbiat Modares Univ., Tehran, Iran, 2:Brain Research Institute, University of Zurich/ETH Zurich, Switzerland

Seizure-induced hyperexcitability can affect synaptic plasticity and thereafter the cognitive functions. Hyperexcitability can also change the modulatory effects of neuromodulators -such as dopamine- on synaptic plasticity in different brain areas including the hippocampus. In this study we tried to investigate the effect of stimulation of dopaminergic fibers of ventral tegmental area (VTA) on exploration, learning and memory in C57BL/6 mice. An optrode was implanted into the VTA to photostimulate the dopaminergic fibers. For PTZ kindling, the animals received sub-threshold dose of PTZ (35 mg/kg, i.p.) on every other day. New frontier test and displaced object-novel object tests were run. Expression of c-fos showed more activity in VTA of kindled mice compared to control. Tonic stimulation of VTA dopaminergic fibers in kindled animals reduced the seizure-induced impairment in exploration behavior and learning and memory. Field potential recording from hippocampal slices of kindled animals also showed that dopamine can return the ability of long-term potentiation to the kindled animals through D2-like dopamine receptors. It may be concluded that activation of dopamine receptors can reduce the seizure-induced impairments in cognitive functions. COI:No

1O-10AM-5

Monkey hippocampal neurons disambiguate overlapping trajectories and reward acquisitions in the virtual environment

Bretas V Rafael, Matsumoto Jumpei, Nishimaru Hiroshi, Takamura Yusaku, Hori Etsuro, Ono Taketoshi, Hisao Nishijo

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Disambiguation of overlapping information is an important element of episodic memory; during navigation, overlapping trajectories and reward events in a space might be differently represented in the hippocampal formation. However, the neural mechanisms of disambiguation is still unknown. In the present study, we recorded monkey hippocampal neurons during performance of three virtual navigation tasks in which a monkey navigated overlapping trajectories and rewarded goal areas. Of 106 hippocampal neurons recorded, 57 displayed place-related activity (place-related neurons), and 18 neurons showed route-dependent activity in the overlapping trajectories. Also 75 hippocampal neurons responded to reward delivery (reward-related neurons), and 56 of these neurons displayed route-dependent reward-related activity in the overlapping trajectories. Ensemble activity of reward-related neurons could represent reward delivery, location, and specific routes in the overlapping areas. Furthermore, neuronal activity patterns of hippocampal neurons more distinctly represented overlapping trajectories. The present results provide neurophysiological evidence of disambiguation in the monkey hippocampus, consistent with a hippocampal role in episodic memory, and suggest that overlapping items are better represented by repeated retrieval with competitive learning, consistent with recent computational studies on neural differentiation rather than those on orthogonalization. COI:No

Oral Presentations
Day 2

March 29 (Thu), 10:30 – 11:30

2O-03AM-1－2O-03AM-5	Sensory function
2O-04AM-1－2O-04AM-5	Circulatory physiology 2
2O-05AM-1－2O-05AM-5	Circulatory physiology 3
2O-06AM-1－2O-06AM-5	Channel・Transporter 2
2O-07AM-1－2O-07AM-5	Cell physiology 3
2O-10AM-1－2O-10AM-5	Neuroscience 2

Oral Session 7

Sensory function

March 29 (Thu) 10:30～11:30 Hall 3

2O-03AM-1

Characterization of nanoscale vibrations in the cochlear sensory epithelium by an advanced laser interferometer

Ota Takeru[1,2], Choi Samuel[2,3], Nin Fumiaki[1,2], Hibino Hiroshi[1,2]

1:Dept Mol Physiol, Sch Med, Niigata Univ, Niigata, Japan, 2:AMED, Niigata, Japan, 3:Faculty of Engineering, Niigata Univ, Niigata, Japan

The cochlea of the inner ear converts sound pressure waves into electrical signals. This process is triggered by nanoscale vibrations induced in the sensory epithelium inside the cochlea. In live animals, the vibration is enhanced nonlinearly as the acoustic stimuli increase; the amplitude is effectively amplified at low sound pressure levels. Although this called "nonlinear amplification" plays key roles in high sensitivity and sharp tuning of hearing, the underlying mechanisms remains uncertain. To address this issue, we developed a laser interferometry that detects the vibration amplitudes at broad frequencies. This technique, named "dual Sinusoidal Phase Modulating (SPM)" method, additionally allows us to visualize the baseline shift of the vibrating object, the parameter unmeasurable with commonly used laser-Doppler vibrometers. The performance of the dual SPM method was tested and verified with a piezo-actuator. When the guinea-pig sensory epithelium was exposed to acoustic stimuli at 21 kHz, the SPM interferometer recorded a nonlinear response of the vibration amplitude, as described elsewhere. Simultaneously, an upward baseline shift of several nanometers was clearly detected. This reaction occurred only with large acoustic stimuli, and it was negligible when the animal was dead. The baseline shift may contribute to establishment of the nonlinear amplification in auditory sensation. COI:No

2O-03AM-2

Development of an imaging system for three-dimensional detection of nanoscale vibrations in sensory epithelium of the inner ear

Nin Fumiaki[1,2], Choi Samuel[2,3], Ota Takeru[1,2], Hibino Hiroshi[1,2]

1:Dept Mol Physiol, Sch Med, Niigata Univ, Niigata, Japan, 2:AMED, Niigata, Japan, 3:Faculty of Engineering, Niigata Univ, Niigata, Japan

Human audition can distinguish frequencies that are only 0.2% apart as well as perceive trillionfold differences in sound pressure level. These properties stem from sound-induced nanoscale vibrations in sensory epithelium of the inner ear. The epithelium is composed of three layers; sensory hair-cell, supporting-cell, and basilar-membrane layers. Although spatially different distribution of the vibrations in each layer seems to be involved in the extraordinary hearing properties, this profile has not yet been precisely measured by conventional technologies. Therefore, we have developed an imaging system that can three-dimensionally detect the object's vibrations. The underlying technique is based on the optical coherence tomography, which is currently applied to medical diagnoses. First, a supercontinuum broadband light source is used to achieve high imaging performance. The spatial and depth resolutions are approximately 3.6 μm and 1.8 μm, respectively. Second, a vibrometry technique and a urtra-speed CMOS camera are incorporated into the system. This arrangement allows us to pursue nanoscale vibrations of up to 30 kHz in a wide area of a radius of 1 mm. Through an equipped microscope we scanned the traveling vibrations within the basilar-membrane layer in a live guinea pig. This system has a potential to determine physical networks across each layer of the epithelium and thereby it may contribute to finding a fundamental mechanism underlying auditory function. COI:No

2O-03AM-3

Electrical synapses of visual cortex pyramidal cells and retinal ganglion cells can enhance excitatory synaptic outputs from these neurons.

Hidaka Soh[1], Umino Osamu[2]

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Electrical synapses are present in many types of visual neurons expressing channel subunit, connexins (J. Neurosci., 2004; J. Integra Neurosci., 2008; Brain Res., 2012; WJET, 2017). Electrical current spread through gap junctions between presynaptic cells is expected to regulate chemical synaptic outputs from these neurons onto postsynaptic cells. In our recent studies, physiological properties of electrical synapses between mammalian α retinal ganglion cells have been characterized (J. Neurosci., 2004; J. Integra Neurosci., 2016). In the present study, we examined electrical synapses of retinal ganglion cells and pyramidal cells in visual cortex of developing rats and primate common marmosets. First, we investigated the localization of gap junctions between visual cells by immunocytochemical studies of connexins. Second, we analyzed functional roles of electrical synapses between the excitatory cells in vision, in relation to excitatory synaptic outputs, under dual whole-cell patch clamp recordings. Connexin-36 electrical synapses occur between visual cells. Synchronous injection of subthreshold currents in two electrically-coupled cells increased Na^+ spiking. Simulation model for coupling resistance of gap junctions between cells demonstrated synchronous generation of Na^+ spikes. These results suggest that visual cells' excitatory synapses onto postsynaptic cells appear to be enhanced through electrical synapses between gap-junctionally connected excitatory cells. COI:No

2O-03AM-4

Physiological function of basal forebrain cholinergic fibers projecting to the olfactory bulb and neocortex

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Dept Auton Neurosci, Tokyo Metropol Inst Gerontol, Tokyo, Japan

Previous study by our research group has demonstrated in rats that activation of the basal forebrain cholinergic neurons projecting to the neocortex produces vasodilative neural regulation of the neocortex by exciting the nicotinic and muscarinic ACh receptors. The olfactory bulb also receives cholinergic basal forebrain input, as does the neocortex. This study investigated the possibility that the basal forebrain cholinergic system in the olfactory bulb also has vasodilation function. We used in vivo microdialysis to measure the extracellular ACh levels in the olfactory bulb of urethane-anesthetized rats. Focal chemical stimulation by microinjection of L-glutamate into the horizontal limb of the diagonal band of Broca (HDB) in the basal forebrain which is the main source of cholinergic input to the olfactory bulb increased extracellular ACh release in the ipsilateral olfactory bulb. When the regional cerebral blood flow was measured using laser speckle contrast imaging, the focal chemical stimulation of the HDB did not significantly alter the blood flow in the olfactory bulb, while increases were observed in the neocortex. The absence of vasodilatory role in the HDB cholinergic input to the olfactory bulb may be due to differences of ACh receptor subtypes in these regions. COI:No

2O-03AM-5

Sound response properties of identified GABAergic and glutamatergic neurons of the inferior colliculus in awake mice.

Ono Munenori, Kato Nobuo

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The inferior colliculus (IC) is an obligatory auditory center that integrates all the auditory information from the brainstem and sends it to the forebrain. In the 20% of neurons are GABAergic and virtually all the IC neurons receive inhibitory inputs that strongly affect the responses to sound. In previous study, we studied the response properties of the GABAergic and glutamatergic neurons in the IC that were identified using optogenetic tool. In anesthetized animals, the GABAergic and glutamatergic neurons were similar in their threshold, spike latencies, rate-level functions and frequency tuning. Further, the responses of both cell classes were found to be affected by their location. However, the response properties of these neurons in unanesthetized state are not known.

Here we recorded the response of IC neurons from the head fixed awake mice. To distinguish GABAergic neurons from glutamatergic neurons in vivo, we used VGAT-ChR2 mice in which inhibitory neurons specifically express channelrhodopsin-2. Also we applied a glass optrode technique to deliver the light to the recorded neurons efficiently. The GABAergic and glutamatergic neurons in the awake mice had diverse response properties as they did in anesthetized mice. However, in contrast to the neurons in the anesthetized mice, many neurons in the awake mice had such low thresholds as 0-10 dB, which were comparable to the behaviorally assessed audiogram of mice. Further, some neurons showed temporally nonlinear responses to the increase of sound intensity that were not observed in the neurons of anesthetized mice. COI:No

Oral Session 8

Circulatory physiology 2

March 29 (Thu) 10:30～11:30 Hall 4

2O-04AM-1
Epac1 deficiency inhibits bFGF-induced neointimal formation via diminished phosphorylation of GSK3β

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Background: Vascular smooth muscle cell (VSMC) migration plays a critical role in neointimal formation after vascular injury. We reported that an exchange protein activated by cAMP 1 (Epac1) promotes VSMC migration and neointimal formation. Since basic fibroblast growth factor (bFGF) plays a pivotal role on neointimal formation, we examined the roles of Epac1 in bFGF-mediated migration and neointimal formation.
Methods: Migration of VSMCs was examined using a primary culture of VSMCs from Epac1-deficient mice ($Epac1^{-/-}$-VSMCs) by the time-lapse microscopy. Lamellipodia formation, which is essential for cell migration, was evaluated by immunocytochemistry. The expression of phosphorylated glycogen synthase kinase 3β (p-GSK3β), which is involved in bFGF-induced cell migration, was evaluated by immunohistochemistry two weeks after femoral artery injury.
Results: The path length of bFGF-induced VSMC migration was significantly shorter in $Epac1^{-/-}$-VSMCs (0.7-fold vs. $Epac1^{+/+}$-VSMCs, $p<0.001$) and lamellipodia-positive cells were significantly decreased in $Epac1^{-/-}$-VSMCs treated with bFGF (0.2-fold, vs. $Epac1^{+/+}$-VSMCs, $p<0.01$). bFGF-induced p-GSK3β expression was diminished in $Epac1^{-/-}$-VSMCs. Furthermore, neointimal formation was attenuated in $Epac1^{-/-}$ mice after femoral artery injury, in which p-GSK3β expression was decreased.
Conclusions: These data suggest that Epac1 is involved in bFGF-induced neointimal formation via diminished phosphorylation of GSK3β. COI:No

2O-04AM-2
Coagulation factor XI induced Ca^{2+} response and cell migration in vascular smooth muscle cells

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Objective: Coagulation factor XI (FXIa) is a serine proteinase that plays a key role in the intrinsic coagulation pathway. FXIa is reported to contribute to the pathogenesis of atherosclerosis; however, the cellular effect remains unknown. Here we investigated whether or not FXIa exerts any cellular effect in vascular smooth muscle cells.Main results: We examined the effects of bovine FXIa on the cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_i$) and cell migration using rat embryo aorta smooth muscle A7r5 cells. In the absence of extracellular Ca^{2+}, FXIa, only at 300 nM, induced a significant but small transient elevation of $[Ca^{2+}]_i$ (0.014 ± 0.003, $n = 4$) due to Ca^{2+} release. The subsequent replenishment of extracellular Ca^{2+} to 2 mM induced a sustained, concentration-dependent elevation of $[Ca^{2+}]_i$ (0.450 ± 0.023, $n = 4$, at 300 nM) due to Ca^{2+} influx. Both Ca^{2+} release and Ca^{2+} influx induced by FXIa were abolished by the pretreatment with 1 μM atopaxar, an antagonist of PAR_1 (proteinase-activated receptor 1) or 50 μM p-APMSF, an inhibitor of proteinase. The FXIa-induced Ca^{2+} influx was completely inhibited by 10 μM diltiazem, a L-type Ca^{2+} channel blocker, when applied at the time of Ca^{2+} replenishment. The FXIa-induced Ca^{2+} influx was inhibited by siRNA targeted to $VOC_{\alpha 1C}$. FXIa increased the rate of migration to 2.5 times of control, which was partly inhibited by atopaxar or diltiazem. Conclusions: We provide the first evidence that FXIa induces Ca^{2+} response, via PAR_1 and $VOC_{\alpha 1C}$, and enhances migration in vascular smooth muscle cells.COI: No COI:No

2O-04AM-3
Tissue-type plasminogen activator contributes to remodeling of the ductus arteriosus

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Aims: Intimal thickening (IT) is necessary for the ductus arteriosus (DA) closure. Although the smooth muscle cells of the DA have been reported to play roles in the IT formation, the roles of endothelial cells (ECs) have not fully investigated. We focused on tissue-type plasminogen activator (t-PA), which was one of the reported DA EC dominant genes, and investigated the role of IT formation. Methods and results: ECs of the DA and aorta were isolated from full-term rat fetuses (21 days of gestation). RT-PCR showed that expression level of t-PA was higher by 2.7-fold in DA ECs than in aortic ECs. A strong immunoreaction for t-PA was detected in DA ECs. t-PA-mediated plasminogen-plasmin conversion activates gelatinase matrix metalloproteinases (MMPs). Gelatin zymography showed that plasminogen supplementation promoted activation of the MMP-2 in rat DA ECs. In situ zymography showed that gelatinase was activated at the site of disrupted internal elastic laminae (IEL) in the DA. In vivo administration of plasminogen to pre-term rat fetuses (19 days of gestation), in which IT is poorly formed, promoted IEL disruption accompanied by gelatinase activation and enhanced IT formation in the DA. Furthermore, the experiments using human DA tissues showed that expression level of t-PA mRNA was higher by 3.8-fold in IT area than in the tunica media. Protein expression of t-PA and gelatinase activation were also detected in the IT area of the human DAs. Conclusion: t-PA expressed in ECs may help to form IT of the DA via activation of MMP-2 and disruption of IEL. COI:No

2O-04AM-4
Fibrinolysis resistant modification of fibrin by carbonylation: its underlying mechanism and pathological role.

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Background: Fibrin formation triggers and enhances plasminogen activation and the following fibrinolysis. Assembly of tissue plasminogen activator (tPA) and plasminogen on fibrin surface, and the following modification of the conformation of plasminogen after binding to the C-terminal lysine residue of partially degraded fibrin are the mechanisms for the enhancement. Recently carbonylation of proteins by oxidized stress are suggested to play roles in aging. Aim: To evaluate the influence of carbonylation of lysine residues in fibrinogen and fibrin on their enhancing ability of fibrinolysis. Methods: Fibrinogen and epsilon amino-caproic acid (EACA) were carbonized by either cigarette smoke extract (CSE) or acrolein, the main element in CSE, respectively, and their effects on fibrinolysis or plasminogen activation were analyzed. Results: Fibrin clots made by CSE-treated fibrinogen were lysis resistant, and the clot lysis time showed positive correlation with the amounts of carbonyl lysine in the lytic solution. Though EACA enhanced tPA-catalyzed plasminogen activation, carbonyl EACA did not. We also analyzed the existence of carbonyl lysine in-vivo, and found those in intra luminal thrombi of abdominal aortic aneurysm by immunostaining. Discussion: Carbonylation made fibrin resistant to fibrinolysis by modifying its binding capacity to plasminogen. Carbonylation of fibrinogen or fibrin under oxidized stress appeared responsible to accumulate fibrinolysis-resistant thrombi in-vivo. COI:No

2O-04AM-5
Exercise improves cardiac Ca^{2+} regulation in ovariectomized obese-insulin resistant rats

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Intracellular calcium ($[Ca^{2+}]_i$) regulation plays an important role for left ventricular (LV) function. Either estrogen deprivation or obesity is known to impair both the LV function and metabolic status, and exercise has been suggested to improve these impairments. However, the effects of exercise on cardiac $[Ca^{2+}]_i$ regulation under estrogen-deprived obese-insulin resistant condition (OVX-IR) has never been investigated. We hypothesized that exercise improves $[Ca^{2+}]_i$ homeostasis, leading to reduced LV dysfunction in OVX-IR rats. Female rats fed with a high-fat (HF) diet for 13 weeks were divided into sham (HFS) operated or ovariectomized (HFO) groups. Six weeks after surgery, rats were divided to sedentary (HFSS and HFOS) or treadmill exercise (HFSEx and HFOEx) group for 6 weeks. The metabolic status, %LV fractional shortening (%LVFS), and [Ca2+]i transients were determined. All HF-fed rats had obese-insulin resistance. HFOS rats had markedly reduced %LVFS [53 ± 2 vs. 60 ± 3] and decreased $[Ca^{2+}]_i$ transient amplitude and decay rate, compared to the HFSS group. However, exercise effectively reduced these impairments by increasing both %LVFS [HFSEx: 64 ± 3, HFOEx: 58 ± 2] and $[Ca^{2+}]_i$ transient amplitude and decay rate. Thus, exercise ameliorated LV dysfunction in estrogen-deprived obese-insulin resistant rats via improved $[Ca^{2+}]_i$ homeostasis. COI:No

Oral Session 9

Circulatory physiology 3

March 29 (Thu) 10:30～11:30 Hall 5

2O-05AM-1

Prefrontal oxygenation correlates to the responses in facial skin blood flows during exposure to pleasantly-charged movie

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Our laboratory have reported that facial skin blood flow may serve as a sensitive tool to assess an emotional status. Cerebral neural correlates during emotional interventions should be sought in relation to the changes in facial skin blood flow. To test the hypothesis that prefrontal activity has positive relation to the changes in facial skin blood flow during emotionally-charged stimulation, we examined the dynamic changes in prefrontal oxygenation with near-infrared spectroscopy and facial skin blood flows with Doppler flowmetry during emotionally-charged audiovisual challenges for 2 min (viewing comedy, landscape, and horror movie) in 14 subjects. The extents of pleasantness and consciousness for each emotional stimulus were estimated by subjective rating from -5 (the most unpleasant; the most unconscious) to +5 (the most pleasant; the most conscious). Comedy stimulation decreased prefrontal oxygenation, especially in the dorsolateral and frontopolar cortices, and facial skin blood flow, whereas either horror or landscape stimulation did not alter or slightly decreased them. The changes in prefrontal oxygenation werecorrelated positively with the changes in facial skin blood flow and negatively with the subjective rating of pleasantness. The reduction in prefrontal oxygenation during positively-charged emotional stimulation suggests a decrease in prefrontal neural activity, which may in turn elicit neurally-mediated vasoconstriction of facial skin blood vessels. COI:No

2O-05AM-2

A limb mechanoreflex decreases the prefrontal oxygenation during motor-driven passive cycling in humans

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Our laboratory has reported that the oxygenated-hemoglobin concentration (Oxy-Hb) of the prefrontal cortex decreased during the later period of voluntary cycling exercise for 1min. Since the similar decrease was observed during motor-driven passive cycling, it is suggested that the decrease in prefrontal Oxy-Hb is evoked due to a feedback related to mechanical limb movement. If so, the decrease in prefrontal Oxy-Hb would be changed depending on the extent of limb mechanoafferent input. To test the hypothesis, the prefrontal Oxy-Hb response to passive cycling was examined at various revolutions of pedal movement. Six males and six females participated in this study. Passive cycling was driven by a motor of the ergometer for 1 min at 35, 50, and 65 revolutions per minute. The relative changes in Oxy-Hb of the prefrontal cortex were measured with near-infrared spectroscopy. The breath-by-breath respiratory variables were simultaneously measured. The decrease in prefrontal Oxy-Hb during the later period of passive cycling was augmented in proportion to the revolutions of pedal movement. Passive cycling did not change end-tidal carbon dioxide tension (ETCO2), suggesting that the changes in ETCO2 could not explain the decrease in prefrontal Oxy-Hb. It is likely that the feedback from limb mechanosensitive afferents decreased Oxy-Hb of the prefrontal cortex during the later period of passive cycling. COI:No

2O-05AM-3

Omega-3 and omega-6 docosapentaenoic acid (DPA) equally inhibit the Ca_2-sensitization of abnormal vascular contractions via inhibiting the Rho-kinase activation and translocation

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Polyunsaturated fatty acids (PUFAs) contain two families of n-3 and n-6 PUFAs, which are thought to have beneficial and harmful effects on prevention of cardiovascular diseases, respectively. We previously identified a sphingosylphosphorylcholine (SPC)/Fyn/Rho-kinase pathway, which mediates the Ca^{2+}-sensitization of abnormal vascular smooth muscle (VSM) contractions leading to vasospasm. After extensive screening, we found that eicosapentaenoic acid (EPA), one of n-3 PUFAs, can specifically inhibit this pathological pathway and thereby block selectively the abnormal VSM contractions, without affecting physiological Ca^{2+}-dependent contractions. Indeed we also found that EPA clinically prevent cerebrovascular vasospasm of the patients. In this study, we clearly demonstrate that docosapentaenoic acid (DPA) strongly inhibit the SPC-induced abnormal contractions in VSM tissue and human coronary artery smooth muscle cells (CASMCs), with little effect on the Ca^{2+}-dependent contractions. Surprisingly n-3 and n-6 DPA equally inhibited the SPC-induced abnormal VSM contractions by blocking the activation and translocation of Rho-kinase from cytosol to cell membrane and the resultant phosphorylation of myosin light chain. In summary, we provide first direct evidence that n-3 and n-6 DPA equally inhibit SPC-induced abnormal contractions by inhibiting Rho-kinase activation and translocation to the cell membrane. **Sci Rep. 7: 36368, 2017**. COI: NO. COI:No

2O-05AM-4

Withdraw

2O-05AM-5

Withdraw

Oral Session 10

Channel・Transporter 2

March 29 (Thu) 10:30～11:30 Hall 6

2O-06AM-1

Molecular mechanism for muscarinic receptor-mediated endocytosis of TASK1 channels in adrenal medullary cells

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Activation of muscarinic receptor in rat adrenal medullary (AM) cells induces depolarization through the inhibition of TWIK-related acid-sensitive K+ (TASK) 1 channels. Here, using pharmacological and immunological approaches, we explored the molecular mechanism for this muscarinic receptor-mediated inhibition. TASK1 channels were mainly located at the cell periphery in dissociated rat AM cells, and its majority was internalized in response to muscarine. The muscarinic translocation was suppressed by MT7, a specific M1 antagonist, and the dose response curves for muscarinic agonist-induced translocation were similar to those for the muscarinic inhibition of TASK1 currents. The muscarine-induced inward current and/or translocation of TASK1 were suppressed by inhibitors for PLC, PKC, and Src. TASK1 channels in AM cells and PC12 cells were transiently associated with Src and were tyrosine phosphorylated in response to muscarinic stimulation. After internalization, TASK1 channels were quickly dephosphorylated even while they remained in the cytoplasm. We concluded that M1R stimulation results in internalization of TASK1 channels through the PLC-PKC-Src pathway with the consequent tyrosine phosphorylation and that this M1R-mediated internalization is at least in part responsible for muscarinic inhibition of TASK1 channels in AM cells. COI:No

2O-06AM-2

Ezrin regulates proximal tubular solute reabsorption by control of membrane localizations of transporters

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Ezrin is highly expressed in the renal proximal tubules, and is known to form multi-protein complex with a scaffold protein NHERF (Na^+/H^+ exchanger regulatory factor 1), and several transporters including Na^+/Phosphate cotransporter NaPi2a. We previously reported that ezrin knockdown (EKD) mice exhibited the hypophosphatemia due to the abnormal membrane localizations of NaPi2a and NHERF1. Since EKD mice exhibited severe growth retardation, we examined the influence of loss of ezrin expression to the membrane localizations of other transporters in the proximal tubules. We performed comprehensive proteomic analysis of renal brush border membrane (BBM) fractions from WT and EKD mice. Totally 1412 proteins including 45 SLC transporters were identified in renal BBM. Interestingly, expressions of Na^+ dependent glucose transporters and several amino acid transporters were down-regulated in BBM from EKD mice. EKD mice exhibited hypoglycemia and increased fractional urinary excretion of glucose ($FE_{glucose}$). Urinary leak of some kinds of amino acids was also observed in EKD mice. Furthermore, uptake of FITC-labeled β_2-microglobulin in the proximal tubules was limited in EKD proximal tubules. These results suggest that ezrin plays important roles in the regulation of membrane targeting of transporters and receptor-mediated endocytosis in the proximal tubules, and dysfunction of ezrin might be associated with the onset of Fanconi syndrome. COI:No

2O-06AM-3

Endocytosis of NKCC2 is impaired in renal tubule in moesin knockout mice

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Moesin is a member of the ezrin-radixin-moesin (ERM) family protein, which is a crosslinker between membrane proteins and actin cytoskeleton. Recently, moesin was reported to interact with $Na^+/K^+/2Cl^-$ cotransporter type 2 (NKCC2) and to play an important role in the apical recycling of NKCC2 by *in vitro* experiments using LLC-PK1 cells (Carmosino *et al. Biol. Cell.* 2012). NKCC2 plays an essential role in regulating body salt levels via the reabsorption in the thick ascending limb of Henle (TALH). However, the physiological roles of moesin in the kidney remain unclear. Here, we examined the physiological roles of moesin in the regulation of renal function *in vivo* by using male moesin-null ($Msn^{-/y}$) mice. We found that apical surface expression of NKCC2 was increased in $Msn^{-/y}$ mice. Internalization assay using tubular suspension showed that the process of NKCC2 endocytosis is impaired in $Msn^{-/y}$ mice. In addition, since the distribution of NKCC2 in lipid raft fractions was decreased in $Msn^{-/y}$ mice, moesin may regulate the NKCC2 distribution to microdomain. To examine the renal physiological roles related to the NKCC2 in $Msn^{-/y}$ mice, we performed biochemical analysis of plasma and urine. Significant increase of plasma Cl^- concentration concomitant with slight increase of plasma Na^+ concentration was observed in $Msn^{-/y}$ mice. Urinary absolute excretions of Na^+ and Cl^- in $Msn^{-/y}$ mice were lower than those of WT mice. These results suggest that moesin regulates the apical surface expression level of NKCC2 by targeting NKCC2 to lipid raft and plays important roles in the renal electrolyte handling. COI:No

2O-06AM-4

Splicing factor RBM20 promotes Cacna1c exon9* inclusion reducing cell surface expression of the cardiac L-type voltage-gated calcium channel.

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In cardiomyocytes, the pore subunit of the L-type voltage-gated Ca^{2+} channel (L channel), CaV1.2, is encoded by the *cacna1c* gene. Alternative splicing of *cacna1c* mRNA generates various CaV1.2 isoforms with different electrophysiological properties. Splice-variants of CaV1.2 are differentially express during heart development or pathologies. The molecular mechanisms of *cacna1c* alternative splicing still remain elusive. A next generation RNA sequencing analysis suggested that *cacna1c* as a potential target for RNA-Binding protein Motif 20 (RBM20), a splicing regulator. Mutations on *rbm20* gene result in sever dilated cardiomyopathy. In the present study, we aimed at identifying the role of RBM20 in the regulation of *cacna1c* mRNA splicing.

First, we found that, in neonatal rat cardiomyocytes (NRCM), RBM20 overexpression promoted the inclusion of exon9* of *cacna1c* but other optional exons. At the opposite, siRNA knockdown of RBM20 increased the skipping of exon9*. Conserved RBM20 consensus sequences were found in the 5' and 3' intronic region of exon9* of *cacna1c*. Functionally, RBM20 overexpression resulted in a decreased L-type Ca^{2+} current density compared to control. Conversely, RBM20 siRNA knock-down increased L-type Ca^{2+} current density. Finally, we found that CaV1.2 cell surface expression was reduced by RBM20 overexpression in NRCM

Taken all together, our results show that RBM20 regulates exon9* inclusion in *cacna1c* mRNA resulting in a reduced cell surface expression of L-channel in cardiomyocytes. COI:No

2O-06AM-5

Myotubularin-related protein 4 (MTMR4), a phosphoinositide 3'-phosphatase, regulates endolysosome integrity and autophagy flux in human lung alveolar epithelial A549 cells

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Phosphoinositides (PIs) regulate vesicular trafficking. The level and turnover of PIs are tightly regulated by a large set of PI-specific metabolizing enzymes (PI-kinases and phosphatases). Myotubularin- related protein (MTMR) family of phospholipid phosphatases are 3'-specific phosphatases for PI(3)P and PI(3,5)P2. In this study, we investigated the intracellular distribution and functions of MTMR4 in A549 cells. MTMR4 was localized mainly in the late endosomes/multivesicular bodies (MVBs), amphisomes and autolysosomes. MTMR4 silencing increased the number and size of PI(3)P+ vesicles, most of which were the Rab7+ MVBs. Live cell imaging showed that MTMR4 silencing impaired motility, fission and fusion of PI(3)P+ endosomes. Starvation stimulated autophagy with increases in autophagosomes and autolysosomes and the nuclear translocation of starvation-stress responsive transcription factor-EB (TFEB). MTMR4 silencing inhibited the lysosome-fusion of autophagosomes and the formation of amphisomes. Furthermore, MTMR4 knockdown almost completely abolished the nuclear translocation of TFEB and, consequently, the upregulation of the TFEB-target genes including v-ATPase. These findings collectively indicate that MTMR4 is essential for autophagy and activation of TFEB, by facilitating the formation of autolysosomes and amphisomes. COI:No

Oral Session 11

Cell physiology 3

March 29 (Thu) 10:30～11:30 Hall 7

2O-07AM-1

Stress-induced microglial activation nullified in β1 and β2 adrenergic receptor double knockout mice

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Microglia has been extensively demonstrated to participate in the neuroinflammatory responses. In the present study, we investigated the mechanism how acute stress could trigger microglial activation in the brain. We first studied the spatial distribution of noradrenaline-synthesizing enzyme, DBH, in comparison with adrenergic receptors (AR), such as $\beta 1$, $\beta 2$ and $\beta 3$ type. Then, we compared the stress-induced microglial activation between wild-type mice and double-knockout mice which specifically lacks $\beta 1$ and $\beta 2$ AR. The results demonstrated that: 1) the microglia activation, as demonstrated with Iba1 protein, occurred in most of these brain regions including the hippocampus, thalamus, and hypothalamus; 2) DBH, noradrenaline synthesizing enzyme, was densely stained in the neuronal fibers located in most of these brain regions including the hippocampus, thalamus, and hypothalamus; 3) the $\beta 1$ and $\beta 2$, not $\beta 3$, AR are detected in the whole brain, and laser-scan microscopic analysis showed that $\beta 1$ and $\beta 2$ AR are co-localized with microglial cells; 4) In double knockout mice, the stress-induced microglial activation is substantially inhibited in the brain. Thus, the present study demonstrates that neuron-microglia may have interactions with noradrenaline via $\beta 1$ and $\beta 2$ AR. We suggest that noradrenaline may be the key neurotransmitter leading the microglial cells throughout the brain to activated status, the condition known as a precipitating factor for the further neuroinflammation. COI:No

2O-07AM-2

Microglia sense systemic immune status to modify activity of neuronal circuit

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Microglia, the primary immune cell in central nervous system (CNS), are known to regulate the formation and homeostasis of neuronal circuit and thus affect on the brain function. Recent accumulated evidences suggested that the patient with systemic immune activation caused by autoimmune disease expresses psychiatric phenotype. It has been thought that the induction of blood-brain barrier (BBB) permeability and microglial activation may actually be the key factors in initiating those psychiatric dysfunction, but the underling mechanism has not been fully known yet. The purpose of the present study was to determine the mechanisms for systemic immune activation to cause behavior abnormalities through the disruption of microglia function. The Results of in vivo imaging suggested that the interaction of microglia and systemic immune cells could cause the change of microglia properties. We now further try to study detailed mechanism for microglia activation to affect on neuronal circuit activity and BBB homeostasis. This research may contribute on understanding the underling mechanism for active systemic immune status to trigger the psychiatric phenotype. COI:No

2O-07AM-3

Exploration of the adrenergic signal common to α1 and β2 adrenergic receptors that contribute to the suppression of pro-inflammatory reaction of the microglia.

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Excessive pro-inflammatory reaction of the microglia (MG) can exacerbates neurodegenerative diseases including the Parkinson's disease and the progressive degeneration after ischemic brain injury. Previously, we reported a suppressive effect of noradrenaline (NA) in pro-inflammatory reaction of the MG induced by lypopolysuccharide accompanies inhibition of nuclear trans-localization of NF κ B. The MG simultaneously expresses $\alpha 1$, $\alpha 2$ and $\beta 2$ adrenergic receptors, and pharmacological analyses revealed that $\alpha 1$ as well as $\beta 2$ functions for the suppression. However, these two adrenergic receptors transduce different intracellular signals in general; indeed $\alpha 1$ and $\beta 2$ receptors on vascular smooth muscle lead different effects such as contraction and relaxation respectively, quite different from the MG. In the present study, we found that neither inhibition of intracellular calcium ion release nor of phospholipase abolished $\alpha 1$ effects. Moreover, inhibition of protein kinase A nor exogenous cAMP did not mimicked NA effects. On the other hand, we found a specific PI3K inhibitor LY294002 suppressed c.a. 90% of the pro-inflammatory reaction, suggesting the pivotal role of PI3K in the signaling. We would like to discuss as to how NA signal can negatively regulate the PI3K signal in the MG.. COI:No

2O-07AM-4

Roles of infiltrated macrophages and activated microglia on pathophysiology of traumatic brain injury: a study using a hypnotic bromovalerylurea

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It has been suggested that both resident microglia and infiltrated leukocytes aggravate traumatic brain injury (TBI). In this study, an old hypnotic bromovalerylurea (BU), which suppresses CCL2 expression and microglial activation, was administered to rats with TBI to elucidate the roles of microglia and leukocytes. BU markedly reduced the lesion cavity size and ameliorated spatial perception and learning ability of TBI rats. BU suppressed expression of mRNA encoding CCL2 and NADPH oxidase subunits, and proinflammatory cytokines in the injured brain tissue at 1.5 days post injury (dpi). BU prevented oxidative injury as revealed by 8-OHdG determination and decreased IL-6 protein in the cerebrospinal fluid. FACS analyses revealed that BU decreased infiltrated macrophages, probably due to the suppressed CCL2 expression. Although BU did not inhibit TBI-caused microglial activation accompanying enlargement of their somata and higher expression of CD11b and CD45, it suppressed the generation of reactive oxygen species (ROS) by activated microglia. BU decreased CCL2 and iNOS mRNA expression at 7 dpi, while BU did not change the numbers of accumulated macrophages and activated microglia. These results suggest that activated microglia are mainly responsible for TBI-induced oxidative damage during acute phase. The main mechanism underlying the ameliorative effects of BU may be the inhibition of ROS generation rather than that of CCL2-CCR2 interaction. COI:No

2O-07AM-5

Pueraria mirifica attenuates microglial activation in vitro

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Chronic neuroinflammation mediated by persistent microglial activation is associated with the pathogenesis of neurodegeneration diseases (ND). Inhibition of microglia-mediated neuroinflammation might be a potential strategy for ND treatment. Here, we investigated the effects of *P. mirifica* extract (PM), a Thai herbal plant which has been reported to elicit anti-inflammatory and neuroprotective activities, on lipopolysaccharide (LPS) and amyloid beta (Aβ)-induced microglial activation. MG6 microglial cells were pretreated with PM for 30 min and then treated with LPS or Aβ for 24 h. Cell viability, nitric oxide (NO) levels and the mRNA expression of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-1 (IL-6) were quantified by MTT, Griess assay and qRT-PCR, respectively. Exposed MG6 to PM at all doses (0.1-10,000 μg/ml) did not alter microglial cell viability. PM afforded protection against LPS- and Aβ-induced microglial cell viability. Additionally, PM attenuated the LPS- and Aβ-induced microglial activation and the production of NO. However, a decrease in TNF-α, IL-1β and IL-6 mRNA expression by treatment with PM was found only LPS-induced microglial activation. Our results revealed that PM could protect microglia against both LPS and Aβ toxicity and serve as anti-neuroinflammation agent, which may have clinical significance in ND treatment. COI:No

Oral Session 12

Neuroscience 2

March 29 (Thu) 10:30〜11:30 Hall 10

2O-10AM-1
Parvalbumin-Positive Interneurons Regulate Population Coding in Cortex

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For efficient cortical processing, neural circuit dynamics must be spatially and temporally regulated with great precision. Although parvalbumin-positive (PV) interneurons can control network synchrony, it remains unclear how they contribute to spatio-temporal patterning of activity, and whether they actually contribute to the information processing. We investigated this by optogenetic inactivation of PV cells with simultaneous two-photon Ca^{2+} imaging from populations of neurons in mouse visual cortex in vivo. For both spontaneous and visually evoked activity, PV interneuron inactivation decreased network synchrony. But, interestingly, the response reliability and spatial extent of coactive neuronal ensembles during visual stimulation were also disrupted by PV-cell suppression, which reduced the functional repertoire of ensembles. Machine-learning based decoding confirmed the importance of PV cells in population coding. Thus, PV interneurons can control the spatio-temporal dynamics of multineuronal activity by functionally sculpting neuronal ensembles and making them more different from each other. In doing so, inhibitory circuits could help to orthogonalize multicellular patterns of activity, enabling neural circuits to more efficiently occupy a higher dimensional space of potential dynamics. Currently, we also investigate the mechanism underlying fear memory. COI:No

2O-10AM-2
High content imaging analysis of drebrin clusters along dendrites regulated by NMDA receptor activity

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Simple measurement method of NMDA-type glutamate receptors (NMDARs) activity is useful for understanding the mechanisms of synapse plasticity and toxicity. We have used an actin-binding protein, drebrin in postsynaptic sites of glutamatergic synapses as a marker for detecting NMDAR activity. Our immunocytochemical analyses have shown that activation of NMDARs reduces accumulation of drebrin in synapses. In contrast, inactivation of NMDARs increased the amount of drebrin in synapses. However, technical requirement and manual operation limits the availability of this methods to high throughput analysis. In this study, we applied this drebrin-based evaluation of NMDAR activity to high-content image analysis using microplates. After 21 days in vitro, cultured hippocampal neurons were fixed and processed for immunocytochemistry to visualize drebrin and MAP2 with nuclear staining. After automated image acquisition, total number of drebrin clusters per fields and drebrin cluster density along dendrites were automatically evaluated using newly-developed algorism. NMDAR activation by NMDA or glutamate reduced the number of drebrin clusters. In contrast, inactivation of NMDARs by D-AP5 or MK-801 increased the number of drebrin clusters. These results suggest that this new high-content imaging analysis of drebrin clusters along dendrites will be useful for understanding the mechanisms of NMDAR activity-dependent regulation of the amount of drebrin in synapses. Supported by AMED 17bk0104077h0001. COI:No

2O-10AM-3
Three-dimensional Single-cell-resolution Whole-brain Atlas Using CUBIC-X Expansion Microscopy and Tissue Clearing

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A three-dimensional single-cell-resolution mammalian brain atlas will accelerate systems-level identification and analysis of cellular circuits underlying various brain functions. However, its construction requires efficient subcellular resolution imaging throughout the entire brain. To address this challenge, we developed a fluorescent-protein-compatible, whole-organ clearing and homogeneous expansion protocol based on aqueous chemical solution (CUBIC-X). The expanded highly-cleared brain enabled us to construct a mouse brain atlas with single-cell annotation (CUBIC-Atlas). The CUBIC-Atlas demonstrated inhomogeneous entire-brain development, revealing a significant decrease in the cerebral visual and somatosensory cortical areas during post-natal development. Probabilistic activity mapping of pharmacologically stimulated Arc-dVenus reporter mouse brains onto CUBIC-Atlas revealed the existence of distinct functional structures in the hippocampal dentate gyrus. Since the CUBIC-Atlas is shareable by an open-source web-based viewer (CATMAID), this pointillistic brain atlas provides a new platform for whole-brain cell profiling. COI:No

2O-10AM-4
Chronological molecular changes in hippocampus of orchidectomy-induced androgen deficient rats

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Though it is recognized that an androgen deficiency is associated with neurodegeneration in male hippocampus, its mechanism of action has not been well understood. We recently performed the experiment in orchidectomy (ODX)-induced androgen deficient rats and could detect changes of expression of genes associated with neurodegeneration in the 9-day ODX rats. This leads us to question further when and what of the earliest molecular changes in the hippocampus of rats could be detected after orchidectomy. Expression of genes associated with the neurodegenerative hallmarks; loss of synaptic plasticity (SP; *Bdnf, Syn, GluN1, nAChR N7* and *mAChR M1*), formation of neurofibrillary tangle (NFT; *Tau4* and *Tau3*) and amyloid plaques (AP; *App, Adam10* and *Bace1*) in the hippocampus of rats after 1, 3, 6 and 9 days of ODX (D_1, D_3, D_6 and D_9, respectively) were determined using a qRT-PCR. Primarily, a sudden loss of androgen induced a remarkable reduction of *Syn* mRNA level (at D_1), the significant increases in GluN1 (at D_3), nAChR N7 (at D_6) and mAChR M1 (at D_6) mRNA levels, and a significant decrease in Bdnf mRNA level (at D_9). The *Tau4* and *Tau3* mRNA levels were significantly increased at D_6 and D_9, respectively. However, no changes of the *App, Adam10* and *Bace1* mRNA levels were detected within these 9 days. From our results, it denotes that an androgen deficiency could induce chronological molecular changes of SP loss, NFT formation and AP in rat's hippocampus, which can finally lead to neurodegeneration in the animals. COI:No

2O-10AM-5
Association between reproductive senescence and cognitive impairment in male rats

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Department of Biology, Faculty of Science, Chulalongkorn University, Thailand

Since the hippocampus, a brain region involves in cognition, can also synthesizes the reproductive hormones as seen in the hypothalamus, it is doubt about the association between these two brain regions. We therefore aim to search for the age of the rats when the declines of systemic reproductive hormones and changes of expression of reproductive-related genes at hypothalamus and hippocampus are detected. Male rats at the age of 4-12 months old were decapitated, accessory sex organs were weighed, blood was assayed for testosterone and LH levels, and hippocampus and hypothalamus (including preoptic area (PoA), anteroventral periventricular nucleus (AVPV), arcuate nucleus (ARC) and median eminence (ME)) were analyzed for gene expression. Testosterone level ($p = 0.09$) and seminal vesicle weight ($p = 0.053$) were marginally decreased in 12 months old rats. An androgen receptor (*Ar*) expression in AVPV and ARC was decreased at 8-12 months and in ME at 12 months. Estrogen receptors (*Esr1* and *Esr2*) expression in AVPV and ARC was decreased at 6-12 months. Kisspeptin (*Kiss1*) expression in AVPV was decreased at 12 months. Conversely to changes in hypothalamus, expression of *Lhβ*, *Ar*, *Esr1* and *Esr2* genes in the hippocampus was increased only at 8 months. In conclusion, male reproductive senescence causes a deterioration of the hypothalamus, which leads to cognitive impairments, while hippocampus up-regulates the expression of those reproductive-related genes in an attempt to maintain the cognitive function. COI:No

Oral Presentations
Day 3

March 30 (Fri), 10:30 – 11:30

3O-03AM-1－3O-03AM-5	Autonomous function
3O-04AM-1－3O-04AM-5	Circulatory physiology 4
3O-05AM-1－3O-05AM-5	Circulatory physiology 5
3O-06AM-1－3O-06AM-5	Cell physiology 4
3O-07AM-1－3O-07AM-5	Cell physiology 5
3O-10AM-1－3O-10AM-5	Neuroscience 3

Oral Session 13

Autonomous function

March 30 (Fri) 10:30〜11:30 Hall 3

3O-03AM-1

Detection of the hypothalamic area exhibiting respiration-synchronized rhythmic activity in the diencephalon-lower brainstem-spinal cord preparation

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It has been suggested that the hypothalamus, higher center for autonomic neural regulation, is involved in respiratory control. We hypothesized that the hypothalamus is involved in respiratory rhythm generation, and conducted mapping analysis to find hypothalamic areas that show respiration-synchronized rhythmic activity by voltage imaging which enabled us to analyze spatiotemporal dynamics of multicellular activities. We used isolated diencephalon-lower brainstem-spinal cord preparations of newborn rats. The preparation was stained with a voltage-sensitive dye di-2-ANEPEQ, fixed with the ventral side up in a recording chamber, and continuously superfused with oxygenated artificial cerebrospinal fluid. We recorded depolarizing optical signals on the ventral surface of the hypothalamus using a voltage imaging system (MiCAM Ultima, BrainVision, Tokyo) connected with an epifluorescence microscope, simultaneously with neural inspiratory output from the C4 ventral roots of the spinal cord. We found neural activities synchronized with inspiratory output in the lateral hypothalamic area. Furthermore, removal of the diencephalon from this preparation decreased respiratory frequency. Our findings suggest that the hypothalamus is involved in respiratory rhythm generation. COI:No

3O-03AM-2

Thermogenic sensitivity to hypothalamic melanocortin signals decreases with age

Oya Manami, Nakamura Kazuhiro

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Obesity is an increasing threat to global health, as it increases the risk of metabolic disorders, such as type 2 diabetes and cardiovascular diseases. Although obesity often develops with age and attenuated energy expenditure likely contributes in part to the development of age-related obesity, the mechanism of age-related alteration of the central neural circuit controlling energy expenditure is unknown. Melanocortin signaling in the hypothalamus plays essential roles in the regulation of appetite and energy homeostasis, and mice lacking melanocortin-4 receptors (MC4Rs) exhibit severe obesity. MC4Rs are expressed in the dorsomedial hypothalamus (DMH), which mediates the controls of sympathetic thermogenesis in brown adipose tissue (BAT) and energy expenditure. In this study, we hypothesized that melanocortin signaling mediated by MC4Rs in the DMH is altered in aged animals. To test this hypothesis, we compared the sensitivity of MC4Rs in the DMH between young (9 weeks old) and aged (6 months old) male rats. In young rats, unilateral nanoinjection of melanotan-2 (MT-2), an MC4R agonist, into the DMH elicited increases in BAT sympathetic nerve activity and temperature in the interscapular BAT pad. On the other hand, the MT-2-induced BAT thermogenesis was attenuated in aged rats. We also found that skin cooling-induced BAT thermogenesis was also attenuated in aged rats compared with young ones. These results suggest that attenuated sensitivity of MC4Rs to melanocortin signals contributes to the age-related decrease in metabolic thermogenesis and energy expenditure, potentially leading to obesity. COI:No

3O-03AM-3

The Role of Ca^{2+}-dependent Hyperpolarization Pathway in Sleep Duration

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During NREM sleep, or slow-wave sleep (SWS), an EEG mostly displays high-amplitude low-frequency fluctuations; during waking, an EEG exhibits low-amplitude high-frequency fluctuations. These two states are mutually exclusive in a normal brain, and their ratio is homeostatically regulated. However, it is still challenging to bridge a gap between the electrophysiological dynamics and the homeostatic dynamics. Recently, a simple computational model predicted that the Ca^{2+}-dependent hyperpolarization pathway may play a role in generating the SWS firing pattern. From this prediction, we hypothesized that this pathway also plays a role in the regulation of sleep duration. To validate our hypothesis, we generated the KO mice by the Triple-CRISPR methods and analyzed these sleep phenotypes by SSS to screen for important molecules for sleep duration. We found that impaired Ca^{2+}-dependent K^+ channels (*Kcnn2* and *Kcnn3*) and voltage-gated Ca^{2+} channels (*Cacna1g* and *Cacna1h*) decrease sleep duration, while impaired plasma membrane Ca^{2+} ATPase (*Atp2b3*) increases sleep duration. In addition, pharmacological impaired NMDA receptors reduce sleep duration. To analyze detailed sleep phenotypes, we conducted the EEG/EMG recording of these mice. As a result, the observed sleep phenotypes were largely attributed to the significant change of NREM sleep duration. We also found that genetic-impaired CaMKII (*Camk2a* and *Camk2b*) decrease NREM sleep duration. Based on these results, we propose a hypothesis that Ca^{2+}-dependent hyperpolarization pathway underlies the regulation of NREM sleep duration. COI:No

3O-03AM-4

Identification of anterograde axonal projections of hypothalamic QRFP neuron by using a novel strain of *Qrfp-iCre* mice

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QRFP is a neuropeptide that was discovered as endogenous cognate ligands for an orphan G-protein coupled receptor GPR103. QRFP-producing neurons were localized exclusively in the hypothalamic regions, including the lateral hypothalamic area (LH), perifornical area (PF), and tuber cinereum (TC). In rodent studies, central administration of QRFP increases food consumption, locomotor activity and grooming response. To examine the target regions of QRFP, we need to identify axon projections of these neurons. To explore axonal projections of QRFP, we used a new mouse strain, *Qrfp-iCre* (iCre-knock-in) mouse, in which codon-improved Cre recombinase (iCre) was knocked-in in the Qrfp locus. We then injected AAV10-EF1α-DIO-ChR2-EYFP, into the hypothalamic area of *Qrfp-iCre* mice to express ChR2-EYFP in QRFP neurons. Following virus transduction, we observed cell bodies of QRFP neurons identified by ChR2 expression in the LH, PF, TC, being consistent with previous characterizations. In addition, we observed abundant ChR2 fibers in various brain areas, such as, basal forebrain, hypothalamus and hindbrain. We also found that moderate levels of ChR2-positive fibers in the bed nucleus of the stria terminalis and other regions. These observations demonstrate that QRFP neurons can send projections to and could physiologically have some effect on a wide range of brain regions. COI:No

3O-03AM-5

Subjective fatigue at wake-up time is associated with low delta EEG activity in the first sleep cycle

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Workers such as nurses with work schedules shifting between day and night suffer from poor sleep and subsequent daytime sleepiness and fatigue. Previous studies have successfully provided the link between sleep loss and several important cognitive functions, such as memory and attention deficits. However, relationships between physiological status of sleep and subjective fatigue remain unknown in shift-workers. The 20 healthy female shift-working nurses participated in the study. Subjective fatigue was assessed using visual analog scale (VAS), and sleep status (sleep quality, sleepiness levels) was assessed by several questionnaires including St. Mary's Hospital Seep Questionnaire (SMHSQ) and Japanese version of Karolinska Sleepiness Scale (KSS-J), and circadian rest/activity rhythm was measured using Actiwatch devices. During the night between two consecutive day shifts, EEGs, ECGs, and proximal and distal skin temperature were recorded at home. The results indicated that the subjects who reported high fatigue levels at wake-up time displayed lower delta power density in the first sleep cycle compared to the subjects with low fatigue levels. The results suggest an important role of slow-wave sleep in recovering from fatigue. COI:No

Oral Session 14

Circulatory physiology 4

March 30 (Fri) 10:30〜11:30 Hall 4

3O-04AM-1

Diastolic overstretch enhanced the gene expression level of fibrosis-related factors in isolated rat papillary muscle

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The mechanism of reactive fibrosis in volume overloaded heart is incompletely understood. We studied the effect of diastolic overstretch on induction of cardiac fibrosis in isometrically contracting muscle preparation from rat right ventricular papillary muscles. We used male SD-rats (BW>350 g) and dissected papillary muscle from right ventricle. A papillary muscle preparation was mounted in a bath chamber and stretched to Lmax, at which active tension reached to the maximal level. We stretched papillary muscle to 110% (110% over stretched; mild-OS) and 115% (115% over stretched; severe-OS) of Lmax for 4 hours with tension measurement (1 Hz, 36°), then compared with Lmax group (non over stretched; Non-OS). Immediately after length change to mild-OS or severe-OS, active tension decreased to 60% or 40% of the initial tension respectively. After measurement of tension, papillary muscles were frozen and qRT-PCR analysis was performed. We measured the mRNA expression levels of fibrosis-related factors, such as TNF-α, TGF-β, connective tissue growth factor (CTGF) and pro-collagen III (PC-3), and also heart failure markers, such as ANP and BNP. The expression levels of CTGF and PC-3 in mild-OS and severe-OS were significantly higher than those in Non-OS. However, we could not get any significant differences of heart failure markers between mild-OS, severe-OS and Non-OS group. These results suggest that overstretch significantly reduced tension, and induce fibrotic transition within 4 hours. COI:No

3O-04AM-2

Donepezil is Superior to Metoprolol for Improving Myocardial Salvage and Preventing the Progression of Cardiac Remodeling after Reperfused Myocardial Infarction in Rats

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Introduction: We have reported that acetylcholinesterase inhibition by donepezil markedly improves the prognosis in chronic heart failure rats with permanent myocardial infarction (MI). This study compared the effects of donepezil and metoprolol on myocardial salvage and cardiac remodeling in reperfused MI (RMI) rats. Methods: RMI rats were created by occluding the left coronary artery (30min) followed by reperfusion. Survived animals were randomly assigned to untreated (UT, n=25), donepezil (DT, n=25, 5mg/kg/day) or metoprolol (MT, n=18, 70 mg/kg/day) group. We performed an immunohistochemical examination at the 8th day, and evaluated hemodynamics, neurohumoral factors and biomarkers after 10 weeks.Results: Compared with UT, MT decreased heart rate and plasma CRP, but did not show changes in myocardial salvage and cardiac remodeling. In contrast, compared with UT, DT decreased heart rate, reduced MI size (12.4± 1.2vs.20.1± 1.4%, p< 0.01) through improving myocardial salvage, prevented cardiac remodeling (2.39± 0.05vs.2.73± 0.13g/kg, p< 0.05), improved cardiac function (cardiac index: 120± 4vs. 98± 3ml/min/kg, p< 0.01; LVEDP: 14± 2vs.23± 2mmHg, p< 0.01), and decreased plasma BNP (335± 22vs.446± 34pg/ml, p< 0.01) and CRP (454± 67vs.825± 67 μ g/ml, p< 0.01).Conclusions: Donepezil significantly improved myocardial salvage, reduced MI size, and prevented progression of cardiac remodeling, suggesting that donepezil may be used as a novel therapy for post-RMI. COI:No

3O-04AM-3

Defective SR Ca^{2+} uptake in the heart of diabetic mouse

Mikami Yoshinori[1], Ito Masanori[1], Hamaguchi Shogo[2], Murakami Shingo[1], Tomida Taichiro[1], Namekata Iyuki[2], Tanaka Hikaru[2], Adachi-Akahane Satomi[1]

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Diabetes mellitus (DM) is a dominant risk factor for heart failure. The defective Ca^{2+} signaling contributes to diastolic dysfunction. The aim of this study was to clarify the underlying mechanisms. To investigate these mechanisms, we used streptozotocin-induced DM model (STZ) mice. The relaxation time of ventricular myocardium from STZ mice was significantly longer. The amplitude and basal Ca^{2+} concentration in the isolated ventricular myocytes from STZ mice were lower and higher than those from control mice, respectively. The Ca^{2+} transient decay rate was slower in STZ mice. Phosphorylation of phospholamban at Ser^{16} (p-PLN) accelerates SR Ca^{2+} uptake through SERCA. In the ventricle of the mice 4 weeks after STZ injection, the level of p-PLN was significantly lower in STZ mouse heart, although expression levels of SERCA and PLN were the same as those of control mice. However, the myocardial response to βAR stimulation as well as βAR expression level was not altered in STZ mice, indicating that βAR signaling is not impaired. We next administered insulin for 3 weeks from 1 week after STZ injection. In insulin-sensitive mice, p-PLN was restored to the control level, while p-PLN was not recovered in hyperglycemic insulin-resistant mice. These results indicate that the insulin signaling plays a crucial role in maintaining the basal p-PLN level and SERCA activity and that impaired insulin signaling in DM causes diastolic dysfunction through defective SR Ca^{2+} uptake. COI:No

3O-04AM-4

Withdraw

3O-04AM-5

High-fat High-carbohydrate diet accelerated cardiometabolic dysfunction faster than high-fat diet alone in obese-insulin resistant rats

Apaijai Nattayaporn, Jaiwongkam Thidarat, Kerdphoo Sasiwan, Chattipakorn C Siriporn, Chattipakorn Nipon

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Although numerous studies demonstrated that both high-fat diet (HFD) and high-fat high-carbohydrate diet (HFHC) consumption induced cardiometabolic dysfunction, the comparative effect of HFD and HFHC on the progression of cardiometabolic dysfunction has never been investigated. We hypothesized that HFD causes worse cardiometabolic dysfunction than HFHC due to severe mitochondrial dysfunction in obese-insulin resistant rats. Male rats were divided into 3 groups to receive normal diet (ND), HFD, or HFHC for 24 weeks. Metabolic parameters, heart rate variability (HRV), and cardiac function were determined every 4 weeks. At week 24, rats were sacrificed and cardiac mitochondrial function and insulin signaling were determined. Both HFD and HFHC rats exhibited obese-insulin resistance at week 8, however, plasma glucose level was increased only in HFHC rats. Myocardial insulin receptor function was markedly reduced in HFHC rats, compared to HFD rats. In addition, depressed HRV was observed in both HFD and HFHC rats at week 8. A decreased % left-ventricular ejection fraction was observed at week 8 following HFHC feeding, and at week 12 following HFD feeding. However, the severity of cardiac mitochondrial dysfunction was comparable between HFD and HFHC rats after 24 weeks of diet feeding. These data suggested that HFHC accelerated cardiometabolic dysfunction faster than HFD in obese-insulin resistant rats. COI:No

Oral Session 15

Circulatory physiology 5

March 30 (Fri) 10:30〜11:30 Hall 5

3O-05AM-1

C1 neurons mediate a stress-induced protection of renal ischemia/reperfusion injury

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C1 neurons, located in the medulla oblongata, mediate adaptive autonomic responses to physical stressors (for example, hypotension, hemorrhage and presence of lipopolysaccharides). We describe here a powerful anti-inflammatory effect of restraint stress, mediated by C1 neurons: protection against renal ischemia-reperfusion injury. Restraint stress or optogenetic C1 neuron (C1) stimulation (10 min) protected mice from ischemia-reperfusion injury (IRI). The protection was reproduced by injecting splenic T cells that had been preincubated with noradrenaline or splenocytes harvested from stressed mice. Stress-induced IRI protection was absent in Chrna7 knockout (α 7nAChR-/-) mice and greatly reduced by destroying or transiently inhibiting C1. The protection conferred by C1 stimulation was eliminated by splenectomy, ganglionic-blocker administration or β2-adrenergic receptor blockade. Although C1 stimulation elevated plasma corticosterone and increased both vagal and sympathetic nerve activity, C1-mediated IRI protection persisted after subdiaphragmatic vagotomy or corticosterone receptor blockade. Overall, acute stress attenuated IRI by activating a cholinergic, predominantly sympathetic, anti-inflammatory pathway. C1s were necessary and sufficient to mediate this effect. COI:No

3O-05AM-2

Acute activation of uterine TRPA1 sensitizes visceromotor response in rats

Lin Tzer-Bin

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Our previous studies have demonstrated activation of uterine TRPA1 expressing afferent fiber reflexively provoked painful colonic motility. Yet, whether irritation of uterine TRPA1 expressing sensory nerve could sensitize nociception of the descending colon remains unclear. To investigate the possible interaction between the uterus and colon, striated abdominal muscle electromyogram activity in response to graded colorectal distension (CRD), an index of visceromotor response (VMR), was recorded before and after intra uterine mustard oil (MO) or corn oil (CO) instillation. The lumbosacral (L6 S1) dorsal of animals was dissected to assess the level and the cellular location of phosphorylated NR2B subunit of N methyl D aspartate receptor (NMDAR) using Western blotting and immunofluorescence analysis, respectively. MO (10 uM, 0.2 ml) instillation into the lower uterine horn dose dependently sensitize the VMR in accompanied with dorsal horn NR2B phosphorylation, an effect that was significantly attenuated by intar uterine pretreated HC 030031 (a selective TRPA1 receptor antagonist; 10 uM, 0.2 ml) and intrathecal pretreated Ro 25 6981 (a selective NR2B subunit antagonist; 1 uM, 10ul). Our results demonstrated that acute irritation of uterine TRPA1 afferent fiber reflexively sensitize VMR caused by CRD. Our findings suggest that he comorbidity of gynecological or obstetrical and gastrointestinal problems is not coincidental but rather causal in nature, and clinicians should investigate for gynecological or urological diseases in the setting of bowel problems with no known pathological etiology COI:No

3O-05AM-3

TRPM4 channel is involved in cellular damage caused by simulated ischemia-reperfusion injury: study of human iPSC-derived cardiomyocytes

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TRPM4 channel is involved in cellular damage caused by simulated ischemia-reperfusion injury: study of human iPSC-derived cardiomyocytesCHEN WANG, HENG WEI, KEIJI NARUSE, KEN TAKAHASHICardiovascular Physiology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama UniversityTRPM4 is a calcium-activated nonselective cation channel expressed in variorus tissues including heart. We previously reported that TRPM4 channel is involved in ischemia-reperfusion (I/R) injury of the heart using rats. In this study, we investigated possible involvement of TRPM4 channel in I/R injury in human using cardiomyocytes which were differentiated from human induced pluripotent stem cells (hiPSC-CMs). Exposure to 22 h-hypoxia (2%-oxygen) and 2h-reoxygenation, which mimics I/R injury, caused decreased cellular viability of hiPSC-CMs detected by MTT assay. Exposure to 750 uM-hydrogen peroxide, which is another simulated condition of I/R, caused similar damage of hiPSC-CMs. However, treatment with 9-Phenonthrol, an inhibitor of TRPM4 channel, protected the hiPSC-CMs from the damage caused by the simulated I/R injuries in a dose-dependent manner. These results suggest that TRPM4 channel may be involved in the pathogenesis of I/R injury in human as well. COI:No

3O-05AM-4

The Role of Renal Afferent Denervation in Chronic Kidney Disease

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Renal sympathetic denervation reduces blood pressure in some patients with hypertension. Chronic kidney disease (CKD) is common comorbidity of hypertension where renal afferent signalling plays a key role in overactivation of sympathetic nervous system.We examined the effects of selective renal de-afferentation (de-aff) in a rabbit model of CKD induced by lesioning of the left kidney and right nephrectomy. Mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) were chronically measured and baroreflex function was studied to compare with those of total renal denervation (RDN) and sham denervation.MAP had increased by 14% to 77±1mmHg after induction of CKD and continued to rise from this level by +8% and +13% after 2 and 4 weeks in sham, respectively, while in rabbits underwent RDN or de-aff, there was no further increase in MAP ($P<0.001$ vs sham). Compared to sham, RSNA was 23% and 25% lower following RDN and de-aff, respectively ($P<0.05$). CKD shifted the RSNA baroreflex towards the higher MAP and this was reversed after RDN with marked reductions in gain (38%) and range (42%), $P<0.05$ vs Sham. These effects were similar in de-aff. Norepinephrine content of kidney was decreased by 98% in RDN, and calcitonin gene-related peptide expression was decreased by 65% in de-aff.Both RDN and de-aff are similarly effective for 4 weeks in ameliorating hypertension and lowering RSNA. Baroreflex resetting was also reversed without altering renal function. Our results suggest that the effects of renal denervation in CKD are likely due to loss of signalling from renal afferents. COI:No

3O-05AM-5

Necrox-5 exerts anti-inflammation and regulates mitochondrial biogenesis in hypoxia-reoxygenation (HR) treated rat hearts

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NecroX compounds have been shown to protect the liver and heart from ischemia-reperfusion injury. In this study, we verified whether the Necrox-5 modulates cardiac proteomic alteration and mitochondrial biogenesis, inflammation and fibrosis responses in a hypoxia-reoxygenation (HR) treated rat heart. Necrox-5 treatment (10 mu;M) and non-treatment were employed on isolated rat hearts during hypoxia/reoxygenation treatment using an ex vivo Langendorff system. Level of mitochondrial biogenesis related proteins has dramatically decreased and level of pro-inflammatory proteins was increased in HR treatment heart. However, treated with NecroX-5 significantly attenuated those HR-induced proteomic alterations, practically which are involved in oxidative phosphorylation and metabolic function. NecroX-5 treatment improved mitochondrial complex activities, markedly higher peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC1 α) expression levels were observed in NecroX-5-treated group. In addition, HR- or LPS-induced TNF-α and TGF β1 and phosphorylation of Smad2 productions were reduced with NecroX-5 supplement. The findings suggested the cardio-protective effect of NecroX-5 against cardiac HR injuries by modulating mitochondrial biogenesis and exerting anti-inflammation actions. COI:No

Oral Session 16

Cell physiology 4

March 30 (Fri) 10:30〜11:30 Hall 6

3O-06AM-1

Reprogramming of fatty acid metabolism is crucial for the inflammatory resolution

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Growing evidence has suggested that chronic inflammation is important for the pathogenesis of numerous diseases, including metabolic disorders and atherosclerosis. Macrophages play pivotal roles in chronic inflammation.We found that macrophages switch their cellular metabolism and functional phenotype throughout the course of inflammatory response. In response to inflammatory activation via Toll-like receptor (TLR4), macrophages rapidly activate glycolysis, increase inflammatory cytokine expression, acquire M1-like, pro-inflammatory phenotype. By contrast, macrophages increase unsaturated, anti-inflammatory fatty acid synthesis to show M2-like, anti-inflammatory phenotype at 24 hours following TLR4 activation. This late program of anti-inflammatory fatty acid biosynthesis is dependent on SREBP1 and results in the uncoupling of NFkB binding from gene activation. Consistent with this, anti-inflammatory omega-3 fatty acids are decreased in SREBP1$^{-/-}$ macrophages, and systemic inflammation was prolonged in SREBP1$^{-/-}$ mice. These findings suggest the functional switch from M1-like to M2-like, and the metabolic switch from glycolysis to lipid metabolism are tightly linked and coordinately regulated during inflammatory response, and these temporal regulatory programs are important for proper inflammatory activation and resolution. Collectively, macrophages have endogenous, temporal programs to switch their function by linking inflammatory signals, and cellular metabolism. This program would be novel therapeutic target for atherosclerosis and metabolic syndrome. COI:No

3O-06AM-2

Mechanism of immune evasion in a mouse model of brain metastasis of lung

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Interaction between tumor cells and glial cells are important in the brain microenvironment. In the previous study, we observed that microglia and astrocytes accumulated around human lung cancer-derived (HARA-B) cells in a rodent model of brain metastasis. In vitro experiments showed that tumor cells and astrocytes stimulate each other by releasing cytokines. On the other hand, tumor cells were harmful for neurons and suppressed microglial immune response (immune evasion). In the present study, we investigated tumor-microglia interaction. We have found that tumor cell-derived factors suppressed microglial activation and the expression of antigen presentation (MHC-II). We identified that one of the tumor-derived factors attenuating microglial expression of MHC-II in the presence of inflammatory cytokines was spermine, an end-product of polyamines. mRNA expression level of the first-step polyamine producing enzyme, ornitine decarboxylase (ODC), was significantly higher in HARA-B cells than that in keratinocyte cell line. These results suggest that the production of tumor-cell derived polyamines could be a good therapeutic target. COI:No

3O-06AM-3

Quantitative imaging and mathematical modeling of JNK activation in inflammatory signaling

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JNK (c-jun N-terminal kinase) is a key mediator of the inflammatory response and plays fundamental roles in multiple cellular processes such as gene expression, migration, differentiation, and cell death. Although the regulatory pathway of JNK has been well documented so far, the dynamics of JNK activation in living cells or organisms have not been well understood. The aim of this study is to reveal JNK dynamics in living cells at a higher resolution to understand its regulatory mechanisms. To quantitatively measure JNK activity in intact cells, we conducted FRET imaging analysis of JNK activity in HeLa cells. In this study, we focused on the input-output (IO) relationship of JNK regulation, in which IL-1β stimulation and JNK activity were defined as an input and an output, respectively. By varying the duration and frequency of input stimuli that is applied to cells, we analyzed the resulting time-course of JNK activation. The IO relationship of JNK signaling pointed that a negatively regulating feedback factor is involved in the pathway. Consistently, we found that the induction of a MAPK phosphatase MKP-1 restricts the duration of JNK signaling. By implementing the mathematical model, we verified that this simple negative regulation can explain the observed complex IO relation of JNK activation dynamics induced by IL-1β. As sustained JNK activity induces cell death, such negative regulation should contribute to keep cells alive and maintain inflammatory response even when cells are exposed to excess cytokines. COI:No

3O-06AM-4

Roles of CD200 in tumor immunity in the suppression of tumor growth and metastasis using a novel rat distant metastasis model

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Prevention of distant metastasis is one of goals for cancer sciences. We have tried to find a novel intervention to suppress distant metastasis by the use of stimulating effects of CD200S on tumor immunity. CD200S is a truncated from of an immunosuppressive molecule full-length CD200, which we call CD200L. We have established tow rat C6 glioma cell lines expressing either CD200S (C6-S) or CD200L (C6-L). We further established a distant metastasis model using immunocompetent Wistar rats. In this model, we transplanted C6 glioma cells into the subcutaneous tissue of the back skin of neonatal rats. All rats with C6-L tumor and 44 % of rats with C6-S tumor developed lung metastasis (n=25). About 20 % of rats bearing C6-S back tumors showed complete disappearance of back tumors and evaded death by 180 days after transplantation, while all C6-L tumor-bearing rats died by 45 days. Analyses using next generation sequencer, C6-S back tumors expressed chemokines and granzyme at much higher levels than C6-L ones. Flow cytometry analyses revealed that C6-S tumor contained more NK cells and CD8+ lymphocytes than C6-L. In particular, multiple subsets of dendritic cells (DCs) expressing CD11c, MHC class II, CD8, and/or CD103 were more abundantly found in the C6-S tumors than those in C6-L ones. These results suggest that CD200S induced accumulation of multiple DC subsets that activate cytotoxic T lymphocytes, leading to elimination of metastasizing tumor cells. COI; no. COI:No

3O-06AM-5

High resolution imaging of proton extrusion in MNK-28 gastric cancer cells by Surface Enhanced Raman Spectroscopy.

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Nowadays, the use of gold nanoparticles (gNP) in physiology is gaining more and more popularity due to the concrete possibility of exploiting these materials as a site-specific drug delivery system or a detection tool for highly localized assays of different entities in intracellular/extracellular compartments. In the present investigation, the gNP surface was specifically functionalized to create a high-resolution sensor for measuring the extracellular pH in the proximity of the membrane of gastric cancer MNK28 cells. The carefully designed surface conjugation protocol was conceived with a twofold purpose: i) to attach the gNP to the membrane surface proteins using Sulfo-NHS-SS-Biotin; ii) to quantify the local pH near the gNP using the Raman spectrum of 4-mercaptobenzoic acid (4-MBA). A 671 nm laser was used to generate plasmon resonance on the gNP surface, which enhanced the otherwise weak and undetectable Raman scattering of 4-MBA. The intensity of the -COOH Raman band of 4-MBA was correlated to the pH by preliminary calibration procedures. Raster scan of Raman spectra collected from the membrane surface of MNK28 cells after gNP attachment allowed us to visualize highly localized variation of pH induced by H^+ extrusion. COI:No

Oral Session 17

Cell physiology 5

March 30 (Fri) 10:30〜11:30 Hall 7

3O-07AM-1

Molecular mechanism of lysophospholipid-induced glucagon-like peptide-1 secretion from enteroendocrine L cells

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Lysophosphatidylinositol (LPI), one of lysophospholipids, is involved in various physiological processes, including cell migration and exocytosis. Although circulating LPI levels and the expression of its receptor, GPR55, are increased in obese and diabetic patients, the relationship between LPI and glucagon-like peptide-1 (GLP-1) secretion, which facilitates insulin secretion from pancreatic β cells, remains unclear. Here we used murine enteroendocrine L cell line GLUTag cells and acutely prepared murine primary small intestinal cells to address the question. Application of LPI caused the increase of intracellular calcium concentration ($[Ca^{2+}]_i$) in GLUTag cells, and induced GLP-1 secretion in both GLUTag and acutely prepared small intestinal cells. Blockage and silencing of GPR55 significantly suppressed LPI-induced $[Ca^{2+}]_i$ elevation. Interestingly, inhibition of transient receptor potential cation channel subfamily V member 2 (TRPV2) activity also suppressed $[Ca^{2+}]_i$ increase and GLP-1 secretion. Moreover, LPI increased the number of focal adhesions, and accelerated transport of TRPV2 channels to the plasma membrane. These data suggest that LPI activates G_q and $G_{12/13}$ signaling pathways and enhances actin reorganization and TRPV2 transport, which stimulates GLP-1 secretion from enteroendocrine L cells. COI:No

3O-07AM-2 (AP5)

Angiotensin II increases Ca^{2+} transients by activating $Ca_V1.2$ Ca^{2+} channels through casein kinase 2 in immature cardiomyocytes

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Angiotensin II (AngII) plays important roles in cardiovascular regulation in perinatal mammalians. Here we found that AngII significantly increased twitch Ca^{2+} transients by robustly activating L-type $Ca_V1.2$ Ca^{2+} channels ($Ca_V1.2$) in mouse neonatal ventricular cardiomyocytes (NVCM) but not adult ventricular cardiomyocytes. This response to AngII was mediated by AT_1 receptors and β-arrestin2. To elucidate possible involvement of protein kinases in this system, we examined the effects of array of kinase inhibitors in NVCM, revealing that AngII activated $Ca_V1.2$ channels through Src-family tyrosine kinases (SFK) and casein kinase 2 (CK2). Overexpression of CK2α' β but not c-Src directly activated recombinant $Ca_V1.2$ channels composed of C-terminally truncated α_{1c}, the distal C-terminus of α_{1c}, β_{2c}, and $\alpha_2\delta_1$ subunits, by phosphorylating threonine 1704 located at the interface between the proximal and the distal C-termini of $Ca_V1.2$ α_{1c} subunits. A cyclin-dependent kinase inhibitor, $p27^{Kip1}$ (p27), inhibited CK2α' β, and AngII removed this inhibitory effect through phosphorylating tyrosine 88 of p27 via SFK in cardiomyocytes. Coimmunoprecipitation revealed that $Ca_V1.2$ channels, CK2α' β, and p27 formed a macromolecular complex. These results indicate that stimulation of AT_1 receptors by AngII activates $Ca_V1.2$ channels sequentially through β-arrestin2, SFK, p27, and CK2α' β in immature but not adult cardiomyocytes, thereby likely exerting a positive inotropic effect in the immature heart. COI:No

3O-07AM-3

Reduction in the mitochondria-associated ER membrane (MAM) plays a crucial role in palmitic acid-induced insulin resistance

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The mitochondria-associated ER membrane (MAM) is a subregion of endoplasmic reticulum (ER) contacting with mitochondria. Although reduction in the contact at MAM affects cellular homeostasis, its pathological significance in insulin resistance (IR) in type 2 diabetes mellitus remains unclear. We elucidated the role of MAM formation in the fatty acid-induced IR in hepatocytes. Palmitic acid (PA) suppressed insulin-stimulated Akt phosphorylation in HepG2 cells within 12 h. Neither ER stress response nor mitochondrial reactive oxygen species is implicated in the repression of Akt phosphorylation as demonstrated by pharmacological inhibition. Even 3-hour-treatment of PA reduced ATP-evoked calcium influx into mitochondria without affecting the calcium influx into cytosol. These results together with the electron microscope images suggested that PA suppressed the functional and structural interaction between ER and mitochondria. Overexpression of mitofusin2, a critical component of the MAM, significantly restored MAM area and partially recovered the PA-elicited IR with Ser473 phosphorylation of Akt selectively improved. These results suggest that the disruption of the MAM contact sites, but not perturbation of homeostasis in the ER and mitochondria, plays important roles in PA-elicited acute Akt inactivation in hepatic IR. COI:No

3O-07AM-4

Asiatic acid inhibits TNF-α-induced diphospho-myosin light chain (MLC) redistribution and structural remodeling of adherens junctions (AJ) in human aortic endothelial cells (HAECs)

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Asiatic acid is an active constituent isolated from *Centella asiatica*, a traditional medicinal herb in tropical Asia which has been shown to suppress TNF-α-induced endothelial hyperpermeability. However, the mechanism underlying the barrier protective effect of asiatic acid has never been clarified. The present study aimed to assess effects of asiatic acid on TNF-α-induced MLC diphosphorylation and reorganization of AJ in HAECs. For methodology, western blot analysis, immunofluorescence staining and confocal microscopy were used. 30 to 40 μM asiatic acid further enhanced TNF-α-stimulated MLC diphosphorylation and prevented diphospho-MLC redistribution. TNF-α disrupted the reticular junctions formed by VE-cadherin and β-catenin and caused formation of linear junctions, which was prevented by asiatic acid. TNF-α also significantly reduced the junctional areas occupied by VE-cadherin and β-catenin, and this was also attenuated by asiatic acid. Furthermore, asiatic acid did not alter total protein expressions of AJ as well as their intracellular redistribution. Asiatic acid prevents TNF-α-induced diphospho-MLC redistribution and structural remodeling of AJ, of which might be associated with its barrier protective effect. This study suggests a potential use of asiatic acid in improving endothelial hyperpermeability, which is a key event in early stage of atherosclerosis. COI:No

3O-07AM-5

NaHS modified ANP secretion in hypertrophied atria

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Hydrogen sulfide (H2S), the third gasotransmitter, is related to the pathogenesis of cardiovascular diseases. However, there are no reports about the relation between H2S and atrial natriuretic peptide (ANP). The aim of this study is to elucidate the role of NaHS on ANP secretion and to unravel its mechanisms using isolated beating atria from normal and isoproterenol (ISP) - treated hypertrophied rats. NaHS dose-dependently augmented high stretch-induced ANP secretion and decreased systolic atrial pressure (SAP). Pretreatment with nitric oxide synthase inhibitor, soluble guanylyl cyclase inhibitor, and KATP channel antagonist blocked these effects. H2S synthesis enzyme inhibitor (DL-propargylglycine, PAG) did not show any significant changes . However, the response of ANP secretion to NaHS markedly attenuated and PAG suppressed ANP secretion in ISP-treated rat atria. The eNOS protein expression was decreased but the expression of cardiomyocyte-specific H2S producing enzyme, cystathione gamma-lyase, was not changed in ISP-treated rat ventricles. These findings clarify that NaHS stimulates ANP secretion through NO-cGMP and KATP channel pathway. The modification of ANP secretion by NaHS and H2S synthesis enzyme inhibitor suggests the possible role of endogenous H2S in the pathogenesis of cardiac hypertrophy. Supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (NO 2017-R1A2B-4002214). COI:No

Oral Session 18

Neuroscience 3

March 30 (Fri) 10:30～11:30 Hall 10

3O-10AM-1

Metabolic energy state dependent synaptic plasticity onto POMC neurons of arcuate hypothalamic nucleus

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Proopiomelanocortin (POMC) neurons of arcuate nucleus in the hypothalamus has well known as first order neuron to regulate satiety and energy metabolism by responding energy state dependent hormonal signals like leptin. These hormonal signals regulate not only neural activity of POMC neurons but also their synaptic rewiring. However, detail mechanism how synaptic transmission changing was unknown. We showed excitatory input onto POMC neurons are regulated via re-composition of AMPA type glutamate receptor subtype expression in the postsynaptic site dependent on energy state. Under fasted condition as negative energy state, amplitude of EPSC was decreased and I-V relationship of AMPAR showed dominant expression of GluR2 contain, calcium impermeable (CI) AMPARs compared with ad lib fed state. On the other hand, short-term (10 days) high fat diet (HFD) feeding as positive energy state promote GluR2 lacking, calcium permeable (CP) AMPAR expression. Leptin, which is increased by HFD, application to the fasted mice eliminated liner I-V relation of AMPARs. In summary, negative energy, low leptin condition promoting GluR2 contained, CI-AMPAR expression suppress activity of POMC neurons whereas positive energy, high leptin condition promoting GluR2 lacking, CP-AMPAR expression facilitate POMC neuronal activity to promote satiety. COI:No

3O-10AM-2

The immediate early reception of optogenetic inputs in the layer 2/3 of the rat barrel cortex

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Rats explore the external world using mechano-reception of whiskers, whose sensory nerves are topographically projected to the layer (L) 4 of the barrel cortex. Here, we investigated whether the L2/3 neurons are involved in the integration of spatiotemporal pattern of whisker deflection. We used transgenic rats that express Channelrhodopsin-2 in the mechanoreceptive neurons of the trigeminal ganglion innervating whisker follicles. The follicles of 16 whiskers were one-by-one connected to the 16 LED-coupled optical fibers and stimulated with a certain pattern while single/multi-unit activities in the L2/3 were recorded. When the main targeting whisker follicle was stimulated, a burst of single unit activity was generated immediately after the onset of light pulse (immediately early reception, IER). The receptive field of IER was often spanned multiple whiskers and enlarged by the simultaneous photostimulation of multiple whisker follicles. It is suggested that the inputs from multiple whiskers are convergent in the L2/3 when they enter synchronously. The IER should be involved in the detection of synchronous touch of multiple whiskers to a large object. The authors have no conflict of interest associated with this research. COI:No

3O-10AM-3

Physiological role of long-lasting intracellular calcium increase in orexin neurons by dopamine

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Orexin neurons are thought to be involved in the regulation of instinctive behavior, such as sleep-wakefulness. Orexin neurons are reported to be active during wakefulness and silent during sleep. The activity of orexin neurons is controlled by variety of extracellular bioactive substances. There have been some reports about substances affect the activity of orexin neurons so far, most of which were based on recording of short-time scale from milliseconds to seconds. However, most physiological state such as wakefulness lasts for more than minutes.
Therefore, we monitored activity of orexin neurons by calcium imaging in a mouse acute brain slice, and screened substances which affect the activity for minutes or hours. We used transgenic mice which express a calcium indicator exclusively in orexin neurons, and screened candidate substances from bioactive molecules which are reported to be implicated in regulation of instinctive behavior.
As a result, we found that dopamine induced long-lasting intracellular calcium increase for several hours after application. It was surprising because dopamine had been reported to suppress orexin neurons activity by electrophysiological short-time scale recording. Examining with adrenaline or dopamine receptor antagonists, we found that at least 3 types of receptors could be involved in the response. Next we will reveal physiological role of the dopaminergic calcium increase in orexin neurons in vivo. COI:No

3O-10AM-4

De novo CaMKIIα/β mutants causing neurodevelopmental disorders upregulate A-type voltage-dependent K^+ currents in hippocampal neurons

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α and β isoforms of calcium/calmodulin-dependent protein kinase II (CaMKII) play a vital role in neuronal plasticity. By whole exome sequencing, we identified *de novo* mutations in the genes encoding CaMKII α/β in five patients with severe neurodevelopmental disorders. All patients have intellectual disability and developmental delay, and three patients with CaMKII α mutants also suffer from epileptic seizures. One mutation caused skipping of an exon encoding a part of the regulatory segment of CaMKII α. Others were missense mutations made at evolutionarily conserved amino acids in the kinase domain or the regulatory segment of CaMKII α/β. Mapping the mutation sites on the crystal structures of human CaMKII α predicted that all the mutations would impair the interaction between the kinase domain and the regulatory segment responsible for the autoinhibition of its kinase activity. In fact, the autophosphorylation level at Thr286/Thr287 was increased in three mutants. Expression of a CaMKII α mutant in primary hippocampal neurons significantly increased A-type voltage-dependent K^+ currents, which accelerated repolarization of single action potentials. Our data highlight the importance of autoinhibitory regulation of CaMKII α/β in human brain function, and suggest the upregulated A-type K^+ currents as a possible pathophysiological basis. All coauthors will be indicated in the presentation. COI:No

3O-10AM-5

Dentate granule cell activity during fear memory extinction in freely moving mice

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Granule cells (GCs) in the hippocampal dentate gyrus are capable of processing the pattern separation of episodic memories. Nevertheless, little is known about how this is achieved in behaving animals. We expressed the calcium sensor GCaMP6f in GCs of adult C57BL/6 mice by means of adeno-associated viral vector. We recorded fluorescent signals representing Ca^{2+} transients in freely moving animals by using a miniature microscope during a five-day procedure of cued fear conditioning and extinction. Control mice were exposed to cues without foot shocks during the conditioning period. After conditioning, freezing behavior was triggered by cue exposure, confirming the successful consolidation of cued fear memory. GC activity seems required for the retrieval of such memory, since chemogenetic disruption of GC activity decreased cue-induced freezing. Along with the extinction of fear memory, cue-triggered freezing behavior decreased significantly in conditioned mice. Analysis of Ca^{2+} imaging data revealed a significant increase in the number of active GCs on the first day of extinction when compared to previous days. This increase was not observed in control mice. Furthermore, the frequency of Ca^{2+} transients in GCs was maintained throughout the extinction sessions in conditioned mice, whereas it significantly decreased in control mice. Overall, our results demonstrate an increased population activity of dentate GCs during the extinction of cued fear memory. This increase may promote synaptic plasticity necessary for encoding the extinguished memory in the dentate gyrus. COI:No

Student Sessions

1SO-08AM-1－1SO-08AM-5	Student Session 1
1SO-09AM-1－1SO-09AM-4	Student Session 2
2SO-08AM-1－2SO-08AM-5	Student Session 3
2SO-09AM-1－2SO-09AM-5	Student Session 4
3SO-08AM-1－3SO-08AM-5	Student Session 5
3SO-09AM-1－3SO-09AM-5	Student Session 6

Student Session 1

March 28 (Wed) 11:00～12:00 Hall 8

1SO-08AM-1

Motor training strengthens the glutamatergic synapses of layers V neurons in the primary motor cortex

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Nerve fibers in the corticospinal tract originate from pyramidal cells in layer V of the motor cortex. To analyze motor learning-induced plasticity at layer V synapses in the primary motor cortex (M1), we trained rats with a rotor rod test up to 2 days (10 trials per day). Rats significantly improved at the final session of the1st day of training. First, we examined the role of local glutamatergic transmission in the behavioral study. Compared with vehicle injected controls, bilateral pretreatment with either CNQX (1 μg/μL, per side) or APV (1 μg/μL, per side) significantly impaired motor performance. Second, we took layer V specific sample from the M1 to quantify the protein level of AMPA receptor GluA1 subunit. In the synaptosome fraction, 2-days trained rats significantly increased the GluA1 subunit level, suggesting long-term increase in the training-induced AMPA receptor delivery into the excitatory synapses. Finally, we made acute brain slices of the M1 to evaluate synaptic plasticity. Vertical fibers from layer II/III neurons to layer V neurons were stimulated to evaluate the ratio of AMPA vs NMDA receptor-mediated current (AMPA/NMDA ratio). Although the ratio was low in untrained rats, 2-day trained rats increased the ratio up to 177 % of untrained rats. Thus, the motor-training clearly induced dynamic change in the plasticity at layers V synapses in the M1. Together with the plasticity at layers II/III synapses (Kida et al, 2016), the plasticity in the M1 circuit may contribute to reorganize the control system of pyramidal tract after training. COI:No

1SO-08AM-2

Effects of corticotrophin releasing factor signals in the bed nucleus of the stria terminalis on the expression of conditioned taste aversion in rats

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Humans and animals acquire aversion and avoidance to a taste followed by a visceral malaise, referred to as a conditioned taste aversion (CTA). Since corticotrophin releasing factor (CRF) in the bed nucleus of the stria terminalis (BNST) plays a role in fear and anxiety, we hypothesized that the CRF signals in the BNST is involved in CTA. We examined the effects of pharmacological blockade of CRF receptors in the BNST on the animal's behavior during retrieval tests. Rats implanted with guide cannulae into BNST received a pairing of 5-mM saccharin with an i.p. injections of 0.15M LiCl. After the conditioning, rats were presented with the CS on three retrieval tests. All behavioral experiments were conducted in a chamber with a bay window. A spout was placed in front of the bay window. The rat's approach and licking to the spout were analyzed. Just before the second test, we injected a CRF-antagonist or saline into the BNST. The rats injected with the CRF-antagonist showed decrease in the number of burst licking (a set of more than 3 licks with less than 250-ms intervals) and extended inter-lick intervals. Their CS intake on the third test was smaller than that in the saline-injected rats. These results suggest that the blockade of CRF receptors in the BNST enhanced the aversion to the CS, and induced longer retention of CTA. The endogenous CRF signal in the BNST may be involved in extinction of learned aversive taste memory. COI:No

1SO-08AM-3

Cortical control of monkey subthalamic nucleus

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The subthalamic nucleus (STN) is one of input stations of the basal ganglia and important for control of voluntary movements. The STN receives cortical inputs through the cortico-STN *hyperdirect* and cortico-striato-external pallido (GPe)-STN *indirect* pathways, and its abnormal activity leads to various movement disorders. Thus, it is important to know how STN activity is controlled by these pathways. To clarify this issue, we recorded STN neuronal activity of awake monkeys. Electrical stimulation of the forearm region of primary motor cortex and supplementary motor area induced early excitation and following late excitation in STN neurons. Local micro-injection of CPP (NMDA receptor antagonist) in the vicinity of recorded neurons decreased amplitude and duration of early excitation. Local micro-injection of NBQX (AMPA-kainate receptor antagonist) decreased amplitude and duration of late excitation. Local injection of mixture of CPP and NBQX significantly suppressed spontaneous activity. Muscimol injection into the striatum decreased amplitude and duration of late excitation. Muscimol or gabazine injection into the GPe decreased amplitude and duration of late excitation, while the former increased spontaneous activity of STN neurons, and the latter decreased that. These data suggest that cortically induced early and late excitation is mediated by the *hyperdirect* and *indirect* pathways, respectively. Cortico-STN transmission is mainly mediated by NMDA receptors. COI:No

1SO-08AM-4

Changes of visual cortex activity with whisker stimulation in monocular deprived mice *in vivo*

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Sensory inputs are essential factors to detect the external environment, but some parts of sensory system are disturbed in the patients of blindness and deafness. Traditionally, the concept of cross-modal plasticity has been raised, which when sensory system failed, impaired sensory input was compensated by the other sensory systems. Through decades, it is studied in several mammals, including humans. Some of the underling mechanism were shown using molecular analysis, electrophysiology, or positron emission tomography (PET). Human studies showed that the blind people use their visual area when they are reading Braille using PET. Reactivation of cortical visual area was observed with the whisker stimulation after the eye enucleation in mice. Those data indicate that the lost sensory cortical area is used for the process of the remaining sensory information. However, the functional changes of lost sensory cortical area with remaining sensory information *in vivo* have not been shown yet. Here we try to unravel the effect of blindness on the activation of the visual area in response to the somatosensory whisker stimulation *in vivo* using two-photon imaging. We identify the axons projecting from the barrel field to the lateral secondary visual area both in control and monocular-deprived mice and analyze the activation of them when the whiskers are stimulated. This study will be an important clue to understand the compensating ability of the cortex for the future therapeutic target. COI:No

1SO-08AM-5

Whisker movement as a possible index of internal states of mice

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Investigating whisker movements in mice performing a whisker detection task, we have noticed that mice often protract their whisker in response to a brief whisker deflection associated with reward. Here we examine whether whisker movement could be used as an index of internal states of mice. We trained mice for an auditory Go/No-Go task and monitored movement of their C2 whisker during task performance. Mice were head-fixed and the whisker was filmed at 200 Hz using a high-speed camera. In expert mice, a brief protraction of the whisker was often observed immediately after "Go" tone presentation (77 $\pm$ 11 % of trials, n = 3), but apparently less frequent after "No-Go" tone presentation (44 $\pm$ 7 % of trials, n = 3), suggesting that reward-anticipating whisker protraction can be seen after presentation of an auditory conditional stimulus associated with reward. Interestingly, whisking bouts were evoked around one second after receiving reward with a high probability in expert mice (83 $\pm$ 7 % of trials, n = 3) which is almost twice larger than whisking probability in "No-Go" trials (45 $\pm$ 7 % of trials, n = 3). Furthermore, whisking at a more protracted mid-point was induced by reward omission with 100 % probability (n = 2). These results suggest that characteristic whisker movements, which are possibly related to the internal states of mice such as pleasure and irritation, can be observed in task-performing mice. We are attempting to build a program decoding internal states of mice from whisker movements. COI:No

Student Session 2

March 28 (Wed) 11:00～12:00 Hall 9

1SO-09AM-1

HS1793 compound, a more potent analogue of resveratrol, improves mitochondrial function and biogenesis

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HS1793, a novel analogue of resveratrol, was previously determined to be more potent at lower dosages by improving mitochondrial function and increased mitochondrial biogenesis-related proteins in breast cancer model. In addition, HS1793 protected rat heart against hypoxia/reoxygenation injury by attenuating mitochondrial damage. We currently focus on targeting mitochondrial function and biogenesis to regulate energy homeostasis through HS1793 in mouse skeletal muscle cells, in order to alleviate dysfunction brought by increased oxidative stress or chemotherapeutic drugs. Dosage screening showed lesser cytotoxicity in dosages lower than 10 μM. HS1793 reduced ROS levels and enhanced cellular and mitochondrial ATP synthesis function, but induced multinucleation in cells as a possible adaptive response. HS1793 upregulated vital mitochondrial biogenesis-related genes and proteins such as PGC1-α, as activated by AKT and mTOR, which are considered important regulators of skeletal muscle function. HS1793 also improved mitochondrial-related markers during cisplatin co-treatment, a known chemotherapeutic drug. Taken altogether, HS1793 restores mitochondrial function and can possibly promote myogenesis, especially in muscle wasting due to chemotherapy. COI:No

1SO-09AM-2

CALHM and maxi-anion channels in mouse bladder urothelial cells

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Extracellular ATP plays important roles in neurotransmission and autocrine/paracrine cell-to-cell signaling in various tissues. In the urinary bladder epithelium, stretch stimulation triggers release of adenosine triphosphate (ATP) which acts as a neurotransmitter to stimulate afferent nerves to signal bladder filling sensation. However, the ATP release mechanism is not yet completely understood. Here we investigated the expression of two recently-identified ATP-permeable ion channels in mouse primary cultured urothelial cells: calcium homeostasis modulator (CALHM) channels and maxi-anion channels (MACs). Among *Calhm* genes, transcripts for *Calhm1* and *Calhm2* were detected in the whole bladder but not in primary urothelial cells. Expression of CALHM1 protein was further tested by immunostaining. Regarding MACs, the patch-clamp technique with the inside-out configuration was employed to detect MAC currents. After pipette excision, a single channel current with a large unitary conductance emerged at the holding potential of -20 mV. We found that this large conductance current has properties that are similar to those of MACs in terms of current-voltage relationship, ion selectivity, inactivation, and pharmacology. Thus, the findings suggest that CALHMs and MACs are potential candidates for urothelial ATP channels contributing to the sensation of bladder filling. COI:No

1SO-09AM-3

Hesperetin inhibits L-type calcium channel through phosphodiesterase 1 and cAMP in human vascular smooth muscle

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Hesperetin, a bioflavonoid in citrus fruits, is vasodilatory in both animals and humans.We aimed to study the mechanism of hesperetin-induced vasorelaxation in human vascular smooth muscle using isometric force measurement in denuded human umbilical vein (dHUV) rings. With 3x10-4 and 10-3 M hesperetin, the tension of dHUV rings (pre-contracted with 35 mM K+) was significantly reduced by 48.5 +/- 4.4% and 67.7 +/- 4.3%, respectively (n=6), compared to matching vehicle (DMSO) concentrations, osmolality changes and time control. Hesperetin could shift to the right the dose-response curve of dHUV contraction in response to Bay K8644, a specific L-type Ca2+ channel opener; the mean EC50 shifted from 1.5 and 1.1 nM with control and DMSO preincubation to 11.3 nM with hesperetin preincubation (n = 5-6). Pretreatment with cGMP and phosphodiesterase (PDE) 3, 4, and 5 inhibitors, did not prevent hesperetin from inducing additional relaxation (i.e., having additive effect), while hesperetin could not significantly increase vasorelaxation in dHUV pretreated with cAMP and PDE1 inhibitors. Our data demonstrated that hesperetin induced human smooth muscle relaxation by inhibiting L-type Ca2+ channel through PDE1 and cAMP COI:No

1SO-09AM-4

Identification of verapamil binding sites in human Kv1.5 channel using mutagenesis and docking simulation

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1:Dept Physiol, Sch Med, Shiga Univ Med Sci, Shiga, Japan, 2:Dept Anesth, Sch Med, Shiga Univ Med Sci, Shiga, Japan

Verapamil prolongs the effective refractory period (ERP) of human atrial myocardium. The present study investigated the effects of verapamil on the human Kv1.5 (hKv1.5) channel that plays an important role in determining ERP in human atrium. Site-directed mutagenesis was used to create mutations in the pore region of hKv1.5 channel. Whole-cell patch-clamp method was conducted to investigate the effect of verapamil on wild-type and mutant hKv1.5 channels, heterologously expressed in Chinese hamster ovary cells. The computer docking simulation was carried out to predict putative binding mode of verapamil within the hKv1.5 channel. Verapamil preferentially blocked hKv1.5 channel in its open state. The blocking effect of verapamil was concentration-dependent with an IC50 of 2.5±1.4 μM. The blocking potency of verapamil was significantly attenuated in T479A, T480A, I502A, V505A, I508A, L510A, V512A and V516A mutants, suggesting that Thr479, Thr480, Ile502, Val505, Ile508, Leu510, Val512 and Val516 are involved in mediating the blocking action of verapamil. The docking simulation analysis predicted that verapamil was stably positioned within the pore cavity of the hKv1.5 channel. Verapamil inhibits hKv1.5 channel, which is presumably due to direct binding to specific amino acids that reside within the pore region of hKv1.5 channel. This blocking action of verapamil on hKv1.5 channel appears to contribute at least partly to the prolongation of ERP of human atrium. COI:No

Student Session 3

March 29 (Thu) 10:30〜11:30 Hall 8

2SO-08AM-1

Anatomical analyses and functional manipulation of sympathetic nerves innervating subcutaneous white adipose tissue

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Background: The beiging (browning) of subcutaneous white adipose tissue (WAT), which is induced by cold exposure, promotes energy expenditure and improves metabolism. The sympathetic nervous system (SNS) and catecholamine signal are important for beiging, but mechanistic details remain elusive. Here we investigated the anatomical and functional relationships between the beige adipocytes in subcutaneous WAT and sympathetic innervation by genetic, histological, and DREADD approaches.Methods: Male WT and Tm mice (SNS-specific colored mice) underwent injection of retrograde neurotracer DiI to inguinal WAT (iWAT) and overnight cold exposure, Their sympathetic ganglion and iWAT were analyzed by tissue clearing (Scale S), H&E staining and Fos immunostaining. Retrograde infection efficiency of 9 different AAV vector serotypes were tested by injection into iWAT of SNS-specific *Dbh-Cre* mice. The expression levels of hM3Dq-mCherry in innervating sympathetic ganglion and iWAT were compared by real-time RT-PCR. *Dbh-Cre* mice received rAAV2-retro DREADD vector injection into iWAT. They were treated with CNO or clozapine for 3 days (q12h), and their SN and iWAT analyzed.Results: We identified SN innervation to beige adipocytes within iWAT. Among 9 serotypes tested, rAAV2-retro showed most efficient retrograde infection to sympathetic ganglion innervating iWAT. Currently we are trying iWAT-innervating SNS activity manipulation by DREADD using this rAAV2-retro vector. COI:No

2SO-08AM-2

Pupillary responses to multisensory stimuli and their relationship with personality traits

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Recent studies have suggested that pupillary size reflects on-going mental activities such as emotion, perception and recognition and its pattern shows individual variability. We hypothesized that the individual pattern of the pupillary response reflects personality traits. In order to test the hypothesis, we presented emotionally arousing auditory and visual stimulus to subjects (n = 260 students in Tohoku University), while measuring their pupil size. Psychological assessments were also conducted to analyze the correlation between their personality traits and pupillary response pattern. We presented auditory stimulus (angry or joyful voice with headphone) and visual stimulus (angry or joyful facial expression images on monitor) individually ("Voice" or "Face" condition, respectively), or in combination ("Face&Voice" condition). The average pupil size change were: +2.27 % to "Voice" (dilated pupil), -0.18% to "Face" (constricted pupil), and +2.03% to "Face&Voice" (dilated). To examine the relationship between these variables, a multiple-linear regression analysis was done:
R(Voice&Face)=0.99+0.48*R(Voice)+0.33*R(Face) (R^2=0.42).
We further examined if the individual deviation from the linear model may reflect personality traits by correlating the fitting errors and psychological test scores. We found that the residues tended to be negatively correlated to Baron-Cohens Systemizing quotient (r=-0.15, p=0.013). This suggests that individuals with high systemizing quotient tend to show a less dilated pupil, when emotionally arousing visual and auditory stimulus are presented. COI:No

2SO-08AM-3

The hypnotic effects of bromovalerylurea; an EEG study

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Bromovarelylurea (BU), an old hypnotic/sedative, is rarely used nowadays. We have found that it has anti-inflammatory actions on microglia cells and macrophages that leads to have curative effects on some inflammatory disorders. However, there is no available literature describing the features of its hypnotic actions. Therefore, we investigated the effects of BU using EEG recordings. BU was administered at zeitgeber time 0 to male Wistar rats (3 months-old) at doses 50, 125 and 250 mg/kg. The 24h-EEG recordings showed BU increases non-REM and total sleeping time at dose of 125 and 250mg/kg. At all doses, BU markedly reduced REM durations during light periods, but increased during the following dark period and did not affect total REM duration. Moreover, depth in non-REM sleep did not change. BU appeared a long acting hypnotic, even in the comparison with a long-acting barbiturate, phenobarbital. However, when administered to aged rats (22 month-old), BU disturbed the circadian rhythm and decreased the delta power. Collectively, BU is a long-acting hypnotic, but suppresses REM sleep similarly to barbiturates or benzodiazepines. BU did not show hypnotic effects at 50mg/kg, the dose that exert marked anti-inflammatory effects. Therefore, BU could be used as an anti-inflammatory agent without affecting sleep-wake cycle. BU may not be suitable for aged people because it affects sleep quality. Our study can contribute to the clinical application of BU, which can ameliorate many kinds of inflammatory disorders. COI:No

2SO-08AM-4

Participation of vestibular system in hypergravity exposure-induced hypothermia

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The vestibular system is the sensory system to sense the liner acceleration and angular velocity. Traditionally, the vestibular system is known to participate in eye movement and body balance. Previous study from our laboratory demonstrated that the vestibular system participates in arterial pressure response via the sympathetic nervous system (vestibulo-cardiovascular reflex). In the other autonomic function, Fuller et al., showed the thermoregulation through the vestibular system in the hypergravity environment (PNAS, 2002); hypergravity exposure-induced hypothermia was blocked by genetic deletion of the peripheral vestibular organ. Since hypothermia requires increase in heat loss and/or decrease in heat production, thus we examined the mechanism of hypergravity exposure-induced hypothermia considering on these phenomena. The mice were divided into 2 groups; Sham (n = 5) and Vestibular lesion (VL) (n = 5). Core body temperature was significantly decreased by -6.6 ± 0.5° C during hypergravity exposure in Sham mice. This drop in core body temperature was significantly suppressed in VL mice (-3.2 ± 0.3° C). Skin temperature measurement using a thermography camera showed increase in tail temperature in Sham but not in VL mice, suggesting that hypergravity-induced hypothermia might be due to heat loss through increasing in blood flow to the tail. This phenomenon could be mimicked using ganglion blocker, hexamethonium. Accordingly, it is possible that autonomic nervous system via the vestibular system might contribute to thermoregulation in the hypergravity environment. COI:No

2SO-08AM-5

Optogenetic manipulation of raphe serotonin neurons altered heart rate in mice

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Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter involved in a multiple brain functions including emotion and autonomic function. Previous studies have suggested that the central serotonergic system regulates the heart rate control; however, the direct causal relationship between serotonin neuron activity and hear rate control has not been clarified yet. To address this, we optogenetically manipulated serotonin neurons and monitored the heart rate in freely moving mice. We generated transgenic mice in which the opsin (step function ChR2 or ArchT) was specifically expressed in serotonin neurons by exploiting KENGE-tet system under the control of Tph2 promoter. An optical fiber was implanted upstream of the dorsal raphe nucleus. EKG was recorded in free moving and the heart rate was calculated. Illumination was applied for 3 min and the light seemed to cover both dorsal and medial raphe serotonin neurons. Wild type mice were used as controls. Our results showed that the blue light illumination increased heart rate in ChR2 expressing mice, while yellow light illumination decreased heart rate in ArchT expressing mice, indicating that the activation of serotonin neuron led to increased heart rate and vice versa. This is the first to demonstrate that serotonin neurons in the raphe nucleus are directly involved in heart rate regulation. COI:No

Student Session 4

March 29 (Thu) 10:30～11:30 Hall 9

2SO-09AM-1

The 1,25(OH) $_2D_3$-induced changes in the short-circuit current and transepithelial resistance across epithelium-like Caco-2 monolayer are inhibited by FGF-23

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Despite being the salient calcium- and phosphate-regulating hormone, 1,25-dihydroxyvitamin D_3 [1,25(OH) $_2D_3$] has been known to modulate other functions of intestinal epithelium, such as epithelial integrity, paracellular permeability, and Na^+/K^+-ATPase activity. The present study aimed to determine whether 1,25(OH) $_2D_3$ was able to alter the epithelial electrical properties in Caco-2 monolayer, as indicated by short-circuit current (I_{sc}) and transepithelial resistance (TER) in Ussing chamber. We found that 10 nM 1,25(OH) $_2D_3$ increased I_{sc} across monolayer, while TER was decreased, suggesting an increase in the electrogenic ion transport. Previous studies showed that 1,25(OH) $_2D_3$-responsive cells could produce fibroblast growth factor (FGF)-23, which in turn acted as a negative feedback regulator to antagonize the action of 1,25(OH) $_2D_3$. 10 and 100 nM 1,25(OH) $_2D_3$-treated Caco-2 cells could upregulate FGF-23 protein levels as demonstrated by western blot analysis. Exposure to recombinant human FGF-23 markedly diminished the effects of 1,25(OH) $_2D_3$ on I_{sc} and TER. Thus, the alterations of I_{sc} and TER across Caco-2 monolayer by 1,25(OH) $_2D_3$ are negated by FGF-23, which may be part of negative feedback loop in the regulation of epithelial integrity and electrogenic transport of ions across the intestinal epithelia. COI:No

2SO-09AM-2

Postnatal change of glutamatergic synaptic transmission in the jaw-closing and jaw-opening motoneurons

Shimono Shinako[1], Teshima Ruri[1], Nakamura Shiro[2], Nakayama Kiyomi[2], Mochizuki Ayako[2], Ikeda Minako[2], Inoue Tomio[2]

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Feeding greatly change during the early postnatal period in mammals in parallel with the orofacial structural development. The property of nervous system involved in jaw movement also possibly changes with orofacial development; however, it is unclear whether synaptic inputs to jaw-closing and jaw-opening motoneurons change during this period. We thus examined the electrophysiological properties of glutamatergic transmission to the rat jaw-closing and jaw-opening motoneurons in early developing rats. The experiments were performed with Wistar rat brainstem slices obtained from three age subgroups: postnatal day (P) 2-5, 9-12, and 14-17. We measured miniature excitatory postsynaptic currents (mEPSCs) mediated by AMPA receptor (AMPAR) and NMDA receptors (NMDAR) in the masseter and digastric motoneurons using whole-cell patch-clamp. There were little differences in the properties of AMPAR-mediated mEPSCs of masseter and digastric motoneurons between three age groups. In contrast, the properties of the NMDAR-mediated mEPSCs evoked at masseter motoneurons changed from P2-5 to P14-17, whereas those of NMDAR-mediated mEPSCs at digastric motoneurons were constant throughout postnatal ages. These results suggest that AMPAR- and NMDAR-mediated inputs may have distinct roles for masseter and digastric motoneurons in postnatal development of neural circuits involved in feeding behavior. COI:No

2SO-09AM-3

Mechanism of acute ethanol effects on potassium currents in human coronary artery endothelial cells

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To explain ethanol(EtOH)-induced coronary vasorelaxation and NO release, we studied whether EtOH affected K+ channels in human coronary artery endothelial cells (HCAECs) leading to NO release. We investigated the effects of acute ethanol application (1-50 mM) on HCAEC currents and its cellular mechanism using whole-cell patch clamp technique. Using whole-cell patch clamp technique, we found that acute application of 20, 30 and 50 mM EtOH significantly increased whole-cell currents at +80 mV by 33.5 + 10.3% (n=10), 50.1 + 30.0% (8) and 45.5 + 17.5% (7), respectively (% increase, mean + SEM), while 1-10 mM EtOH did not. The EtOH-induced currents were significantly inhibited by blockers of IKCa or SKCa (intermediate- and small-conductance Ca2+-activated K+ channels). When IKCa was mainly recorded (all other known HCAEC channels were blocked), EtOH significantly increased the IKCa currents by 98 + 25.11%, but could not enhance SKCa currents that were similarly isolated. Pretreatment with a non-selective adenosine receptor (AR) blocker, a specific A2AR blocker, a Gs blocker, or a PKA blocker, significantly prevented the EtOH stimulatory effect. Moreover, EtOH-induced NO release from these cells was inhibited by IKCa blocker clotrimazole. Our study is the first to demonstrate that EtOH acutely activates endothelial IKCa channel via A2AR-Gs-PKA signaling pathway, leading to enhanced NO release. COI:No

2SO-09AM-4

Zebrafish possesses three functional ROMK channels with different sensitivities to Ba^{2+}

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ROMK channel is a renal potassium channel which is responsible for K^+ recycling and secretion in human kidney. It is encoded by a single gene named *KCNJ1*. Mutations in this gene can cause Barter syndrome typeII, characterized by hypokalemic alkalosis. Zebrafish is a small freshwater fish widely used in biology and medical sciences. Both embryonic and adult zebrafish have kidneys, and they are composed of nephron-like functional units, therefore, zebrafish can be good animal models of renal diseases. Interestingly, zebrafish possesses seven *KCNJ1* orthologs while human has only one *KCNJ1* gene. We isolated all seven genes (*kcnj1a.1-6* and *kcnj1b*) and confirmed that three of them were functional when they were expressed in Xenopus oocytes. These three functional zebrafish ROMK genes (*kcnj1a.1*, *kcnj1a.2* and *kcnj1b*) and human *KCNJ1* are relatively well conserved, however, V (valine) residue right before the potassium channel selectivity filter motif "TIGYG" in human KCNJ1 is not conserved at all; "S" in *kcnj1a.1*, "A" in *kcnj1a.2* and "T" in *kcnj1b*. Because this amino acid residue is known to be responsible for external Ba^{2+} block, we examined Ba^{2+} sensitivities on the three ROMK orthologs. Among them, *kcnj1b* was the most sensitive to external Ba^{2+}, and *kcnj1a.2* was the least sensitive. Interestingly, blockade of *kcnj1a.1* was not voltage dependent at all while other two channels have an apparent voltage dependency. Although physiological functions of three ROMK channels are not yet identified, they clearly showed functional differences in external cation sensitivity. COI:No

2SO-09AM-5

Effect of sFlt-1 and preeclamptic serum on umbilical vein endothelial inward rectifier potassium current alterations

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Preeclampsia is associated with endothelial dysfunction, high soluble vascular endothelial growth factor receptor-1 (sFlt-1) levels in serum, and inward rectifier potassium (Kir) current decrease in human umbilical vein endothelial cells (HUVECs). We studied whether sFlt-1 could alter Kir currents in HUVECs. Using whole-cell patch clamp technique, we demonstrated that Kir currents were present in freshly dissociated HUVECs. Then we compared HUVEC Kir currents cultured in normal pregnant (NP) and preeclamptic (PE) sera (10%), 2.5 ng/ml sFlt-1, or 10% fetal bovine serum (control). The results showed that, at -100 mV, the control group had the largest Kir currents (-4.06 +/- 2.09 pA/pF, n = 6), followed by NP serum-treated groups (-3.42 +/- 2.40 pA/pF, 3), PE serum-treated group (-1.74 +/- 0.10 pA/pF, 3) and sFlt-1-treated group (-1.22 +/- 0.47 pA/pF, 7), respectively. Furthermore, Kir current was inversely correlated with sFlt-1 levels and sFlt-1/PlGF ratio. (PlGF = placenta growth factor.) All groups had similar cell morphology. The present study is the first to suggest that sFlt-1 had an inhibitory effect on HUVEC Kir channels, which may participate in the pathogenesis of preeclampsia. COI:No

Student Session 5

March 30 (Fri) 10:30〜11:30 Hall 8

3SO-08AM-1

The properties of spontaneous spinal activity in isolated spinal cord preparation from neonatal rat

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The spontaneous spinal activities (SSA) as fetal movements existed only fetal period and disappeared after birth, because glycine inhibited SSA (Shimomura et al, 2015). In the present study, we examined to compare the properties of SSA using isolated spinal-cord with arms and legs preparation. We compared SSA both under Ringer solution (control) and under strychnine condition (depressed glycinergic inhibition) in isolated spinal-cord preparations with arms and legs using 0-4 days-old neonatal Wistar rats. Under control condition, the arms and the legs of this preparation moved individually and observed the behavior like a roll-over. Under strychnine-condition, both right and left arms and legs of this preparations moved at same time because the alternation of arms and legs were caused by glycinergic inhibition at behavior level. It is well known that strychnine induces seizure-like activity in adult rat behavior. Interestingly, the behavior of isolated spinal-cord preparation with arms and legs did not seemed seizure-like behavior under strychnine. The movements of arms and legs were rather the rhythmic movement-like an epilepsy than shaking like a seizure-activity. These results suggested that the SSA generated in spinal cord appeared from fetal period to neonatal period continuously but disappeared because of glycinergic inhibition after birth. If the glycinergic inhibition balance was breaking after birth, abnormal SSA would become a repeated rhythm activities such as juvenile epileptic stroke. COI:No

3SO-08AM-2

Relationships between dopamine contents in the striatum and motor deficits of 6-OHDA-induced hemi-Parkinsonism model rat.

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Parkinson's disease (PD) is a neurodegenerative disease characterized by loss of dopaminergic (DArgic) neurons in the substantia nigra (SN) pars compacta. Despite that rat PD models are widely used, the correlation between DArgic neuron loss and severity of behavioral impairments of the models has not fully been elucidated. In this study, 6-hydorxydopamine (6-OHDA) was injected into the right medial forebrain bundle (MFB) to prepare hemi-PD model rats. Two weeks after injection, motor deficits were evaluated by rota-rod, open field, forepaw adjusting steps (FAS), cylinder, beam and apomorphine rotation tests. Furthermore, body weights were measured. After the evaluations, rat brains were dissected and the striatum and the ventral midbrain containing the SN were collected for DA determination by HPLC as well as preparation of total RNA for quantitative RT-PCR. We found significant correlation of apomorphine rotation test ($r=-0.57$) with striatal DA contents. However, FAS ($r=0.80$) and cylinder ($r=0.84$) tests showed stronger correlation with DA contents ($r>0.80$). Expression levels of tyrosine hydroxylase mRNA in the SN was well correlated with striatal DA levels, the mRNA may well reflect the severity of the PD model. There was also strong correlation between DA contents and apomorphine rotation test ($r=0.84$). Collectively, FAS and cylinder tests may be sensitive tests to monitor the motor deficits in the PD models and also good for quantitative evaluation of DArgic neurons loss. COI:No

3SO-08AM-3

Senescent changes in cerebral cortices of aged rats: relationships with decline in higher nervous system functions.

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The decline in cognitive, memory and motor functions in aged rats is widely known. We investigated senescent changes while comparing the cerebral cortices of the young (2 month-old) and aged (22-24 month-old) rats using biochemical and morphological techniques. Morris water maze, passive avoidance and open-field tests were employed to evaluate the decline of higher nervous system functions. The behavioral tests showed the significant decline in the spatial perception and learning ability in the aged rats. Immunohistochemical examination revealed characteristic changes at the cellular levels in the aged brains; the slight decrease in neuronal cell numbers and their irregular arrangements, increased staining of GFAP, decreased numbers of NG2 cells and microglial cells. Among these, the changes in astrocytes and microglia were prominent. For further evaluation, young and aged rat brains were dissociated into individual cells for flow cytometry analyses that showed marked decreased number of microglia and increase in the proportion of activated microglia. Although it may be probable that neurons are affected by the oxidative stresses caused by microglia in the aged brain, generation of reactive oxygen species (ROS) was less significant by microglia in aged brains than that in young ones. ROS-scavenging activity may be impaired along with aging. Collectively, these results suggest that aging of brains impair glial cell functions rather than neuronal ones. COI:No

3SO-08AM-4

Hypoxia-ischemia induced factor in the neonatal brain, insulin-like growth factor-2, promotes the differentiation of oligodendrocyte progenitor cells

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A model for neonatal white matter (WM) injury is made by hypoxic-ischemia (H-I) for 1 hour at P3, showing a specific damage of oligidendrocyte progenitor cell (OPC) and imbalance of motor coordination. We revealed that insulin-like growth factor 2 (IGF-2) was increased in the H-I area and the receptor expression was detected on the OPC in the WM. In this study, we challenged to investigate the role of IGF-2 on cultured OPC. OPC was obtained from mixed glial culture that was prepared from P1 rat brain, plated on PLL-coated slide glass at a density of $5{,}000/cm^2$, expanded with FGF-2 and PDGF for 4 days and allowed to differentiate for 6 days with CNTF and T3. IGF-2 (100 ng/ml) was applied to OPC from the start of the differentiation, followed by fixation at 2 or 6 days later. We first confirmed serial expressions of immunological markers on OPC: NG2, PDGFRα, CC-1 and CNPase. We found that the proportion of NG2-positive cells and PDGFRα-positive cells were significantly decreased while the propotion of CC1-positive cells and CNPase-positive cells were increased on the second day. A similar tendency was also observed on day 6 of differentiation. These data suggest that IGF-2 that is upregulated in the neonatal H-I brain promotes the differentiation of OPC, which is more prominent in the presence of CNTF and T3. COI:No

3SO-08AM-5

Mood stabilizing drugs activate adult neural cell-neurogenesis system

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Neural stem cells (NSCs), which produce all neurons and glial cells in the developing brain and provide new neurons in the adult brain for the olfactory bulb and the dentate gyrus of the hippocampus. These adult-born neurons play significant roles in the olfaction and some types of memory. NSCs attract much attention as therapeutic method for impaired central nervous system. However, efficient and clinically feasible strategy to activate endogenous NSCs has never been established. We have previously demonstrated that mood stabilizing drugs, which are used to treat patients with bipolar disorder, enhance the self-renewal of mouse NSCs in vitro at therapeutically relevant concentrations in the cerebrospinal fluid. The pharmacological effects of classical mood stabilizers are mediated by the activation of Notch signaling in the NSC. In this study, we examined effects of lamotrigine (LTG), a novel type of mood stabilizer, on the self-renewal of NSCs in vitro and neurogenesis in the subependymal zone, the dentate gyrus and other regions such as the cortex after chronical administration of LTG in vivo. Our data suggest that LTG promotes the self-renewal of NSCs, which are similar to classical mood stabilizers, such as valproate and carbamazepine. We are also assessing whether or not LTG-induced neurogenesis possesses impact on the mouse behavior. COI:No

Student Session 6

March 30 (Fri) 10:30〜11:30 Hall 9

3SO-09AM-1

Distant metastasis model established in immunocompetent Wistar rats: relationship between CLIC2, MAP3K7CL and distant metastasis.

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Prevention of distant metastasis is a critical goal for cancer research. We have established a distant metastasis model using immunocompetent rats. In this model, we subcutaneously transplanted rat C6 glioma cells into the back skin of neonatal rats. Almost all rats developed one or more visible tumor masses in the lung by 5 weeks after the transplantation. C6 glioma cells expressing green fluorescent protein (GFP) also developed metastatic tumors in the lung, when they were transplanted in the back. To investigate the characteristic distinctions between metastatic tumor cells and non-metastatic ones, GFP+ C6 glioma cells were isolated using a cell sorter from the primary and metastatic tumors. The total RNA was prepared from the both tumor masses and served for analyses by next generation sequencer. Among many characteristic changes in mRNA expression, we addressed two factors expressed prominently in primary back tumors; chloride intracellular channel protein 2 (CLIC2) and MAP3K7 C-terminal like (MAP3K7CL). The results suggest that CLIC2 and MAP3K7CL are supposed of their critical involvement in the suppression of distant metastasis. To investigate whether these factors are really suppressive for metastasis, we are trying to establish both transgenic and knock-down cell lines that express the two factors at high or low levels. It is planed to transplant the established cells to the back to evaluate the effects of the two factors on metastasis. COI:No

3SO-09AM-2

Genistein Attenuated Steatohepatitis and Hepatic Apoptosis in High-fat High-fructose Diet-induced Non Alcoholic Steatohepatitis with Estrogen Deficiency Rats

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1:Dept. Physiol., Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, 2:Dept. Patho., Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

To investigate the anti-lipidemic, anti-inflammatory and anti-apoptosis effects of genistein on bilateral ovariectomized and NASH rats. Sprague-Dawley female rats (n=48) were randomly into ovariectomized (OVX) and non-ovariectomized (non-OVX) group, then divided into 3 subgroups; controls, fed with high-fat high-fructose (HFHF) diet (NASH group), and fed with HFHF diet plus daily 16 mg/kg genistein (GEN group). Liver were used for H&E, oil-red-o fat stained, FFA using colorimetry, apoptosis and NFkB expression by IHC. Serum TNF-α was evaluated by ELISA. NASH increased serum TNF-α, hepatic FFA, %NFkB positive cells, and apoptosis when compared with control ($p<0.01$). The most severe hepatic fat accumulation and necro-inflammation found in OVX with NASH. Genistein treatment decreased serum TNF-α compared with NASH groups in both non-OVX and OVX ($p<0.01$), however, apoptosis decreased only in non-OVX ($p<0.01$). Genistein reduced %NFkB positive cells in NASH rats with non-OVX and decreased hepatic FFA levels in OVX with NASH rats ($p<0.01$). Genistein decreased NASH score and more improved liver damage in OVX than non OVX group. In conclusion, estrogen deficiency is the aggravating factors that worsen steatohepatitis. Genistein attenuated hepatic fat accumulation, inflammation and apoptosis. Moreover, genistein demonstrated more effective in estrogen deficiency status. COI:No

3SO-09AM-3

α-melanocyte stimulating hormone attenuates dexamethasone-induced atrophy of C2C12 myotubes

Inamori Haruka[1], Yamaguchi Ayano[1], Kumazawa Akari[1], Sugiura Yusuke[1], Hikosaka Seiya[1], Yokoyama Shingo[1], Goto Katsumasa[1,2]

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Sarcopenia is aging is aging-associated skeletal muscle atrophy, which is caused by complexed factors including increased oxidative stress. On the other hand, α - melanocyte stimulating hormone (α -MSH), an essential factor for melanin production, is not only anti-inflammatory factor via down-regulation of pro-inflammatory cytokines but also anti-oxidant molecules. However, the effects of α MSH on skeletal muscle cells remain unclear. In the present study, therefore, we investigated the effects of α -MSH on skeletal muscle atrophy using a dexamethasone-induced muscle atrophy model. On 3 rd day of differentiation of Mouse myoblasts-derived C2C12 cells, α -MSH and/or dexamethasone were administrated for 48 h. The diameter, protein content and myosin heavy chain (MHC) expression of myotubes were evaluated. Dexamethasone caused to decrease in the diameter, total protein content and fast type MHC of C2C12 myotubes. α -MSH rescued dexamethasone-associated changes in C2C12 myotubes. Evidences suggest that α -MSH may be an anti-atrophic factor on oxidative stress-associated skeletal muscle atrophy. This study was supported, in part, by JSPS KAKENHI (16K13022), the All Japan Coffee Association, the Science Research Promotion Fund from the Promotion and Mutual Aid Corporation for Private Schools of Japan, and Graduate School of Health Sciences, Toyohashi SOZO University. COI:No

3SO-09AM-4

Insulin treatment does not restore type 2 diabetes-impaired bone microstructure in Goto-Kakizaki rats

Aeimlapa Ratchaneevan[1], Wongdee Kannikar[2,4], Charoenphandhu Narattaphol[1,2,3]

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Type 2 diabetes mellitus (T2DM) is a common metabolic disorder that negatively affects calcium and bone metabolism. Although chronic hyperglycemia is detrimental to osteoblasts and can aggravate osteoporosis, the effects of T2DM on bone mineral density (BMD) are still controversial due to the increased body weight or obesity that can increase BMD. Non-obese T2DM Goto-Kakizaki (GK) rats were used in the present study to investigate calcium homeostasis and bone microstructure during diabetic progression. In young adult GK rats, growth retardation, hyperglycemia, low plasma calcium, decreased trabecular BMD, and osteopenia were clearly observed. Since insulin is able to alleviate T2DM and induce anabolic effect on bone, we further demonstrated whether insulin treatment could alter bone structure in GK rats. Although 8- to 12-week insulin administration was found to decrease blood glucose and partially increased plasma ionized calcium in GK rats, it could not restore bone structure in trabecular or cortical sites. Thus, it is likely that early prevention of pre-diabetic condition and T2DM is better than late treatment for diabetic osteopathy. COI:No

3SO-09AM-5

5-Aminolevulinic acid attenuates atherosclerotic plaque progression.

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1:NDMC, Tokorozawa, Japan, 2:SBI Pharmaceuticals Co., Tokyo, Japan

Background: 5-Aminolevulinic acid (ALA) is an intrinsic amino acid and is metabolized in mitochondria into heme. Recently, it has been reported that ALA can suppress inflammation. Therefore, we examined whether ALA inhibits the formation of atherosclerotic plaque.
Methods: LDL receptor knockout (LDL-R KO) mice were fed the following diets for 12 weeks after 16 weeks of normal chow diet (CD): normal CD, CD with 1.25% cholesterol (Ch), CD with 1.25% cholesterol and ALA of which the content was estimated to correspond to 10 mg/kg/day (Ch+ALA), and CD with 1.25% cholesterol and ezetimibe (10 mg/kg/day) (Ch+EZ). At the end of the protocol, the area of atherosclerotic plaque was evaluated by oil red O staining. Serum levels of total cholesterol (T-C), LDL cholesterol (LDL-C), triglyceride (TG) and oxidized LDL cholesterol (ox-LDL-C) were also measured.
Results: The total area of atherosclerotic plaque in the aorta was significantly smaller in mice fed Ch+ALA (32±5% of the whole aortic surface) than in mice fed Ch (39±4%)*. However, this anti-atherosclerotic effect was more modest than that seen in mice fed Ch+EZ (12±5%). Serum lipids in mice fed Ch were increased (LDL-C: 379±49 mg/dl, TG: 195±49 mg/dl, ox-LDL-C: 422±64 pg/ml), while Ch+ALA retained intermediate levels of LDL-C (325±70 mg/dl) *, TG (141±44 mg/dl)* and ox-LDL-C (384±39 pg/ml). These results suggest that ALA intake exerts an antioxidant effect on LDL cholesterol. *; $p<0.05$.
Conclusions: ALA attenuates atherosclerotic plaque progression in LDL-R KO mice. COI:No

Poster Presentations

Day 1

March 28 (Wed), 12:30 – 14:00

1P-001－1P-007	Behavior Science･Biorhythm (1)
1P-008－1P-023	Neuron･Synapse (1)
1P-024－1P-033	Sensory Function (1)
1P-034－1P-042	Neurochemistry
1P-043－1P-059	Ionic Channel・Receptor (1)
1P-060－1P-069	Cell Physiology・Molecular Physiology (1)
1P-070－1P-075	Membrane Transport
1P-076－1P-092	Heart・Circulation (1)
1P-093－1P-098	Oral Physiology (1)
1P-099－1P-104	Digestion・Absorption
1P-105－1P-112	Nutrition・Metabolism・Thermoregulation (1)
1P-113－1P-117	Development・Growth・Aging
1P-118－1P-122	Environmental Physiology (1)
1P-123－1P-130	Pathophysiology (1)
1P-131－1P-135	Others (1)

1P-001

Cortical mechanisms underlying perceptual memory consolidation during NREM sleep

Hirai Daichi[1], Miyamoto Daisuke[1,2], Oisi Yasuhiro[1], Odagawa Maya[1], Matsubara Chie[1], Hayashi-Takagi Akiko[3], Murayama Masanori[1]

1:Behav Neurophysiol, BSI, RIKEN, Saitama, Japan, 2:Dept Psychiatry, Univ Wisconsin-Madison, WI, United States, 3:Lab Medical Neurosci, Gunma Univ, Maebashi, Japan

Non-rapid eye movement (NREM) sleep is essential for the consolidation of motor and sensory learning experiences in animals. Recently, we found that top-down cortical information flow during NREM sleep is required for perceptual memory consolidation (Miyamoto et al., 2016, *Science*). We also found that the top-down projection from the M2 secondary motor cortex neurons to the S1 primary somatosensory cortex initiated dendritic activity and persistent firing of S1 layer 5 (L5) neurons (Manita et al., 2015, *Neuron*). The dendritic activity may cause an increase in intracellular concentrations of Ca^{2+}, which acts as a secondary messenger in neurons and induces an activity-dependent increase or decrease in synaptic strength. Therefore, we hypothesized that the activation of such top-down circuits during NREM sleep induces dendritic activities and the subsequent growth of dendritic spines in individual pyramidal neurons. To test this hypothesis, transgenic mice with cortical layer 5-specific expression of GCaMP6s (*Rbp4-Cre; syn-flex-GCaMP6s*) were used to perform a perceptual memory task. We measured Ca^{2+} activity during NREM sleep in single dendrites of L5 pyramidal neurons in these animals. In the present study, we will discuss the influence of the relationship between dendritic activities and spine growth on memory consolidation. COI:Properly Declared

1P-002

Unusual social behavior in HPC-1/syntaxin1A knockout mice

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HPC-1/syntaxin1A (STX1A) is known as a neuronal t-SNARE which regulates synaptic transmission. Previously, we reported that STX1A gene knockout mice (STX1A KO) showed reduction of monoamine release and neuropeptide release but GABAergic and glutamatergic transmission was almost normal. STX1A KO showed neuropsychological abnormalities, suggesting relation between STX1A and human disorders. Interestingly, we found that some ASD patients were haploid for the STX1A gene (Kofuji eta al 2017).

Here, we focused on the social behavioral profiles in STX1A KO. In social interaction test, STX1A KO did not showed a characteristic decline in the time interacting with intruder mouse unlike in WT (Fujiwara et al 2010). In social novelty preference test, discrimination of novel and familiar mouse was impaired (Fujiwara et al 2016). We also analyzed social buffering (SB) which is a phenomenon that presence of affiliative conspecific reduces stress response. In WT, conditioned fear response was reduced in the subject mice housed with cage mate compared with the subject individually housed. However, in STX1A KO, the SB was suppressed in both of male and female. These results suggested that STX1A KO exhibited unusual social behavioral profiles in their home cage. In our previous study, unusual social behavior behavioral in social novelty preference test was partially recovered by DA receptor agonist or oxytocin. Then, we also analyzed if these drugs could also improve suppression of SB in STX1A KO. And we also studied the home cage behavior using Nanotag. COI:No

1P-003

The role of brain mast cells in mood and behavior

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Mast cells (MCs) are immune cells expressed in whole body. In the peripheral system, MCs play an important role in allergic reactions and immune responses. Recent studies report that the activation and population of MCs in the brain are changed in response to a wide variety of environmental stimuli. We found that MCs deficient mice (WBB 6F1 / Kit W/Wv) showed anxiety-like and depression-like behavior. However it is unclear that MCs would itself contribute to the regulation of mood and behavior, because Kit W/Wv mice present varying degrees of impairment related to intrinsic c-kit receptor function. Therefore, we investigated the role of brain MCs in behavioral modulation using by Mas -TRECK (Toxin Receptor-mediated Conditional cell Knock out) system that can selectively remove MCs by administion of diphtheria toxin (DT). We injected DT intraperitoneally into Mas-TRECK mice and confirmed deletion of MCs in both peripheral and brain. In the behavioral evaluation, we found anxiety-like and depression-like behavior in DT-injected Mas-TRECK mice. These result suggest that MCs are involved in the regulation of higher brain function including mood change. COI:No

1P-004

The functions of subclassified corticotropin-releasing factor neurons in the hypothalamic paraventricular nucleus

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CRF neurons in the PVH send axons to the median eminence as neuroendocrine cells, but they also send axons to various other brain regions. Thus, these neurons are heterogeneous and can be divided into several groups. In the present study, we classified PVH CRF neurons according to their differential projections to brain regions. First, we injected AAV [FLEX-GFP-t2A-WGA (wheat germ agglutinin)] vector to the PVH of CRF-Cre mice. GFP and WGA were selectively expressed in CRF neurons; GFP was used for tracking neural pathways and WGA for the target neurons. Potential target regions were found to be the solitary nucleus (NTS), locus coeruleus (LC), parabrachial nucleus (LPBN), dorsal raphe (DR), and lateral hypothalamus (LH). Secondly, we injected HiRet [FLEX-GFP] retrograde viral vector to each target region. GFP was expressed only in CRF neurons projecting to the injected regions. Populations of CRF neurons projecting to each target region were distributed separately. These neurons were mostly non-endocrine. Thirdly, we expressed functional protein (tetanus toxin) selectively in each type of CRF neurons to find specific physiological functions. Thus, this strategy is effective in classifying PVH CRF neurons, tracking each neural pathway, and finding specific neural functions. COI:No

1P-005

Retrieval-Induced Forgetting in Aged Mice

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Retrieval-induced forgetting (RIF) is a phenomenon that retrieval practice on a subset of target items leads to forget the other, non-target items. We have previously reported that RIF occurs in adult mice in a modified spontaneous recognition test. However, the effect of aging on RIF occurrence has not yet well known. Here, we examined the RIF occurence in aged mice. Male C57BL/6J mice, 22 months old, were used in this study. The test consisted of three phases; sample, retrieval-practice (Rp) and test. The intervals between the sample and Rp phase and between Rp and test phases were 1 h. Mice were assigned to two experimental conditions (Rp+, Rp-). In the sample phase of the Rp+ condition, mice explored a field in which two objects (A, B) were placed. In the Rp phase, one of the two objects was replaced to an object identical to the other (A, A). In the test phase, one of the objects used in the retrieval phase was replaced again to a novel object (A, X). In the Rp- condition, after the Rp phase, mice were explored two objects different from those in Rp phase, but one of which was identical to the one in the sample phase (B, Y). The discrimination index in the Rp+ condition was significantly higher than that in the Rp- condition. These results indicate that RIF also occurs in aged mice. COI:No

1P-006

Hippocampal place cell activities in the freely behaving monkey

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The hippocampal place cell increases the firing rate while animals are traversing a particular location (a place field) in an environment. The activity of place cell assemblies is considered to be a neural substrate for "the cognitive map". The place cell activity is altered by proprioceptive and vestibular inputs generated by self-motion. Although earlier studies in primates suggested the presence of place cells in primate hippocampus, these studies have not performed under freely behaving conditions. In the present study, we recorded the activity of hippocampal neurons in the macaque monkey performing a shuttle-movement task on a linear track under freely behaving condition, and investigated their spatial responsiveness and firing periodicity. As results, 18 neurons out of 83 pyramidal neurons in the CA1 showed an activity depending on monkey's location on the track and these activities were not tuned by monkey's movement-direction. Monkey's running speed was correlated positively with peak firing rate and negatively with place field size. The power spectrum of the spike autocorrelogram showed that only 2 place cells were theta-modulated. The presence of place cells in the monkey hippocampus and self-motion effects on place firing provide compelling evidence that primates also have a similar cognitive map mechanism to that of rodents; the absence of theta modulation, which is recognized as the encoding carrier of spatial information in rodent hippocampus, indicates that the primate hippocampus has a different information encoding protocol than rodents. COI:No

1P-007

Behavioral analysis of single prolonged stress-induced anxiety-related behaviors in mice

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We tried to clarify the availability of post-traumatic stress disorder (PTSD) model in mice instead of rats produced by single prolonged stress (SPS) paradigm because it's more useful that mice not only require less space to keep and smaller experiment equipments to test, but also provide a technique for useful in the field of genetic engineering. SPS paradigm is conducted in three stages: animals are restrained for 2hr and immediately afterwards undergo a 20 min forced swim. Following recuperation for 15 min, mice are exposed to ether until the loss of consciousness. To confirm the pathophysiology of PTSD, we firstly examined the hypothalamo-pituitary-adrenal (HPA) negative feedback testing at 1, 4, 8 or 12 weeks. Nextly, to investigate the PTSD-like characteristics, light / dark box test (anxiety), Y-maze task (working memory), cliff avoidance test (visual cognition), and open field test (locomotor activity) were measured at 1, 4, 8 or 12 weeks. In the light / dark box test, the spent time of light box of SPS model mice significantly decreased at 8 or 12 weeks. In the cliff avoidance test, the spent time of open-area of SPS model mice significantly decreased at only 1 week. However, both in the Y-maze test and open field test, SPS model mice showed slightly reduced tendency in the time-dependent manner until 12 weeks. Therefore, SPS mice may be useful for characteristics relevant to PTSD coincides. COI:No

1P-008

Developmental role of the spontaneous depolarization wave transiently expressed in the embryonic brainstem

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One of the earliest activities expressed within the developing central nervous system is a widely propagating wave-like activity, which we referred to as the depolarization wave. Despite considerable consensus concerning the global features of the activity, its physiological role is yet to be clarified. We previously reported that chronic inhibition of the depolarization wave by *in ovo* application of bicuculline/strychnine resulted in a reduction of polysynaptic EPSPs in the higher-order nucleus of the vagus nerve (the parabrachial nucleus: PBN), suggesting that the wave plays an important role in the synaptic network organization in the brainstem. To obtain further support for the result and to clarify whether the cholinergic wave at the early stages plays a significant role in the synaptic network organization, we performed similar experiments using a nicotinic acetylcholine receptor blocker, *d*-tubocurarine. Although monosynaptic EPSPs in the first-order nuclei of the vagus nerve were not affected by *d*-tubocurarine, polysynaptic EPSPs in the higher-order nucleus, the PBN, were significantly reduced by *in ovo* blockade of the wave. The results confirmed that the depolarization wave, especially that at the early stages, plays a significant role in the development of functional synaptic networks along the brainsem sensory pathway and that the organization of synaptic networks is not dependent on specific neurotransmitters or receptors, but rather requires a global activity pattern of the wave. COI:No

1P-009

Optical analysis of functional development of the facial motor nucleus in the embryonic rat brainstem

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In the embryonic rat brainstem, facial motoneurons are first generated in rhombomere 4 and then migrate toward the caudo-ventral direction. This migration forms a unique axonal trajectory called the "genu", a loop of facial motor axons around the abducence nucleus. However, it is still unclear when and how this unique structure is established during ontogenesis. Using voltage-sensitive dye recording, we detected neural responses evoked by facial nerve (N.VII) stimulation and examined developmental processes of the facial motor nucleus in E13-E17 rat brainstems. From comparison with morphological data obtained using the DiI staining method, we identified two types of fast spike-like signals; a long-duration signal, which corresponded to the action potential in N.VII soma, and a short-duration signal, which reflected the action potential in N.VII axons. The long-duration signal was detected as early as E13 at the level of the N.VII root, suggesting that the N.VII motoneuron is already excitable at the beginning of cell migration. The response area of the long-duration signal extended caudally at E13-E14, and moved in a ventral direction at E15. At E16-E17, the long-duration signal was concentrated in the caudo-ventral area, which was comparable to the location of the adult facial motor nucleus. These results show that the excitability of facial motoneurons is generated at a very early stage, and that the developmental processes of cell migration and nuclear organization can be visualized and identified functionally. COI:No

1P-010

Expression of aggrecan-positive perineuronal nets in the mouse cortex

Ueno Hiroshi[1], Takao Keizo[2], Fujii Kazuki[2], Suemitsu Shunsuke[3], Okamoto Motoi[4], Ishihara Takeshi[3]

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To preserve region-specific functions, certain areas of the cortex are associated with high and low plasticity over the course of development. It is thought that perineuronal nets (PNNs) regulate plasticity, but labeling for Wisteria floribunda agglutinin (WFA), which is widely used to detect PNNs, exists throughout the cortex. Considering that the plasticity of specific brain regions is highly variable over the span of an organism lifetime, it is unlikely that WFA-positive PNNs control plasticity. Some studies have suggested the possibility that aggrecan molecules regulate plasticity. However, a quantitative analysis of aggrecan-positive PNNs in the cortex has not been conducted. In this study, we focused on the quantitative measurement of aggrecan-positive PNNs and glycosylated aggrecan-positive PNNs in the mature mouse cortex. Our findings revealed the selective expression of both aggrecan-positive and glycosylated aggrecan-positive PNNs in the cortex. WFA-positive PNNs expressed aggrecan in a region-specific manner in the cortex. Furthermore, we observed variable distributions of PNNs containing WFA- and aggrecan-positive molecules. Together, our findings suggest that PNN components and function differ depending on the cortical region, and that aggrecan molecules may be involved in determining region-specific plasticity and vulnerability in the cortex. COI:No

1P-011

Activation of the metabotropic glutamate receptor subtype 1 is necessary for visual experience-dependent maintenance of synaptic connectivity in the dorsal lateral geniculate nucleus.

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In the dorsal lateral geniculate nucleus (dLGN), retinal afferents make eye-specific innervation pattern that is established around birth. During postnatal development, redundant retinogeniculate (RG) synapses are eliminated and matured synapses are maintained in a visual experience-dependent manner. The maintenance phase opens around postnatal day 20 (P20) since one week of visual deprivation from P20 causes abnormal remodeling of RG synapses. A few molecule such as MeCp2 was reported to be involved in the maintenance phase (Noutel et al., 2011) but further mechanisms were unclear. In this study, we found age-dependent increase of the metabotropic glutamate receptor subtype 1 (mGluR1) expression that reached a plateau by P20 in the dLGN. In mGluR1 knock-out (KO) mice, formation and elimination of RG synapses was normal until P20 but the synapses were remodeled in the mice older than P28 even in normal rearing condition. Importantly, pharmacological blockade or knock down of mGluR1 in the dLGN of wild-type mice triggered remodeling and activation of mGluR1 in the dLGN rescued remodeling induced by visual deprivation. These results clearly indicate that mGluR1 is crucial for the experience-dependent maintenance of mature RG synaptic connectivity. COI:No

1P-012

Establishment of the co-culture system of motor neuron-like cells NSC-34 and immortalized Schwann cells IFRS1

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Co-culture systems of neurons and Schwann cells have been used for the study of myelination and demyelination; in most of the previous studies, however, these cells were obtained by primary culture with embryonic or neonatal animals. A spontaneously immortalized Schwann cell line IFRS1 from long-term cultures of adult rat peripheral nerves has been shown to retain fundamental ability to myelinate neurites in co-cultures with adult rat dorsal root ganglion neurons and PC12 cells. Our current investigation focuses on the establishment of stable co-culture system with IFRS1 cells and NSC-34 motor neuron-like cells. NSC-34 cells were seeded at a low density ($2 \times 10^3/cm^2$) and maintained for 5-7 days in serum-containing medium supplemented with non-essential amino acids and brain-derived neurotrophic factor (BDNF; 10 ng/mL). The cells were then exposed to an anti-mitotic agent mitomycin C (1 μg/mL) for 12-16 hours, and co-cultured with IFRS1 cells ($2 \times 10^4/cm^2$). The co-culture was maintained in serum-containing medium supplemented with ascorbic acid (50 μg/mL), BDNF (10 ng/mL), and ciliary neurotrophic factor (10 ng/mL). Double-immunofluorescence staining carried out at day 28 of the co-culture showed myelin protein zero-immunoreactive IFRS1 cells surrounding βIII tubulin-immunoreactive neurites. This co-culture system can be a beneficial tool to study the pathogenesis of motor neuron diseases (e.g. amyotrophic lateral sclerosis, Charcot-Marie-Tooth diseases and immune-mediated demyelinating neuropathies) and novel therapeutic approaches against them. COI:No

1P-013

Function of metabotropic glutamate receptors in the marginal zone of neonatal rat hippocampi

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Type I metabotropic glutamate receptors (mGluRs) are 7 transmembrane-spanning receptors which couple to G_q protein and cause intracellular Ca^{2+} mobilization. Two type I mGluR subtypes, mGluR1 and mGluR5, are detected in central nervous system. These receptors regulate neuronal excitability and concern with synaptic plasticity. Hippocampal marginal zone contains early-generated neurons including Cajal-Retzius cells (C-R cells), which play an important role in migration of neurons through secretion of glycoprotein, reelin. Though function of secreted reelin has been well studied, less is known about regulation of C-R cell excitability. In the present experiments, characteristics of the Ca^{2+} mobilization by activation of mGluR1 in hippocampal marginal zone were determined. Ca^{2+} imaging was performed using acute slices of neonatal rat hippocampus. Elevation of intracellular calcium concentration ($[Ca^{2+}]_i$) was induced by glutamate application in C-R cells, and this elevation was not prevented by blockade of ionotropic glutamate receptors. Type 1 mGluR-specific agonist increased $[Ca^{2+}]_i$ in the presence of mGluR5 specific antagonist. Contrarily, mGluR1-specific antagonist prevented the $[Ca^{2+}]_i$ increment induced by type I agonist. These results demonstrate that neonatal hippocampal C-R cells express functional mGluR1. Effects of application of other excitatory neurotransmitters on $[Ca^{2+}]_i$ were also measured and compared with that of mGluR agonist in mGluR1-expressing neurons of hippocampal marginal zone. COI:No

1P-014

Novel open skull method for *in vivo* two-photon imaging of living mouse brain by utilizing fluoropolymer nanosheets

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For observing deeper regions of living mouse brain by *in vivo* two-photon microscopy, open skull method is employed to dig a hole in the skull of the mouse and to seal it with a glass cover slip. However, this surgical operation is so tricky and requires a high-level skill to make such chronical cranial windows. On the other hand, polymer thin films, or nanosheets, are known as novel nanomaterials for bioimaging and biomedical applications. Thus, we propose the use of nanosheets for living mouse brain imaging. In this study, we employed newly-developed nanosheets composed of an amorphous fluoropolymer CYTOP, that have a high level of flexibility, adhesion strength, and transparency. Such nanosheets would make surgical operations for the open skull method more simplified and facilitate long-time stable *in vivo* observations. In addition, using the nanosheets instead of the cover slip, we improved observing condition compensating the index mismatch between the brain tissue and the immersion solution in the optical path. We hope that this technique will be combined with adaptive optics devices in order to realize high-resolution deep tissue imaging. COI:No

1P-015

Wide field two-photon microscopy with cellular resolution for in vivo Ca^{2+} imaging

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Imaging a specimen with higher pixel numbers and faster sampling rate from a wide field of view (FOV) has been challenging, not only for biology, but also for other disciplines. To accomplish this mission, we selected a straightforward approach, that is, developed two-photon microscopy with a novel objective lens, tube lens and scan lenses with large pupil diameters and low aberrations over a FOV. Our microscope enables structural imaging of apical dendritic tufts and somas of Layer V pyramidal neurons in transgenic mice (Thy1-YFP line H), and also achieves in vivo two-photon functional imaging of neuronal activity with single-cell resolution. We labeled cortical excitatory and inhibitory neurons with Ca^{2+} sensor by adeno-associated virus injection into the cerebral lateral ventricles of neonatal mice. After more than 4 weeks, in vivo imaging was performed in the awake state. We also developed a new fast and accurate algorithm to automatically detect activating neuron during imaging. This algorithm could extract activities from tremendous neurons in layer 2. This microscope will give us a new possibility to understand the information processing represented by neural network consisting of single neurons. COI:No

1P-016

Drebrins critically regulate mGluR- and NMDAR-dependent LTD induction

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Drebrin is an actin-binding protein that is preferentially expressed in the brain. The embryonic-type (drebrin E) is expressed in the embryonic and early postnatal brain and is replaced by the adult-type (drebrin A) during development. In parallel, long-term depression (LTD) of synaptic transmission is dominant in the immature brain and decreases during development. Here, we report that drebrin regulates LTD induction in the hippocampus. While low-frequency stimulation (LFS) induced NMDA receptor (NMDAR)-dependent LTD in the developing hippocampus in wild-type mice, LFS induced robust metabotropic glutamate receptor 5 (mGluR5)-dependent LTD in both developing and adult brains of drebrin A knockout (DAKO) mice, in which drebrin E is expressed throughout development and adulthood. Agonist-induced mGluR-dependent LTD was normal in wild-type and drebrin null knockout (DXKO) mice; however, it was enhanced in DAKO mice, suggesting that abnormal drebrin E expression in adults induces mGluR5-dependent LTD. Furthermore, we found that LFS did not induce NMDAR-dependent LTD in developing DXKO mice, indicating that drebrins are required for NMDAR-dependent LTD. Therefore, developmental conversion from drebrin E to drebrin A prevents robust mGluR5-dependent LTD, while drebrin expression is critical for NMDAR-LTD induction. COI:No

1P-017

M1 receptor-mediated presynaptic inhibition of GABAergic transmission from striatal medium spiny neurons onto cholinergic interneurons

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We have reported M1 muscarine receptor-mediated inhibition of GABAergic transmission onto striatal cholinergic interneurons. However, the underlying mechanism is still unknown. In the present study, we examined the site of modulation a cholinergic receptor agonist, presynaptic or postsynaptic. Acute coronal brain slices were obtained from transgenic mice with restricted expression of channelrhodopsin-2 in the striatal medium spiny neurons. GABAA receptor-mediated inhibitory postsynaptic currents (IPSCs) were evoked by blue light stimulation in the presence of glutamatergic and glycinergic antagonists. Light-evoked IPSCs were inhibited by bath application of carbachol, a musucarine receptor agonist (1 μM: by 49.5 $\pm$ 7.8%, n = 5). To determine the role of postsynaptic M1 receptors in the inhibition of IPSCs by carbachol, G-protein inhibitor, GDP-β-S (2-3 mM), were loaded into cholinergic interneuron to inhibit G-protein-coupled intracellular cascade. In this condition, IPSCs were still inhibited by carbachol (by 61.3 $\pm$ 3.9%, n = 8). Finally, to examine changes in the GABA release probability, we calculated paired-pulse ratio (PPR) at before and after the application of carbachol. The average value of PPR before application of carbachol was 0.75 $\pm$ 0.04 (n = 30). The PPR was significantly increased after carbachol application (0.97 $\pm$ 0.05). These results suggest that M1 muscarine receptors inhibit GABA release from medium spiny neurons onto striatal cholinergic interneurons. COI:No

1P-018

The role of cholinergic transmission and electrical coupling in the synchronous oscillatory network of the invertebrate olfactory center.

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Synchronous oscillatory activity in a laminar structure is common in the central nervous system of both vertebrates and invertebrates. In the terrestrial slugs, periodic oscillation is recorded from the surface of the laminar structure of procerebrum (PC), olfactory center, and its frequency changes are suggested to encode the olfactory information and memory. We recently found that in vitro oscillatory neuronal network was formed from dispersed cell culture of PC neurons. In the present study, we thus examined what role cholinergic system plays in synchronized oscillatory network of cultured PC neurons. First, increases in neurite arborization and neurite connection were observed after a week in culture. Second, in calcium imaging for the PC neuron network, nicotine or acetylcholine esterase inhibitor increased the number of spontaneous calcium transients in PC neurons and induced synchronous oscillatory activity in the network. Previous our studies show acetylcholine increased frequency of LFP oscillation in the PC may be via nicotinic ACh receptors. Thus, these results suggest cholinergic system can function as a major chemical transmitter in cultured PC neuron network. It may play an essential role, such as driving force for synchronous oscillation in the olfactory neuron network. Furthermore, we report on the effects of arachidonic acid or niflumic acid for studying the electrical coupling in the PC oscillatory network. COI:No

1P-019

Involvement of syntaxin 1B in the fever-associated epilepsy syndromes.

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Two syntaxin 1 (STX1) isoforms, HPC-1/STX1A and STX1B, are coexpressed in neurons and function as neuronal target membrane (t)-SNAREs. STX1 also binds and regulates neurotransmitter transporter belonging to the solute carrier 6 gene family which include GABA transporter. We previously reported that STX1B is primarily involved in the regulation of spontaneous and evoked release of glutamatergic and GABAergic synaptic transmission. Recently, mutations in the STX1B gene have been shown to cause a broad spectrum of fever-associated epilepsy syndromes. In the present study, in order to examine involvement of STX1B in pathogenesis of fever-associated epilepsy syndromes, we assessed for susceptibility to seizures induced by systemic administration of PTZ or kainic acid in STX1B gene-ablated mice. We found that STX1B heterozygote mice showed increased susceptibility to the drugs. In vivo microdialysis showed extracellular GABA level was decreased in hippocampus of STX1B heterozygote mice. We also examined the effect of acute high temperature to burst activity of cultured neuronal networks which resembling epileptiform seizures. High temperature decreased burst frequency in wild-type neurons but not in STX1B heterozygote neurons. To clarify the influence of temperature on synaptic functions of STX1B, we examined presynaptic properties of neurotransmitter release and transporter kinetics in GABAergic synapses of hippocampal neurons. COI:No

1P-020

Analysis of synaptic functions in valproate-induced autism model marmosets

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Autism is a disorder characterized by impaired social communication. In the brain of autism patients, neurons develop abnormally high number of spines, often with irregular morphologies. We have developed an autism model common marmoset (*Callithrix jacchus*) by exposing to valproic acid (VPA) in utero. Pyramidal cells of VPA-exposed marmosets had a higher spine number than those in unexposed animals. We made acute slices of the prefrontal cortex (areas 8B and 9) of VPA-exposed and unexposed animals at 3 months of age, and performed whole-cell recording to analyze synaptic functions. The frequency of miniature EPSCs (mEPSCs) was higher in VPA-exposed animals than in unexposed animals. The amplitude of mEPSCs was smaller in VPA-exposed animals than in unexposed animals. The frequency and amplitude of miniature IPSCs (mIPSCs) were not significantly different between VPA-exposed and unexposed animals. The higher mEPSC frequency in VPA-exposed animals may reflect the increased synaptic number, while the smaller mEPSC amplitude may reflect a reduced synaptic efficacy. These results suggest functional alterations in the cortical synapses of VPA-exposed marmosets. COI:No

1P-021

The readily releasable synaptic vesicle pool in cultured superior cervical ganglion neurons

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he active zone (AZ) is a slightly electron dense region beneath the presynaptic plasma membrane where synaptic vesicles dock, fuse, and release their neurotransmitter contents into the synaptic cleft. Although each presynaptic bouton in cultured hippocampal neurons shows many vesicles, only a small fraction of which are available for immediate release of transmitters. These synaptic vesicles are classified as the readily releasable pool (RRP). Under long-term culture, sympathetic superior cervical ganglion (SCG) neurons form a well-characterized cholinergic synapse, however, the ultrastructure of AZ and the distribution presynaptic vesicles have not been analyzed. Here, we examined these by the transmission electron microscopy (TEM). The presynaptic boutons contain clear synaptic vesicles with the diameter 40-50 μm. The AZ size was estimated by the electron dense region, while the PRP size was estimated from counting the number of vesicles bridged by a filament to the AZ within 60 nm from the AZ. The data suggest that the presynaptic structure of the SCG neuron boutons resembles to that of hippocampal neurons. COI:No

1P-022

Orexin B enhances glycinergic spontaneous inhibitory transmission in adult rat spinal substantia gelatinosa neurons

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A hypothalamic peptide orexin B produces a membrane depolarization and/or a glutamatergic spontaneous EPSC frequency increase by activating orexin-2 receptors with EC_{50} values of about 0.05 μM in adult rat spinal substantia gelatinosa (SG) neurons that play a pivotal role in regulating nociceptive transmission from the periphery. The orexin B activities may increase the excitability of the SG neurons, resulting in spontaneous inhibitory transmission enhancement, a cellular mechanism for antinociception produced by orexin B, as shown for another hypothalamic peptide oxytocin. To reveal this possibility, we examined the effects of orexin B (0.05 μM) on GABAergic and glycinergic spontaneous inhibitory transmissions in the SG neurons. The whole-cell patch-clamp technique was applied to SG neurons in spinal cord slices of adult rats. In 71% of the neurons tested, superfusing orexin B for 2 min repeatedly increased the frequency and amplitude of glycinergic spontaneous IPSC. This activity was sensitive to a voltage-gated Na^+-channel blocker tetrodotoxin and orexin-2 but not orexin-1 receptor antagonist (JNJ10397049 and SB334867, respectively). On the other hand, GABAergic transmission was hardly affected by orexin B. These results indicate that orexin B produces glycinergic inhibitory transmission enhancement occurring as a result of an increase in the excitability of glycinergic neurons through orexin-2 receptor activation. This effect could contribute to the antinociception produced by orexin B. We declare no conflict of interests. COI:No

1P-023

Synaptotagmin isoforms-mediated readily releasable pool recovery in distinct dynamin isoform-dependent synaptic vesicle recycling

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Presynaptic nerve terminals must maintain stable neurotransmission despite encountering wide fluctuations in the number and frequency of incoming action potentials (APs). We have demonstrated that three isoforms of dynamin, an essential endocytic protein, work individually to match vesicle reuse pathways, having distinct rate and time constant with physiological AP frequencies. However, the molecular mechanism underlying the relationship of dynamin isoforms and sensing cell activity remains to be elucidated. Thus, we examined the role of synaptotagmin, one of the candidate Ca^{2+} sensor molecules in fast endocytosis, in activity sensing in the presynaptic superior cervical ganglion (SCG) neurons. Synaptotagmin 1 or 2 expressed in SCG neurons was acutely knocked down by microinjection of the specific siRNA. Two days later, after depletion of synaptic vesicles in the readily releasable pool by a train of 4-min APs at 5 Hz, the refilling rate was monitored by measuring the EPSP amplitude every 1 s. We found that the recovery rate of the fast phase was delayed with synaptotagmin 1 or 2 knockdown (KD), and the rate of the slow phase was delayed with synaptotagmin 2 KD. These results suggest that, in sympathetic neurons, synaptotagmin 1 mediates recovery with fast kinetics following dynamin 1-mediated endocytosis, whereas synaptotagmin 2 mediates recovery with both fast and slow kinetics following dynamin 1- and 3-mediated endocytosis, respectively. COI:No

1P-024

Effect of Acupuncture Stimulation on the Long Latency Reflexes

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The present study aimed to clarify the details of the impact of the acupuncture stimulation on the motor function. For this purpose, we examined the effect of the acupuncture stimulation on long latency reflexes (LLR).16 healthy right-handed subjects participated in the present study. We used a crossover design, with 2 acupuncture group (right LI 4 , left LI4) and control group. The acupuncture group received an acupuncture needle 180 μm in diameter at the LI4. The needle was inserted to a depth of about 10 mm and kept there for 5 min. The control group received no needle. LLR evoked by the electrical stimulation on the right median nerve at the wrist were recorded from the right adductor pollicis muscle during 20% of the maximal voluntary isometric contraction. The stimulus intensity to evoke the LLR was an intensity of the 120 % M wave threshold. LLR were measured before and during acupuncture stimulations. The appearance frequency of LLR and the amplitude ratio of LLR/M were significantly decreased during acupuncture stimulation in the acupuncture group, while these were not significantly changed in the control group. These results suggest that the supraspinal pathway may be involved in the effect of the motor function induced by acupuncture stimulation. COI:No

1P-025

Cross-modal sensory interactions in the thalamic reticular nucleus: a possible neural basis for cross-modal modulation of attention and perception

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Thalamic reticular nucleus (TRN), a cluster of GABAergic cells projecting to thalamic nuclei, plays a pivotal role in regulating sensory information processing in the thalamocortical loop circuitry. Although the TRN is composed of anatomical domains of different sensory modalities, our previous studies of anesthetized rats have revealed robust cross-modal alterations of sensory response in the TRN. The findings are summarized here to provide the overall view of cross-modal sensory interactions that were examined in TRN cell (n = 388) activities evoked by uni- or combined-sensory stimuli (white LED light, noise burst and cutaneous electrical stimulation) of two different modalities (visual-auditory, auditory-somatosensory, somatosensory-visual). TRN cells basically showed unimodal responses except for a small subset of cells responsive to both sound and skin stimulation. In either of the combinations, stimuli of a given modality altered early (onset) and recurrent late responses of the counterpart modality in the majority (75-90 %) of cells with regard to response magnitude, latency and/or burst spiking. Modulation was bidirectional and suppression predominated. Either type of TRN cell projecting to first- or higher-order thalamic nuclei showed modulation. Alterations were largely similar across sensory modalities, but there appeared to be modality-dependent effects of cross-modal modulation. Such cross-modal sensitivity of the TRN could be a crucial neural basis for cross-modal attention and perception. COI:No

1P-026

Attenuation of pCREB and Egr1 expression in the insular and anterior cingulate cortices associated with enhancement of CFA-evoked mechanical hypersensitivity after repeated forced swim stress

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In several animal models chronic stress has been reported to produce lasting hyperalgesia, which is termed stress-induced hyperalgesia (SIH). The insular cortex (IC) and anterior cingulate cortex (ACC), which are typically activated by noxious stimuli, affect pain perception through the descending pain modulatory system. In the present study, we examined the expression of phospho-cAMP response element-binding protein (pCREB) and early growth response 1 (Egr1) in the IC and ACC at 3 h (the acute phase of peripheral tissue inflammation) after complete Freunds adjuvant (CFA) injection in naive rats and rats preconditioned with forced swim stress (FS) to clarify the effect of FS, a stressor, on cortical cell activities in the rats showing SIH induced by FS. The CFA injection into the hindpaw induced mechanical hypersensitivity and increased the expression of the pCREB and Egr1 in the IC and ACC at 3 h after the injection. FS (day 1, 10 min; days 2-3, 20 min) prior to the CFA injection enhanced the CFA-induced mechanical hypersensitivity and attenuated the increase in the expression of pCREB and Egr1 in the IC and ACC. These findings suggested that FS modulates the CFA injection-induced neuroplasticity in the IC and ACC to enhance the mechanical hypersensitivity. These findings are thought to signify stressor-induced dysfunction of the descending pain modulatory system. COI:No

1P-027

Regeneration of orofacial sensory circuits following inferior alveolar nerve transection

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An accidental nerve injury in the orofacial area gives rise to various sensory disorders. However, the relationship between the symptom and regeneration process of neural circuits following the axonal injury is largely unknown.To clarify this, we developed an IAN transection (IANX) model in mice. IANX induced a significant increase in head-withdrawal threshold to noxious stimuli. The hypoalgesia persisted for 1 month after IANX, although it was partially recovered. The axotomy led to decrease of IB4-binding and CGRP immunoreactivity (IR) in trigeminal ganglion (TG) and within trigeminal subnucleus caudalis (Vc). At 2 weeks after IANX, the reduction of IB4-binding and CGRP-IR in TG was recovered to the control level, however IB4-binding within Vc did not. A retrograde tracer, Fluorogold (FG) injected was transferred to a subset of IB4-binding or CGRP-IR TG cells at 2 weeks after IANX. Three days following IANX, decreased activation of ERK to noxious stimuli was observed in Vc. Two weeks after IANX, the noxious mechanical or heat stimuli induced-ERK activation was restored to the control level, but the distribution pattern of noxious mechanical dependent ERK-activated neurons was changed in Vc.The present findings suggest the possibility that sustained reduction of IB4-biniding in TG central axon results in impaired activation of secondary neurons in the medulla after pinch stimulation, thereby lasting of sensory dysfunction associated with nerve injury. COI:No

1P-028

Deficiency of 2-methylthio modification of tRNALys(UUU) causes neuropathy

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Neuropathy is a major diabetic complication, but the molecule mechanism has been largely unknown. Recently, genetic variations in CDKAL1 gene have been associated with the development of type 2 diabetes. CDKAL1 is a methylthiotransferase that catalyzes 2-methylthio (ms2) modification of the adenosine at position 37 of tRNALys(UUU). The ms2 modification is important for accurate decoding of Lys codon in Proinsulin, and thus contributes to the glucose metabolism. In addition to Proinsulin, Lys is also critical for the processing of various neurotropic factors. Because dysregulation of neurotropic factors has been implicated in the development of neuropathy, we hypothesized that the deficiency of ms2 modification might cause aberrant translation of neurotropic factors, and leads to the development of neuropathy. To test this hypothesis, we investigated the sensory functions of peripheral nerves in Cdkal1-knockout mice. Constitutive Cdkal1 knockout mice showed hypoalgesia, which was accompanied with the loss of nerve fibers and reduction of neurotrophic factors. In contrast, the pancreatic β-cell specific Cdkal1 knockout mice did not exhibit hypoalgesia despite of glucose intolerance. These findings suggest that deficiency of Cdkal1 in nerve system is involved in the development of neuropathy. COI:No

1P-029

Involvement of the HCN channel of GN neurons in abnormal behavior after peripheral nerve injury in rats

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The aim of this study is to clarify that hyperpolarization activated cyclic nucleotide gated channel type 2 (HCN 2) of neurons in the gracile nucleus (GN) is involved in abnormal behavior following peripheral nerve injury (CCI). Abnormal behavior (flinching) during night was automatically detected and objectively evaluated using a MicroAct apparatus (Neuroscience, Tokyo, Japan). Frequencies of flinching were increased at the14, 21 and 35 days after CCI in compared with that before CCI. Almost of neurons in GN projected to the ventral posterolateral nucleus of thalamus (VPL) were immunohistochemically positive for HCN2 after CCI. Frequencies of flinching decreased for one and half one hour after intrathecal injection of ivabradine (HCN channel inhibitor, 2 μM, 20 μL) to GN. These results suggest that HCN channel of GN neurons may be involved in abnormal behavior after nerve injury. COI:No

1P-030

Recognition of the components of the binary taste mixtures in the rats denervated taste nerves

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When the rats are negatively conditioned to the binary mixed taste solutions, they can recognize the components of conditioned stimulus (CS) (Katagawa *et al.*, 2016). In the present study, we conducted behavioral studies by using the rats denervated taste nerve, such as chorda tympani nerve (CT) or glossopharyngeal nerve (GL), to investigate how these nerves play a role in this recognition of the components of taste mixtures. Wistar male rats, which were denervated CT or GL, were negatively conditioned to 30 μM quinine hydrochloride (Q) or 30mM sucrose (Suc) as CSs by using conditioned taste aversion (CTA) technic. As unconditioned stimulus (US), 0.15M LiCl (2% bw, i.p.) was used. Control rats were injected physiological saline instead of LiCl. As results, the rats denervated GL could acquire the conditioning to Q and Suc by only one pairing of CS-US. The rats denervated CT could not acquire these conditioning by one pairing of CS-US, but 2 pairing let them acquire these conditioning. CT or GL-denervated rats could find that CSs were contained in the tested mixture solutions. The number of licks in the control rats without CT or GL, which were injected physiological saline, were lower than those of non-denervated control rats. These result suggest that denervation of taste nerve does not affect the recognition of component of taste mixture, but that denervation of CT affects to acquisition of CTA. In addition, it is possible that the denervaion of taste nerve suppresses the number of licks for taste solutions in both conditioned and unconditioned rats. COI:No

1P-031

Possible roles of parabrachial neurons in pain-respiratory response in the neonatal brainstem-spinal cord-unilateral forelimb preparation

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Most of the sensation of pain signals projects to the lateral parabrachial nucleus (LPB) of the pons via the dorsal horn; and LPB is also known as the system of inspiratory-expiratory (I-E) phase switching that contributes to the control of respiratory rate. In our previous study, we suggested that nociceptive stimulation caused by capsaicin injection increased C4 inspiratory rate via LPB using 0-3-days-old rats' brainstem-spinal cord-unilateral forelimb preparations. In the present study, we investigated the properties of LPB neurons contributing to pain-respiratory relay mechanism. The respiratory activity was recorded from C4 ventral nerve, and LPB neurons were recorded by whole-cell patch-clamp method. The inspiratory neuron with I-E phase depolarization changed to the I-E neuron when it received excitation caused by nociceptive stimulation. This inspiratory neuron labeled by neurobiotin had the dendrites projected to the medial and internal PB. Non-respiratory neurons changed to tonic neurons when the excitation was received by nociceptive stimulation. These results suggested that 1) non-respiratory neurons which received excitation from nociceptive stimulation might drive the pain-respiratory relay circuit, 2) the inspiratory neuron, which switched to I-E neuron, might be contributing to pain-respiratory relay mechanism. COI:No

1P-032

Yokukansan, a Kampo medicine, prevents the development of morphine tolerance by inhibiting the secretion of orexin A

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Objectives: Yokukansan (YKS), a traditional herbal (Kampo) medicine, is effective in the treatment of pain disorders, such as headache, post-herpetic neuralgia, fibromyalgia, and trigeminal neuralgia. We have previously shown that YKS is effective against morphine analgesic tolerance in rats with morphine tolerance. It is reported that the administration of orexin receptor antagonist prevents the development of morphine tolerance and that YKS inhibits the secretion of orexin A in the hypothalamus. The present study examined whether the administration of YKS inhibited the secretion of orexin A.

Methods and results: Male Wistar rats received a subcutaneous injection of morphine hydrochloride (10 mg/kg/day) for 5 days, and the withdrawal latency following thermal stimulation was measured daily using a hot plate test. The pre-administration of YKS (started 3 days before the injection of morphine) prevented the development of morphine tolerance. The repeated administration of morphine significantly increased the serum and midbrain levels of orexin A, and the activation of astrocyte; however, these increases were significantly inhibited by the pre-administration of YKS.

Conclusion: These results suggest that the pre-administration of YKS attenuates the development of antinociceptive morphine tolerance, and that the inhibition of orexin A may be one mechanism underlying this phenomenon.

COI: No COI:No

1P-033

Intramusculary injected lidocaine reduced the repeated cold stress-induced muscular mechanical hyperalgesia through activation of TRPA1 in rats

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Intramuscular injection, such as trigger point injection, is used for the treatment of chronic myofascial pain. Several drugs such as local anesthetics, botulinum toxin and neurotropin were used. However, the analgesic mechanisms through this route are not yet clarified. Therefore, we examined the effects of i.m. lidocaine-induced analgesia (LIA) using repeated cold stress (RCS) model regarding to the extent of spread of the analgesic effect, and whether TRPA1 channel activation is involved in i.m. LIA. Furthermore we examined transmitter systems in the spinal cord involved in i.m. LIA.

RCS was applied to rats according to the previous report (Nasu et al, 2010). Muscular mechanical withdrawal threshold (MMWT) was measured by Randall-Selitto apparatus. We confirmed that MMWT in the gastrocnemius (GC) muscle was decreased after RCS. I.m. injection of lidocaine (0.01- 0.1 mg/rat in 20 μl) to GC and trapezius muscle reversed the reduced MMWT after RCS in the bilateral GCs for 1 hour. LIA was inhibited by i.m. injection of TRPA1 antagonists AP-18 and HC030031. TRPA1 agonist AITC induced muscular analgesia. These results suggest an involvement of peripheral TRPA1 channel activity in LIA. Intrathecal administration of antagonists for GABA receptors (R), serotonin R, acetylcholine R, and $\alpha_2$adrenergic R, and intraperitoneal injection of naloxone also inhibited LIA. These results suggest that i.m. lidocaine activates muscular TRPA1 channels, and this afferent input triggers endogenous pain inhibitory systems in the spinal cord. COI:No

1P-034

The role of FXIII-A activation following zebrafish optic nerve injury and its involvement in wound healing

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Generally, fish central nervous system (CNS) neurons can regenerate even after nerve transection, whereas mammalian CNS neurons cannot. We have identified many nerve regeneration associated genes (RAGs) in the fish visual system after optic nerve injury. Coagulation factor XIII A subunit (FXIII-A), a cross-linking enzyme, was identified as one of the RAGs which shows very rapid activation at the lesion site of optic nerve and injured retina in zebrafish. During the regeneration process, FXIII-A induces neurite outgrowth from injured retinal ganglion cells, and stimulates neurite elongation of the optic nerve in early stage of regeneration. However, the rapid activation mechanism of FXIII and its physiological roles are not well known. Following optic nerve injury, many glial cells accumulated at injured site and they express FXIII-A protein. Additionally, some extracellular matrix, including fibronectin, immediately upregulated after injury. In this study, we focused FXIII-A and other acute phase reactants involved in wound healing after optic nerve injury using zebrafish visual system. COI:No

1P-035

Pathophysiological Roles an Actin-Binding Protein Moesin in Primary Mouse Microglia

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Microglia (MG) are immune cells in the central nervous system. In response to inflammation, MG migrate toward the injury site and secrete nitric oxide (NO) and cytokines. Although MG represent large morphological changes between resting (ramified) and activated (ameboid) states, the regulation mechanisms have not been clear. Here, we focus on an actin-binding protein moesin, which connects between actin cytoskeleton and membrane proteins, and is involved in morphological changes and movement of cells. Recently, moesin was reported to be essential for neutrophil polarization and chemotaxis by using moesin knockout (MKO) mice (Liu X et al. J. Exp Med. 212(2): 267-280, 2015). Moesin is highly expressed in MG also. We examined the physiological roles of moesin in primary MG prepared from wild type (WT) and MKO mice. Primary culture MG were prepared from the whole brains of newborn (1 or 2 days after birth) WT and MKO mice, and collected as floating cells by gentle shaking. MG were identified by immunofluorescence with anti-Iba1 antibody. By the treatment with LPS, NO and TNF-α secretion was stimulated, and iNOS expression was up-regulated in the WT MG. However, the levels of NO secretion and iNOS expression were significantly lower in MKO compared with WT MG. Transwell assays showed that migration and chemotaxis response toward adenosine diphosphate (ADP) were significantly decreased in MKO MG. These results show that moesin is involved in expression of iNOS and NO secretion stimulated by LPS, and migration and chemotaxis toward ADP in MG cells. COI:No

1P-036

Contribution of opioids to the responses of dopamine release in the nucleus accumbens to tactile stimulation in rats.

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Tactile stimulation produces positive emotions such as pleasantness; however, its brain mechanisms had not been elucidated. In this regard, we have shown in rats that tactile stimulation increases dopamine (DA) release in the nucleus accumbens (NAc) (Maruyama et al., 2012), which plays a pivotal role in the occurrence of the positive emotions. On the other hand, brain opioids are also involved in the positive emotions. Thus, present study aimed to clarify the involvement of brain opioids in the responses of DA release in the NAc to tactile stimulation. Especially, we focused on the opioids in the NAc. For this purpose, naloxone, a non-selective opioid receptor antagonist, was administered not only systemically but also locally into the NAc. The present study was performed in conscious rats, and employed in vivo microdialysis technique and high-performance liquid chromatography for measuring DA release in the NAc. Tactile stimulation was applied to the abdominal area for 5 min. Local infusion of naloxone into the NAc significantly diminished the tactile stimulation-induced DA response, while systemic treatment with naloxone totally blocked the DA release. The present study demonstrates that opioids in the NAc and those other than NAc are involved in the responses of DA release to tactile stimulation in the conscious rats. COI:No

1P-037

Generation and characterization of glutamate decarboxylase knockout rats

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GABA is a major neurotransmitter in the mammalian CNS. Glutamate decarboxylase (GAD) is the enzyme responsible for the production of GABA. The two isoforms GAD65 and GAD67 are encoded by separate genes. They are distinguished by their molecular masses and their subcellular distributions. GAD65 knockout mice showed an increase in susceptibility to seizures and approximately 25% of the knockout mice died till 6 months of age. On the other hand, GAD67 knockout mice were shown to die of cleft palate. However, the size of the mouse is a potential limitation for some types of physiological monitoring, behavioral testing, brain mapping and repeated blood sampling. To overcome the problems, we generated GAD65 and GAD67 knockout rats using TALEN and CRISPR-Cas genome editing, respectively. Western blot analysis demonstrated that GAD65 and GAD67 proteins were not detected in the GAD65 and GAD67 knockout brains, respectively. GAD65 knockout pups began to exhibit spontaneous seizures during the third postnatal week, and more than 80% of GAD65 knockout rats died until postnatal day 23. GAD67 knockout rats survived after 8 weeks of age, but had smaller body than their wild-type littermates. The different severity in phenotypes between GAD knockout rats and mice will be discussed. COI:No

1P-038

A novel biosensor for real-time measurement of dopamine is stable at least 7 days in vivo

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Fast-scan cyclic voltammetry (FSCV) is a technique for measuring monoamine neurotransmitter. Because of the electrochemical similarity among monoamines, it is difficult to differentiate them using FSCV. We aimed to produce a novel electrode that can discriminate the dopamine (DA) from other monoamines such as serotonin (5-HT) and norepinephrine (NE). A novel electrode coated with a double membrane comprising the ion exchanger Nafion and MAO-B-diluted cellulose was evaluated the discriminative capacity of the electrode for DA against 5-HT and NE in vitro. The currents in vitro when we added 1 μM each of DA, 5-HT, and NE to PBS revealed that the biosensor selectively detected DA. The probe was implanted in the striatum to investigate whether it could selectively detect changes in the DA content in vivo. It revealed that the probe could detecte both the tonic change induced by methamphetamine administration and the phasic change induced by electrical stimulation of the medial forebrain bundle. In contrast, the electrode in the 6-hydroxydopamine-lesioned striatum did not respond to systemic selective serotonin or serotonin/norepinephrine reuptake inhibitors, confirming its selectivity. It was also revealed that the probe in the striatum could still detect changes in the DA level 1 week after electrode implantation. Data suggest that the novel biosensor can measure real-time changes in DA levels in vivo with a relatively high signal-to-noise ratio at least for 1 week. COI:No

1P-039

RFP-dependent Cre recombinase and its application to glucocorticoid receptors

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Glucocorticoid receptors (GRs) are deeply involved in stress responses. Social defeat stress induces GR activation, resulting in expression of GR-related genes. After binding of ligands, GRs form dimer and translocate into the nucleus. Fluorescent proteins have been be used to detect translocation of GRs, but its observation of freely moving animals is still difficult. In this study, we used dimer-dependent RFPs (ddRFPs) in order to detect dimerization of GRs by fluorescence intensity. ddRFPs are fluorgenic proteins that emit red fluorescence upon its dimerization. We also generated RFP-dependent Cre recombinase using RFP-specific nanobodies and DARPins. Dimer formation of ddRFP by GR activation induced dimeric RFP-dependent Cre activity. These results may provide a new method to detect GR activation in living animals. COI:No

1P-040

Variant differentiation of cultured neurosphere cells in glia- or VPA-treated glia-conditioned medium

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The abnormality of interaction between neuron and glia is one of the causes ofdevelopmental disorders such as autism spectrum disorder (ASD). Valproate (VPA) is awidely used anti-epileptic drug, whereas it is known as an inducer of ASD. Weobserved some abnormalities of cerebellar cortex in ASD model rats maternallyadministered 600 mg/kg VPA p.o. at embryonic day 16 (E16). In this study, weexamined the effect of glia and VPA-treated glia to the differentiation of neurospherederived from E16 rat cerebrum. Neurosphere was differentiated on the confluent ratcerebellar glial cells derived from native or VPA-administrated newborn rats. Glia-conditioned differentiation medium (GCM) were prepared with each confluent glial cellmedium. We observed neurosphere cells migrated away through the glial fibrousscaffold and accelerated their differentiation. Neuronal differentiation in GCM wasdifferent from it in normal differentiation medium. We would compare this glia-relatedneurosphere differentiation with the VPA-administered glia-related one. COI:No

1P-041

Altered gut microbiota observed in valproate-administrated autistic model rats

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Autism spectrum disorder, ASD is a severe neurodevelopmental disorder which includes a wide range of symptoms and levels of disability. There are many candidate chemicals which induce ASD in the administrated offspring. One of the inducers is valproate, VPA which is an anti-epileptic drug. Recently the link between gut microbiota with brain disease was reported. In this research, we aim to reveal the alteration in the gut microbiota of autistic model rat administrated 600 mg/kg of VPA to gestational day 16, G16. The stools from the rats were collected during their pregnancy. Then, DNA was prepared and purified from the stool samples by using QIAmp DNA Stool Mini Kit. Purified DNA was then subjected to 16S rRNA sequencing using MiSeq (Illumina), and the data were then analyzed using a DADA2 package as described by Callahan et al., 2016. The result reveals that the gut microbiota is different in between the control and the autistic model rat. The phylum *Bacteroidetes* makes up most of the gut microbiota in control while the phylum *Firmicutes* is more abundant in the autistic model rat. *Firmicutes* is known to contribute to inflammation in the gastrointestinal tract. This shows the correlation between ASD with altered gut microbiota which causes inflammation during pregnancy. COI:No

1P-042

Signal crosstalk and complex formation of mGluR1 and $GABA_BR$.

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Various G protein-coupled receptors (GPCRs) localize at the synaptic membrane of neurons and play crucial roles such as regulation of synaptic transmission. Many reports suggest that multiple GPCRs form heteromeric complexes and cooperatively trigger atypical signaling that cannot be initiated by individual GPCRs on their own. Mammalian cerebellar Purkinje cells express GPCR for glutamate and gamma-amino butyric acid, type-1 metabotropic glutamate receptor (mGluR1) and B-type GABA receptor ($GABA_BR$), respectively. Our previous studies suggested that these GPCRs form complexes at the postsynaptic membrane of Purkinje cells and that agonist binding to $GABA_BR$ leads to the facilitation of mGluR1-mediated neuronal responses including the long-term depression of the postsynaptic glutamate-responsiveness, a cellular basis for cerebellar motor learning.To dissect the molecular mechanism of this inter-GPCR modulation, we created HEK293 cells heterologously expressing mGluR1 subunit and $GABA_BR$ subunits (GBR1 and/or GBR2). Co-immunoprecipitation on the extracts of the HEK293 cells and FRET analysis in the living HEK293 cells showed that mGluR1 subunit formed complexes with GBR1. These results suggest that mGluR1 and $GABA_BR$ may have an intrinsic property to form complexes and that GB1 is important for the binding of $GABA_BR$ to mGluR1. Furthermore, we showed that these receptors mutually modulate each other by direct or indirect interaction. Our finding indicates GPCR signal crosstalk occur independently of the neuron-specific cellular environment. COI:No

1P-043

Involvement of thermosensitive TRP channels in temperature-dependent microglia movement

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Microglia maintain the homeostasis of the central nervous system (CNS) through baseline surveillance and protective roles. In their activated state, microglia have an amoeboid phenotype and migrate *via* chemotaxis. Interestingly, hypothermia was shown to reduce the microglial migration induced by ischemia, suggesting a modulation of microglia movement by temperature. Although, several ion channels and transporters are known to support the microglia movement, the molecular mechanism behind the temperature-dependent movement of microglia has not yet been elucidated. Some members of the transient receptor potential (TRP) channels superfamily which exhibit thermosensitivity, constitute strong candidates for the mediation of this phenomenon. Here, we report a clear regulation of mouse microglia movement by temperature. Time lapse experiments showed a decreased microglia movement by exposure to mild cooling, while mild heating had the opposite effect. The expression pattern of thermosensitive TRP channels in mouse microglia revealed several potential targets. Among them, TRP Melastatine 4 (TRPM4) and TRP Vanilloid 4 (TRPV4) were of particular interest, and shown to be functional in microglia. By using 9-phenanthrol, an inhibitor targeting both TRPM4 and TRPV4 channels, and TRPV4 KO microglia, we observed a significantly reduced microglial movement induced by heat. All together these results suggest a role of TRPM4 and TRPV4 in the temperature-mediated microglia movement and further investigation will be required to discriminate their precise involvement. COI:No

1P-044

Characterization of TRPA1 channel from disease vector mosquitoes

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Mosquitoes have long been recognized as crucial vectors for transmission of malaria and other epidemic diseases in tropical and subtropical areas. Transient receptor potential, subfamily A, member 1 (TRPA1) channel of mosquito is a key transducer for various sensory stimuli and plays an important role in nociception. Thus, it has been thought to be a specific target for repellents. Nevertheless, physiological characteristics of TRPA1 from disease vector mosquitoes have not been systematically compared among different species. In this project, functional properties of TRPA1 isoforms from *Anopheles gambiae* (Ag), *Anopheles stephensi* (As) and *Aedes aegypti* (Aa) were characterized. Responses of mosquito TRPA1 isoforms to heat or chemicals were examined with calcium-imaging and whole-cell patch-clamp recording methods. The activity of TRPA1 splicing variants were different; TRPA1B showed higher responses to heat stimulus comparing with TRPA1A in all species. Temperature thresholds for heat-evoked activation of mosquito TRPA1B channels have been found to be quite similar among species. We also found that 14 additional amino acids are located at the N-terminus of TRPA1B, which affected its channel property. Finally, three novel mosquito TRPA1 agonists were discovered. Better understanding of functional properties of mosquito TRPA1 may inspire the design of novel mosquito repellents. COI:No

1P-045

Temperature dependent gating of TRPA1 in lipid bilayers

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Transient receptor potential ankyrin 1 (TRPA1) also known as "wasabi receptor" is unique among other thermosensitive TRP channels with both, cold and heat sensing in mice and different species, like, snake species, green anole lizard, frog and chicken. TRPA1 exhibits an unusual large number of ankyrin repeats (16-17) and is expressed in dorsal root ganglion (DRG) and trigeminal ganglion (TG) neurons. Although heat- and chemical-evoked TRPA1 activation was well studied in heterologously expressed cells, distinct heat-evoked activation of TRPA1 remained unknown. We previously reported the effect of extracellular Ca^{2+} on the heat-evoked activation of green anole lizard TRPA1 (gaTRPA1) and proposed a new model for heat-evoked activation of TRPA1. To determine heat-evoked activation mechanism of TRPA1 we focused on purified gaTRPA1 protein function upon heat stimulation in planar lipid bilayers. We expressed 2 *myc*-tagged gaTRPA1 in HEK 293T cells and purified it using magnetic beads. We observed purified gaTRPA1 protein in silver staining and western blotting. We also found gaTRPA1 activation by heat and allyl isothiocyanate (AITC), but not cold in planar lipid bilayers in the presence of Ca^{2+} ions. These results suggest that purified gaTRPA1 is functional and gated directly by heat and AITC. COI:No

1P-046

Electric fields guide axons through Ca^{2+} binding to integrin β1 subunit

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The axons of embryonic brain, spinal cord, and retina extend along the extracellular voltage gradient towards the cathode in a process known as galvanotropism. In embryonic nervous tissues, positive direct current (DC) potentials are generated by neuroepithelial cell's sodium transport, of which disruption results in erroneous path-finding of newborn neurons' axons. This suggests that electric fields play a pivotal role in orienting axons in the early period of embryonic development. I have already shown that integrin and the extracellular Ca^{2+} mediate the electric axon guidance (Yamashita, J Physiol Sci, 2017; Yamashita, J Neurol Neurophysiol, 2017). However, the role for Ca^{2+} in the regulation of integrin activities was unknown. Here I show that Ca^{2+} binding to integrin β1 subunit is crucial for axon orientation. Retinal strips of chick embryos were embedded in Matrigel, and cultured in the electric field of the same strength as that *in vivo* (15 mV/mm). Retinal ganglion cell axons extended towards the cathode. A monoclonal anti-chicken β1 antibody (TASC and W1B10) enhanced the cathodal growth. The addition of 500 μM Mn^{2+} abolished the electric effect. Since Mn^{2+} activates integrins non-specifically by binding to "ADMIDAS" site of β subunit, and Ca^{2+} inhibits integrin by binding to this site, it was suggested that the inhibition of integrin by Ca^{2+} binding to β1 subunit underlies the electric axon guidance. Electric fields cause Ca^{2+} flow, which redistributes Ca^{2+} on the surface of axons. The asymmetric distribution of Ca^{2+} may lead to the asymmetric inhibition of integrin. COI:No

1P-047

Modulation of the voltage dependence by phosphoinositides in Two-Pore Na^+ Channel 3 (TPC3)

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Two-pore Na^+ channels (TPCs) have 2-repeats of 6 transmembrane helices. Each of the repeats corresponds to a functional unit of tetrameric voltage-gated cation channels, composed of a voltage sensor domain (S1-S4 helices) and a pore domain (S5-S6 helices). TPCs have three homologues, two of which (TPC1 and TPC2) are activated by $PI(3,5)P_2$, a phosphoinositide (PI). However, the other one, TPC3, has been believed not to be sensitive to any PIs. In this study, we re-examined whether TPC3 is modulated by PIs or not, using the TPC3 derived from *Xenopus tropicalis* (XtTPC3) by two-electrode voltage-clamp in *Xenopus laevis* oocytes expression system. XtTPC3 shows the characteristic feature, called "induction", in which long duration of depolarizing stimulus makes the activation kinetics faster. We observed that the co-expression of XtTPC3 with voltage-sensitive phosphatase from *Ciona intestinalis* (Ci-VSP), which catalyzes PIs in a voltage-dependent manner, dramatically changed the "induction" kinetics. This indicates that the voltage dependence of XtTPC3 is modulated by some of PIs. We also searched for the critical amino acid residues for the PI modulation of XtTPC3. There is a cluster of positively charged amino acid residues in S4/S5 linker in repeat-1. Arg187Gln mutant did not show the "induction" and became less sensitive to modulation by Ci-VSP. These results suggest that some of PIs modulate the voltage dependence of XtTPC3 by binding to the positively charged cluster in repeat-1. COI:No

1P-048

Role of TRPV3-ANO1 interaction in keratinocyte wound healing

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TRPV3 is a member of highly calcium-permeable nonselective cation channels. This channel is strongly expressed in skin keratinocytes, and involved in warmth sensation, itch, wound healing, and several cytokine secretions. Previous studies have shown that anoctamin1 (ANO1), a calcium-activated chloride channel (CaCC), is activated by calcium influx through TRPV1, TRPV4 or TRPA1. These TRPs-ANO1 interactions are important for TRPs-induced physiological functions. ANO1 is also expressed in epithelial cells. Therefore, ANO1 could have physiological significance with TRPV3 in keratinocytes. The aim of this study is elucidation of interaction and physiological function of TRPV3-ANO1 in keratinocytes. We investigated TRPV3-ANO1 interaction in HEK293T cells, and observed ANO1-mediated currents upon TRPV3 activation. Furthermore, we studied their functional interaction in normal human epidermal keratinocytes (NHEK). We observed chloride currents in NHEK, which were inhibited by an ANO1 blocker following camphor-induced TRPV3 activation. Moreover, these chloride currents depended on extracellular calcium. This result suggests that ANO1 interacts with TRPV3 in keratinocytes. Then we investigated effect of an ANO1 blocker with in vitro wound-healing assay by using NHEK. ANO1 blocker inhibited cell migration. Low chloride medium (4 mM Cl-) also inhibited the wound-healing process. These results indicate that chloride influx through ANO1 activity enhances the wound healing in keratinocytes. COI:No

1P-049

Early activation of volume-sensitive Cl^- current in the rabbit knee ACLT OA model.

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Background: The frequently used anterior cruciate ligament transection (ACLT) model of osteoarthritis (OA) makes use of a permanent trigger (joint instability) for inducing degenerative changes. The degeneration process is complex, but includes chondrocyte apoptosis and OA-like loss of cartilage integrity. Previously, we reported that activation of volume-sensitive Cl^- current ($I_{Cl,vol}$) mediates cell shrinkage, triggering apoptosis in rabbit articular chondrocytes.
Objective: To evaluates whether $I_{Cl,vol}$ was activated in the early stages of the rabbit ACLT OA model.
Design: Adult Rabbits underwent ACLT. Rabbits were euthanized at 2 or 4 weeks. Samples were analyzed histologically and with assays of cell volume, apoptosis and electrophysiological characterization of $I_{Cl,vol}$.
Results: At 2 and 4 weeks post ACLT cartilage appeared histologically normal. In cell-volume experiments, exposure of chondrocytes to hypotonic solution, led to a greater increase in cell size in ACLT compared to controls. Caspase-3/7 activity, an indicator of apoptosis, was elevated. Whole-cell currents were recorded with patch clamp of chondrocytes in iso-osmotic, hypo-osmotic external solutions. ACLT treatment resulted in a large increase in hypotonic-activated chloride conductance.
Conclusion: These results demonstrate the usefulness of a physiologically approach using a patch clamp analysis. It is suggests that these analysis methods would have efficacy for early diagnosis of OA. COI:No

1P-050

Functional interaction of metabotropic glutamate receptor mGlu2 with Gq-coupled monoamine receptors

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Monoamine neurotransmitters, such as serotonin, noradrenaline and dopamine, are known to play important roles in the high order brain function. Recently, Gq-coupled serotonin receptor 2a (5-HT2aR) has been shown to form a molecular complex with Gi/o-coupled metabotropic glutamate receptor 2 (mGlu2) in mammalian brain. The crosstalk between 5-TH2aR and mGlu2 has been reported to alter their signaling profiles. However, controversial results with regard to the crosstalk were also reported. Here we analyzed the functional coupling of mGlu2 with 5-HT2aR as well as other Gq-coupled receptors in HEK293T cells, by monitoring the Gq and Gi/o signaling. Gq signaling was optically evaluated as the activity of phospholipase C (PLC) which induces the translocation of pleckstrin homology (PH) domain from membrane to cytosol. Activation of mGlu2 did not induce the translocation of PH domain when expressed alone, whereas it induced the translocation when co-expressed with 5-HT2aR. Similar results were observed when mGlu2 was co-expressed with Gq-coupled adrenergic receptors (α1A-AR and α1B-AR) but not with Gq-coupled muscarinic receptors (M1R and M3R). Gi/o signaling was analyzed by recording the current through G protein gated inwardly rectifying potassium channel upon the activation of mGlu2. The amplitude of the mGlu2-induced current density was significantly decreased when co-expressed with 5-HT2aR or α1B-AR, but not with α1A-AR, M1R or M3R. These results show that the signaling profile of mGlu2 changes by the interaction with 5-HT2aR and α1-ARs, which may play some physiological roles in the high order brain function. COI:No

1P-051

Double nanodomain coupling of calcium channels, ryanodine receptors and BK channels controls generation of burst firing

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Action potentials clustered into high-frequency bursts play distinct roles in many neural computations. However, little is known about ionic currents that control the duration and probability of these bursts. We found that, in cartwheel inhibitory interneurons of the dorsal cochlear nucleus, the likelihood of bursts and the interval between their spikelets were controlled by Ca^{2+} acting across two nanodomains, one between plasma membrane P/Q Ca^{2+} channels and endoplasmic reticulum (ER) ryanodine receptors and another between ryanodine receptors and large-conductance, voltage- and Ca^{2+}-activated K^+ (BK) channels. Each spike triggered rapid Ca^{2+}-induced Ca^{2+}-release from ER immediately beneath somatic, but not axonal or dendritic, plasma membrane. Moreover, immunolabeling demonstrated close apposition of ryanodine receptors and BK channels. Our results indicate that double nanodomain coupling between somatic plasma membrane and hypolemmal ER cisterns compose an organelle for rapid control of burst generation in neurons. COI:No

1P-052

Actions of hinokitiol-related compounds on compound action potentials in the frog sciatic nerve

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We previously reported that a seven-membered ring compound hinokitiol inhibits fast-conducting compound action potentials (CAPs) recorded from the frog sciatic nerve with an IC_{50} value of 0.54 mM. Based on the observation that kojic acid and guaiazulene have no effects on CAPs, we concluded that an interaction among the isopropyl, carbonyl and hydroxyl groups of hinokitiol plays a role in the inhibitory action. In order to advance this idea, we examined the actions of hinokitiol-related six-membered ring compounds on frog sciatic nerve CAPs by using the air-gap method. Biosol and 4-isopropylphenol, which have isopropyl and hydroxyl groups bound to their six-membered ring, reduced CAP peak amplitudes with the IC_{50} values of 0.58 and 0.85 mM, respectively, which were comparable to that of hinokitiol. On the other hand, 4-isopropylcyclohexanol had an IC_{50} value (1.5 mM) smaller than that of 4-isopropylphenol, suggesting a role of pi electron systems in the CAP inhibition. Cumene and phenol, which lack the hydroxyl and isopropyl groups of 4-isopropylphenol, respectively, at concentrations less than 1 mM had no effects on CAPs. 4-tert-Butylphenol and 4-tert-amylphenol reduced CAP peak amplitudes with the IC_{50} values of 0.60 and 0.28 mM, respectively, indicating that the CAP inhibition increases in extent with an increase in the number of $-CH_2$ added to the isopropyl group. It is suggested that the hydroxyl and isopropyl groups bound to pi electron systems are important in the inhibitory action of hinokitiol on CAPs. We declare no conflict of interests. COI:No

1P-053

Crystallographic structure analysis of the ligand-binding domain of calcium-sensing receptor

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Calcium-sensing receptor (CaSR) is activated by the increase in the extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) from its normal range (1.1~1.3 mM), and induces a cell signaling to maintain the homeostasis of $[Ca^{2+}]_o$ in living bodies. CaSR belongs to the Class C G-protein coupled receptors, and the sensing of $[Ca^{2+}]_o$ increase is achieved by direct binding of Ca^{2+} at its large extracellular ligand-binding domain (LBD), followed by signal transmission to cytosol through the transmembrane domain. The Ca^{2+}-binding affinity of CaSR (EC_{50}: 2.8 mM) is favorably adjusted to detect the change in physiological $[Ca^{2+}]_o$. To reveal the mechanism of Ca^{2+}-binding, we solved the crystal structure of human CaSR-LBD in complex with Ca^{2+} at 3.3 angstrom resolution. During our structure analysis, other groups reported crystal structures of the extracellular domain of CaSR (Zhang *et al.* 2016. *Sci Adv.*; Geng *et al.* 2016. *eLife*). Our structure adopts the conformation similar to the ones assumed as an active conformation. Ca^{2+}-binding sites and binding mode are shared with the published structures, where Ca^{2+} is coordinated by partial negative charges of main chain carbonyl oxygens. On the other hand, in our structure, Cl^- is likely bound to the sites where polyatomic anions, such as phosphate ion, occupied in the reported structures. Since the polyatomic anions have been reported to serve as allosteric effectors of CaSR, our structure suggests that Cl^- also has a role in the regulation of CaSR activity. COI:No

1P-054

Functional expression of TRPV3 in the superficial layer of stratum granulosum of mouse epidermis

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In mammalian epidermis, the stratum corneum (SC), which is composed by dead cells, plays a critical role in barrier function. However, the molecular mechanism by which SC is formed from the superficial layer of the stratum granulosum (SG) is totally unknown. In this study, we focused on TRP channels that have been reported to sense physical or chemical cues such as mechanical stress, chemical exposure or a change in pH or temperature. Our RNA-seq analysis indicated that among TRPV, M, A subfamilies, TRPV3 was predominantly expressed in the superficial layer of the stratum granulosum, SG1 cells. Whole-cell patch-clamp recordings showed large TRPV3-like currents but not TRPV1, V4, M8, A1 currents in isolated SG1 cells. These results suggest that TRPV3 is functionally expressed in SG1 cells, which might play a role in the formation of SC in response to the outside cues. To our knowledge, this is the first report showing membrane currents from SG1 cells that place very superficial layer of the skin. COI:No

1P-055

FRET analyses of the effect of Phe860Glu mutation on the interaction between the N- and C- terminal cytoplasmic domains in hERG channel

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hERG channel has the EAG domain (EAGD) and the cyclic nucleotide binding homology domain (CNBHD) in the N- and C- terminal cytoplasmic regions, respectively. The interaction between the two domains is known to be critical for its characteristic slow deactivation. It was shown that the side chain of phenylalanine (Phe) 860 fills the ligand binding pocket of CNBHD in hERG channel, like cyclic nucleotides fills CNBD in CNG and HCN channels. We previously observed that Phe860Glu mutation accelerated the deactivation kinetics of hERG channel. We hypothesized that the interaction between EAGD and CNBHD in hERG channel is changed by mutation of Phe860. To examine this hypothesis, we made a construct for FRET analyses in which two fluorescent proteins are introduced just after EAGD and CNBHD. We expressed it in HEK 293 cells and performed acceptor photo bleaching analyses to evaluate FRET efficiency. In the hERG Phe860Glu mutant, the FRET efficiency was approximately two-thirds of that of WT, and the level was similar to those of mutants in EAGD (Arg56Asp) and in CNBHD (Asp803Arg) whose interaction between EAGD and CNBHD is known to be lost. The result suggests that the acceleration of the deactivation kinetics by Phe860Glu mutation is caused by the collapse of the interaction between EAGD and CNBHD. COI:No

1P-056

TRPV4 activation in Muller glia accelerates disease progression of retinal detachment

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We successfully developed an acute retinal detachment model by region specific injection of hyaluronic acid in mice. By utilizing this specific model system, we investigated the molecular mechanisms how cell death of photoreceptors was triggered by retinal detachment. We focused on the TRPV4 ion channel, which is a thermosensor, an osmosensor, a mechanosensor or a lipid sensor. We analyzed the retinal expression of TRPV4 by *in situ* hybridization, immunohistochemistry or a whole cell patch clamp recording, and confirmed the TRPV4 was functionally expressed in Muller glia as previously shown (J. Neurosci. 2012, 2014). We hypothesized that TRPV4 channels might contribute to the transduction of mechanical stress associated with retinal detachment. Retinal detachment in wild type mice induced apoptosis of rod photoreceptors but the numbers were significantly reduced (approximately 50%) in TRPV4KO mice. These results indicate that the TRPV4 activation might be involved in the photoreceptor cell death by the retinal detachment. Furthermore, we found that the TRPV4 in Muller glial cells can be activated by mechanical stimuli, resulting in release of several cytokines. We propose that retinal detachment adversely impacts the viability of photoreceptors via TRPV4-dependent cytokine release. Our study identified a novel molecular pathway that could exacerbate the effects of hypoxia on photoreceptor survival following detachment of the retina. COI:No

1P-057

Palmitoylation of CALHM3 positively controls activity of the CALHM1/3 channel

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A heteromeric calcium homeostasis modulator (CALHM) 1/3 complex consisting of CALHM1 and 3 is a voltage-gated ATP-permeable channel and serves as the *bona fide* neurotransmitter release channel in taste cells. Our previous study showed that palmitoylation, a reversible lipid modification, of CALHM1 controls the voltage-gating and lipid raft association of the homomeric CALHM1 channel. However, how palmitoylation controls the heteromeric CALHM1/3 in its life cycle is unknown. We show that CALHM3 is reversibly palmitoylated at three intracellular cysteines. Two protein acyltransferases that catalyze CALHM3 palmitoylation were identified. Palmitoylation-deficient mutation in CALHM3 alters none of its protein lifespan and formation and forward trafficking of the heteromeric CALHM1/3 channel. In contrast, the activity of CALHM1/3 channel was lowered by loss of CALHM3 palmitoylation. These results - demonstrating upregulation of CALHM1/3 channel activity by CALHM3 palmitoylation - provide an insight into dynamic regulation of physiological processes involving the channel. COI:No

1P-058

Membrane tension-assisted gating of the KcsA potassium channel revealed by the membrane sterol effects

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Membrane cholesterol is one of a major regulator for the function of ion channels. The cholesterol effects are brought by direct binding to channel molecules and/or by indirect manner such as via changes in physical properties of membrane. In this study, cholesterol effects on the KcsA channel were evaluated by applying the contact bubble bilayer (CBB) method. Various types of membrane sterol were administered into the KcsA-embedded CBB transiently and repeatedly, and the responses of the channel current were analyzed. The activation gate of the KcsA channel closed immediately upon administration of all the sterols tested. Effects of sterols on physical properties of membrane, such as bilayer thickness and tension, were also analyzed. We found that the sterol effects on the KcsA channel were non-stereospecific and the closure of activation gate correlated with reduction of the membrane tension. We estimated minimum required membrane tension as 2 mN/m for the KcsA channel to maintain its activation gate open. Our results indicate that the KcsA channel possesses inherent mechanosensitivity, and its activation is assisted by intrinsic membrane tension. COI:No

1P-059

Cav1.2 channel regulation by calmodulin and ATP: A study on genetically mutated channels

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Activity of Cav1.2 channels is regulated by intracellular Ca^{2+}. This mechanism requires the interaction of the Ca^{2+}-binding protein calmodulin (CaM) with the channels and the underlying molecular mechanisms have been extensively investigated. Although conformations of the channel-CaM complex in the resting, facilitatory and inactivated state have been proposed, they are different in various studies. In this study, we have investigated the mechanisms of the effects of CaM and ATP in distal carboxyl-terminal tail deleted channels ($\alpha 1C\,\delta$) and CaM-linked channels ($\alpha 1C\,\delta$ CaM) using the inside-out mode of patch-clamp technique. We have previously reported that the wildtype $\alpha 1C$ channel required CaM and ATP to induce its activity. The $\alpha 1C\,\delta$ with CaM (but not without CaM) and $\alpha 1C\,\delta$ CaM maintained the channel activity. These results suggest that CaM plays a crucial role in maintaining activity of Cav1.2 channels through a dynamic interaction with the channel, even without the distal carboxyl-terminal tail. ATP enhanced activity of the mutant channels. Okadaic acid, an inhibitor of PP1 and PP2A, mimicked the effect of ATP on the wildtype $\alpha 1C$ channel but not mutant channels. Fostriecin (PP2A inhibitor), but not cyclosporine A with cyclosphilin A (PP2B inhibitor mixture) substituted for ATP. Thus ATP bound to the channels seems to have 2-fold effects: first, to maintain the channels in a conformation that can be reprimed by CaM, and second, to inhibit dephosphorylation of the channels. COI:No

1P-060

Glucocorticoid receptor positively regulates transcription of FNDC5 in the liver

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Irisin is secreted by skeletal muscle during exercise and influences energy and metabolic homeostasis. This hormone is a cleaved and secreted fragment of fibronectin type III domain-containing 5 (FNDC5). Elucidation of the FNDC5 gene regulation mechanism is necessary to clarify the function of irisin as a potential therapeutic target in human metabolic diseases. Thus, we investigated the genetic and epigenetic mechanisms that regulate expression of the FNDC5 gene. FNDC5 mRNA was strong expressed in major energy-dependent human tissues, including heart, brain, liver, and skeletal muscle. Promoter analysis of the FNDC5 gene revealed that the core promoter region of the FNDC5 gene contained one CpG island that was located just upstream of the transcriptional start site for variants 2 and 3. Treatment with the histone deacetylase inhibitor sodium butyrate and the demethylating agent 5-azacytidine increased mRNA expression of FNDC5 in Huh7 cells. Prediction of transcription factor binding sites suggested that the glucocorticoid receptor was involved in the regulation of FNDC5 expression, and indeed, cortisol treatment increased mRNA expression of FNDC5 in Huh7 cells. Collectively, these findings offer insight into the genetic and epigenetic regulation of FNDC5. COI:No

1P-061

High apical calcium diminishes 1,25(OH) $_2D_3$-enhanced calcium transport across the epithelium-like Caco-2 monolayer by activating calcium-sensing receptor

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The intestinal epithelium can restrict calcium absorption when the luminal calcium concentration is markedly increased, but the underlying mechanism remains elusive. We hypothesized that calcium may activate calcium-sensing receptor (CaSR) in the apical membrane of the enterocytes, thereby inhibiting the transcellular calcium transport. Caco-2 cells were cultured on Snapwells in order that they could form an epithelium-like monolayer, and then transepithelial calcium transport was determined by Ussing chamber technique. The intestinal epithelial cells were found to abundantly express CaSR. The transepithelial calcium transport was significantly enhanced by 1,25-dihydroxyvitamin D_3 [1,25(OH) $_2D_3$]. Interestingly, the 1,25(OH) $_2D_3$-enhanced calcium transport was diminished after exposure to high apical calcium in a concentration-dependent manner. On the other hand, the negative effect of high apical calcium concentration was completely prevented by CaSR inhibitor (2.5 μM Calhex231). Therefore, it can be concluded that apical exposure to high calcium concentration inhibited the 1,25(OH) $_2D_3$-induced calcium absorption through CaSR activation, thereby preventing excessive calcium uptake as well as its detrimental consequences. COI:No

1P-062

Subtraction analysis using new orexin-Flp knock-in mice reveals physiological importance of orexin peptide

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Orexin/hypocretin neurons have been implicated for their multiple roles in the physiology which includes regulation of sleep/wakefulness and energy homeostasis. However, orexin is reported to be co-localized with dynorphin, glutamate, etc. Thus, cellular inhibition or ablation cannot subtract the function of orexin neurons from that of orexin peptide. To address this, we generated a new knock-in (KI) mice expressing flippase (Flp) recombinase and EGFP protein exclusively in orexin neurons. Histological experiment confirms that orexin was knocked out (oKO) in homozygous mice. Then we compared the active and passive electrophysiological properties of orexin neurons. Orexin neurons without orexin peptide were found to be hyperpolarized, have lower discharge rate, lower input resistance, and higher capacitance. The voltage clamp recording reveals that orexin neurons without orexin peptide receive lower glutamatergic inputs. We next compared the behavioral output of orexin neuronal activation. Physiological hallmarks of orexin neuronal activation like increased wakefulness, feeding and drinking behaviors, and metabolic parameters were found to be absent in oKO mice after pharmacological activation of orexin neurons. Taken together, our data suggest that orexin neuropeptide, not other co-transmitters, perform the vital roles of orexin/hypocretin system in physiological condition. COI:No

1P-063

Microarray analysis of electrically-modulated molecules that regulate bone marrow stromal cell (BMSC) proliferation

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Bioelectricity such as membrane potential may control stem cell's fates, proliferation and differentiation of BMSCs. We previously reported that depolarization induced by TRPC6 channel activation effectively facilitated the cell cycle progression of BMSCs. Periodic electrical manipulation of resting membrane potential (RMP) via platinum electrodes was also found enhance BMSC proliferation as well as its migration, but did not affect the expression levels of TRPC6 and Orai1 proteins or translocation of β-catenin to nuclei. In this study, to further deepen our knowledge about this effect of electrical stimulation, we performed a comprehensive analysis of electrically-modulated molecules in BMSC by using the DNA microarray technique. At the early stage of electrical stimulation (before proliferation is affected), 40 molecules were up-regulated and 12 molecules were down-regulated. These include a few miRNAs, but none which directly regulates cell proliferation cycle. These results suggest that electrically driven BMSC proliferation may involve multiple complicated processes. COI:No

1P-064

Manipulating intracellular concentration of inositol trisphosphate by photoactivatable enzymes

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A family of inositol phosphates serves as intracellular messengers to regulate a variety of cellular functions. Among them, inositol 1,4,5-trisphosphate (IP_3) is the key molecule that links the inositol phosphate metabolism pathway to the calcium signaling, by mobilizing Ca^{2+} from the intracellular stores. To investigate the physiology of IP_3/Ca^{2+} signaling in the cell of interest, an experimental method that can freely manipulate the concentration of IP_3 in living cells will be highly useful. Currently we are developing optogenetical tools to enable us to increase or decrease the intracellular IP_3 concentration by light irradiation. Phospholipase Cζ (PLCζ), a sperm-borne IP_3-producing enzyme, was split into two halves, and N- and C-terminal domains were fused with a plant photoreceptor protein phytochrome B (PhyB) and its ligand that binds to PhyB in a light-dependent manner, respectively. When expressing these proteins in mouse eggs, repetitive increases in intracellular Ca^{2+} concentration, or Ca^{2+} oscillations, were induced by red light irradiation, suggesting that the intracellular IP_3 concentration was increased due to the activation of split PLCζ by light. We constructed several fusion proteins that consist of IP_3 5-phosphatase and a light-sensitive domain of another photoreceptor protein, aiming to create a tool to decrease IP_3 concentration by blue light irradiation. The results of experiments that examined the light-dependent suppression of Ca^{2+} oscillations by the constructed proteins will be presented and discussed. COI:No

1P-065

Characterization of monoclonal antibodies against Phycocyanin

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Spirulina platensis has been studied for several biological activities. Previous studies have shown that C-phycocyanin (PC) containing protein extract of *Spirulina platensis* has anticancer effects, but its molecular mechanism remains unclear. Therefore, to reveal the molecular mechanisms, we determined making monoclonal antibodies (mabs) against PC in addition to investigating the function of PC. We obtained two established mabs (5C2 and TI) from mice immunized with C-PC. Immunocytochemistry revealed that 5C2 and TI recognized intact *Spirulina platensis*. Interestingly, these mabs appeared to recognize osteosarcoma U2OS cells. Western blotting of C-PC with these mabs also suggested that both mabs bound these constituents, revealing a few bands corresponding to the major components of PC. ELISA indicated that both mabs reacted with the entire PC fraction, whereas those almost did not bind phycocyanobilin. A mouse isotyping antibody kit showed that the isotypes of both mabs are IgM and κ. Furthermore, we are investigating the antiproliferative effect of U2OS cells treated with various concentrations of PC. COI:No

1P-066

Rapid oscillations of phagosomal pH in RAW264-derived osteoclasts

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Osteoclasts contribute to bone-remodeling by degrading bone tissues. The plasma membrane faced to bone surface is called "ruffled border", where exocytosis and endocytosis occur dynamically. The degradation products accumulated in resorption pits are endocytosed through the ruffled membrane, transported to the basal membrane, and are secreted into blood. As osteoclasts are macrophage-lineage cells, it is speculated that they develop efficient phagocytic machineries. In this study, we monitored pH of single phagosomes continually following uptake of fluorescein-conjugated zymosan particles in osteoclast-like cells generated from a macrophage cell line, RAW264. The endocytosed vesicles containing zymosan were acidified to pH < 5.6. After this initial acidification, the pH of some phagosomes was oscillated, often accompanying rapid and large transient increases of pH (pH spike). The rates of pH spikes were 50 to 100-fold faster than that of passive proton leak. The peak pH depended on the extracellular pH (6.5-7.6). Phorbol 12-myristate 13-acetate (PMA) increased the frequency of pH spikes, but did not affect the peak magnitudes. Neither extracellular pH nor PMA affected basal phagosomal pH. Addition of 10 mM NH_4Cl increased the basal pH and increased the frequency of pH spikes. Other than pH spikes, smaller and slower oscillations of phagosomal pH were often observed. These data revealed that phagosomal pH oscillates in osteoclasts, which may reflect variable modulations of phagosomal kinetics. COI:No

1P-067

Involvement of MARCKS and PLD in parotid and pancreatic amylase release

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Exocytotic amylase release occurs in parotid gland and exocrine pancreas. β-agonist-cAMP and cholecystokinin (CCK)-Ca^{2+}/diacylglycerol signalings contribute to the amylase release in parotid and pancreatic acinar cells, respectively. We have previously demonstrated that myristoylated alanine-rich C kinase substrate (MARCKS) phosphorylation is observed in both parotid and pancreatic amylase release signalings. However, a commonality of these signalings have been unclear. As a typical example, phospholipase D (PLD) is thought to be involved in parotid amylase release. In contrast, there are few reports in exocrine pancreas. Recently, it has been reported that glucagon-like peptide-1 (GLP-1) induces cAMP-dependent amylase release in mouse pancreas. Here, we investigated the commonality of the cAMP-dependent amylase release signaling, which MARCKS and PLD are involved in, between rat parotid and pancreatic acinar cells. Rat parotid and pancreatic acinar cells were prepared using trypsin and collagenase. MARCKS phosphorylation was detected by Western blotting. In parotid acinar cells, isoproterenol (IPR), a β-agonist, induced amylase release, and this effect was inhibited by MANS peptide and FIPI, inhibitors of MARCKS and PLD, respectively. IPR induced MARCKS phosphorylation and this effect was also inhibited by FIPI. In pancreatic acinar cells, amylase release was induced by GLP-1 as well as CCK. MANS peptide and FIPI inhibited the GLP-1-induced amylase release. These results suggest that the involvement of MARCKS and PLD in cAMP-dependent amylase release signaling is common in both parotid and pancreatic acinar cells. COI:No

1P-068

MAPK pathways are involved in the stimulatory effects of IFN-γ and TNF-α on K^+ channel activity in human proximal tubule cells

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The kidney proximal tubule reabsorbs about 70% of the filtered Na^+ load. The basolateral K^+ channels, as well as Na^+-K^+ ATPase, in proximal tubule cells provide a driving force for the transepithelial Na^+ reabsorption. Although the proximal tubular K^+ channels play such an important role, it is known that acceleration of K^+ channel activity could result in renal cell injury. We previously reported that proinflammatory cytokines, IFN-γ and TNF-α, stimulated the activity of an inwardly rectifying K^+ channel (Gi: 40pS, Go: 7pS) in cultured human proximal tubule cells (RPTECs). In this study, we investigated the mechanisms underlying the actions of these cytokines. In the cell-attached mode of the patch-clamp technique, an ERK inhibitor, U0126 (20 μM), blocked the stimulatory effect of IFN-γ (20ng/ml) on K^+ channel activity, whereas a p38 inhibitor, SB203580 (10 μM), blocked the effect of TNF-α (20ng/ml). In inside-out patches, ERK directly increased channel activity, suggesting that IFN-γ stimulated the channel via activation of ERK. In fact, Western blotting revealed that IFN-γ increased phosphorylated ERK. On the other hand, p38 had no effect on channel activity in inside-out patches although TNF-α caused phosphorylation of p38, suggesting that the stimulatory effect of TNF-α on channel activity was brought about by the sequential activation of p38 and its downstream factors. Taken together, the effects of IFN-γ and TNF-α on K^+ channel activity in RPTECs were differentially mediated by the MAPK pathways. COI:No

1P-069

A method to measure the amplitude of ciliary beating, CBD, in ciliated human nasal epithelial cells: enhancement of the amplitude by a [Cl-]i decrease.

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The activities of airway ciliary beating are assessed by two parameters, frequency (CBF) and amplitude of the ciliary beating. In the ciliated human nasal epitherial cells (cHNECs) in primary culture, we applied a new method measuring ciliary bend distance (CBD), which is an index of ciliary beating amplitude. In the CBD measurement using cHNECs, the replacement of Cl- with NO3- in the extracellular solution, which diminished the intracellular Cl- concentration ([Cl-]i), increased CBD, but not CBF. As decrement of [Cl-]i, CBD increased in a concentration-dependent manner. To the contrary, the addition of NPPB (a Cl- channel blocker), which increased [Cl-]i, decreased CBD and CBF. Thus, the effects of [Cl-]i on CBD appears to be different from those on CBF, that is, an [Cl-]i decrease has more potential to influence CBD than CBF. The intracellular Cl- may affect signals regulating CBD (inner dynein arms) and also CBF (outer dynein arms). COI:No

1P-070

Xenin evokes anion secretion through non-cholinergic secretomotor neurons in rat ileum

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Xenin-25 is a 25 amino acid neurotensin-related peptide produced by enteroendocrine cells and it increase the insulin release at physiological glucose concentrations. Recently, we have reported that Xenin augments duodenal bicarbonate and chloride secretion through neurotensin receptor1 (NTS1) activation on intrinsic and extrinsic afferent nerves [J. Pharmacol. Exptl. Ther., 361:151-161, 2017]. In this study, we analyzed efferent limb of xenin-induced anion secretion in rat middle ileum. Middle ileum was mounted between halves of an Ussing flux chamber. The mucosal and submucosal surfaces of the tissue were bathed 10 ml of Krebs-Ringer solution. Short-circuit current (Isc) was continuously measured and recorded on a Power-Lab System 4/26. Serosal application of Xenin-25 concentration-dependently increased in Isc (10-11 ~ 10-6 M). TTX abolished the Xenin 25-induced an increase in Isc. However, serosal Xenin-25-induced an increase in Isc was not altered by atropine or hexamethonium. These results suggest that Xenin-25-induced an increase in Isc might be linked with the activation of non-cholinergic secretomotor neurons. To identify the non-cholinergic neural pathway, VPAC1 antagonist was used. VPAC1 antagonist PG97-269 (10 μM) significantly inhibited the Xenin-25-induced response. These results suggest that Xenin 25 evokes an increase in Isc through non-cholinergic secretomotor neurons in rat ileum. (COI. No) COI:No

1P-071

Accumulation of Mn^{2+} by kidney of bivalve

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In order to detect accumulation of a heavy metal, manganese (Mn^{2+}), in the kidney of mussel, the kidney of *Mytilus galloprovincialis* was imaged by T_1-weighted magnetic resonance imaging (T_{1w}-MRI) by 7 T MRI at 20° C after immersion in Mn^{2+} containing seawater. As concentration of Mn^{2+} in seawater ($[Mn^{2+}]_{SW}$) were increased from 1 to 50 μM, T_1 relaxation rate of kidneys (R_{1K}) were increased from 3 μM $[Mn^{2+}]_{SW}$. When the mussels were immersed in 3 μM $[Mn^{2+}]$SW or higher, Mn^{2+} in the kidney ($[Mn^{2+}]_K$) was 10-15 folds concentrated compared to ambient $[Mn^{2+}]_{SW}$. In a range of $[Mn^{2+}]_{SW}$ from 10 to 50 μM, R_{1K} reached to a plateau level that corresponds to $[Mn^{2+}]_K$ of ca. 200 μM. In frog and rat heart, cardiac function decreased from Mn^{2+} concentration around 100 μM. Therefore, 200 μM Mn^{2+} might be the maximum concentration that enable the kidney to keep normal function. Using 3D T_{1w}-MRI, changes in Mn^{2+} concentration were measured in minutes duration, and found that $[Mn^{2+}]$ increased almost the same timing along the long axis of the kidney, not started from the position of connection with the renoperivascular canal of the kidney. From these results, the kidney of *Mytilus* is not only storage of the urine, but could concentrate a heavy metal ion, Mn^{2+}. COI:No

1P-072

Computer simulation of intracellular pH changes induced by NH_4^+ and AcO^- pulses in pancreatic duct cell

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Pancreatic duct cell produces HCO_3^--rich fluid secretion. The mechanisms for changes in intracellular pH (pH_c) induced by NH_4^+ and AcO^- pulses were analyzed in a mathematical cell model constructed by MATLAB/Simulink. The model was composed of bath, cell, intercellular space, and lumen. Cell stimulation was mimicked by increasing the permeabilities of Na^+-HCO_3^- cotransporter and K^+ channel in the basolateral membrane and CFTR and Cl^-/HCO_3^- exchanger in the apical membrane. Addition of 20 mM NH_4Cl to the bath caused rapid cellular alkalinization and that of 80 mM NaAcO caused rapid acidification followed by partial recovery. Subsequent removal of NH_4Cl caused rapid acidification (overshoot) and that of NaAcO caused rapid alkalinization (overshoot) followed by recovery to the baseline. Those changes were due to membrane diffusion of NH_3 or AcOH, consumption/production of H^- by intracellular buffering, and membrane H^+/HCO_3^- transport. When NH_4^+ pulse was performed under stimulation, the initial rate of pH_c recovery from acidification was accelerated from 0.56 to 1.05 pH unit/min due to the increase of basolateral HCO_3^- uptake from 38 to167 nmol/min/cm^2. When AcO^- pulse was performed under stimulation, the initial rate of pH_c recovery from alkalinization was accelerated from 0.10 to 0.25 pH unit/min due to the increase of apical HCO_3^- secretion from 39 to 166 nmol/min/cm^2. These suggest that NH_4^+ and AcO^- pulse techniques are useful to study the regulation of H^+/HCO_3^- transport in pancreatic duct cell. COI:No

1P-073

Differential regulation of Na^+/K^+-ATPase in the colonic epithelium in salt-sensitive hypertension

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We have previously demonstrated electrophysiologically that high-salt diet decreases basolateral Na^+/K^+-ATPase (NKA) activity which might lead the increased secretion of Na^+, Cl^-, and water in normotensive Sprague Dawley (SD), but not in hypertensive Dahl salt-sensitive (DSS) rats. The aim of this study is to investigate biochemically whether high-salt diet induce a differential regulation of functional NKA membrane expression in the colonic epithelium of hypertensive DSS rats compared to that of SD rats. Male DSS and SD rats were divided into two groups; one fed on a high-salt diet (DSSH, SDH), and the other fed on a regular diet (DSSR, SDR) for 4 weeks. Rats were then perfused with 4% paraformaldehyde, and the fixed colon was removed, frozen, and sliced at 10 μm in a cryostat. The cellular distribution of NKA in colonic epithelium was examined by immunolabeling techniques and the deconvolution software. The fluorescence intensity profile across the basolateral membrane and cytoplasm was compared between the colonic epithelium of SDH and SDR, or DSSH and DSSR. The relative fluorescence intensity of membrane region to the central region was lower in SDH compared with SDR, however no significant difference was found between that in DSSH and DSSR. These results imply that high-salt diet stimulates internalization of NKA in colonic epithelium of normotensive SD rats, but not in hypertensive DSS rats, mirroring our previous electrophysiological results using Ussing chamber system. COI:No

1P-074

Anion exchanger in the luminal membrane of guinea pig pancreatic duct cells

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Introduction: The HCO_3^- transport across the luminal membrane of the pancreatic duct has been proposed to be mediated by SLC26A Cl^--HCO_3^- exchangers. However, few studies have investigated the electrophysiological property of the conductance across the luminal membrane of native pancreatic duct cells. **Objective:** The aim of the present study was to identify electrogenic HCO_3^- conductance which is important for pancreatic secretion. **Methods:** We microdissected the interlobular duct from the pancreas of guinea pig and split open it to allow patch-clamp access to the luminal membrane. And then, we measured macroscopic current using inside-out patch configuration. Expression and localization of SLC26A family were determined by RT-PCR and immunohistochemical analyses. **Results:** The inward conductance was dependent on the intracellular HCO_3^- concentration and was reduced when intracellular HCO_3^- was replaced with Cl^- or gluconate or extracellular Cl^- was replaced with gluconate. The inward conductance was decreased in the presence of H_2DIDS, an inhibitor of Cl^--HCO_3^- exchangers. The interlobular duct expressed all SLC26A family members except *Slc26a5* and *Slc26a8*. SLC26A1, SLC26A4, SLC26A6, and SLC26A10 were found to be localized to the luminal membrane of the pancreatic ducts. **Conclusion:** These results demonstrate that these SLC26A Cl^--HCO_3^- exchangers may mediate the electrogenic HCO_3^- transport through the luminal membrane and may be involved in pancreatic secretion in guinea pig ducts. COI:No

1P-075

Action of protein tyrosine kinase inhibitors on the trafficking kinetics of epithelial Na^+ channels (ENaC) in renal epithelial cells: Analysis using a mathematical model

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Epithelial Na^+ channels (ENaC) play crucial roles in control of blood pressure by determining the amount of renal Na+ reabsorption, which is regulated by various factors such as aldosterone, vasopressin, insulin and osmolality. The intracellular trafficking process of ENaC regulates the amount of the ENaC-mediated Na^+ reabsorption in the kidney mainly by determining the number and the time of ENaCs existing at the apical membrane of renal epithelial cells. Using the mathematical model recently established in our laboratory, we studied the effect of tyrphostin AG1296 and AG1478 (protein tyrosine kinase inhibitors: PTKI) on the rates of intracellular ENaC trafficking. We found that PTKI significantly reduced the insertion rate of ENaC to the apical membrane by 56%, the recycling rate of ENaC by 83%, the cumulative time of an individual ENaC staying in the apical membrane by 27%, the whole life-time after the first insertion of ENaC by 47%, and the cumulative Na^+ absorption by 34%, while the degradation rate was increased to 3.8-fold by application of PTKI. These observations indicate that protein tyrosine kinases contribute to the processes of insertion, recycling and degradation of ENaC in the intracellular trafficking process. COI:No. COI:No

1P-076

Inhibition of cardiac mitochondrial fission protects against arrhythmias susceptibility in acute cardiac ischemia/reperfusion injury through increased connexin43 phosphorylation

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Excessive cardiac mitochondrial fission is a predominant cause of cardiac dysfunction during ischemia-reperfusion (I/R) injury by increased oscillation of mitochondrial membrane potential and decreased gap junctional communication via connexin43 phosphorylation. We hypothesized that the mitochondrial fission inhibitor, Mdivi-1, reduces these complications in cardiac I/R injury in rats. Twenty Male Wistar rats were subjected to I/R injury and randomly to receive either Mdivi-1 (1.2 mg/kg) or normal saline solution prior to LAD ligation. During the I/R protocol, arrhythmia scores, and mortality rate were determined. The heart was then removed to determine infarct size, cardiac mitochondrial membrane potential and cardiac connexin43 phosphorylation. Mdivi-1 attenuated the incidence of arrhythmias along with reduced mitochondrial depolarization, decreased the infarct size and increased connexin43 phosphorylation, leading to preserved cardiac function during I/R injury. These findings indicate that mitochondrial fission inhibition exerts cardioprotection by attenuating cardiac arrhythmia during acute cardiac I/R injury via increased connexin43 phosphorylation and reduced an oscillation of mitochondrial membrane potential. COI:No

1P-077

Role of phosphate transporters in vascular calcification

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Inorganic phosphate (Pi) plays an essential role in cell signaling and energy metabolism. However, elevated serum Pi results in a variety of serious disorders including cardiovascular complications. Until now, the underlying molecular mechanisms of how Pi induces vascular calcification have not been clearly elucidated. Here we investigated whether mitochondrial Pi uptake followed by reactive oxygen species (ROS) generation acts a critical role in high Pi-induced vascular calcification in rat aortic smooth muscle cells. Type III Na^+-Pi cotransporters (PiT-1/2) which are the predominant plasmalemmal Pi transporters expressed in vascular smooth muscle, were upregulated by high Pi incubation. Cellular Pi uptake elicited cytosolic alkalinization that further facilitated Pi transport into mitochondrial matrix. Increased mitochondrial Pi uptake accelerated superoxide generation (ROS), upregulation of osteogenic genes and calcific changes in aortic smooth muscle cells. Vascular calcification by high Pi was effectively prevented by mitochondrial ROS scavengers or pharmacologic blocking of mitochondrial Pi transporter. We propose that Pi transport across mitochondrial inner membrane could be a novel therapeutic target for vascular calcification and cardiovascular morbidities. COI:No

1P-078

Vagus Nerve Stimulation Exerts Cardioprotection Against Myocardial Ischemia/Reperfusion Injury Predominantly Through its Efferent Vagal Fibers

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Vagus nerve stimulation (VNS) has been shown to exert cardioprotection against myocardial ischemia/reperfusion (I/R) injury. We hypothesized that VNS exerted cardioprotection predominantly through its efferent vagal fibers. Thirty swine (30-35 kg) were randomized into 5 groups: no VNS (I/R), both vagal trunks intact (LC-VNS), left vagus nerve transection (LtVNX), right vagus nerve transection (RtVNX) and atropine (Atropine). VNS was applied at the ischemic onset (60 min) and continued until the end of reperfusion (120 min). Cardiac function, infarct size, arrhythmia score, myocardial connexin43, apoptotic, oxidative stress, inflammatory markers and cardiac mitochondrial morphology, function, biogenesis and fatty acid oxidation (MFN2, OPA1, DRP1, PGC1α and CPT1) were determined. LC-VNS exerted cardioprotection, which were abolished by atropine, via attenuation of mitochondrial dysfunction, decreased mitochondrial fission and shifted cardiac fatty acid metabolism toward beta oxidation. However, LC-VNS and LtVNX produced more profound cardioprotection, particularly infarct size reduction, decreased arrhythmia score and apoptosis and attenuated mitochondrial dysfunction compared to RtVNX. Our findings suggest that selective efferent VNS may potentially be effective in attenuating myocardial I/R injury. Moreover, VNS also required the contralateral efferent vagal activities to fully provide its cardioprotection. COI:No

1P-079

Modified Starling's hypothesis is applicable for the microcirculation in small intestine

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Starling's classical study has indicated that the difference between microvascular pressure (usually referred to as capillary pressure) and plasma colloid osmotic pressure determines the movement of fluid between the vascular system and the interstitium. For many years, tissue fluid pressure and tissue colloid osmotic pressure were thought to be unimportant because they appeared to be small and insensitive to changes in tissue hydration. On the other hand, plasma albumin is known to leak out from venular walls and then recirculate through lymph vessels to blood stream. The finding may suggest that the colloid osmotic pressure of tissues will be higher in the vicinity of the venular portion and the tissue hydrostatic pressure will be more negative, resulting in always positive net filtration force through the capillaries.To address the hypothesis of modified Starling's concept, we have attempted to (1) analyze the changes of color in the chylocyst with intravenous administration of Evans blue dye in in-vivo rabbit experiments and then (2) evaluate the effects of intragastric administration of water on lymph formation of the jejunum in in-vivo rat experiments.In conclusion, in the microcirculation of small intestine the diffusive leakage of plasma albumin is markedly observed, which produces a large amount of lymph formation. So it may be impossible for Starling's hypothesis to operate in tissues in which π_T and P_T change. COI:No

1P-080

The Roles of Interleukin-6 in the Cardiomyopathy Associated with Nuclear Envelopathy

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Backgrounds: Emery-Dreifuss muscular dystrophy (EDMD) is an inherited disorder caused by gene mutations encoding nuclear envelope proteins. EDMD is characterized by clinical triad of joint contractures, muscle weakness, and fatal cardiomyopathy with conduction defects, although the detailed mechanisms remain unknown. It was reported that interleukin-6 (IL-6) promotes cardiac fibrosis. In addition, the antibody of IL-6 receptor (MR16-1) was reported to suppress inflammation of the heart in a mouse model of the myocardial infarction, and then improved the remodeling. In this study, we investigated the roles of IL-6 in cardiomyopathy of an EDMD mouse model and the therapeutic effects of MR16 -1. **Materials and Methods**: We used C57BL/6J male mice as a wild type (WT) and Lmna p.H222P knock-in mice (H222P). We performed histological analyses of hematoxylin & eosin and Masson's trichrome staining, qRT-PCR to check gene expression of IL-6, IL-6 receptor α, gp130, and western blotting of STAT3 (Signal transducers and activator of transcriptions 3) and pSTAT3. We will compare these changes before and after the MR16-1 treatment. **Results**: Severe fibrotic changes were observed in the heart of H222P mice. mRNA levels of IL-6 was increased and STAT3 protein was increased at 24 weeks old H222P mice. The effects of MR16-1 are now examining. **Conclusions**: We suggest IL-6 involves in a part of the cardiomyopathy associated with nuclear envelopathy. COI:No

1P-081

Visualization of abnormal automatism in isolated rat atrial preparation using the cMOS camera and a voltage-sensitive absorption dye

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Using a **cMOS image sensor** (camera) together with a fast **voltage-sensitive absorption dye (NK2761)**, we have visualized the spatiotemporal pattern of abnormal automatism in the isolated rat atrial preparation. We have been recording optical action potentials simultaneously from many sites and mapped the excitation spread pattern in the isolated rat atrial preparation during the event of experimental tachyarrhythmia (**tachycardia-like excitation, TE**) using a scientific cMOS camera (Andor Zyla 5.5 10-tap) as the photodetector which was newly introduced. Using this camera, we could recorded the optical action potentials with the temporal resolution of 100 frame per second and the S/N ratio of >3. We evoked the event of TE by several shocks of tetanic stimulation, and recorded the propagation of optical action potential optically. Finally, we made movie clips of the excitation spread during events of TE. In the most cases, the circus movement of excitatory waves (i.e. **re-entry**) was observed. However, among these clips, we found the event of TE with the *abnormal automatism*, in which the optical action potential first appeared at the specific point (*ectopic focus*) in the preparation, and propagated in the preparation. The appearance of excitation was almost rhythmically (i.e. "*abnormal pacemaker*"). In small number of cases, two ectopic foci appeared simultaneously and generated excitatory waves independently ("*double pacemakers*"). The disturbance of intracellular Ca^{2+} dynamics seems to be the background of this phenomenon. COI:No

1P-082

Analysis of cardiovascular disease with ECG in nonhuman primates.

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Cardiovascular disease (CVD) is one of the common death factor in humans. Especially arrhythmia which is a problem because it cause sudden death. The Guideline of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) describes the importance of animal models including monkeys for nonclinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) induced by human pharmaceuticals. As monkeys and humans show similar ionic mechanisms of repolarization, differing from those of laboratory mice, interest in using monkeys for pharmaceutical studies has recently increased. However, there are few reports on standard values of electrocardiogram in cynomolgus monkeys, and such studies are indispensable for research. In this study, we used over 300 monkeys and the electrocardiographic data were obtained from there. As a result, we establish the electrocardiogram in cynomolgus monkeys, and establish QTc formula that can correct most accurately. In this study, QTc was clearly prolonged in cardiac disease such as dilated cardiomyopathy, and the diagnostic ability could also be confirmed. Result is very important, and using it will further develop future CVD studies including arrhythmias. COI:No

1P-083

Multi-scale regulatory mechanism of cardiomyocyte proliferation/regeneration based on low oxygen environments.

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Mammalian cardiomyocytes (CMs) lose proliferative/regenerative capacity soon after birth. Meanwhile, some amphibians such as axolotls retain regenerative capacity in adulthood. Based on the shared feature of proliferative CMs across species, we have recently shown that low oxygen (O_2) environments are crucial for active proliferation, and in mammals, O_2 elevation at birth by the onset of breathing stops cell cycle, which was mediated by novel gene Fam64a. But the functional link between external O_2 environment and internal gene regulatory networks is unclear. Here we focused on the energy source such as monosaccharides, amino acids, and fatty acids, because energy metabolism is tightly coupled with O_2 availability, and entirely switches at birth from anaerobic glycolysis to oxidative phosphorylation in CMs. We analyzed abundance of these molecules (39 amino acids and 24 fatty acids) in blood at fetal, neonatal, and adult stages in mammals (rats). Interspecies difference was also studied among amphibians (axolotls), reptiles (turtles), birds (quails), and mammals (rats). Axolotls and turtles have strong and weak CM regenerative capacity, respectively. It was considered lost in quails. Some candidate molecules were identified, which showed drastic changes across stages or species, and greatly impacted CM proliferation. These data pave the way to unravel multi-scale regulatory mechanism of CM proliferation/regeneration originating from low O_2 environments that is converged into gene regulatory networks. COI:No

1P-084

A new simple antegrade perfusion technique for isolating single cardiomyocytes from neonatal to aged mouse heart without using Langendorff perfusion system

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Langendorff-based retrograde perfusion with enzyme solution through coronary artery has been commonly utilized to isolate cardiomyocytes, because the heart comprises extensive extracellular matrix and cardiomyocytes are tightly associated with neighboring cells and highly sensitive to the mechanical stress. However, it is not technically easy to mount the aorta from mouse, especially juvenile, onto the perfusion system. We have developed a simple Langendorff-free antegrade perfusion method by clamping the aorta of excised mouse heart followed by infusion of enzyme solution via left ventricle. The all procedure was performed under microscope, which allowed to isolate single heart cells from neonatal to aged mouse. We demonstrate the quality of the isolated cells that the atrial and ventricular myocytes are morphologically normal and electrophysiologically functional, and interstitial cardiac progenitor cells (atypically-shaped cardiomyocytes ACMs) start to beat spontaneously after several days of culture. The results suggest that our new method is available to promote the research in single heart cells from mouse of any age. COI:No

1P-085

Regulation of intracellular Ca^{2+} concentration by angiotensin II in human cardiac fibroblasts

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Fibroblast is a heterogeneous cell population derived from different origins. Although fibroblasts derived from heart (cardiac fibroblasts, CFs) play a key role in fibrosis in the heart, it is not fully clarified the function of these cells. We investigated the regulation of intracellular free Ca^{2+} concentration by extracellular ATP and angiotensin II in cultured human CFs loaded with Fluo-4. Bath application of ATP caused a transient increase in intracellular Ca^{2+} in most of the cells even in the absence of extracellular Ca^{2+}, indicating a release of Ca^{2+} from intracellular stores. Angiotensin II also evoked transient increase in Ca^{2+} in CFs depending on the cell culture term. Immunostaining of angiotensin II type 1 receptor (AT1) revealed that the expression of AT1 gradually increased and spread towards the plasma membrane along with the cell proliferation. The results suggest that the sensitivity of CFs to angiotensin II is not stable but changes during the proliferative state of the CFs. COI:No

1P-086

Formation process of multinuclear beating cardiac progenitor cells, atypically-shaped cardiomyocytes (ACMs): Irregular nuclear division and occasional cell fusion

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The adult heart comprises heterogeneous cell lineages including cardiac stem or progenitor cells. We recently discovered a novel cardiac progenitor cell population in adult mouse cardiac ventricles that spontaneously develops into beating cardiomyocyte, defined as atypically-shaped cardiomyocytes (ACMs). ACMs express cardiac specific proteins and continue beating during a long time culture. However, many of the characteristics are still unclear. One of the largest characteristics of these cells is the presence of multiple nuclei although cell division and proliferation has yet to be detected. The present study examined the process of formation of multinuclear ACMs. ACMs were found to fuse with each other to become multinuclear and more complex shaped beating cells. Furthermore, using a high resolution microscope, we observed the cluster of different sizes of nuclei in the cells, caused by the irregular division of nucleus. The results suggest that the abnormal division of nucleus makes one of the obstacles to proceed cytokinesis in ACMs which is quite different from the ventricular myocytes and also other types of the cells. COI:No

1P-087

Modified regulation in the non-neuronal acetylcholine in the heart by circadian rhythmicity and sexual dimorphism

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We have thus far reported that cardiomyocytes possess a non-neuronal cardiac cholinergic system (NNCCS) regulated by a positive feedback system, indicating that cardiomyocytes produce ACh through an ACh-induced ChAT (a rate limiting enzyme gene) upregulating manner and moreover, that the system plays a critical role for maintenance of cardiomyocyte functions; however, its other regulatory mechanisms remain to be elucidated, which include the epigenetic control or regulation by the female sex steroid, estrogen. In this study, we have clarified that the NNCCS was shown to possess a circadian rhythm; its activity transactivating ChAT mRNA was upregulated in the light-off phase (a night time) via histone acetyltransferase (HAT) activity and downregulated in the light-on phase (a day time). However, disrupting the circadian rhythm altered the physiological choline acetyltransferase (ChAT) expression pattern. The NNCCS circadian rhythm may be regulated by miR-345, independently of HAT, causing decreased cardiac ChAT expression. Additionally, murine cardiac ChAT expression and ACh contents were increased more in female hearts than in male hearts. This upregulation was downregulated by treatment with the estrogen receptor antagonist tamoxifen and ovariectomy, and in contrast, estrogen reciprocally regulated cardiac miR-345 expression. These results suggest that the NNCCS is regulated by the circadian rhythm and is affected by sexual dimorphism, and it can be a therapeutically novel target to be focused. COI:No

1P-088

Mapping of hindquarter resistance responses to stimulation with glutamate or an ionotropic excitatory amino acid candidate L-cysteine in the rat ventrolateral medulla

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The rostral ventrolateral medulla (RVLM) includes lumber and adrenal pre-sympathetic motor neurons, which activation will produce opposite responses of vasoconstriction versus vasodilation via adrenaline. A pressor response site in the RVLM is usually defined by glutamate microinjections. However, there has been less focused on a site for vasodilation of adrenaline. Therefore, we aimed to find sites within the rat RVLM where microinjections of glutamate or an ionotropic excitatory amino acid (EAA) candidate L-cysteine produce the vasodilation, by mapping of their responses. Anesthetized ventilated rats were opened a window above the ventral medulla and measured terminal aortic blood flow, monitoring arterial blood pressure (ABP). Buffer nerves were cut for only rats receiving glutamate. Glutamate microinjections produced vasodilator and/or depressor responses in several sites within the RVLM pressor area in seven baroreflex-blocked rats, while L-cysteine microinjections produced similar responses in 11 baroreflex-intact rats. A final summarized map showed intermingled sites for vasodilator and vasoconstrictor responses in either experiment. Those results indicate that there are the possible distinguishable sites for adrenaline release within the RVLM, but intermingled with sites for vasoconstriction. Ionotropic EAA receptors in the RVLM could be involved in adrenal medulla regulation. COI:No

1P-089

Effects of stretch-induced reactive oxygen species on single cell mechanics in mouse ventricular cardiomyocytes.

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In quiescent cardiomyocytes, cellular stretch activates NADPH oxidase (NOX) 2 to produce reactive oxygen species (ROS), which stimulates ryanodine receptors to increase Ca2+ spark rate. Although the stretch-induced ROS modulates Ca2+ handling, its role in excitation-contraction coupling is not clear. In the present study, we investigated the effects of stretch-induced ROS on single cell mechanics.Ventricular cells were enzymatically isolated from either 8-12 week old mice (WT) or NOX2 knock out (KO) mice hearts. The cells loaded with 2`,7`-Dichlorodihydrofluorescein were exposed to 10% axial stretch using computer-controlled piezo-manipulated carbon fibers, attached to both cell ends, to measure cellular ROS production. In single cell mechanics study, cells were superfused at 37° C and paced at 4 Hz, then 5 steps of stretch were applied to obtain slopes of end-systolic and end-diastolic force-length relation curves that represent cellular contractility and diastolic stiffness, respectively.The stretch significantly increased ROS production in WT group, while not in NOX2 KO group. The cellular contractility was significantly lower in NOX2 KO group, while the diastolic cellular stiffness was not statistically different between the groups. The results suggest that stretch-induced ROS contributes to contraction against increased load without affecting cellular diastolic stiffness. COI:No

1P-090

Dose-dependent energy-saving action of a new myosin activator, omecamtiv mecarbil on LV mechanical work and energetics

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A novel myosin activator, omecamtiv mecarbil (OM) is a cardiac inotropic agent with increasing in the number of myosin heads interacting with actin filament without changing myosin ATPase activity and calcium transient. Thus, it is possible to exert the positive inotropic action without increasing oxygen consumption. We previously reported that OM improved the oxygen cost of PVA (1/contractile efficiency), although OM did not show obvious positive inotropic action in both of the hearts in normal and heart failure rats. In this study, we investigated a dose-dependent action of OM on LV mechanical work and energetics in the excised, cross-circulated rat heart preparations. We analyzed the LV end-systolic pressure-volume relation (ESPVR) and the linear relation between the myocardial oxygen consumption per beat (VO_2) and systolic pressure-volume area (PVA; a total mechanical energy per beat) in isovolumically contracting rat hearts at 300-bpm pacing in a dose-dependent action of OM (final concentration: 0.1 to 1.0 μM) in normal hearts. The ESPVR slightly shifted upward and each data point on the VO_2-PVA in VO_2-PVA linear relation shifted to the right-downward direction in OM dose-dependent manner. These results suggested that OM improved the contractile efficiency in dose-dependent manner with a slight positive inotropic action. COI:No

1P-091

Effect of enforced bite-opening on heart in mice

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Purpose: Autonomic nerves system plays important roles in maintaining heart function. A chronic activation of sympathetic nerves system is considered to be an important factor for the pathogenesis of life-threatening arrhythmias and heart failure. It is suggested that malocclusion has various adverse effects not only on the oral cavity area but also on the heart at least partly via imbalance of autonomic nervous system. To obtain an insight into the molecular mechanism in response to malocclusion, we investigated the effect of enforced bite-opening (BO, a mechanical overload) on the morphology and signal transduction of cardiac myocytes in mice. **Methods:** C57BL/6 male mice (16-week-old) were divided into three groups: control, BO, and BO mice treated with 1 mg/mL of propranolol, a β-adrenergic receptor antagonist, via drinking water. Two weeks after initiation of each treatment, the measurement of atrial weight and immunoblot analysis using protein extracts from atria were performed. **Results & Conclusion:** There was no significant difference in the atrial weight/tibia length ratio. The phosphorylation level of Akt was significantly decreased in BO group as compared to control. The amount of phosphorylated CaMKII and ryanodine receptor was significantly increased in BO than control. The Bax/Bcl-2 ratio of BO was significantly greater than that of control. Blockade of β-adrenergic receptor had a tendency of inhibiting the BO-induced alterations. These results suggested that BO treatment may activate sympathetic nerves system and affect Akt-mediated signaling, calcium handling and apoptosis in atrial myocytes in mice. COI:No

1P-092

Low air temperature around the tympanic membrane decreases heart rate.

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The vestibular system consists of otolith and semicircular canals. Otolith stimulation modifies the sympathetic nerve activity. In the present study, we stimulated only semicircular canals using caloric stimulation, and continuously measured heart rate (HR) and arterial pressure (AP), nystagmography, and analyzed stroke volume (SV) from the waveform of AP. For15 healthy subjects, 6 L/min of airflow at 15 ° C was applied to the left ear for 1 min. Nystagmus was observed in all subjects around the end of the stimulation, and continued about 1 min after the end of stimulation. Mean AP did not change significantly through the measurement. However, HR decreased at the onset of the stimulation, and maintained during the stimulation. SV increased during stimulation, probably due to extended cardiac filling time and mechanical increase in contractility. After the end of stimulation, HR recovered immediately. No relationship between HR and the nystagmus was observed, thus, decrease in HR might not be related to semicircular canals function. Furthermore, similar stimulation just in front of the ear did not did not induce nystagmus and decrease in HR. With airflow of 37 ° C for the ear did not induce nystagmus and decrease in HR, neither. Thus, the decrease in HR was not induced by mechanical stimulation against the ear, but by cold stimulation. Afferent nerves might not be trigeminal nerves, but chorda tympani nerves, dominant for the tympanic membrane, and around tissues. COI:No

1P-093

Two neuronal groups with different sensitivities to salt concentrations and the amiloride effects in the rostral nucleus of the solitary tract in rats.

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A previous study has reported that in taste receptor cells (TRCs) that respond to a low concentration of NaCl, their responses are reduced by amiloride (epithelial sodium channel, ENaC antagonist). But TRCs that only respond to a high concentration are unaffected by amiloride. In the present study, we investigated whether two neuronal properties as well the TRCs were maintained for the first-order taste relay, the rostral nucleus of the solitary tract (rNST) in the medulla. Here, we recorded extracellular single unit activities in the rNST neurons using multi-barrel glass micropipettes while under urethane anesthesia. Taste solutions were applied to the tongue and the oral cavity, and rinsed by distilled water (water-condition) or amiloride solution (10 μM). The rNST neurons that the net responses were reduced by amiloride exhibited the sufficient sensitivity to a low concentration (0.1 M). In contrast, the neurons unaffected by amiloride responded only to the higher concentrations of NaCl under water-condition. As a results, the amiloride-sensitive neurons showed a low threshold (0.1M), and the amiloride-insensitive neurons had the higher thresholds (0.4, 0.8 and > 0.8 M). The higher sensitivity to the hypotonic/isotonic NaCl would be important to ingest vigorously palatable foods. The property originated the ENaC would be principally maintained in the rNST. No conflicts of interest, financial or otherwise, are declared by the authors. COI:No

1P-094

Psychophysical stress increases nociceptive neural activities evoked by masseter muscle injury in the rostral ventral medulla (RVM) in rats.

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Chronic stress causes long-term neural changes in the brain that can change nociceptive responses. The aim of this study was to test if psychophysical stress had modulatory effects on neural activity evoked by masseter muscle (MM) injury in the rostral ventral medulla (RVM), which is known to play critical roles in descending pain controls. Male rats were subjected to repeated-forced swim stress (FST) or sham (non stress) treatments for 3 days (10 min/day). On Day 4 neural activity indicated by Fos expression in RVM was determined 2 hours after MM injury with saline or formalin injection. The number of Fos positive cells was quantified at several regions within RVM including the nucleus raphe magnus (NRM) and reticularis gigantocellularis pars alpha (GiA). MM injection of formalin significantly increased Fos expression in NRM and GiA regions compared to that of saline injection in FST and Sham groups. In addition FST rats showed greater Fos expression in NRM and GiA than sham rats, indicating that neural excitability in these regions was enhanced under FST condition. These findings supported the hypothesis that increased nociceptive response under psychophysical stress condition could be mediated by functional changes in descending pain controls in RVM. COI:No

1P-095

Regulation of transport pathway of cystatin D in salivary acinar cells

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The mechanism for selective transport of cargo proteins into the regulated and constitutive secretory pathways in exocrine cells such as salivary acinar cells remains to be solved. It is expected that endogenous cargo proteins have the specific signal for selective transport in their amino acid sequence. To examine the mechanism of loading saliva proteins to secretory granules, we constructed fusion proteins of full-length and signal peptide sequences of cystatin D, a saliva cystatin, with reporter protein HaloTag (fCst5H and ssCst5H, respectively). When the fusion proteins are expressed in salivary acinar cells, both fCst5H and ssCst5H were localized in the secretory granules, however, the both coefficients of overlapping and correlation of fCst5H with amylase were higher than those of ssCst5H. The secretion of the both fusion proteins was enhanced by addition of β-adrenergic receptor agonist as well as endogenous saliva amylase. On the other hand, the secretion of ssCst5H in the absence of secretagogue was significantly higher than that of fCst5H and amylase, suggesting that ssCst5H was partially secreted via constitutive secretory pathway. Complex formation of fCst5H and amylase was not detected by using blue native PAGE analysis. In contrast, precipitation of fCst5H was increased at low pH, which may mimic milieu of trans-Golgi networks. There is a possibility that addition of full-length sequence of cystatin D facilitates efficient selective transport into regulated pathway by aggregation under low pH in the trans-Golgi networks. COI:No

1P-096

Anticancer drugs-induced hyposalivation disturbs healing of oral ulcerative mucositis in rats

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Salivary clearance and antimicrobial effect of saliva are essential for maintenance of oral health. In a recent animal study, an anticancer drug 5-fluorouracil (5-FU) has been reported to induce hyposalivation due to atrophy of salivary glands. Hence, the hyposalivation may be involved in exacerbation of experimental oral ulcerative mucositis (OUM) in 5-FU-administered rats in our previous report. In the present study, we investigated effects of anticancer drugs on salivary glands and severity of OUM in the major salivary glands-extracted rats. Representative anticancer drugs 5-FU and cisplatin were intraperitoneally administered in rats. As a control, saline was administered in the same manner. OUM was experimentally induced by topical acetic acid treatment in the labial fornix region of the inferior incisors 2 weeks after extraction of the salivary glands. Sham procedure was performed without removal of salivary glands. In both 5-FU- and cisplatin-administrated rats, salivary glands weights were decreased compared with control. Level of mRNAs for cystatin-3 and lysozyme-2 (antimicrobial components) in the submandibular gland in cisplatin-administered rats were significantly increased compared with control. In 5-FU-administered group, those mRNA levels were same as control. Extraction of the salivary glands exacerbated severity of OUM, prolonged healing process and enhanced bacterial loading into the ulcerative region. These results suggest that anticancer drugs-induced hyposalivation disturbs healing of OUM due to loss of salivary washout function. COI:No

1P-097

Identification of apoptosis related factors in the salivary gland of periodontitis model rats

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Salivary dysfunction is known as a risk factor for periodontitis, whereas the experimental model exhibits apoptosis of the salivary glands (SG) and reduction of salivary secretion. Although the infiltration of B cells and TNF-α have been reported to induce apoptosis of the SG, the relationship with periodontitis is unclear. In the present study, we examined apoptosis-related factors in the SG of experimental periodontitis model rats (PerioM). The unilateral second maxillary molars of rats were tied with ligatures. In sham rats, ligature was removed just after the ligation. After 4 weeks, we measured weights of the parotid (PG), submandibular (SMG) and sublingual (SLG) glands, and performed real time RT-PCR and immunofluorescence. We also measured TNF-α levels of serum of experimental rats. Weights of the PG, SMG and SLG in PerioM were reduced compared with sham. Levels of CD19 mRNA, a B cell marker, was enhanced in the PG and SMG of PerioM. In immunohistochemistry, fluorescence intensity of IgA increased in duct of the PG of PerioM. TNF-α levels in the blood of PerioM were enhanced compared with sham, whereas there were no difference in TNF-α expression in PG between PerioM and sham. These results suggest that periodontitis-induced salivary apoptosis is induced by infiltration of B cell in to the SG and enhancement of circulating TNF-α. COI:No

1P-098

The effect of vitamin C deficiency on the peripheral taste organ

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Vitamin C (VC) is organic compound required by an organism as a vital nutrient. VC cannot be stored the body in large amounts, and must be obtained from daily diet. Otherwise, VC deficiency syndrome, called scurvy, may occur. It has been reported that scurvy often presents itself initially as symptoms, such as malaise and lethargy, followed by formation of spots on the skin and bleeding from the mucous membranes. However, the effect of VC deficiency on peripheral taste organ is unknown. In the previous study, we showed that the magnitudes of whole chorda tympani nerve (CTN) responses to VC, HCl and NaCl in Shionogi ODS/Shi Jcl-od/od (ODS) rats with the deficiency of VC were less markedly than those in non-deficient ODS rats. In this study, the responses of CTN were recorded to evaluate the amiloride-sensitive (AS-) NaCl, amiloride-insensitive (AI-) NaCl and some other organic acid responses in the VC deficient and non-deficient ODS rats. AS-NaCl responses in VC deficient rats were significantly decreased than that in non-deficient rats. On the other hand, there was no significant difference in the AI-NaCl responses between VC deficient and non-deficient rats. The magnitudes of the CTN responses to citric acid, acetic acid and tartaric acid in the VC deficient rats were significantly smaller than those in the non-deficient rats. These data suggest that VC deficiency may affect the AS-NaCl and organic acid responses on peripheral taste organ. Furthermore, we will present our recent data on the mRNA expression level of taste signaling elements in the taste cells obtained from VC deficient ODS rats. COI:No

1P-099

Lower esophageal sphincter receptive relaxation plays a key role in successful LES relaxation

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Background and Aim: The coordinated lower esophageal sphincter (LES) relaxation is indispensable for successful swallowing, which is critical to sustaining life. Recently developed high-resolution manometry (HRM) enabled us to evaluate esophageal motility function precisely. This study aimed to investigate how successful LES relaxation was achieved. Method: Fourteen participants with normal HRM results were enrolled. The participants were asked to perform a dry swallow 5 times and then a wet swallow when drinking 5 ml of water 5 times. Result: It was considered that two mechanisms cause LES relaxation: LES receptive relaxation and swallow-induced LES relaxation. There was no significant difference in basal LES pressure (BLESP) between before (30.9 ± 3 mmHg) and after (29.6 ± 2.4 mmHg) dry swallowing. The extent of LES relaxation in dry swallowing was smaller than that found in wet swallowing since LES receptive relaxation was not observed in dry swallowing, but seen in wet swallowing. After the first wet swallowing, during repeated wet swallowing, LES pressure did not return to BLESP before starting the protocol. In contrast, there was no significant difference in the extent of swallow-induced LES relaxation between dry swallowing (13 ± 1.4 mmHg) and wet swallowing (11.4 ± 1.2 mmHg). Conclusion: Dry swallowing possesses only swallow-induced LES relaxation. Dry swallowing cannot achieve full EGJ relaxation, which is important for protecting against gastro-esophageal reflux. LES receptive relaxation is indispensable for successful EGJ relaxation in food intake. COI:No

1P-100

Regulation of esophageal motility by purinergic signaling in rats

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The external muscle layer of the mammalian esophagus consists of striated muscle fibers and smooth muscle fibers. Striated muscle is mainly regulated by cholinergic signaling, whereas smooth muscle is regulated by cholinergic and non-cholinergic signaling in the esophagus. ATP is a representative non-cholinergic extracellular transmitter, which control smooth muscle motility in the blood vessels and gastrointestinal tracts via purinergic receptors. However, it is unclear whether purinergic signaling can regulate esophageal motility. Therefore, the aim of the present study was to clarify the effects of ATP on the motility of the esophageal muscle in rats. An isolated segment of the rat esophagus was placed in an organ bath and the mechanical responses were recorded using a force transducer. Exogenous application of ATP did not affect basal tone of the esophageal preparations. After contraction of smooth muscle in the muscaris mucosa of the esophagus was induced by carbachol, we applied ATP. ATP evoked relaxation of smooth muscle, which was blocked by pre-treatment with suramine, a purinergic receptor antagonist. RT-PCR revealed the expression of mRNA of purinergic receptors in the esophageal tissue. These findings suggest that purinergic signaling might regulate the motor activity of the esophageal smooth muscle. COI:No

1P-101

Study on inhibitory mechanism of glucose absorption by cabbage vinegar on isolated small intestine of mice

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Authors have been studying about functionality of cabbage vinegar (CV), and reported that CV inhibited glucose absorption and acetylcholine-induced motility on isolated intestines of mice with acidity-dependently. As a comparison of CV, grain vinegar (GV) and acetic acid (AA) solution were used and similar inhibitory effects by GV and AA were obtained. That is, the main factor of these inhibitions is thought to be AA. The present study aimed to clarify inhibitory mechanism of glucose absorption by CV, but various unknown components were included in CV. For simplify, AA was used for experiments of this study. Short circuit current (Isc), flown between mucosa and sub-mucosa tissues of isolated small intestine of mice, was measured by using Ussing-chamber method. Glucose-induced Isc was inhibited in the presence of AA. Moreover, by using everted sac specimen of isolated small intestine, AA concentration at a serosal side was measured and determined by using F-kit AA (J.K.International). Acidity-dependent AA absorption occurred. Experiments and analysis about interactions between AA and glucose are in progress. COI:No

1P-102

Luminal chemosensing and effects of mucosal functions (fluid secretion and barrier function) in the intestine

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Intestinal mucosa is exposed to many chemicals originated from food and microbiota-fermented products in the intestine. To maintain the intestinal homeostasis, intestinal mucosa is considered to sense luminal chemical environment. The aim of present study is to search and investigate the chemicals sensed by intestinal mucosa and affecting mucosal functions. Intestinal mucosa-submucosal preparations isolated from humans and experimental animals, and intestinal epithelium model monolayer cultured cell-line, caco-2, were mounted on Ussing chambers, and short-circuit current (Isc) and tissue conductance (Gt) were continuously recorded. In this poster presentation, the effects of several polyphenols on Isc and Gt in several segments of intestine. Mucosal, but not serosal, applications of resveratrol and its dimer, viniferin, and a flavonol, quercetin, induced a reduction of Gt in lower concentrations and an increase in Gt and Isc in higher concentrations. These results suggest that the existence of some receptors for polyphenols in the apical membrane of epithelial cells. It is hypothesized that such polyphenol receptors will be a clinical target of mucosa protective effects in the intestine. COI:No

1P-103

Dietary regulation of intestinal Na-dependent glucose transporter SGLT1 in mouse small intestine

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Major nutrients, such as glucose and amino acids, absorption capacity in the small intestine are proportional to changes in parallel with the amount of ingested nutrients. The effect of starvation on the intestinal glucose absorption has been reported to decrease glucose absorption in vivo experiments. It is generally accepted that sodium-dependent glucose transporter SGLT1mediates uptake of glucose in the brush-border membrane and that glucose leaves enterocytes across the basolateral membrane via facilitated glucose transporter GLUT2. It has been shown recently that, at high luminal glucose concentrations, facilitated glucose transporters were inserted into the apical membrane. To identify the intestinal segment which is responsible for changes of amount of diet, we measured glucose-induced short-circuit currents (δ Isc) in each intestinal segment in 48 h fasted mice and compared them with in fed mice in Ussing chambers. The results showed that, in fed mice, luminal application of glucose resulted in an increase in δ Isc in the ileum but not in the jejunum. However, in 48 h fasted mice, a robust increase in δ Isc was observed both jejunum and ileum, suggesting SGLT1 up-regulation in jejunum. To clarify the mechanism of elevation of SGLT1 in 48 h fasted mice, we conducted immunofluorescence experiments. There was no discernable change in SGLT1 immunofluorescence between 48 h fasted mice and fed mice. These apparently paradoxical findings may be explained by other than SGLT1 involves in fed mice. COI:No

1P-104

Changes in breath hydrogen (H2) after the ingestion of soybean by the cooking methods

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Hydrogen molecules (H2) play an important role of inactivating hydroxyl radicals in the body. Several evidences lead us speculate that H2 production by the colonic fermentation also play a role of anti-oxidative effects. It is well known that soybean contains a considerable amount of fibers and that ingestion of soybean increases breath H2 via colonic fermentation. In the present study, we examined whether or not cooking methods of soybean alter colonic digestion and fermentation. Healthy adult volunteers participated in the study. We compared effects of soybean flour and well-boiled soybean on the breath H2. Both of the test meals consisted of 100g soybeans. After starvation of 12 hours, breath H2 concentrations were analyzed every 1 h for 10 h by gas chromatography with a semiconductor detector. After ingestion of boiled soybean, levels of exhaled H2 were gradually increased in parallel with those after ingestion of soybean flour. However, 6 hours after the ingestion, the ingestion of boiled soybean caused a marked rise in breath H2 up to 30 to 40 ppm, whereas soybean flour did not. These results suggested that cooking methods affected on colonic fermentation of soybeans. Ingestion of boiled soybean may be beneficial, in terms of H2 increase in the body. COI:No

1P-105

Study on hypothalamic phospholipids affected by hyperglycemia

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Brain plays a critical role in regulating whole body energy homeostasis. The hypothalamus is able to monitor blood glucose level by using glucose sensing neurons, then regulates glucose production and glucose utilization in peripheral tissues. It has been reported that the hypothalamic fatty acid plays a key role in the peripheral energy metabolism. However, distributions of lipids and phospholipids in the hypothalamus were remained to be investigated. In this study, we performed imaging mass spectrometry and found that there is a specific distribution on the hypothalamus in each lipid. Phosphatidylinositol (PI) is one of the most important phospholipids to affect PI3 kinase pathway, IP3 pathways and prostaglandin production. The intraperitoneal injection of glucose (3g/kg) dramatically decreased the amount of PI in the hypothalamus. To elucidate the role of PI in glucose metabolism, glucose tolerance tests were performed after the injections of inhibitors into the hypothalamus. Hypothalamic injections of phospholipases A2 or COX1/2 inhibitor decreased glucose tolerance, while phospholipases C inhibitor or IP3 receptor antagonist did not change it. The results suggest that the PI-arachidonic acid-prostaglandins pathway play a role in peripheral glucose homeostasis. COI:No

1P-106

EID1 induces the energy expenditure in visceral and subcutaneous adipose tissue

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The obesity is related with the increased lipid accumulation in WAT (white adipose tissue) which is a major risk factor for cardiovascular diseases. In contrast, the use of stored lipids for promoting the thermogenesis and energy expenditure as BAT (brown adipose tissue), would help to prevent the metabolic disorders. EID1 is a protein able to induce the browning phenotype in-vitro. Here we demonstrate the role of EID1 in the energy expenditure, using a transgenic mouse model. Our results showed that both subcutaneous and visceral adipose tissue from EID1 transgenic mice increased the expression levels of PGC1a, UCP1 and PRDM16, which are markers of brown adipose tissue involved in the thermogenesis. On the other hand, adipocyte precursors were isolated and induced to mature adipocyte from both adipose tissue, in order to analyze the adipogenic capacity and proteins expression related with energy expenditure. Adipocytes from EID1 transgenic mouse reduced significantly the accumulation lipids and increased the expression of brown fat markers. These results demonstrate that EID1 modulates both the proinflammatory visceral adipose tissue phenotype and white adipose tissue, which induce characteristics of brown fat in the transgenic mouse, therefore it can be considered as a future therapeutic target against obesity. COI:No

1P-107

Effect of estradiol administration on thermoregulatory responses induced by application of cinnamaldehyde in ovariectomized rats

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INTRODUCTION Estradiol (E_2) contributes to maintain body temperature (T_b) by modulation of autonomic thermoregulatory responses via hypothalamus in the cold in female rats. E_2 facilitates thermoregulatory behavior in the cold using a new behavioral indicator (tail-hiding behavior; the behavior which rats place their tails underneath their bodies) which we reported. Cinnamaldehyde (CN) is agonist for TRPA1 which is one of cold receptors in cutaneous sensory nerve endings. The aim of the present study was to examine the effect of E_2 on thermoregulatory responses via TRPA1 in female rats. **METHODS** Ovariectomized rats were implanted a silastic tube with or without E_2 (22.3mg) underneath the dorsal skin (E_2 (+) and E_2 (-) groups) and data loggers into peritoneal cavity. After application of 30% CN or vehicle to skin of whole trunk of rats, rats were exposed to 16° C or 27° C for 2 hours. T_b, tail temperature (T_{tail}), and tail-hiding behavior were measured with data logger and thermography. **RESULTS** At 16° C, change in T_b in the E_2 (+) group was greater than in the E_2 (-) group in rats applied CN; however change in T_{tail} and the duration of tail-hiding behavior was not different between the E_2 (-) and E_2 (+) groups. **CONCLUSION** Since administration of E_2 increased T_b in rats applied CN at 16° C, E_2 might affect thermoregulatory responses via TRPA1 in female rats. **ACKNOWLEDGMENTS** We are grateful to Dr. K Morimoto. KAKENHI No. 26870417, 17K17882; Urakami Foundation; Nara Womens Univ; Intramural and Mental and Physical Health Project Research Grants COI:No

1P-108

Glucagon-like peptide-1 diminishes the suppressive effect of orexin-A on the reflex swallowing in anesthetized rats

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Orexin-A has an appetite enhancing effect. Glucagon-like peptide-1 (GLP-1), which is incretin hormone, has an anorectic effect. We previously reported that orexin-A and GLP-1 independently suppress swallowing reflex elicited by the electrical stimulation of the superior laryngeal afferent nerve. It seems to be inconsistent that both appetite-enhancing peptide and anorectic peptide suppress reflex swallowing. It is however possible that appetite-enhancing peptide and anorectic peptide interact antagonistically. Our previous study revealed that orexin-A suppressed GLP-1-response of the reflex swallowing by way of orexin-1 receptors situated in the cNTS. In the present study, we examine whether GLP-1 affects the suppressive response of the reflex swallowing induced by orexin-A in urethane-chloralose anaesthetized rats. Pre-administration of GLP-1 abolished the suppressive response of the reflex swallowing induced by the administration of orexin-A. The effect of pre-administration of exendin (5-39), GLP-1 receptor antagonist, was evaluated. The administration of the small dose of orexin-A, which did not affect the reflex swallowing, induced the suppression of swallowing reflex after the administration of exendin (5-39). Taken together, it was suggested that GLP-1 and orexin-A mutually inhibit the suppressive effect on swallowing reflex. This work was supported by JSPS KAKENHI Gant Number 15K00818. COI:No

1P-109

Effects of intranasal insulin administration on fat oxidation during exercise in normal-weight and overweight young individuals

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We investigated the effects of nasal insulin administration on the maximal fat oxidation rate during exercise in 11 normal-weight (N group) and seven overweight (O group, BMI > 25 kg/m^2) young individuals. On two separate days, either 40 IU regular insulin (INS) or normal saline, as a placebo (PL), was intranasally administered after an overnight fast in random order, then a graded exercise test was administered to each participant. The maximal fat oxidation rate (max FOR) during exercise and the exercise intensity at which max FOR was observed (FATmax) were assessed using indirect calorimetry. Neither blood insulin nor glucose levels changed after insulin administration. The max FOR tended to decrease in the INS trial (3.43 ± 0.30 vs. 2.79 ± 0.21 mg/kg/min, p = 0.050); the FATmax and total amount of fat oxidation during exercise were significantly smaller in the INS trial than in the PL trial in the N group. The max FOR in the O group (2.48 ± 0.22 mg/kg/min) was significantly smaller than in the N group (p = 0.021) and was not influenced by insulin administration. Intranasal insulin administration reduces fat oxidation during exercise without increasing peripheral insulin levels; however, these effects are diminished by an increase in body weight in healthy young individuals. COI:No

1P-110

Oral perceptions and preferences to sweet taste and fat stimuli are modulated by mood rather than Body Mass Index or daily eating behavior in young females.

Aso-Someya Nami

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The purpose of the present study was to evaluate the relationship between mood, Body Mass Index (BMI) and daily eating behavior and perceptions and preferences to sweet taste and fat. Thirty-eight females (18-22 yrs) participated in this study. We assessed mood by the Brief Japanese Version of POMS™, and daily eating behavior by the restraint eating sub-scale of the Japanese version of Dutch Eating Behavior Questionnaire (DEBQ-R). Sucrose solutions (25, 50, 100, 150, 200 and 250 mmol/l) and mixtures of non-fat milk and heavy cream (2, 4, 8, 12, 16 and 20 % fat) were used for oral stimulations of sweet taste and fat, respectively. The perceived intensity and preference were rated on general Labeled Magnitude Scale and Visual Analog Scale, respectively. The intensity rating of sweetness for sucrose solutions were negatively correlated with Tension-Anxiety (TA), Fatigue (F) and Confusion (C) scores of POMS™. The intensity rating of 20 % fat milk was also negatively correlated with TA, F and C scores of POMS™. In addition, the preference rating to the higher concentration of sucrose solution and high fat milk were positively correlated with TA, F and C scores of POMS™. On the other hand, neither BMI nor DEBQ-R showed significant relationship with perceptions and preferences to sweet taste and fat. These results suggested that sweet taste and fat perception and preference were affected by mood but not by BMI or eating behavior, and imply that negative mood lower the perceptions of sweet taste and fat, resulting in higher preference for sweet high-fat food. COI:No

1P-111

Effects of body-mass index on cold-induced vasodilation in young men and women

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Purpose The aim of the present study was to clarify whether body-mass index (BMI) is involved in cold-induced vasodilation (CIVD) in young male and female. ***Methods*** 28 male students and 41 female students were divided into four groups: low-BMI males (LM, n = 12), low-BMI females (LF, n= 22), normal-BMI males (NM, n = 16), and normal-BMI females (NF, n= 19). The CIVD during finger immersion of 3° C water was conducted to evaluate local cold tolerance. Finger temperature and thermal and pain sensations were recorded. Questionnaires regarding the cold constitution, height, and body weight were conducted. ***Results*** Finger temperature in the LF group was lower than in the NF group at pre, 7-17 min, and post exposure. Finger temperature in the LM group was higher than in the NM group at 6-7 min. The pain sensation was higher in the LF group than in the NF group, although the thermal sensation was not different among four groups. The scores of cold constitution were higher in the LF group than in the NM group. ***Conclusion*** It was suggested that low-BMI females had a low CIVD reaction. COI:No

1P-112

Mechanical stimulation causes PKC activation and subsequently rises intracellular Ca^{2+} in mouse brown adipocytes

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Brown adipocyte is a thermogenic organ. Mitochondrial uncoupling by β_3-adrenergic activation or an uncoupler (FCCP) causes Ca^{2+} release from mitochondria and subsequently Ca^{2+} release from the endoplasmic reticulum (ER) and further evokes plasmalemmal Ca^{2+} entry in rodent brown adipocytes. These rises of $[Ca^{2+}]_i$ promote thermogenesis via activation of Ca^{2+} -dependent dehydrogenase. Recently we indicated a new mode of $[Ca^{2+}]_i$ rises in brown adipocytes elicited by mechanical stimulation using dynamic water pressure measured by fluorometry of $[Ca^{2+}]_i$. Mechanical stimulation evoked Ca^{2+} rises in brown adipocytes. These rises in $[Ca^{2+}]_i$ induced by mechanical stimulation were promoted by the application of R59022 (a PKC activator) and were depressed by ML-9 (an inhibitor of phosphokinase C (PKC)) and not by a nominally Ca^{2+} free. RT-PCR suggested the expression of PKC$_\alpha$, PKC$_\beta$, PKC$_\delta$, PKC$_\varepsilon$, PKC$_\eta$, and PKC$_{\lambda/\iota}$among the nine PKC subtypes. Immunoblotting confirmed the expression of PKC$_\alpha$, PKC$_\beta$and PKC$_\varepsilon$. These results showed the expression of PKC$_\alpha$, PKC$_\beta$and PKC$_\varepsilon$in mouse brown adipocytes. The roles of PKC subtypes in the thermogenesis are discussed. COI:No

1P-113

The effect of synchronized circadian rhythm on the differentiation of human iPS cells

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In order to apply iPS cells to regenerative medicine, various differentiation induction methods have been reported. However, the differentiation efficiency is not fully complete and undifferentiated iPS cells remains throughout the differentiation, namely, there is heterogeneity in the differentiation capacity. In this study, we screen the cause of the heterogeneity of the endodermal differentiation. We focused on the difference between the embryonic environment in which organ development occurs and the environment during culturing in dishes, especially the circadian rhythm. Circadian rhythms are synchronized in fetal environment, whereas rhythm synchronization is not seen in culture environment. In human fibroblasts, rhythm was synchronized by horse serum or forskolin, but not in iPS cells. These stimulations have been reported to increase the expression of *FOS* and *PER 1/2*. Therefore, when the induction rate of *FOS* and *PER 1/2* expression was examined, it was about one-tenth of fibroblasts in iPS cells. In contrast to those stimulations, when the cells were cultured in circadian cycling of temperature, the rhythm in *DBP* and *CRY2* was observed in iPS cells, but not *BMAL1*. The unsynchronized rhythm in *BMAL1* is likely due to the low expression of *NR1D1* and *NR1D2* associated with its transcriptional regulation. Next, we performed the endodermal differentiation culture on iPS cells under temperature cycling, whereas the efficiency of differentiation was not improved. The advanced lineage, the efficiency of pancreatic differentiation is currently under investigation. COI:No

1P-114

Motor and learning ability of adult rats after dexamethasone administration at late preterm period

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Problems. Effects of steroid administrations to human late preterm infants (between 34 and 36 wks of gestation) have been unclear although its medication before 34 wks produces brain side effects. We examined steroid effects after growth using late preterm rat models. Methods. We gave dexamethasone (Dex) to rats equivalent to late preterm, and evaluated motor and learning abilities by behavioral tests. We gave Dex solutions (0.01mL/body weight) to 10-day-old rats once and established three groups; D0.5 (Dex 0.5mg/kg, n=5), D2.5 (Dex 2.5mg/kg, n=4), and the control (saline, n=5). Behavioral tests. Suspension test. We suspended a 3-week-old rat by its forelegs on a high horizontal bar and measured suspension time (ST) for 5 days. In step-down type passive avoidance test, we placed a 6-week-old rat on the rubber platform on a metal grid floor and gave an electrical shock when it stepped on the metal floor. On next 5 consecutive days, we placed the rat on the platform, and measured retention time (RT) until stepping on the floor once a day. Results. The ST at day3 of D2.5 (2,898 ± 439 sec, mean ± SE) was longer ($p<0.05$) than those of D0.5 (206 ± 156) and the control (137 ± 41). The RTs at day3 of D2.5, D0.5, and the control were 68 ± 50 sec, 127 ± 29, and 95 ± 18, respectively, and without statistical significance. There was no difference in brain sizes among the groups. Conclusion. Dex medications to rats at late preterm were anabolic without intellectual impairment. Effects of steroids on late preterm rats may differ from those on preterm rats. COI:No

1P-115

Dynamic reconstruction of Golgi apparatus during mammalian oocyte maturation

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The intracellular organelles such as mitochondria and the endoplasmic reticulum are known to change their size and localization dynamically during the maturation of mammalian oocytes. The adequate organization of organelles is prerequisite for the mature egg to fertilize and develop normally, and therefore it is important to deduce the regulatory mechanism of the organelle reconstruction during oocyte maturation. In the present study, we investigated the changes in the intracellular organization of Golgi apparatus (GA), by continuously observing mouse oocytes throughout the process of in vitro maturation under the confocal fluorescence microscope. By expressing fluorescent protein TagRFP targeted to the GA with localization signal sequence, it was revealed that the small granules of GA were distributed evenly in the cytoplasm of the oocyte at germinal vesicle stage, and that they were fragmented into smaller size as maturation proceeded. The GA granules were fused and became larger temporally at around the period of emitting the first polar body, and thereafter were fragmented again. Cytoplasmic actin filaments, which were successfully visualized by a GFP probe without affecting the functionality, were observed to be in close contact with each GA granule, suggesting the participation of the actin cytoskeleton to the process of the dynamic reconstruction of the GA during oocyte maturation. The results of experiments using the inhibitors of some actin binding proteins and of protein kinases that are known to be involved in the fragmentation of the GA in somatic cells will be presented and discussed. COI:No

1P-116

Functional characterization of various ion channels-expressing airway epithelial cells generated from induced pluripotent stem cells

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Background: Primary airway epithelial cells have been used for understanding of ion channel properties and airway disease such as cystic fibrosis caused by mutations of cystic fibrosis transmembrane conductance regulator (CFTR) gene. However, it is not easy to acquire an adequate quantity of cells and maintain in culture for long time. Therefore, airway epithelial cells generated from iPS cells are expected to be useful cell source instead of primary airway epithelial cells. The aim of this study is to examine functional properties of iPS cell-derived airway epithelial cells. Methods: We have generated airway epithelial cells from iPS cell based on serum-free conditions and air-liquid interface culture. iPS cell-derived airway epithelial cells were characterized by gene expression, immunoreactivity, ciliary movement, and measurement of CFTR activity using yellow fluorescent protein molecule sensitive to halide ions. Results: The expression of airway epithelium markers and various ion channel markers including CFTR was detected in the cells generated from iPS cell. Furthermore, iPS cell-derived airway epithelial cells showed the ciliary movement. Additionally, CFTR activity was confirmed in iPS cell-derived airway epithelial cells. Conclusion: Airway epithelial cells generated by our method have physiological function and will be useful cell source for molecular mechanisms of airway function and disease. COI:No

1P-117

In vitro recapitulation of adult hypothalamic neurogenesis using embryonic stem cell culture

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Although adult mammalian neurogenesis has been well described in the hippocampus and olfactory bulb, recent studies has also identified the hypothalamus as a neurogenic region in the mature brain. It is reported that adult hypothalamic neurogenesis affects energy balance and is promoted by a high-fat diet. Furthermore, *in vitro* studies using neural stem cells (NSCs) isolated from the perinatal hypothalamus have demonstrated that proliferation and differentiation of the NSCs are facilitated by nutritional signals, such as leptin and insulin. However, there is currently no adequate experimental system to evaluate direct action of peripheral signaling molecules on hypothalamic neurogenesis in adulthood. Here we report that murine embryonic stem cell-derived hypothalamic tissue culture (ES-Hypo) can recapitulate adult neurogenesis-like events *in vitro*. After long-term culture, ES-Hypo was largely composed of differentiated neurons and glial cells, but simultaneously contained a small number of NSCs. These NSCs were comparable to the ones found in the adult hypothalamus in terms of morphology and marker profile. Analysis of cell division using EdU, a thymidine analog, indicated that these NSCs have proliferated slowly and generated new neurons. Our findings suggest a novel utility of ES-Hypo as a semi-*in vivo* system to investigate the regulatory mechanisms of adult hypothalamic neurogenesis. COI:No

1P-118

Organic arsenic diphenylarsinic acid transfers from mother to pups via the placenta in rats

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Organic arsenic exposure by drinking well water containing diphenylarsinic acid [DPAA(V)] was reported in Kamisu city, Ibaraki prefecture in 2003. To investigate the possibility of exposure at fetal stages, the amounts of DPAA(V) in the umbilical cord of residents exposed to DPAA(V) were measured and found in a part of their umbilical cords. Based on this fact, we further examined the transition rate (placental rate) of DPAA(V) between mother and child in mammals. DPAA(V) was orally administered to pregnant SD rats [Crl:CD(SD)] at doses of 0.25, 0.5, 1.0 mg/kg between embryonic day (E) 7 to 19. After laparotomy of pregnant rats at E20, the samples of the blood and brain were collected from maternal rats (3 in each, total 9) and their fetuses (5 from each mother, total 45). Then, DPAA(V) concentrations in these samples were determined by liquid chromatography-tandem mass spectrometry. As a result, the DPAA(V) concentrations in the fetal blood were about 40% of those of the maternal blood, regardless of the dose of DPAA(V). On the other hand, DPAA(V) concentrations in the fetal brain were less than one tenth of those in the maternal brain, regardless of the dose of DPAA(V). (COI: NO) COI:No

1P-119

Early-life-stress induces cognitive disorder

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Early-life stress can induce several neuropsychological disorders in adulthood. However, the underlying mechanisms inducing such disorders are still not fully understood. Furthermore, the effects of early-life stress on the changes in cognitive function with age are still not clarified. In this study, we used maternal deprivation (MD) to examine the cognitive function in middle-aged mice using a touchscreen-equipped operant chamber. In the visual-discrimination task, the aged (1.4 years old) control mice could accurately learn to discriminate between different visual stimuli. In contrast, the correct response rate of aged MD mice increased to approximately 60% by day 10, it was still significantly lower than that of the control mice (85%). In the hippocampus of aged MD mice, the expression level of the N-methyl-d-aspartate receptor subunit GluN1 decreased significantly as compared to that in control mice. On the other hand, no significant difference in GluN1 expression level was detected in young (2.5 months old) mice. These findings indicate that early-life stress accelerates cognitive impairment in middle-aged mice. We also show the effect of breeding condition on cognitive function in young mice. COI: No COI:No

1P-120

The role of leak K^+ channels in the regulation of sleep duration

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It is known that during the slow-wave sleep, the cortical neurons show the slow-wave-sleep (SWS) firing patterns. A recently-proposed computational model, the averaged-neuron model, succeeded in recapitulating the SWS firing patterns and predicted that the Ca^{2+}-dependent hyperpolarization pathway plays a role in generating the SWS firing patterns. In addition, that study reported that knockout mice of the Ca^{2+}-related channels showed decreased sleep duration. Therefore, revealing the mechanisms of generating the SWS firing patterns could provide clues about the mechanism of the regulation of sleep duration. However, the mechanism of generating the SWS firing patterns is elusive except for the Ca^{2+}-dependent hyperpolarization pathway. Therefore, to reveal the mechanism of generating the SWS firing patterns more precisely, we simplified the averaged-neuron model by eliminating channels inessential for generating the SWS firing patterns. As a result, we constructed the simplified-averaged-neuron (SAN) model, which recapitulates the SWS firing patterns with five channels and a pump. The current analyses, bifurcation analyses, and mathematical analyses with the phase portrait in the SAN model suggested that the leak K^+ channel and the Ca^{2+}-dependent K^+ channel regulate the transition from the bursting phase to the silent phase of the SWS firing patterns cooperatively. Based on these results, we proposed the hypothesis that the leak K^+ channel plays a role in generating the SWS firing patterns, and hence in the regulation of sleep duration. COI:No

1P-121

Possible involvement of central oxytocin on cisplatin-induced anorexia in rat

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During cancer chemotherapy, drugs such as 5-HT_3 receptor antagonists have typically been used to control vomiting and anorexia. However, these drugs cannot entirely control anorexia and nausea during chemotherapy. We examined the effects of oxytocin (OXT), which has been linked to appetite, on cisplatin-induced anorexia in rats. Fos like immunoreactivity (Fos-LI) expressed in the supraoptic nucleus (SON), the paraventricular nucleus (PVN), the area postrema and the nucleus of the solitary tract (NTS) after intraperitoneal (ip) administration of cisplatin. We also examined the fluorescence intensity of OXT-monomeric red fluorescent protein 1 (mRFP1) after ip administration of cisplatin in OXT mRFP1 transgenic rats. The mRFP1 fluorescence intensity was significantly increased in the SON, PVN and NTS after ip administration of cisplatin. The cisplatin-induced anorexia was abolished by pretreatment with OXT receptor antagonist (OXTR-A). In the OXT-LI cells, cisplatin-induced Fos expression in the SON and PVN was also suppressed by OXTR-A pretreatment. These results suggested that central OXT may be involved in cisplatin-induced anorexia in rat. COI:No

1P-122

Investigation of mechanisms for the resistance to hypothermia in a mammalian hibernator, Syrian hamster

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Most mammals maintain body temperature (T_b) at around 37° C, and deep hypothermia can lead to cardiac arrest or tissue injury. However, mammalian hibernators can survive during hibernation (HIB), in which their T_b drops to below 10° C. To address physiological and molecular mechanisms responsible for the resistance to hypothermia, we examined organ function and global gene expression in a mammalian hibernator, Syrian hamster. We found that hamsters did not exhibit obvious renal or hepatic dysfunction during HIB period. RNA-seq revealed that 42 genes were differentially expressed between hypothermic state during HIB and normothermic state in both kidney and liver. To examine whether these genes are regulated in a HIB-specific manner or in a hypothermia-inducible manner, we compared their expression changes among normothermic, HIB and artificially induced hypothermic (AIH) animals. Some of the genes exhibited similar expression changes in HIB and AIH compared to normothermic animals, suggesting that they are regulated in a hypothermia-inducible manner. Another group of genes, in contrast, were differentially expressed between HIB and AIH, raising the possibility that they are regulated in a HIB-specific manner. We established primary culture of hamster hepatocytes to examine their resistance to cold culture and involvement of the focused 42 genes in the cold resistance. The data will be discussed. COI:No

1P-123

Mechanism of regulation of the lipid droplet formation through the transfer and synthesis of phospholipids

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NASH is characterized by lipid accumulation with inflammation and fibrosis in the liver. We have previously shown that STARD10 (StAR related lipid transfer domain containing 10) is highly expressed in the liver. We also showed that the STARD10 interacts with LPCAT1 (Lysophosphatidylcholine acyltransferase 1) and that STARD10 and LPCAT1 promote lipid droplet (LD) formation in Hepa1-6 cells. LPCAT1 is an enzyme which catalyzes the conversion of lysophosphatidylcholine to phosphatidylcholine (PC). The purpose of this study was to clarify the role of STARD10 and LPCAT1 in lipid accumulation. We examined the effect of lipid accumulation by choline-deficient L-amino acid-defined diet (CDAA) on LPCAT1 expression in the liver. The expression level of LPCAT1 was elevated by CDAA diet, while no difference was observed between wild type (WT) and *Stard10*$^{-/-}$ mice. However, the liver of *Stard10*$^{-/-}$ mice was smaller in size and the area of LD in hepatocytes of *Stard10*$^{-/-}$ mice was significantly smaller than those of WT mice. Surprisingly PC compositions of LD were not different between WT and *Stard10*$^{-/-}$ mice although significant difference was observed between normal diet and CDAA diet. We will report whether the difference of PC compositions could be explained by the LPCAT1 expression level by use of *Lpcat1* knockout mice. These results indicate that STARD10 could accelerate LPCAT activity to promote LD formation without influence on its substrate specificity. COI:No

1P-124

Withdraw

1P-125

Increase in blood-brain barrier permeability does not directly induce neuronal death but accelerates ischemic neuronal damage

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It is established that the increase in the blood-brain barrier permeability (BBBP) of is associated with ischemic stroke, which is thought to trigger neuronal damage and deteriorate ischemic stroke outcome. Here, we investigated the effect of increased BBBP on brain damage, using a combination of photochemically-induced thrombotic brain damage model (PIT-BD, a focal brain ischemic model) and transient bilateral carotid artery occlusion model (CAO, a whole brain ischemic model), in mice. In PIT-BD, BBBP increased in the region surrounding the ischemic damage after 24 hours. On day 4, the damaged region was not expanded at the region of increased BBBP with PIT-BD alone or in combination with CAO, applied before, PIT-BD, but with CAO after PIT-BD. The increase in the damage size was paralleled the increase in the number of apoptotic cells at the peripheral region of the damage. These findings indicate that the increase in BBBP alone does not cause direct neuronal death but it accelerates ischemic neuronal damage with ischemic stroke, which was attributed, at least partially, to acceleration of apoptotic cell death. COI:No

1P-126

Mucopolysaccharidosis in captive Japanese monkey at Primate Research Institute, Kyoto University

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We found a male monkey (Wk2389) with a unique face similar to gargoylism, a protruded tongue, bone deformation and rigid joints in extremities, leading to gait disturbance. Wk2389 also showed osteoporosis and secrete a high level of mucopolysaccharide in urine. Wk2389's mother has 7 children and some of them share similar characteristics with Wk2389. A sister of Wk2389 showed enlargement of cerebral sulci and lateral ventricles. These data strongly suggest that they have a genetic disorder similar to mucopolysaccharidosis. Genetic analysis revealed that Wk2389 have a homozygous SNP in alpha-L-iduronidase (IDUA), the responsible gene for mucopolysaccharidosis type I. Distribution of homozygous and heterozygous SNP of IDUA in this family and local group individuals is also shown. COI:No

1P-127

The duration of the preemptive analgesic effect of transcutaneous electrical nerve stimulation (TENS) in rats with acute inflammatory pain

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Preemptive analgesia is an analgesic intervention initiated before the surgical procedure to reduce sensitization. We previously demonstrated that transcutaneous electrical nerve stimulation (TENS) had a preemptive analgesic effect on acute inflammatory pain. However, it is not clear how long this effect lasts. We investigated the duration of the preemptive analgesic effect in a rat model of acute inflammatory pain.

Wistar rats were divided into five groups: a control group, a formalin-treated (F) group, a group treated with formalin immediately after TENS (T+F), a group treated with formalin 30 minutes after TENS (T+30+F), and a group treated with formalin 60 minutes after TENS (T+60+F). TENS was applied with a frequency of 4 Hz for 30 minutes. Formalin (50 μl, 1%) was injected into the hindpaw, and the duration spent licking, biting or lifting was measured for 1 h. In addition, the expression of phosphorylated-ERK (pERK) in the spinal dorsal horn was analyzed by immunofluorescent staining.

The pain-related behavior was significantly more frequent and the number of spinal pERK (+) cells was significantly greater in the F group than in the other groups. However, such increases were markedly suppressed in the T+F and T+30+F groups. There were no marked differences in these values between the F and T+60+F groups.

These results suggest that the preemptive analgesic effect of TENS on acute inflammatory pain lasts for at least 90 min after the TENS treatment. COI:No

1P-128

The novel rat model of vascular dementia by microsphere embolism

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[Introduction] Vascular dementia (VD) is one of severe cerebrovascular disease involving risk of progression to Alzheimer's disease. However, the suitable animal models is absent in researching VD. In this study, we propose the novel rat model of VD that exhibit multiple cerebral infarction and memory dysfunction by microsphere (MS) embolism.

[Methods] Cerebrovascular embolism were induced by MS (2,500 to 4,000 particles) injection into right internal carotid artery of rats. Neurological deficit was scored at 24hr after MS injection. Cognitive function was evaluated by Morris water maze test (MWM) between 7 to 11 days after MS injection. Motor function was evaluated by rotarod test at 1, 2, 4 and 7 days after MS injection. Brain damage was evaluated in hematoxylin eosin stained sections at 7 days after MS injection. Cerebral blood flow (CBF) in hippocampus and cortex were measured by Laser doppler flowmeter before, during and after MS injection.

[Results] MS injection dose-dependently aggravated the physiological deficit score, memory function (MWM), motor function (rotarod) and infarct area. CBF of both hippocampus and cortex were significantly decreased by MS injection.

[Conclusion] MS injection dose-dependently induced multiple cerebral infarction and memory dysfunction. This model is considered to represent VD like symptoms. COI:No

1P-129

Cytosolic Ca^{2+} dynamics through the SR is associated with pathology of muscular dystrophy

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Duchenne (DMD) and the less severe Becker (BMD) muscular dystrophy due to mutations in the DMD gene. Patients with DMD display increased cytosolic Ca^{2+} ($[Ca^{2+}]_c$) levels in skeletal muscle. However, the relationship between disease severity and $[Ca^{2+}]_c$ levels remains unclear. Based on our earlier findings about dystrophinopathy with very mild or asymptomatic phenotype, we generated the exons 45-55 deleted dystrophin transgenic/*mdx* (Tg/*mdx*) mice. Muscle function and pathology of Tg/*mdx* mice were restored close to those of wild type mice. On the other hand, the localization of the neuronal type of nitric oxide synthase (nNOS) was changed from the sarcolemma to the cytosol in Tg/*mdx* mice. This altered location led to hyper-nitrosylation of the ryanodine type-1 receptor (RyR1) and similar to mdx mice, subsequent increased Ca^{2+} release from the sarcoplasmic reticulum. However, Tg/*mdx* mice displayed restored Ca^{2+} uptake by sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) after activation of RyR1, suggesting that their Ca^{2+} dysregulation can be corrected by SERCA activation. We found that the expression level of sarcolipin, a SERCA-inhibitory peptide, was elevated in mdx mice, but normal in Tg/*mdx* mice. These findings suggest that sarcolipin may be a novel target for DMD therapy via improved SERCA function. COI:No

1P-130

A peptide from coagulation factor IX regulates endothelial barrier function and improves prognosis in traumatic brain injury model.

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Recently, coagulation factor IX (FIX) and its activation peptide have been reported to regulate vascular permeability. The activation peptide protects endothelial barrier function in lung of septic model with endotoxin. The aim of this study is to investigate if the treatment with a chemically synthesized peptide (F9-AP) from FIX activation peptide effectively protects barrier function of vasculature in traumatic brain injury (TBI). TBI model rats were generated by controlled cortical impact (CCI). After CCI, rats were intravenously injected with 350 μg/kg of F9-AP or PBS every day for a month. Neurogical functions were examined during a month of observation. Water content was used to assess brain edema. To assess the function of BBB, Evans Blue method was employed. For morphological analysis, macro- and microscopic observation were performed. In beam walking test, the treated rats delivered significantly better scores than control rats. The treatment significantly reduced brain edema by 34% and extravascular leakage of Evans blue by 91%. CCI finally made 333.4 ± 147.1 and 58.9 ± 22.0 mm^3 of recessus in control and F9-AP treated rats, respectively ($P<0.01$). Nissl staining revealed that neural cells adjacent to recessus by CCI were lost in control rats, but saved in F9-AP treated rats. The intravenous injection with F9-AP significantly protected vascular barrier function, reduced the damage of neural tissue, and improved neural function. COI:No

1P-131

Effects of Buffer Components on pH Dependency of Recombinant Human Neprilysin Activity

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Brain deposition of the amyloid β protein ($A\beta$) reflects an imbalance between the rates of $A\beta$ production and clearance. The causes of $A\beta$ elevation in Alzheimer's disease (AD) are largely unknown. However, the $A\beta$-degrading protease neprilysin (NEP) is down-regulated in normal aging and AD. AD would be due to one of the emerging complication of type 2 diabetes. Our previous study indicates that the interstitial fluid pH in ascites and metabolic tissues of OLETF rats, a model of type 2 diabetes, is lower than that of control rats. Therefore, the effects of the change of interstitial fluid pH on the NEP activity are one of the most important factor leading to AD. The activity of NEP is pH dependent as it is with most enzymes. So many experiments are performed to evaluate the optimal pH for the NEP activity. However, the pH optimum for NEP activity is still controversial and may be affected by buffer compositions. In this study, to determine effects of pH and buffer components on the activity of NEP, we performed *in vitro* NEP activity assays at various pH values in buffered solutions by using different buffer agents (NaH_2PO_4, KH_2PO_4, HEPES, MOPS and Tris). The present study demonstrated that the pH dependency of NEP enzymatic activity was affected by buffering components in reaction buffer. COI:No

1P-132

Intracellular ratiometric imaging of Ca ion and pH

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Metal ion is known as essential material in physiological phenomena and metabolism in vivo. Calcium ion, especially, has important role in second messenger system which related with neurotransmission and so on. The intracellular pH behavior is also important in order to investigate the reaction environment of various biomolecules. The visualization of dynamics of metal and hydrogen ion concentration is difficult because their original concentration is usually low. However fluorescence probe and microscopic system make it possible to observe the intracellular ion and pH behavior with high sensitivity. On the other hand, it is known as artifact that the difference of optical distance and the probe concentration inside of cells which has non-uniform thickness makes artifact for florescence imaging. This means the fluorescence intensity has no correlation between the metal ion and pH concentration. The ratio-imaging is one of the solutions, but there is the problem that the observation depends on the switching time of the filter wheel because multiple wavelengths are needed for ratio-imaging. We solved this problem by using monochromator which enable to select multiple wavelengths. In this presentation, we would like to show the results of intracellular ratiometric imaging of Ca ion and pH. COI:No

1P-133

Influence of CO_2-water bath on gene expression in the skin of anesthetized rats

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Human skin vasodilation by bathing in CO_2-hot spring is reproducible in the rat immersed in the water containing comparable amount of CO_2 (>1g/L) to the hot-spring water. This vascular response to CO_2-water immersion appears to be mediated by arachidonate metabolites because pretreatment with arachidonate metabolism inhibitor suppressed the response. Moreover, prostaglandin (PG) E_2, a vasodilatory metabolite of arachidonate, was significantly larger in skin tissue during CO_2-water immersion than that during tap-water immersion. PGE_2 production seems to be stimulated by CO_2-water immersion. In this study we investigated influence of CO_2-water immersion on gene expression in the rat skin tissue by comprehensive analysis using cDNA microarray chip technique. Male Wistar rats were anesthetized, fleeced with cut machine, equipped with probes for measuring of the rectal and skin temperatures and skin tissue blood flow. Rats were immersed into the tap-water (35° C) or the CO_2-water of same temperature in head out position. A portion of the skin immersed was sampled at 30 min of immersion, frozen in liquid nitrogen, and stored at -80° C until analysis. Total RNA in skin homogenate was extracted and purified for analysis. As for the PGE_2- and PGI_2-synthase genes expression, CO_2-water immersion group was larger by 2-3% than tap-water group, but as for its upper stream COX-2 gene, tap-water immersion group was 4% larger than CO_2-water group, so far. COI:No

1P-134

Physiological response analyses related to formation of "familiarity" for objects

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How human confirm emotion of "familiality" for non-life object? This is a simple question in human life observation. I tried a task study with some physiological reponce analyses to directed at resolve this question. Particularly, comparing physiological responses during obseving a certain kind of object at the time of the first contact and observing after seeing for a certain period of time. In this present study, I selected "kinakina" kokeshi-doll manifucturerd in Hanamaki, Iwate as providing object. Because it have simplified human feature ,curvilinear form and small head with movable neck, without painting. A wood cylinder that manufactured in same material and surface finishing as the kokeshi was used for control object. The participants was seated on a chair placed in a air conditioned space and measured brain and autonomic nerve responses by optical topography, skin conductance response, heart rate recording during observing the two kind of object. Some subjective impression evaluations of participants were checked after the measurement. As a result, I defined some slight difference during obsevation of the object in participants' physiological response between the first contact and after possesion for one week. These differences suggests that the emotion of familiarity with the object were evaluated. This result may provide a basical knowledge in consideration of favorable emotional formation process of the human. This research was supported by JSPS KAKENHI Grant Numbers JP 26540139 Grant-in-Aid for Challenging Exploratory Research. COI:No

1P-135

Expression patterns and feeding control function of LGR4 in mice brain

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LGR4 (leucine-rich repeat containing G-protein coupled receptor 4) is a membrane receptor with the common stracture of G-protein coupled receptor. Some research groups reported that R-spondins (Roof plate-specific spondins) were possible ligands for LGR4, with enhancing function on Wnt signaling pathways. LGR4 is widely expressed in several organs such as kidney, hair follicle, gut, reproductive organs, mammary tissue and tooth germ. The expression of LGR4 in brain was also detected in olfactry bulb, cortex, hippocampus, amygdaloid nuclei and hypothalamus, however only limited study suggesting physiological function of LGR4 signal in brain was reported. In the present work, to elucidate the possible mechanism to control feeding behavior by Rspo1/LGR4 system, we carried out molecular-genetic work using hypothalamus specific LGR4 gene deficient mice. Resaltantly, we found that Rspo1/LGR4 regurate POMC expression via noncanonical Wnt pathway. Additionally, we identified the expression pattern of LGR4 in brain at embryonic and neonatal stage. LGR4 null mice exhibit neonatal lethality, we then generated neural specific LGR4 null mice using Nestin-Cre mice. We report the analysis results of the mice. COI:No

Poster Presentations

Day 2

March 29 (Thu), 12:30 – 14:00

2P-001－2P-007	CNS Function (1)
2P-008－2P-014	Behavior Science･Biorhythm (2)
2P-015－2P-031	Neuron･Synapse (2)
2P-032－2P-043	Sensory Function (2)
2P-044－2P-053	Autonomic Nervous Systems (1)
2P-054－2P-071	Ionic Channel・Receptor (2)
2P-072－2P-080	Cell Physiology・Molecular Physiology (2)
2P-081－2P-082	Study Methodology
2P-083－2P-099	Heart・Circulation (2)
2P-100－2P-101	Blood
2P-102－2P-107	Respiration
2P-108－2P-113	Physical Fitness・Sports Medicine (1)
2P-114－2P-120	Muscle Physiology (1)
2P-121－2P-125	Motor Function (1)
2P-126－2P-132	Oral Physiology (2)
2P-133－2P-140	Nutrition・Metabolosm・Thermoregulation (2)
2P-141－2P-144	Reproduction
2P-145－2P-151	Environmental Physiology (2)
2P-152－2P-154	Drug Actions
2P-155－2P-167	Pathophysiology (2)

2P-001

Physiological effects in CNS and the autonomic nervous system by listening classical music

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To investigate physiological effects by listening classical music, especially so-called α-music, we measured EEG and the autonomic nervous system by measuring heart rate and heart rate variability. Psychological conditions were monitored by describing two psychological tests, i.e. the multiple mood scale (MMS) and the General Arousal Checklist (GACL). Subjects were young healthy 14 students. The subjects listened the classic music selected by commercial CD. By listening the music, amplitude of α band in frequency analysis (mFFT) of EEG was changed. Two patterns of responses were observed. One was that an amplitude of α band was enhanced 5 min after listening music (14 times in 7 subjects). The other pattern was that amplitude of α band was reduced but amplitude of δ band was enhanced (7 times in 4 subjects). In this case, the music seemed to induce a higher relaxation level and eventually leaded to reduce an arousal level. Heart rate was reduced and the sympathetic nervous activity indicated by LF/HF was also reduced. These data indicated that listening the music suppressed the sympathetic nervous activity. The parasympathetic nervous activity (HF) was contrarily enhanced. Well-being of mind in MMS was increased after listening the music. Deactivation-sleep of energetic arousal in GACL was tended to enhance by the music. These data suggested that listening the classic music, especially so-called α -music, rested general brain activity, namely leading to relaxation in the central nervous activity, the autonomic nervous activity and feelings in the multiple mood scale. (COI:No) COI:No

2P-002

Salicylate-induced changes of the tonotopy in the primary auditory cortex of guinea pigs observed by optical recording

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The influence of salicylate on the tonotopy map, which shows the spatial arrangement of the processing to sounds of different frequencies, within the primary auditory cortex (AI) of the guinea pig was investigated using optical imaging with a voltage-sensitive dye (RH795). Seven guinea pigs were anesthetized with ketamine (80 mg/kg) and xylazine (40 mg/kg). Activity patterns to the tones (0.5, 1, 2, 4, 8, 16 kHz, 200ms duration at 75 dB SPL) were recorded from the AI on both sides at 1-4 and 8-11 hours after the intraperitoneal injection of 200 mg/kg salicylate. When the 0.5 kHz tone was supplied, the active spot was appeared at the rostral AI and moved to and expanded toward the dorsal to ventral part of AI. From the movement of the active spot to 0.5 kHz tone, 0.5-kHz frequency band (FB) was determined. The 16-kHz FB was also determined by the same methods. The distance between the 0.5 and 16-kHz FBs measured 4 hours after the salicylate injection was longer than that after 1 hour. However, the distance between 0.5 and 16-kHz FBs after 11 hours was shorter than that after 8 hours. These findings may correspond to the enhancement of the spontaneous activity of AI at the early stage after the salicylate injection and the increment of the ringing generation (tinnitus) by salicylate. COI:No

2P-003

Effect of early weaning on relationship between anxiety trait in the elevated-plus maze and the prefrontal dopamine release in the open-field

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Early-weaned rodents exhibited changes in behavioral emotion traits and myelin formation in the anterior basolateral amygdala, which the prefrontal efferents terminated reportedly. Our recent studies in the SD rats suggest that the early-weaning up-regulates basal release of the amygdalar dopamine (about 200%), increasing reactivity (about 150%) of the prefrontal dopamine release in the open-field without changes in the basal release. The prefrontal dopamine transmission (both D1/2 receptors) reportedly consolidates extinction of conditioned fear (Hikind & Maroun, 2008; Mueller et al. 2010). We previously found the early-weaned SD rats statistically spent more time in the closed arms of an elevated-plus maze than controls for the last 5 min of 10 min experimental period. On the other, these in the open-field insignificantly increased the prefrontal dopamine release for 1 hour of experimental day 1 and 2. We planned here to analyze correlation between the anxiety trait and the prefrontal dopamine reactivity. The study was supported by JSPS KAKENHI grant number 23530972 and 15K04202 to M.T, and 15H02479 and 25660258 to T.K and AIST grant for neurorehabilitation research to M.T. COI:No

2P-004

Evaluation of emotion-induced brain activity measurement using wearable NIRS

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In the present study, we aimed to evaluate the emotion-induced brain activity by using a 2channel type wearable NIRS (near infrared spectroscopy), HOT-1000 (Hitachi High-technologies Corporation). This simple wearable NIRS (HOT-1000) is a hairband type NIRS and far small as comparing to PET, fMRI and multi-channel NIRS which are used for various emotional researches. The wearable NIRS, thus, is less restrictive and suitable for the art viewing. Ten healthy subjects (5 males, 5 females, mean of 22.3 years) continuously watched a series of the affective photo pictures, IAPS (International Affective Picture System) [1]. We measured the stimulus-induced changes in the cerebral blood flow in the prefrontal cortex of the subjects during watching the IAPS pictures. We detected stimulus-dependent changes in the cerebral blood flow. Based on the present results, we concluded that the simple wearable NIRS, HOT-1000 could capture the change in the brain activity during IAPS appreciation.[1] Lang, P.J., Bradley, M.M., and Cuthbert, B.N. (2008). International affective picture system (IAPS): Affective ratings of pictures and instruction manual. Technical Report A-8. University of Florida, Gainesville, FL. COI:No

2P-005

Task-dependent modulation of the local field potentials in the dorsolateral prefrontal cortex of monkey

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The lateral premotor cortex (lPFC) of monkey is consider to play a crucial role in executive function. Although it separates into the dorsal and the ventral parts from an anatomical point of view, their functional segregation has not been well investigated. Here we recorded local field potentials (LFPs) from the lPFC while monkeys were performing a shape-manipulation task. This task requires planning multiple movements based on differences in size and direction between the first (sample) and second (test) shapes presented on a monitor. The subjects were rewarded when they fit the test shape to the sample one by rotating, expanding, and/or contracting it. In this study, we examined the task-dependent modulation of LFPs in the dorsal lPFC and compared them with those recorded from the ventral lPFC.This research is supported by the following grants: #24120703, #26350991, #26120703 and #15H05879 from MEXT. COI:No

2P-006

Changes in human prefrontal activity during exposures to emotionally-charged movies: a preliminary study by simultaneous recordings of NIRS and fMRI

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We have recently reported that facial skin blood flow decreased during viewing of pleasant movies in association with a decrease in the prefrontal oxygenation (Matsukawa et al. 2017, in press). These results might reflect autonomic regulation associated with the changes in the emotional status by exposures to emotional-charged movies in humans. In this preliminary study, we examined the changes in human prefrontal activity by simultaneous recordings of near-infrared spectroscopy (NIRS) and functional magnetic resonance imaging (fMRI). The experimental task involved three types of video clips; positive (comedy), neutral (landscape), and negative (horror) movie. All movies were edited to 30 secs. For one session, three different movies with each type (positive, neutral, or negative) were adopted, so totally nine movies were presented randomly to the subject. Two movie sessions were conducted in the present study. During viewing positive movies, the decrease in the prefrontal Oxy-Hb concentration recorded by NIRS was negatively correlated with the subjective pleasantness score, while that had no correlation with the subjective consciousness score. A positive or negative correlation was detected between the oxygenation (NIRS) and BOLD signals (fMRI) depending on the brain areas. The results in detail regarding the correlation between the NIRS and fMRI will be presented and discussed. COI:No

2P-007

Episodic-like experiences alter shape/size of ripple-like events of hippocampal CA1 neurons

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Generation of ripple-like events in hippocampal CA1 during slow-wave sleep is required for memory formation for episode-like experience (Girardeau et al. Nat Neurosci 2009), but dynamic changes during and after the experience has not been investigated in behaving animals. Since emotional experiences increase the generation of ripple-like events in male rats (Ishikawa et al, Soc. Neurosci 2016), we hypothesized that ripple-like events might code encrypted intra-brain information of their experiences. To address the issue, we recorded multiple-unit spike activity of CA1 neurons while rats were experiencing either one of the four experiences for 10 min (restraint stress, contact with a female rat, contact with a male rat, or exposure to a novel object), and investigated whether shape/size of ripple-like event could be altered after experiences, and could be different depending on type of experiences. Shape/size of ripple-like events were significantly altered after restraint stress or contact with female as compared with those before each experience, whereas those were not altered by contact with male or novel object. Moreover, shape/size of ripple-like events after restraint stress or contact with female were significantly different from those after contact with male or novel object. Shape/size between contact with male rat and novel object were also different significantly. These results further support our hypothesis and provided a clue to clarify the function of ripple-like event during awake stage. COI:No

2P-008

Establishment of a visual detection task for head fixed rodents and modulatory effect of caffeine on contrast sensitivity

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Caffeine (1, 3, 7-trimethylxanthine) is a well-known CNS stimulant, and thought to enhance arousal level and releases of neuromodulators including acetylcholine via antagonizing adenosine receptors.Recently, we found that intraperitoneal administration of caffeine improves the behavioral contrast sensitivity (CS) of freely moving rats depending on spatial frequency (SF) of visual gating stimulus. Moreover, in the head-fixed and passively viewing condition of awake rat, the same treatment of caffeine was found to shift upwardly the contrast-response functions in neurons of the primary visual cortex (V1), which is called a baseline control. However, baseline control enhances not only visual responses as a signal but also spontaneous discharges as a noise, and those behavioral and electrophysiological studies were conducted separately.Therefore, it remains unknown whether and how behavioral CS improvement by caffeine could be mediated by the neuronal modulation in V1. To answer these questions, we newly established a simultaneous measurement system for behavioral and neural CSs, in which neural activities were recorded in V1 of head-fixed rats performing visual detection task in Go-No-Go paradigm. By using this system, we measured CS-SF relationship, confirming that the SF tuning of the CS is low-pass which is the same as our previous results. We are currently performing the multi-unit recordings from V1 of task-performing rats with or without administration of caffeine and acetylcholine. COI:No

2P-009

Aversive stimuli-induced wake from hypnotics-induced sleep in mice: Comparison between a dual orexin receptor antagonist and triazolam

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Retention of the ability to wake from sleep in response to dangerous situations is an ideal characteristic of safe hypnotics. Here we studied effect of a dual orexin receptor antagonist (DORA22) and the GABAA receptor modulator, triazolam (TRZ), on the ability to wake in response to aversive stimuli. We examined three modalities of sensory inputs, namely, olfactory (predator odor), auditory (ultrasonic sound), and vestibular (trembling)-stimuli. When the mouse was asleep, one of the three stimuli was applied for 30 sec. In the case of olfactory stimulation, latency to arousal following vehicle, DORA22, and TRZ administration was 3 +/- 0, 5 +/- 4, 330 +/- 84 sec (mean +/- SEM, n=10), respectively. Latency to re-sleep after cessation of the stimulation was 445 +/- 80, 156 +/- 19, 57 +/- 7 sec, respectively. Similar results were obtained for auditory and vestibular stimuli-induced wake. These results are consistent with the distinct mechanisms of these sleep promoting therapies; GABA receptor activation by TRZ is thought to induce widespread CNS suppression while DORA22 more specifically targets sleep / wake pathways through orexin receptor antagonism. These data support the notion that DORA22 preserves the ability to wake in response to aversive stimuli, regardless of modality, while remaining effective in the absence of the threat. COI:No

2P-010

Adjustment of diural feeding pattern reverse estrogen deficiency-induced hyperphagia in ovariactomized rats.

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Estrogen deficiency causes hyperphagia and obesity. We have previously reported that estrogen deficiency attenuates c-Fos expression at the suprachiasmatic nucleus (SCN) and increases food intake specifically during the light phase. We hypothesized that disturbance of circadian feeding rhythm is one of mechanisms for estrogen deficiency-induced hyperphagia and obesity.Here we examined whether the adjustment of diurnal feeding pattern by food deprivation during the light phase can attenuate daily food intake and body weight gain in estrogen deficient rats. Female Wistar rats were ovariectomized and given estradiol (E2) or cholesterol (Veh) via a subcutaneous silicon tube. With ad lib feeding, food intake during the light phase and body weight gain were greater in the Veh group than in the E2 group.Food deprivation during the light phase increased food intake during the dark phase in both the E2 and Veh groups. However, food deprivation during the light phase decreased daily food intake and body weight gain in the Veh group, but did not alter these in the E2 group.These indicate that estrogen deficiency-induced hyperphagia can be reversed by the adjustment of diurnal feeding pattern, and suggest that estrogen deficiency-induced hyperphagia is, at least in part, caused by disturbance of circadian feeding rhythm. Anorectic action of estrogen is possibly accomplished by the regulation of circadian feeding rhythm. COI:No

2P-011

The analysis of rat fetal movement in the ultrasonic tomography using non-anesthesia pregnant rat

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Fetal movement of human has been analyzed using ultrasonic tomographic analysis (UTA). However, that of rat has not analyzed by UTA, because there is no equipment for analysis of rat fetal movement in pregnant rat without anesthesia. We tried to create the equipment for analysis of pregnant rat without anesthesia for investigating the comparison of rat fetal movement and human fetal movement. The fetal movement of human fetus was observed around 18-weeks-gestation in human, and the % of rolling-movement of the total observation time (30 minutes) is significantly decreased at 10 months. We analyzed the fetal movement of the embryonic day 18 (E18), E19, E21 of Wistar rat fetus. The % of fetal movement of the observation time was 40% (E18), 30% (E19) and 16% (E21). We observed rolling movement, arm and leg movement and shaking head at E18-E19, but rolling movement was significantly decreased at E21 like human. Moreover, we analyzed the developmental changes of isolated brainstem-spinal cord preparation from fetal rat (E18-E21). The fetal movement was recorded from C8 ventral root in E18-19; and fetal movement was disappeared at E20. This phenomenon was same as UTA data in the pregnant rat without anesthesia. The fetal-like movement was recovered by strychnine application at E20. These results suggested that the fetal movement might be controlled by glycinergic system. This UTA system is between human baby behavioral study and rat fetal movement study. COI:No

2P-012

Sex difference in neuronal activity of the ventral part of the principal nucleus of the bed nucleus of the stria terminalis (BNSTpv) in parent mice

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We recently reported that the BNSTpv shows female-biased sex differences in volume, neuron number, and estrogen receptor alpha (ER α) expression. Additionally, we showed that the expression of c-Fos, a neuronal activity marker, increases with exposure to pups in virgin female mice exhibiting lower performance of maternal behavior, but not in primiparous mothers exhibiting higher performance of maternal behavior. In this study, we performed c-Fos immunohistochemistry and measured the number of c-Fos cells in the BNSTpv of sexually naive male mice and fathers with or without pup exposure as well as virgin females and primiparous mothers. We also performed a parental behavior test and confirmed that parent mice displayed parental behavior when they encountered pups. Primiparous mothers exhibited higher performance of parental behavior than did fathers. Virgin females and sexually naive males showed few parental behaviors. Sexually naive males displayed aggressive behavior against pups. Counting c-Fos cells resulted in that the number of c-Fos cells in the BNSTpv increased with exposure to pups in sexually naive males, fathers, and virgin females. However, the number of c-Fos cells in the BNSTpv of primiparous mothers did not change with exposure to pups. These results suggest that neuronal activity of the BNSTpv changing in response to pup exposure differs between sexes in parent mice, although the neuronal activity in nonparent mice is similar between sexes. COI:No

2P-013

Relationship between sensory stimulation and respiration in isolated neonatal rat brainstem-spinal cord preparation

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The coordination between respiration and locomotion has been well understood, but the relationship has not much studied. We examined whether the respiratory rhythm was synchronized by sensory input from forelimb. We used isolated brainstem-spinal-cord preparation from neonatal rat, this preparation is suitable for analysis of sensory-respiratory interaction including the respiratory center and the spinal sensory system. The respiratory activity was recorded from the C4 ventral nerve root; and spinal sensory system was regarded by the C8 dorsal nerve root stimulation (spinal stimulation) as a sensory input. We observed whether the entrainment of respiratory rhythm was induced by the spinal stimulation. The condition of spinal stimulation by 65% ratio of the respiratory cycle was most inducible for the entrainment of respiratory rhythm. The spinal stimulation in below 55% ratio of the respiratory cycle decreased the entrainment. The stimulation of longer respiratory cycle did not synchronize the respiratory rhythm, but increased respiratory frequency because of the respiratory activity induced by the stimulation. The pons-medulla-spinal cord preparation showed the entrainment of respiratory cycle caused by spinal stimulation but removal pons preparation did not. These results suggested that the entrainment of respiratory cycle caused by a slightly-faster-respiratory-cycle spinal stimulation, and pons might play an important role for locomotion-respiration coupling. COI:No

2P-014

Effects of *Rev-erbα* deficiency on the serotonergic system and mood regulation in mice

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REV-ERB α is a powerful circadian transcriptional repressor that not only comprises a negative limb of the core circadian clock but also participates in diverse physiological processes, including energy metabolism and mood regulation. We and others have previously reported that *Rev-erbα* knockout (KO) mice show both depressive- and mania-like behaviors. However, molecular mechanisms underlying such mood swings in *Rev-erbα* KO animals remain unclear. Here we demonstrate that *Rev-erbα* KO mice show a reduction in serotonin levels in the prefrontal cortex although levels of dopamine, another critical mood-associated monoamine neurotransmitter, are not altered in the same area of KO mice. Accordingly, KO animals show a decreased mRNA expression level of *Tph2* (*tryptophan hydroxylase 2*), the rate-limiting enzyme in serotonin synthesis, in the raphe nuclei that contain a cluster of neurons producing serotonin and projecting to the prefrontal cortex. Our results indicate that *Rev-erbα* plays an important role in stabilizing mood through the serotonergic system. COI:No

2P-015

Sub-region specific difference in learning-induced plasticity at both temporoammonic and Schaffer's synapses: dorsal CA1 pyramidal neurons along with the proximodistal axis

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Hippocampus interplays with entorhinal cortex during learning. Pre-processed information streams via indirect route, trisynaptic pathway from EC layer II and unedited information via direct route, the temporoammonic (TA) pathway from EC layer III. Although contextual learning requires GluA1-containing AMPA receptors into hippocampal CA1 synapses (Mitsushima et al., PNAS, 2011), pathway-specific synaptic plasticity at CA1 neurons is still unclear. While prominent functional differences occur dynamically along the proximodistal axis of CA1 area (Igarashi K. M. et al., FEBS Letters, 2014), the detail of this heterogeneity is still unresolved. Here we examined the learning-induced synaptic plasticity at TA-CA1 and CA3-CA1 synapses along the proximodistal axis of dorsal CA1 in male SD rats. After inhibitory avoidance (IA) task, we made acute hippocampal slices for whole cell patch clamping. We stimulated either pathway to analyze their AMPA/NMDA (A/N) ratio. Compared with untrained control, the trained rats exhibited greater A/N ratio at both TA-CA1 and CA3-CA1 synapses all along the proximodistal axis. Moreover, significant learning-induced increased variance was seen at both synapses of proximal third, TA-CA1 synapse of middle third and CA3-CA1 synapse of distal third of CA1 area. In conclusion, contextual learning may strengthen both Schaffer's Collateral and TA pathway all along the proximodistal axis of dorsal CA1 with different pattern of coding density. COI:No

2P-016

X-irradiation on Immature Neurons (In Vitro) causes decreases of drebrin at mature stage

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Neurons are regarded as post-mitotic cells and thought to be relatively radioresistant. The effect of X-irradiation, less than 1 Gy, on immature neurons when most of the cells are still in stage 3 or days in vitro (DIV) 1 neurons and their function is unknown. To determine the effect of low doses of X-rays on immature neurons, in this study, we used primary hippocampal cultured neurons. The DIV 1 neurons were irradiated using 0.5 Gy and 1 Gy of X-rays with dose rate 1.3 Gy / min. The cells were incubated for 3, 6, 13 and 20 days following the irradiation. Then the number of neurons was examined using MAP2 positive cells in the selected region of interest / ROI. We also analyse the number of drebrin, a postsynaptic marker at DIV 14 and DIV 21. There was a significant decrease in the number of cells starting from 6 days following 1 Gy irradiation, and after 0.5 Gy the decrease started at 13 days compared to sham-irradiated cells. The number of drebrin clusters and its expression on surviving neurons was found to be decreased at DIV 21 in 1Gy irradiated neurons. This study reveals that small doses of 0.5 Gy and 1 Gy of X-irradiation at DIV 1 may affect neurons and their function.This study is supported by AMED with grant number 17bk0104077h0001. COI:No

2P-017 (AP1)

Measuring dynamics of releasable synaptic vesicles and their plastic changes at hippocampal mossy fiber boutons.

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Neurons use chemical synaptic transmission to communicate with each other. In response to the arrival of an action potential at the presynaptic active zone, synaptic vesicles undergo exocytosis and discharge their cargo contents, neurotransmitters. Extensive electrophysiological studies have revealed detailed kinetics of exocytosis itself, but little is known about the kinetics of the preceding steps, such as tethering, docking and priming at mammalian CNS synapses. We managed to visualize single synaptic vesicles near the plasma membrane, and examined their exocytosis and the kinetics upstream at the hippocampal mossy fiber boutons (hMFBs). We employed total internal reflection fluorescence (TIRF) microscopy to directly visualize dynamics of single synaptic vesicles adjacent to the plasma membrane at high spatial resolution. In addition, we have combined high temporal resolution measurements of presynaptic capacitance and EPSCs to measure the kinetics of exocytosis. Readily releasable vesicles mostly consisted of already-tethered vesicles in the TIRF field. Vesicle replenishment had fast and slow phases, and TIRF imaging suggests that the fast phase depends on vesicle priming from already-tethered vesicles. Application of cAMP, a molecule crucial for long-term potentiation (LTP), mainly increases the vesicular release probability rather than the number of readily-releasable vesicles or their replenishment rate, likely by changing the coupling between Ca^{2+} channels and synaptic vesicles. COI:No

2P-018

Neuropsin dependent and independent late associativity.

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Synaptic plasticity is widely accepted to provide a cellular basis for learning and memory. Synaptic associativity could be involved in activity-dependent synaptic plasticity, because it distinguishes between local mechanisms of synaptic tags and cell-wide mechanisms that are responsible for the synthesis of plasticity-related proteins. An attractive hypothesis for synapse specificity of long-term memory (LTM) is synaptic tagging: synaptic activity generates a tag, which captures the plasticity-related proteins derived outside of synapses. Previously we have been reported that neuropsin, a plasticity-related extracellular protease, was involved in synaptic tag setting. In the present study, we tested the hypothesis that neuropsin was engaged in behavioral tagging for LTM in vivo. Behaviorally, weak training inhibitory passive avoidance task (IA) or spatial object recognition task (SOR), which induces short-term memory (STM) but not LTM, can be consolidated into LTM by exposing animals to novel but not familiar environment 1 h before training. We found that neuropsin deficient mouse impaired such transformation short-term into long-term memory by exposure to novelty in IA, but not SOR. These results suggest that the presence of neuropsin-dependent and -independent late associativity in vivo. COI:No

2P-019

Visualization of spatiotemporal Ca^{2+} dynamics in astrocyte-neuron interaction

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P2Y1 receptor (P2Y1) is a typical Gq protein coupled receptor that promotes Ca^{2+} signals in astrocytes. However, P2Y1 is also expressed in other cell types in the brain. To understand specific role of astrocytic P2Y1 in neuronal circuits, we generated transgenic mice, in which P2Y1 is overexpressed specifically in astrocytes (P2Y1-astrocytes) using Tet-off system. Using acute hippocampal slices, we measured Ca^{2+} signals in both astrocytes and neurons in the dentate gyrus using two different colors of genetically encoded Ca^{2+} indicators (GCaMP6f for astrocytes and R-CaMP2 for neurons) by AAV injection. P2Y1-astrocytes showed larger Ca^{2+} signals than control astrocytes when stimulated with P2Y1 agonists. An electrical stimulation of the perforant path also showed larger Ca^{2+} signals in P2Y1-astrocytes, suggesting an importance of P2Y1 in excitatory neuron-to-astrocyte communication. Interestingly, the electrical stimulation-evoked Ca^{2+} signals in neurons were rather increased by P2Y1 receptor antagonists. The results suggest that excitatory synaptic transmission between the perforant path and granule cells should be suppressed by P2Y1-astrocytes, but such suppression could be observed only when astrocytes show excess Ca^{2+} signals as seen in P2Y1-astrocytes. We also succeeded in simultaneous dual Ca^{2+} imaging of neurons and astrocytes, and will discuss their spatiotemporal relationship as well. COI:No

2P-020

Effect of exogenous coenzyme Q treatment on fEPSPs of the motor cortex of aged mice

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Brain mitochondrial function is impaired by not only disease like Parkinson's disease (PD) but also aging. Takahashi et al (2016) found that mitochondrial oxygen consumption rate (OCR), coenzyme Q (CoQ) content, and motor activities were reduced in aged (15-month-old) mice compared to those in young (6-month-old) mice. In this study, we examined whether normal aging affects electrophysiological activities of the motor cortex. Field excitatory postsynaptic potentials (fEPSPs) of primary (M1) and secondary (M2) motor cortices in brain slices of young or aged mice were recorded by multi-electrode array (MEA). In M1 region of aged mice, fEPSPs in a range from 20 to 80 μA current stimli were significantly small. The administration of exogenous water-soluble-CoQ_{10} to aged mice via drinking water restored the mitochondrial OCR, motor function, and phosphorylated α-synuclein and vesicular glutamate transporter 1 levels in the motor cortex (Takahashi et al, 2016). We also examined whether reduced fEPSPs of the motor cortex of aged mice were rescued by exogenous CoQ_{10}. These results suggest that age-associated motor impairment would be ameliorated by the exogenous CoQ_{10} treatment. COI:No

2P-021

The ubiquitin proteasome system regulates presynaptic long-term potentiation in the anterior cingulate cortex

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The ubiquitin proteasome system plays important roles in synaptic transmission. An E3 ubiquitin ligase, SCRAPPER regulates synaptic transmissions and long-term potentiation (LTP) in the hippocampus, but no report is available for the cortex. Here, we provide genetic evidence for critical roles of SCRAPPER in excitatory transmission and presynaptic LTP (pre-LTP) of the anterior cingulate cortex (ACC). We used in vitro whole-cell patch-clamp methods from layer II/III pyramidal neurons in adult mice ACC. First, we examined if transmitter releases of glutamate were changed or not among SCRAPPER knockout (SCR-KO), heterozygous (SCR-Hetero) and wild type (SCR-WT) groups. Next, we tested if the UPS including SCRAPPER could regulate the LTP in the ACC. The low frequency stimulation clearly induced the cortical pre-LTP. In the presence of a proteasome inhibitor, MG-132 in the bath solution, we applied the stimulation to induce pre-LTP. The induction of pre-LTP was reduced by MG-132. Finally, we studied if SCRAPPER contribute to the pre-LTP. SCR-KO mice inhibited the pre-LTP in the ACC. Our results thus provide direct evidence for SCRAPPER and the UPS in both spontaneous release and pre-LTP in the ACC. COI:No

2P-022

Analgesic effect of isoliquiritigenin on oral ulcer-induced pain through specific blocking of nociceptive peripheral nerves

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The traditional Japanese herbal medicine (Kampo) hangeshashinto has analgesic effect on oral ulcer-induced pain. Previously, we found that isoliquiritigenin (ILG), which is one of ingredient in hangeshashinto, showed strong inhibitory effect on $Na_V1.8$. In this study, to clarify the analgesic mechanism of ILG, we investigated effects of ILG on pain-related behaviors in naive and an oral ulcer model rats and on cultured rat sensory neurons. Oral ulcer was experimentally induced by topical acetic acid treatment on the oral mucosa of the labial fornix region. In our proprietary assay system for conscious rats, mechanical allodynia and spontaneous pain were significantly suppressed by swab application of ILG. Pain-related behaviors following subcutaneous injections of TRPV1, TRPA1 and TRPV4 agonists into the hind paw of naive rat were suppressed by co-injection of ILG. In patch-clamp recordings, ILG inhibited action potential generation in all small-sized and half of medium-sized sensory neurons, but did not in the remained half of medium-sized neurons. These findings suggest that the analgesic effect of ILG on oral ulcer is induced by blocking of specific sodium channel subtypes on nociceptive peripheral nerves. COI:No

2P-023

Arsenic and its metabolites affect synaptic plasticity mediated by AMPA type glutamate receptor trafficking in neurons

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Arsenic toxicity has been sporadically reported in entire world. In an effort to test if drinking of arsenic contaminated water may cause psycho-behavioral symptoms through AMPA type glutamate receptor (AMPA-R) function; major fast excitatory neuronal transmitter receptor in central nervous system, we initiated study of arsenic toxicity and neuronal malfunctions in neurons. The present study was aimed to evaluate the effects of four different types of arsenic, such as standard arsenic (As-V, As-III) and their metabolites Di-methylarsonous acid (DMA), Mono-methylarsonous acid (MMA) on receptor trafficking and function of AMPA-R, particularly focusing on GluA1/A2 subunits using primary cultured neurons. Two arsenics, As (III) and DMA, induce significant AMPA receptor internalization in primary culture neurons. Receptor internalization was partly mediated by GluA1 S845 phosphorylation. Internalized GluA1 contained synaptic and extra-synaptic receptors. Whole-cell patch-clamp recording from mouse hippocampal slices revealed that AMPAR-mediated excitatory postsynaptic current (EPSC) amplitudes were decreased by bath-application of DMA. Miniature EPSC analysis also demonstrated that exposure of DMA to the slice reduced the amplitude without changing the frequency. In addition, long-term potentiation (LTP) induction was significantly lower in the presence of DMA. COI:No

2P-024

Reduced synaptic transmission in neurons of mouse medial prefrontal cortex by knockdown of a schizophrenia-related molecule, Setd1a, during postnatal development

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Schizophrenia (SZ) is a heritable psychiatric disorder that causes impairment of perception, cognition, and social interaction. Some postmortem analysis of SZ patients shows changes in synapse density in the medial prefrontal cortex (mPFC) that is considered to be a center for the above higher brain functions. Although recent studies on common and rare genetic variants of SZ patients have identified numerous risk candidate genes, their roles in the development of mPFC neural circuits remains largely unknown. Here we focused on a SZ-associated gene, *SETD1A*, which codes a histone methyltransferase. There are some clinical reports showing mutations of *SETD1A* in SZ patients, but how the loss of function in *SETD1A* affects synapses has not been investigated. We examined possible roles of *Setd1a* in synapse development of mouse mPFC with RNA interference (RNAi)-mediated knockdown by in utero electroporation at embryonic days 14. Green florescent protein (GFP) was used as a marker for RNAi transduction. We then measured excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) from layer 2/3 pyramidal cells in mPFC slices by whole-cell recoding at postnatal days 16-25. We compared EPSCs and IPSCs between GFP-positive and -negative cells in the same mPFC slices. We found that both EPSCs and IPSCs were significantly reduced in GFP-positive knockdown cells compared with GFP-negative control cells. The results suggest that the lack of Setd1a may affect development of synapses in mPFC. COI:No

2P-025

The effect of chronic diazepam administration on hippocampal long-term potentiation and spine morphology

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Diazepam (DZP, a benzodiazepine) is widely prescribed for anxiety, epileptic discharge, insomnia, muscle-relaxing, and anti-convulsants. The long-term use of DZP is limited due to adverse effects such as tolerance, dependence, withdrawal effects. Additionally, clinical reports have shown that chronic DZP treatment increases the risk of dementing disorder. The adverse effects following chronic DZP treatment are complex processes that remain incompletely understood. Previously, we found that chronic administration of DZP upregulated lipocalin 2 (Lcn2) in hippocampus. It is known that Lcn2 controls neural excitability by regulating dendritic spine formation and maturation. In this study, we investigated the effect of chronic DZP administration on spatial memory, hippocampal long-term potentiation (LTP) and spine density in young (8 weeks-old) and aged (12 months-old) mice. The spatial memory was impaired by chronic DZP administration in aged mice but not in young mice. LTP was attenuated by DZP administration in young mice. The spine density of CA1 neuron was decreased by chronic DZP administration. These results suggested that cognitive dysfunction and LTP attenuation induced by chronic DZP administration were likely to be affected by a decrease of dendritic spine density in hippocampal neurons. COI:No

2P-026

Functional roles of src kinase activity in presynaptic terminals of avain cochlear nucleus

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Src kinase is expressed in many neurons and thought to be involved in the regulation of synaptic transmission. In the hippocampal neurons, long-term potentiation is induced by increasing the expression of NMDA receptors at postsynapse via activity dependent regulation of src kinase. However, it is not well understood whether and how src kinase regulates synaptic transmission of presynaptic terminals. In this study, we examined the contribution of src kinase to the synaptic transmission at avian cochlear nucleus, where an auditory nerve fiber forms a large end-bulb synapse. We made brainstem slice preparation from posthatch chicks and recorded EPSC in cochlear nuclear neurons, while stimulating the auditory nerve. We found that application of src kinase inhibitor (PP2) to the bath increased the evoked EPSC amplitude. On the other hand, mEPSC amplitude did not change with PP2, suggesting that the increase of EPSC would be mediated by presynaptic mechanisms. Then, the factors for EPSC quantal content, N (number of releasable vesicles) and P (release probability), were estimated from the plot of cumulative EPSC amplitude during train stimulation. After application of PP2, the N increased in parallel with EPSC amplitude, whereas the P did not change. Thus, we concluded that the src kinase suppresses the neurotransmitter release by decreasing N in avian cochlear nucleus. We will further examine the effects of physiological stimulation on the src kinase regulation and discuss the physiological roles of it. COI:No

2P-027

Reproducing scotopic electroretinogram wave using one-dimensional retinal circuit model

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Electroretinogram (ERG) is an electrical potential generated by the retina in response to a change in illumination. Since pattern of temporal change in the electric potential represents retinal function, ERG is useful for evaluating physiology and pathophysiology of retina. Although it has been suggested that photoreceptor and bipolar cells are predominantly responsible for generating the ERG wave, electrical mechanism explaining how these cells contribute to a wave form of ERG remains obscure. Detailed mathematical model of retinal cells would be a powerful tool for quantitative understanding of the ERG and underlying electrical interactions in the visual system. To reproduce scotopic ERG wave, we constructed a mathematical model of a one-dimensional retinal circuit consisting of photoreceptor and bipolar cells. About 350 grid points were assumed for the retinal circuit, for the tip of photoreceptor cell and the end of the bipolar. Using the bidomain equation, and electrical potentials and currents on both intra- and extracellular sides we calculated at every grid point. The current retinal circuit model well reproduced ERG in good agreement with the experimentally data. COI:No

2P-028

PKCγ contributes to the motor coordination through the modulation of voltage gated sodium channel properties in mature Purkinje cells.

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Protein kinase C γ (PKC γ) is expressed exclusively in neurons of the brain and spinal cord. PKC γ-deficient mice have been shown to exhibit normal cerebellar long-term depression (LTD) but deficient pruning of climbing fibers (CFs) onto developing Purkinje cells (PCs) and impaired motor coordination. These suggest a critical role for PKC γ in brain development and a possible contribution to developmental abnormalities leading to motor deficits. However, the physiological significance of PKC γ in mature animals has remained unknown. To clarify this, we compared the electrophysiological properties of PKC γ-null PCs with those of wild-type (WT) PCs. After confirming no difference in CF-PC (except for multiple innervation) and PF-PC synaptic transmission between WT and KO mice, we found the significantly higher threshold for action potential generation in KO PCs than in WT PCs. Then, we examined whether viral vector-mediated re-expression of PKC γ in the PCs of matured PKC γ-KO mice rescued the defects seen in KO mice. The rescue significantly restored the action potential threshold and the motor performance, while the multiple CF innervations of PCs remained unaltered. Additionally, we found the impaired voltage gated sodium channel (VGSC) currents in the KO PCs, that were rescued by the re-expression of PKC γ.The results suggest that, via the regulation of VGSCs, PKC γ modulates the threshold for action potential generation, potentially shapes the firing property in PCs and critically regulates motor function in adult mice. COI:No

2P-029

Activity of dorsal striatum during sleep-wake transition

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Sleep/wake transition is controlled by several brain regions. Among these, the ventral striatum (VS) is known to enhance sleep and the dorsal striatum (DS) is known to enhance wakefulness. Recently, Lazarus group at Tsukuba elegantly showed that the pharmocogenetic activation of striatopallidal neurons in the VS induced NREM sleep and the inactivation of same neurons enhanced wakefulness, indicating the direct causality of VS striatopallidal neurons and sleep/wake transition. However, little is known about how these neurons behave during sleep/wake transition. Regarding the DS, both cell type specific manipulation and monitoring are lacking. Here we motivated to understand the role of the VS on sleep/wake transition and started to monitor DS neuron activity. We generated transgenic mice in which the calcium indicator (Yellow Cameleon nano 50) was selectively expressed in striatopallidal neurons and recorded the compound activity of these cells in the DS by using a ratiometric fiberphotometry system. Simultaneously we recorded EEG and EMG to identify the sleep stage. DS striatopallidal neurons showed high levels of compound Ca2+ during awake period and low levels during sleeping period in general. We observed the fluctuation of Ca2+ signals regardless of the basal activity. According to these activity profiles, we move to optogenetic manipulation to understand causal relationship between DS striatopallidal neuron activity and wakefulness. COI:No

2P-030

The estimate of spatial distribution of readily-releasable vesicles at a central presynaptic terminal

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The timing and probability of synaptic vesicle fusion from presynaptic terminals is critically controlled by the strength of coupling between voltage-gated Ca^{2+} channels (VGCCs) and Ca^{2+} sensors for exocytosis. This coupling distance can be estimated from the fractional block of vesicular release by exogenous Ca^{2+} chelators such as EGTA (Neher, 1998; Eggermann et al., 2012). However, when multiple readily-releasable synaptic vesicles (SVs) are simultaneously coupled to single VGCC or a VGCC cluster at different distances, simple mean distance does not stand due to the nonlinearities of Ca2+ gradient at the vicinity of VGCC and Ca^{2+} sensors. To estimate overall spatial distribution of readily-releasable SVs around a VGCC cluster, we used deconvolution analysis of EPSCs evoked by voltage-step of various duration, and simulations of presynaptic Ca^{2+} and release probability in at the active zone in the calyx of Held presynaptic terminal in the absence/presence of EGTA. These experiments and analysis allowed us to reconcile the exact timing of exocytosis with distances from a VGCC cluster. Our results indicate that the ~50% of readily-released SVs are tightly coupled with VGCC cluster (< 50 nm) contributing to a fast component of exocytosis, and the remaining is widely distributed over 60-200 nm mediating a slower component at this high fidelity auditory synapse. COI:No

2P-031

Anatomical pathway for perioral sensory signals to the inferior olive in the mouse

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Perioral tactile signals from the infraorbital nerve (ION) are initially transmitted to trigeminal nuclei. Cerebellar Purkinje cells (PCs) receive these signals via the inferior olive (IO), generating complex spikes (CSs). The IO directly receives inputs from contralateral spinal trigeminal nuclei, but it was not clear whether the perioral tactile signals are transmitted through this pathway or not. We examined the anatomical pathway for perioral sensory transduction from trigeminal nuclei to the IO in mice. The CS generation evoked by ipsilateral ION stimulations was completely suppressed by a $GABA_A$ receptor agonist, muscimol, injections into the contralateral area parafascicularis prerubralis (PfPr) in the mesodiencephalic junction. CSs evoked by ipsilateral whisker stimulations by air-puffs were also suppressed. In contrast, inhibition of the primary motor or somatosensory cortex did not suppress the CS generation by ION stimulations, suggesting that the cerebral cortex was not directly involved in the signaling pathway. These results indicate that the PfPr is a relay area for tactile signals to the IO. COI:No

2P-032

Developmental change of the prefrontal cortex for tactile stimulation in infancy: A Longitudinal study

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Touch is considered to facilitate normal mental development in humans from several behavioral and physiological studies. In fact, previous studies have exhibited that touch, especially pleasant touch, activates reward-related cortical regions including the anterior prefrontal cortex (APFC) in adults. Although it is known that glucose uptake increases in the dorsal and medial frontal cortices by 10 months, the developmental neural system underlying affective touch during the age is still unclear. The current study used near infrared spectroscopy (NIRS) to examine activation of the APFC by gentle touching of the hand and the waist of infants 3-10 months after birth, who were classified into four groups (3, 6, 8 and 10 months old). Results indicate that 10-month-olds, but not 3- and 6- 8- month-olds, showed activation of the APFC by gentle touching of the palm with a sensuous pleasant material. The present finding therefore demonstrates that developmental neural system underlying affective touch in infancy is reflected to the activation of the APFC and that the critical point is approximately 10 months after birth. COI:No

2P-033

Spinal nociceptive sensitization in a rat model of chronic fatigue syndrome

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Pain is one of the debilitating symptoms in patients with chronic fatigue syndrome (CFS), but the underlying mechanisms are poorly understood. We investigated the peripheral and spinal mechanisms using a rat model of CFS. The model was made under continuous stress by keeping rats in a cage filled with water (1.5 cm in depth) for 5 days (Yasui et al., Glia, 2014). Under urethane anesthesia extracellular recordings of the superficial dorsal horn (SDH) was performed in vivo. The SDH cells were found to be sensitized to mechanical stimulation applied with von Frey filaments (vFFs). Spontaneous firings of the SDH neurons were also increased, and the firing rate was significantly correlated with the rate induced by vFFs in the CFS group. C-Fos-immunoreactive nuclei were significantly increased in the SDH. In the peripheral nervous system, neither general characteristics nor responsiveness of C-fiber nociceptors to noxious stimuli was changed in the CFS model. These results suggest that spinal, but not peripheral, nociceptive sensitization is involved in the pain mechanisms of CFS. COI:No

2P-034

Serotonergic modulatory effects on behavioral and neural contrast sensitivity of rats

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Serotonin (5-HT) is one of the neuromodulators and released from serotonergic neurons in the dorsal raphe nuclei to almost the whole brain including cortical visual areas in a behavioral context-dependent manner.Recently, we found that 1) the intraperitoneal administration of fluoxetine (FLX), selective serotonin reuptake inhibitor improves the behavioral contrast sensitivity (CS) of freely moving rats performing a grating detection task and 2) modulates the CS of neurons in the primary visual cortex (V1) of head-fixed and passively viewing, awake rats. These results suggest that FLX improves behavioral CS via serotonergic modulation of V1 neurons. Since the behavioral and electrophysiological studies were conducted separately in different experimental conditions, it remain unsolved whether and how serotonergic modulation of behavioral CS correlates with that of neural CS of V1 neurons. To answer the question, we newly developed the system which enables to measure both behavioral and neural CS simultaneously for head-restraint rat. Rats performed a grating detection task, making Go/No-Go reactions by lever manipulation. We adopted "method of constant stimuli" procedure to obtain the two functions of behavioral detection ability and the neuronal response in relation to grating contrast. We are currently conducting the experiments with and without FLX administration using this system. COI:No

2P-035

Peripheral visual field stimuli for SSVEP-based brain-machine interface.

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Brain-machine interface (BMI) or brain-computer interface (BCI) is an interface technology that utilizes neurophysiological signals from the brain to control external machines or computers. Steady-state visual evoked potential (SSVEP) is one of the main methods for the BMI, and the SSVEP-based BMI has been used in the situations, whose eye movements are freely available. In this study, we investigate the attentional modulation of the SSVEP signals elicited by the peripheral visual field stimuli.

Seven able-bodied participants (age 31.0 years old, 4 male) participated in this study. We prepared a green/blue LED flicker (Sakurada, et al., 2015). The LED was placed in the peripheral visual field at 5, 10, 15, 30 and 45 degrees in the opposite side of the dominant eye. Participants were asked to gaze at a central fixation point, and attend to or ignore the LED (attention/ignore tasks). EEG signals were measured from Oz, PO7 and PO8. We calculated the power of the EEG data.

A two-way ANOVA was applied to the power of the EEG, and a significant main effect was shown between the attention/ignore tasks (attention: $F(1,6) = 5.38$ ($p<0.05$)).

The result suggests that the peripheral visual field stimuli are potentially useful for the SSVEP-based BMI. COI:No

2P-036

Comparison between ulnar nerve crush- and anesthesia-induced acute change in the propagating excitation wave pattern on the rat somatosensory cortex

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Using our original optical system for recording membrane potential, we have reported that neural response to a somatic stimulation spreads from the somatotopically corresponding site to whole somatosensory cortex as a wave. In this study, we evaluated the acute change in the spatiotemporal pattern of this wave derived from two kinds of ulnar nerve disorder; crush (UC) or anesthesia (UA). The right ulnar nerve of rats was crushed by forceps (UC) or anesthetized by 2% lidocaine (UA). We electrically stimulated the hypothenar pad of the right forepaw. The optical recordings were performed 3 times; before (PRE), immediately after (UC0) and 30 min after (UC30) the nerve crush in UC group, or before (PRE), 5min after anesthesia (UA5) and recovery (30 min after wash: UR30) in UA group, respectively. After ulnar nerve disorder, the propagation velocity exhibited the most notable change around the initiation site. Both at UC0 and UA5, the velocity in the medial-posterior direction was significantly lower than that at PRE. At UR30 it was remarkably increased, even higher than that at PRE, but there were no changes between at UC0 and UC30. Thus, the decreased velocity lasted at least as long as 30 min after the crush while recovered 30 min after washout of anesthetic. These results indicate that the acute changes in the propagating wave pattern after the nerve disorder as well as the recovery might play an important role in the cortical reorganization observed after peripheral nerve injury. COI:No

2P-037

Effects of motor cortex tetanic-stimuli on the spontaneous single-unit activity and the motor cortex single-square-pulse-stimulus-induced field potentials in the rostral ventromedial medulla in chronic pain model (spared nerve injury) rats

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Motor cortex stimuli lead to anti-nociceptive effects in chronic pain model rats, however, the precise mechanism remains to be unknown. The purpose of this study is to examine whether the neurons in the rostral ventromedial medulla (RVM) are involved in cortex-stimuli-induced anti-nociception. We tested the effects of motor cortex tetanic stimuli (50Hz, 30 min) on the spontaneous single unit activity and the field potentials in the RVM in chronic pain model (spared nerve injury (SNI) model) rats. Spontaneous single unit activity, and the field potentials evoked by single square pulse stimulus to the motor cortex were recorded with the same tungsten microelectrode located in the RVM. The recorded single unit activity was classified into the on, off and neutral cells, based on their responses to nociceptive pinch stimuli applied to the skin. We found that in SNI rats the off cells increased their spontaneous single unit activity, and the on cells decreased their spontaneous single unit activity, for at least 30 min after the motor cortex tetanic stimuli. We also found that in SNI rats the field potentials recorded at the on cell expressing sites were depressed. These results suggest that the RVM is involved in the motor cortex stimuli-induced anti-nociceptive effects, and synaptic plasticity underlies these anti-nociceptive effects. COI:No

2P-038

Live imaging of calcium dynamics in human epidermis in response to point laser stimulation

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Changes of epidermal calcium ion concentration are involved in regulation of barrier homeostasis and keratinocyte differentiation. Moreover, intracellular calcium dynamics might play a role in skin sensation. Although calcium dynamics of cultured keratinocytes in response to mechanical stresses has been well studied, calcium propagation in stimulated human epidermis is still poorly understood. In this study, we demonstrated a novel method for real-time measurement of calcium dynamics in response to point stimulation of human epidermis.We examined calcium propagation in living human epidermis ex vivo, as well as in cultured human keratinocytes, using two-photon microscopy after stimulating cells in stratum granulosum. Cells in stratum basale showed the greatest elevation of intracellular calcium. Calcium propagation from stratum granulosum to stratum basale was inhibited in the presence of apyrase, which degrades ATP (adenosine triphosphate), or gap-junction blockers. These results suggested that ATP and/or gap junctions might play an important role in calcium propagation induced by point laser stimulation of the uppermost layer of epidermis. Our method should be broadly useful to study calcium dynamics, epidermal physiological mechanisms, and mechanisms of skin sensation at the single-cell level. COI:No

2P-039

In vivo imaging of visual response dynamics from the SC of awake mice.

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The superior colliculus (SC) is a brainstem center which has key roles in generating spatial attention and mediating the signal for sensory-motor translation. It is hypothesized that integrating spatial information is essential for selecting the target and paying an attention to it; however, it was difficult to examine spatial processing of visual information via capturing small number neuronal activities at a time. Recently, we have shown a method of *in vivo* population imaging of visual responses from the SC of anesthetized mice. Here we improve the previous method and present a new method for stable and chronic imaging from the SC of awake head-fixed mice. To achieve longterm optical access to the SC, a small glass cube are implanted. GCaMP6f, Ca^{2+} sensitive fluorescent indicator, is delivered in the SC neurons via AAV vector injection and their population responses are recorded using two-photon laser microscopy through the implanted imaging window. To monitor the behavior of mice during visual stimulus presentation, we simultaneously recorded saccadic eye movements and locomotion activities.We first compared visual population responses between anesthetized condition and awake behaving condition. We found that individual neuronal responses are much stronger in the awake condition and increases tuning sensitivity to stimulus sizes. We also found widely spreading Ca^{2+} signal in the superficial layer of the SC at the beginning of locomotion behavior. Our chronic imaging method from deep site should be applicable in other brain area and useful for examining neural implementation of sensory-motor interaction. COI:No

2P-040

Optogenetic analysis of nociceptive mechanism mediated by 5-HT neurons in the nucleus raphe magnus

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The descending 5-HT-mediated pain inhibition pathway originates in the nucleus raphe magnus (nRM) of the rostral ventromedial medulla. However, the effects of selective 5-HT neuron stimulation in the nRM remain unclear. The descending inhibition induced by morphine was damaged in a mouse model of fibromyalgia. Therefore, we investigated whether selective excitation of 5-HT neurons in the nRM influences nociceptive behavior using an intermittent cold stress (ICS) mouse model of fibromyalgia. Genetically modified mice [Tph2-tTA::tetO-ChR2(C128S)] expressing channel rhodopsin 2 mutation [ChR2(C128S) step function opsin] on brain 5-HT neurons were used. An optical fiber was inserted above the nRM, and nociception tests were performed using von Frey and hot plate tests. The optical fiber was illuminated using a Teleopto stimulator. The ICS model mice were treated for 4 days, including 3 nights at 4 ºC and 2 days with room temperatures alternating from 24 ºC to 4 ºC every 30 min. Illumination of the nRM increased the threshold of the hot plate test, but not the von Frey test, in the control and ICS model mice. The ICS treatment and intraperitoneal 5-HT1A antagonist decreased the threshold of the von Frey test, but not the hot plate test. These results suggest that in freely moving mice, 5-HT neurons of the nRM mediate thermal and mechanical nociceptive behavior in different manners. COI:No

2P-041

Neural basis for chronic headache and photophobia after mild traumatic brain injury

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Mild traumatic brain injury (mTBI) accounts for the majority of head injuries, and posttraumatic headache (PTH) is the most common adverse effect. The most PTH has migraine-like phenotype (Hyperalgesia, Photophobia, Throbbing pain) and is difficult to resolve. The goal of this study was to establish a new model for the post-TBI headache.The parietal region of male rats was irradiated with laser-induced shock wave (diameter 3mm, 4J/cm2) under barbiturate anesthesia. At first, in awake rats, eye wipe behavior and light-aversion were tested. Second, under isoflurane anesthesia, single cornea/dura responsive neurons were recorded at the Vc. Hypertonic saline (0.15-5M) and blight light (irradiance=50, 300, 500 W/m2) selectively activated ocular surface and intraocular (neurovascular system), respectively. Third, under isoflurane anesthesia, light evoked blood flow change was monitored in arteries of the exposed cranial dura mater and the parietal cortex. Eye wiping and light-aversion behavior were enhanced in ten days after post mTBI (TBI rat). In TBI rats, Vc units had enhanced responses to hypertonic saline and blight light compared to naive rats. Bright light enhanced the magnitude of dural blood flow in TBI rat but not naive rat, and this blood flow increases evoked dura -responsive Vc units activities.It is concluded that mTBI produces a chronic state of hyperalgesia and light evoked vessels dilation that is reflected in the sensitization of trigeminal -parasympathetic circuit. This model may be suitable for future studies of migraine. COI:No

2P-042

Nicotinic regulation of sound-evoked activities by protein kinase A in mouse primary auditory cortex

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Systemic nicotine exposure increases the characteristic frequency (CF) tone-evoked current sinks but decreases nonCF tone-evoked current sinks via nicotinic acetylcholine receptors. This nicotinic regulation lasted for at least 30 minutes in the primary auditory cortex (A1) (Kawai et al., 2011, J. Neurosci., 31, 14367-14377) and was mediated by extracellular signal-regulated kinases ERK1/2 (Intskirveli and Metherate, 2012). Since ERK1/2 is activated by protein kinase A (PKA), we investigated whether the nicotinic enhancement is mediated by PKA. We recorded white noise (WN)-evoked local field potentials (LFPs) in A1. Nicotine (2 mg/kg mouse) increased initial slope reliability and subsequent LFP gamma oscillation power in two phases: early (<10 min) and late (>10 min) sustained phases. A PKA inhibitor myristoylated PKI 14-22 amide mainly inhibited the late phase. The initial slope increase in the late phase was correlated with the phosphorylation of the GluA1 subunits of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) at serine 845 by PKA. The results suggest that PKA mediates the sustained nicotinic enhancement at excitatory synapses. Since excitatory inputs to inhibitory neurons have an important role for gamma oscillation (Salkoff et al., 2015, J. Neurosci., 35, 10236-10251), PKA at excitatory synapses may contribute to the nicotinic regulation of sound-evoked cortical processing in A1. COI:No

2P-043

Visualization of odor- and taste-evoked cortical responses by *in vivo* optical imaging with flavoprotein autofluorescence

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The integration of the gustation and olfaction is important to form the basis of flavor. Two sensory pathways are generally thought to converge in the same secondary sensory cortex, the orbitofrontal cortex. Recently, some human fMRI and our previous studies indicated a possibility that the insular cortex, including the primary gustatory cortex, processes not only taste information but also odor information. However, little is known whether and how two sensory inputs converged in the insular cortex.To address these issues, we observed mouse cortical responses to either or both of two stimuli, odorants and tastants, by *in vivo* optical imaging with flavoprotein autofluorescence. Taste stimulation on the tongue of anesthetized mouse evoked obvious responses in the extensive area of the insular cortex. On the other hand, when the air flow including odors were administered to the nasal cavities, the evoked-responses were observed in the piriform cortex. The areas and amplitudes of such odors-evoked responses increased in a concentration-dependent manner. Simultaneous stimulation by odorants and tastants evoked additional responses in the agranular insular cortex, area of which tended to increase toward the its rostral part. This spatiotemporal change in cortical responses suggest that the insular cortex really take part in integration of taste and odor information, especially the agranular insular cortex might be a key center for flavor formation. COI:No

2P-044

TRPV4 involvement in gender differences of blood pressure control in SHR

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Pre-menopausal women exhibit a lower arterial pressure (AP), a lower sympathetic outflow and a greater baroreceptor reflex than age-matched men but the molecular mechanisms are still not well understood. We hypothesized that a different neuronal function between men and women at the level of the nucleus tractus solitarius (NTS), a pivotal region of the medulla oblongata for regulating the set-point of AP, could contribute to the gender difference in cardiovascular homeostasis.We performed gene expression profiling of the NTS of Spontaneous Hypertensive Rats (SHRs), a strain that clearly exhibits a gender difference in AP levels, by using microarray technique and gene ontology/pathways analysis. Target molecules were microinjected into the NTS of SHRs to analyze their functional role on AP level. Ovariectomized SHR females were treated with estrogen for one month and RT-qPCR was used to assess the effect of estrogen on candidate genes expression in the NTS.The expression of TRPV4 (Transient receptor potential cation channel subfamily V member 4), a channel involved in brain fluid and ion homeostasis control, was found significantly up-regulated in the NTS of female SHRs compared to males. TRPV4 agonist 4α-PDD decreased AP when injected into the NTS of female SHRs. The expression of TRPV4 transcript was not affected by estrogen level. Thus, TRPV4 might be involved in the gender difference of AP control via the estrogen-independent alteration of its expression level in the NTS of female SHRs. COI:No

2P-045

Age-related attenuation of parasympathetic nerve-evoked blood flow response in the orofacial area of rats

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Aging has received significant attention among the public in the super-aged Japanese society. It is known to cause blood vessel aging, resulting in symptoms such as retardation of wound healing and sensitivity to cold. Hence, functional disorders triggered by aging may be related to the blood flow within the affected region, although the presence of such a relation in the facial area remains unclear. Age-related changes in parasympathetic nerve-evoked blood flow response are particularly appealing because of the speedy and wide blood flow increases controlled by parasympathetic nerves in the facial area. Here, we compared the changes in hemodynamics evoked by the trigeminal afferents of the masseter muscle (cholinergic) and the lower lip (non-cholinergic) between two age groups of rats in order to examine the effects of aging in the orofacial area. Stimulation of the lingual nerve (LN) resulted in significant increases in blood flow in the lower lip (LBF) and masseter (MBF) in young rats. In the older rats, no significant difference in MBF was observed; however, LBF was significantly increased. The dose of exogenous acetylcholine required for the increase in MBF was higher in the older rats when compared with the young rats. Muscarinic receptor (M3) mRNA expression in the young rats was higher than that in the older rats. Our results indicate that aging attenuates the parasympathetic cholinergic nerve-evoked increase in blood flow in the orofacial area, and suggests that the reduction in M3 expression may be involved in this response. COI:No

2P-046

Pharmacological analysis of autonomic nerve innervation for colonic transit in conscious rats.

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We reported that colonic transit(CT) was measured for time-course analysis by a method using radiopaque markers. Furthermore, it allows a calculation of CT by the geometric center(GC). The aim of this study is to investigate the autonomic nerve innervation of CT with pharmacological technique in conscious rats. The operation had been demonstrated under the isoflurane in 5 days before measurement time for set the cannula into the cecum to connect the proximal colon. Other side of the cannula was through under the back skin to inject makers for the measure of CT that had been repeated twice in DAY1 and DAY2. On DAY1, all rats were intraperitoneally administered with saline as a control. Rats were dived into 4 groups and administered different agonist or antagonist intraperitoneally for each group on DAY2: atropine, neostigmine, phentolamine or propranolol. Twenty markers were administrated into the proximal colon with saline. It was visible throughout the GI tract via soft X-ray from just after injection of markers to 240min every 30min. CT was calculated by the GC on the images of those. CT was accelerated by neostigmine and phentolamine compared with control. However, atropine and propranolol did not change CT. It is concluded that a remarkable accelerated CT is induced in the enhanced parasympathetic nerve activity and the spontaneous CT is modulated by sympathetic nerve activity via α-adrenoceptor. COI:No

2P-047

Change in blood pressure by inhalation of fragrance of essential oil and muscle sympathetic nerve activity.

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We recently reported that inhalation of fragrance of Grapefruit essential oil (GF) increased blood pressure (BP) while that of Marjoram essential oil (MJ) decreased BP. We tested the hypothesis that changes in BP induced by inhalation of the GF or MJ were associated with changes in muscle sympathetic nerve activity (MSNA).Thirteen healthy subjects participated in this study. The experiment was performed in the supine position. While subjects in the supine position, they breathed blank air through a face mask for 10 min as baseline. Then, they inhaled air with the GF or MJ from Douglas bags for 10 min. Throughout the trial, MSNA (peroneal nerve, microneurography), beat-by-beat heart rate (HR) and BP, and breath-by-breath respiratory variables were recorded continuously.By inhalation of the GF, diastolic BP (DBP) increased at 8 to 10 min of inhalation (64.0 $\pm$ 8.8 mmHg) compared to baseline (62.1 $\pm$ 9.5 mmHg), whereas MSNA burst rate remained unchanged at the period. Also, by inhalation of the MJ, DBP increased at 8 to 10 min of inhalation (61.1 $\pm$ 7.3 mmHg) compared to baseline (59.7 $\pm$ 7.5 mmHg) against our previous findings, while MSNA burst rate remained unchanged at the period. HR and respiratory variables remained unchanged in both trials.Increase in BP induced by inhalation of the GF was not associated with the mechanisms of increase in MSNA. COI:No

2P-048

Responses in facial skin blood flow and prefrontal oxygenation during exposures to emotionally charged negative and positive odors

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We examined the responses in facial skin blood flow and prefrontal oxygenated-hemoglobin (Oxy-Hb) (Peach odor, Butyric acid odor, and Placebo) in humans. The concentrations of Oxy-Hb in prefrontal cortices were measured with near-infrared spectroscopy to monitor regional cerebral blood flow. Simultaneously, regional facial skin blood flows were assessed with noninvasive two-dimensional laser speckle flowmetry. The extents of pleasantness and consciousness for each emotional stimulus were estimated by the subjective ratings of pleasantness and consciousness from -5 (the most unpleasant; the most unconscious) to +5 (the most pleasant; the most conscious). As soon as butyric acid odor (negative odor) was exposed, both the Oxy-Hb of the prefrontal cortices and facial skin blood flow increased. The increase in the prefrontal Oxy-Hb had a significant correlation with the increase in facial skin blood flow. On the other hand, the prefrontal Oxy-Hb did not change during exposure to peach odor (positive odor). The present findings suggest that negative emotion induces increase in prefrontal Oxy-Hb, which may in turn elicit an increase in facial skin blood flow. To examine the responses among the different subdivisions of the prefrontal cortex, a study using a multichannel NIRS will be conducted and the results will be discussed. COI:No

2P-049

Role of Orexin neurons in the hypothalamus on the cardiovascular response during chronic social defeat stress in the rat

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Neurons in the hypothalamus play an essential role on the autonomic cardiovascular evoked by psychological stress such as changes in interpersonal issue. In addition, orexin and orexin neurons are localized within a restricted region around the hypothalamus and it is indicated that the orexin neurons may be involved in the autonomic response during the stress. In the present study, we investigated the distributions of expression of c-Fos (a marker of neuronal activation) in orexin neurons at hypothalamus during social defeat stress in conscious Wistar rats. The Wistar rat (an intruder) was moved into a home cage of a Long Evans rat (a resident). After the social-defeated relationship was established between the intruder and the resident, the rats were separated with a wire-mesh in the same cage for 55 min. The chronic social defeat stress was made to the Wistar rat for 2 weeks every day. In the intruder rat, blood pressure and heart rate were maintained at higher level than those in the non-stress control animal throughout the defeat stress period. The c-Fos expressions in orexin neurons in the hypothalamus were strongly increased in the stress group compare to the control group. These results suggest that the orexin neurons in the hypothalamus may play important role on the cardiovascular response evoked by the chronic social defeat stress. COI:No

2P-050

Central alteration of baroreceptor reflex during anaphylactic hypotension

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Anaphylaxis, a systemic allergic reaction, causes shock symptoms such as hypotension and consciousness disturbance when it becomes severe. Various opinions have been suggested sympathetic regulatory reactions during anaphylactic hypotension, but a consensus opinion has not been obtained. In this study, we investigated the sympathetic baroreceptor response and its central mechanism during anaphylactic hypotension in acute and chronic experiments. An allergy rat model was made by injecting ovalbumin (OVA) subcutaneously. Two weeks later, re-sensitization of OVA was made by intravenous (iv) injection of re-sensitization of OVA with recording pressure (BP), heart rate (HR), and renal sympathetic nerve activity (RSNA) in urethane anesthetized sensitized rat. In the anaphylactic group, the re-sensitization caused large decrease in BP. In the control group, continuous iv administration of sodium nitroprusside, vasodilator agent produced BP decrease to the level similar to the anaphylactic hypotension. The re-sensitization of OVA caused decrease in RSNA compared to the control group. Therefore, these results suggest that anaphylactic sensitization with OVA may centrally inhibit the baroreceptor reflex function of sympathetic nervous system by some mechanism. We also report the distribution of c-Fos immunoreactive neurons in the medulla that were obtained in chronic experiment. COI:No

2P-051

Blood pressure regulation by neurons projecting from the mesencephalic locomotor region to the rostral ventrolateral medulla in rats

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We previously reported that rat rostral ventrolateral medulla (RVLM) C1 neurons were specifically excited by voluntary exercise (Kumada *et al.*, in press). Here, we attempted to identify the upstream pathway to the RVLM, thereby underlying exercise-elicited autonomic adjustments. Retrograde neuronal tracing experiments revealed that the mesencephalic locomotor region (MLR), which abundantly contains cholinergic neurons, sends direct projections to the RVLM in rats. Very few neurons that exhibited choline acetyltransferase immunoreactivity in the MLR were RVLM-projecting (0.6 ± 0.3%, N=3), suggesting that the MLR-RVLM neurons are not cholinergic. Next, we examined the role played by MLR-RVLM neurons in blood pressure regulation. Rats received bilateral microinjections into the MLR with an AAV vector that encodes the channelrhodopsin variant, ChIEF-tdTomato. In the anesthetized rats (N=7), two-min bilateral photostimulation (473 nm wavelength, 10 mW, 40 Hz) of the RVLM significantly ($p<0.05$) increased blood pressure (+7 ± 1 mmHg). Moreover, confocal imaging showed that MLR-derived, tdTomato-labeled axons containing VGLUT2 were closely associated with tyrosine hydroxylase-positive RVLM neurons. Based on these observations, we propose the hypothesis that MLR-RVLM glutamatergic neurons are involved in autonomic adjustments to exercise. Further studies are required to test this hypothesis. COI:No

2P-052

Segmental modulation of cortical cerebral blood flow by acupuncture-like stimulation in anesthetized rats

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Acupuncture to the auricular region is useful in improving symptoms accompanied by cerebral circulation disturbances. The mechanism underlying the beneficial effects remains unknown. We hypothesized that acupuncture to the auricular region innervated by multiple somatic afferent nerves increases cerebral circulation. To explore the hypothesis, we investigated whether acupuncture to the auricular region increases the cortical regional cerebral blood flow (CBF) and whether there is a segmental organization in this response. Cortical CBF was measured by laser speckle contrast imaging, in urethane-anesthetized rats. Acupuncture-like simulation was manually performed at the auricular concha or the abdomen. The auricular stimulation significantly increased in CBF of the bilateral cerebral cortex in the frontal, parietal and occipital lobes, without changes in systemic arterial pressure. In contrast, abdominal stimulation did not change the cortical CBF and systemic arterial pressure. The increase in cortical CBF induced by auricular stimulation was completely abolished by severance of somatic nerves innervating the auricular region, comprising the trigeminal nerve, facial nerve, auricular branch of vagal nerve, glossopharyngeal nerve and great auricular nerve. This study concludes that acupuncture to the auricular region induces an increase in the cortical CBF via somatic afferent nerves in a manner of segmental organization. COI:No

2P-053

Sex-specific regulation of colorectal motility via descending inhibitory pathway in rats

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Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by abdominal pain and altered bowel habits. While there is ample evidence for sex differences in altered bowel habits (constipation is common in women whereas diarrhea is common in men.), the underling mechanism is unclear. We have previously demonstrated that activation of the descending pain inhibitory pathway by intracolonic noxious stimulation enhances colorectal motility. In this study, we aimed to examine sex differences in the regulatory mechanism controlling colorectal motility via the descending pathway. Anesthetized rats were cannulated in the colorectum, and changes of intraluminal pressure and expelled volume were measured. In males, application of noxious stimulant capsaicin into the colorectal lumen enhanced colorectal motility. The effect of capsaicin was abolished by transection of the thoracic spinal cord, suggesting that the noxious stimulation promotes an intrinsic activation of descending pathways from the brain to spinal defecation center located at the L6-S1 level of the spinal cord. In contrast to males, intracolonic capsaicin had no effect in females. The effect of capsaicin in males was mediated by dopamine and/or serotonin at the spinal defecation center. These monoamines administrated into the lumbosacral cord enhanced colorectal motility even in female rats. Thus, the notable sex differences in the regulatory mechanism of colorectal motility would be due to differences in the central neural circuit. (COI:No) COI:No

2P-054

4-AP long-term treatment facilitates Kv1.5 channel expression on cell surface through its action as a chemical chaperone

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Human Kv1.5 underlies the cardiac ultra-rapid delayed rectifier potassium current (I_{Kur}), which only functional expresses in human atria but scarcely in ventricle. However, Kv1.5 loss-of-function mutations have been reported to produce kindred atrial fibrilation, which suggest that the genetic alteration of Kv1.5 may substantially enhance arrhythmia susceptibility. As a relatively selective blocker of Kv1 family members, 4-AP (4-aminopyridine) suppresses Kv1.5 with acute treatment. Using HEK overexpressing hKv1.5, we investigate hKv1.5 channels in forward trafficking and protein degradation with 4-AP long-term treatment. Our studies show long-term 4-AP incubation facilitates Kv1.5 glycosylation rate and promotes Kv1.5 functional expression (stability of cell surface-expression). Residues of I502 and I508 locate in the pore (outer pore or pore helix) of hKv1.5 channels, which providing structural components for blockade of the channel. Our results show 4-AP not only fails to inhibit mutant of I508 current under acute exposure and also loses the ability of rescuing after long-term treatment, which suggest that I508 would be the chaperone point of 4-AP on Kv1.5 channel. COI:No

2P-055

Analyses of the structural rearrangements of P2X2 receptor by voltage-clamp fluorometry using fUAA fluorophore - quencher pairing

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P2X2 is a ligand-gated ion channel activated by extracellular ATP. This receptor shows a complex gating depending on both [ATP] and voltage, in spite of the absence of a canonical voltage sensor domain. It remains unknown how the structural rearrangements occur during voltage dependent gating. The detail of the structural rearrangements upon ATP binding in the pore region also remains controversial. Thus, we aim to analyze in this study the structural rearrangements upon (1) voltage and (2) ATP dependent gating, by voltage-clamp fluorometry (VCF). We used a fluorescent unnatural amino acid (fUAA) named Anap as a fluorophore which can be directly incorporated into the channel protein in *Xenopus* oocytes. First, we observed a subtle Anap fluorescent signal change associated with voltage changes at Ala337 and Thr339 in the 2nd transmembrane domain (TM2). The changes imply the movement of pore region especially in TM2 during voltage dependent gating. To enhance the fluorescent signal change, we paired Anap with Tryptophan (Trp) which is known to quench the fluorescent signal of fluorophores. We used this Anap-Trp pairing by introducing additional Trp residues by mutagenesis to intensify the Anap fluorescent signal. We observed Anap fluorescent signal changes associated with voltage dependent gating in Ala337Anap/Leu334Trp. These results provide us with a clue to reveal the first insight of the voltage dependent structural rearrangements of P2X2 receptor. COI:No

2P-056

De-energized mitochondrial function in permeabilized myocytes.

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Mitochondria are organelles which play a critical role in the generation of metabolic energy in cells. In previous study, we showed de-energized mitochondria in absence of mitochondrial substrates have also a membrane potential. To understand mitochondrial bioenergetics, the effects of Pi and ATP on the de-energized mitochondria were investigated. In this study, NADH, FAD and ψm were monitored using permeabilized ventricular myocytes of the rat. The ψm of de-energized mitochondria was about -42 mV. The addition of Pi could hyperpolarize further about -2mV. Surprisingly, when we add both Pi and ATP, $\delta\psi$m was dramatically hyperpolarized to about 51mV. In addition, FAD signal was greatly increase which reflected FADH consumption. NADH signal was also increased, however, very small compared to FAD change. Interestingly, the addition of diazoxide could inhibit Pi/ATP-induced hyperpolarization and FAD increase, but not completely. The cytosolic K+ replacement with NMDG also attenuated Pi/ATP-induced effects, however, the states of the de-energized mitochondria was not changed by NMDG, that is, the FAD signal and the ψm were not changed. A KATP channel blocker, 5-HD, did not show any effect. Therefore K+ flux via KATP channel may not participated in Pi/ATP-induced effects. The treatment of Oligomycin A like diazoxide could block the Pi/ATP-induced changes. From the above results, we postulated cytosolic K+is essential to generate Pi/ATP-induced changes. Mitochondrial KATP channel may not be related to those changes. Somehow, F1,F0-ATPase may control the FADH/FAD conversion with K+ion.Funding: R0005739and2014M3A9D7034366 COI:No

2P-057

TRPV4 is functionally expressed in cultured mouse Schwann cells

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Schwann cells are the primary glial cells in peripheral nerves system. Myelinating Schwann cells wrap the peripheral axons and form the myelin sheath. Sensing and detecting the changes in the external microenvironment is very important for retaining the phenotypic plasticity of Schwann cells. TRP channels were reported as sensors for temperature, osmotic pressure, volume, stretch, and vibration, which will be expressed and activated throughout the body. However, whether TRP channels are expressed and function in Schwann cells are still unknown. We isolated Schwann cells from adult mice, and checked the functional expression of TRPV1, TRPA1, TRPV4 and TRPM3. The results indicated that Schwann cells were activated by the TRPV4 activator GSK1016790A, but not by capsaicin, allyl isothiocyanate, or pregnenolone sulfate, the TRPV1, TRPA1, or TRPM3 activator, respectively. It suggests that TRPV4 is functionally expressed in primary Schwann cells from adult mice. COI:No

2P-058 (AP3)

Novel regulation mechanisms of the GIRK channel activity by small molecules

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G-protein-gated inwardly rectifying K^+ (GIRK) channels control various physiological functions. For example, GIRK1/2 heterotetramers in the brain regulate neuronal excitability; GIRK1/4 heterotetramers in the heart regulate heart rate. We previously identified a novel GIRK activator, ivermectin (IVM), and more recently two novel GIRK inhibitors, astemizole (AST) and terfenadine (TER). We reported that IVM directly activates GIRK current in a PIP_2-dependent, $G_{\beta\gamma}$-independent manner. Here we present novel findings of the regulation mechanisms of GIRK channels by these small molecules. (1) By using *Xenopus* oocytes expressing M2 muscarinic receptors with GIRK channels, we observed that IVM potentiates the ACh-induced current, suggesting that IVM not only acts as an activator but also an allosteric modulator of GIRK channels. (2) By examining the effects of AST and TER in the oocytes expressing different GIRK subunits, we observed that AST- and TER-mediated inhibitions are GIRK1 dependent. (3) Mutation of a GIRK1-specific amino acid residue located in the pore helix close to the selective filter, Phe137, to Ser abolished the inhibition of GIRK current by AST and TER, suggesting that the Phe137 in GIRK1 may play important roles in the channel gating. Taken together, the present data shows the effects of the novel activator and inhibitors on GIRK channels and the structural determinants for the regulations. The results provided us with a clue toward the identification of the novel gating mechanisms of GIRK channels by small molecules. COI:No

2P-059

Inhibition by NSAIDs having various chemical structures of frog sciatic nerve compound action potentials

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Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit fast-conducting compound action potentials (CAPs) recorded from the frog sciatic nerve. Salicylic acid-based NSAID (aspirin; 1 mM), propionic acid-based NSAIDs (ketoprofen, ibuprofen, naproxen and loxoprofen; each 1 mM) and an oxicam-based NSAID (meloxicam; 0.5 mM) had no effects on CAPs, while acetic acid-based NSAIDs (indomethacin and etodolac; each 1 mM) inhibited the peak amplitudes of the CAPs by 15-40%, indicating that only acetic acid-based NSAIDs may exhibit CAP inhibition. To address this issue, we further examined how other NSAIDs affect frog sciatic nerve CAPs. The experiments were performed by applying the air-gap method to the frog sciatic nerve. Acetic acid-based NSAIDs (diclofenac and aceclofenac) reduced the peak amplitude of the CAP with the IC_{50} values of 0.94 and 0.47 mM, respectively in a partially reversible manner. Fenamic acid-based NSAIDs (tolfenamic acid and meclofenamic acid), that are similar in chemical structure to diclofenac, reduced CAP amplitudes with the IC_{50} values of 0.36 and 0.19 mM, respectively; their derivatives (mefenamic acid and N-phenylanthranilic acid) were less effective in inhibiting CAPs. These results indicate that not only acetic acid- but also fenamic acid-based NSAIDs inhibit CAPs in a manner dependent on their chemical structures. This result may serve to develop NSAIDs having an ability to inhibit nerve conduction, possibly contributing to a part of their antinociceptive actions. We declare no conflict of interests. COI:No

2P-060

Coupling mechanisms of voltage-sensing phosphatase: a role of membrane interaction

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Voltage-sensing phosphatase (VSP) consists of a transmembrane voltage sensor domain and a PTEN-like cytoplasmic catalytic region. VSP has phosphatase activity toward $PI(4,5)P_2$ regulated by membrane potential change (Murata et al. 2005). The mechanism of the coupling between the two modules in VSP has still been enigmatic. We previously reported that the hydrophobic membrane interacting region in the cytoplasmic catalytic region (L284 and F285 in Ciona intestinalis VSP), which we call hydrophobic spine, plays an important role in the phosphatase activity. We also showed that the hydrophobicity of this region is critical and mutation into aromatic amino acids facilitated the voltage-dependent enzyme activity. In this study, we extended this research to understand mechanisms of coupling from voltage sensor to enzyme in VSP. In vitro phosphatase assay of purified cytoplasmic catalytic region of Ci-VSP showed that hydrophilic amino acids reduced the phosphatase activity. The voltage clamp fluorometry study of fluorescent reporter attached to extracellular side near S4 and fluorescent unnatural amino acid (Anap) introduced into the cytoplasmic region showed that alteration of amino acid into hydrophilic or aromatic amino acids in hydrophobic spine remarkably altered motions of both of the voltage sensor and the enzyme region.These indicate that the hydrophobicity in hydrophobic spine is required both for the coupling between the voltage sensor domain and cytoplasmic catalytic region and the innate phosphatase activity of cytoplasmic catalytic region. COI:No

2P-061

Effects of peripheral nerve injury on GABA receptor-mediated currents in GABAergic and non-GABAergic neurons of the mouse spinal dorsal horn

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The spinal cord superficial dorsal horn (SDH) contains various types of neurons including GABAergic inhibitory and glutamatergic excitatory neurons. In the present experiments, we characterized the GABA receptor-mediated currents in inhibitory and excitatory neurons in the SDH of partially sciatic nerve-ligated (PNL) and sham-operated mice. Experiments were performed on adult ICR mice. Tight-seal whole-cell recordings were made from SDH neurons of spinal cord slices. Membrane currents evoked by a puff application of GABA and GABA receptor-mediated inhibitory postsynaptic currents (GABA-IPSCs) evoked by electrical stimulation were recorded. After whole cell recordings, the neurons were subjected to single-cell RT-PCR analysis for GAD67 and VGLUT2 mRNAs. The amplitude of currents by the GABA puff was significantly increased by PNL in GABAergic neurons. The PNL fastened the rate of decay time of GABA-IPSCs in glutamatergic neurons. In contrast, the PNL slowed the decay time of the GABA-IPSCs in GABAergic neurons. Additionally, the quantitative RT-PCR analysis indicated that the PNL increased the expression of $\alpha 3$ and $\alpha 5$ but decreased that of $\alpha 1$ and δ of the GABA receptor subunits. The present results indicate the PNL attenuated GABAergic inhibitory influence on excitatory neurons while the PNL strengthened it on inhibitory neurons. Furthermore, such changes in GABAergic synaptic activity may be associated with alterations of the expression profile of GABA receptor subunits. COI:No

2P-062

Evaluation of activity of amiloride-blockable epithelial Na+ channel in cement glands by hanging behavior in young bullfrog tadpoles

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Young tadpoles of frogs, including bullfrog and Xenopus laevis, spend most of its time hanging from surface of water or solid object by its cement glands. Our previous studies showed epithelial sodium channel (ENaC) expressed specifically in embryonic cement glands in bullfrog. Also in Xenopus tadpole, we studied behavioral change by addition of amiloride, a blocker of ENaC, in tank water and confirmed that ENaC in embryonic cement glands were functionally active in this species. In the current study, we examined activity of ENaC expressed in cement glands in bullfrog tadpoles using the same behavioral examination. Twenty bullfrog tadpoles in stage 21 were divided into two beakers with 200 ml water. Each beaker was stirred for 10 sec after addition of amiloride (0.1mM) or same amount of water and number of hanging subjects were counted 2 h after the stirring. In another set of experiment, Steinberg solution, a typical ringer for young amphibian, was used as tank water. As in the case of Xenopus, number of hanging subjects was suppressed in Steinberg solution compared with water (MilliQ water). In both condition, different from Xenopus, there was no effect of Amiloride. For the case of undetectably low activity of ENaC, overnight treatment of aldosterone (activator of ENaC) in tank water introduced in the same experiment. No effect of aldosterone was observed in this experiment.From these results we conclude, in bullfrog tadpole, ENaC have no clear role in hanging behavior and we cannot detect amiloride-blockable response in this experiment. COI:No

2P-063

SLCO2A1 is a pore-forming component of Maxi-Cl channel

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The molecular entity of Maxi-Cl which is an ATP-release anion channel had long been elusive. We reported the identification of *Mac-1* as a core factor of Maxi-Cl in the last PSJ meeting (Hamamatsu, 2017). Here, we showed that the identity of *Mac-1* is *Slco2a1* (solute carrier organic anion transporter family member 2A1), encoding a prostaglandin transporter (PGT). The endogenous Maxi-Cl currents in a murine cell line, C127, were significantly inhibited by three known PGT antagonists, bromosulfophthalein (BSP), bromocresol green (BCG), and indocyanine green (ICG), as well as by a PGT substrate, prostaglandin E2 (PGE2). Maxi-Cl-like currents became induced by exogenous expression of SLCO2A1 in HEK293T cells, which lack endogenous Maxi-Cl currents and *Slco2a1* expression, in a manner sensitive to BSP. We then examined the effects of point mutations in the transmembrane domains of SLCO2A1 on Maxi-Cl activities. Forced expression of the charge-neutralized mutant (K613G) generated markedly reduced macro-patch currents with a reduced single-channel conductance, and reversed its ion selectivity from anion- to cation-selective in HEK293T cells. In contrast, exogenous expression of a disease-causing mutant, G222R, failed to generate evident Maxi-Cl activities. These results showed that SLCO2A1 is a pore-forming component of Maxi-Cl channel. (COI:NO) COI:No

2P-064

Effects of an artificial lipid, dipalmitoyl sulfobetaine, on the function of ion channel

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Biological membranes are consisted of variety of lipids, and function of ion channel is modulated by the lipid composition of the membrane. To examine the effect of artificial lipid having distinct property from that of ordinary lipids, such as phosphatidylcholine (PC), a neutral lipid having oppositely oriented dipole at the head group was synthesized: dipalmitoyl sulfobetaine (DPSB). First, lipid bilayers were formed using the contact bubble bilayer (CBB) technique at 25° C, and the electric resistance of the DPSB membrane was low, whereas the bilayer was failed to be formed with dipalmitoyl phosphatidylcholine (DPPC). The phase transition temperature of DPSB is 40.8° C and 56.8° C, and that of DPPC is 41.4° C. Thus, a bilayer was formed above the phase transition temperature at 60° C. A bilayer membrane with high electric resistance was successfully formed for both DPSB and DPPC. A peptide channel, polytheonamide B (pTB), was inserted into the lipid bilayer, and single channel current recording was performed. Single-channel conductance of pTB in the DPSB membrane was lower than that in the DPPC membrane, probably because of the altered dipole potential at the membrane surface. The open probability was high both in the DPSB and DPPC, and sub-conductance levels were frequently observed in the DPSB membrane. COI:No

2P-065

Muscarinic receptor and KCNQ channel mediated 2-second memory of medium spiny neuron in the striatum

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The medium spiny neuron in the striatum has a remarkable electrical property where a depolarizing current pulse induced action potential is delayed several hundred milliseconds after the onset of the pulse. The spike delay is reduced by pre-pulse stimulation induced depolarization. Although it is known that the acceleration of the spike timing depends on the inter pulse interval (IPI), neurochemical modulations of the IPI dependent spike acceleration have not been investigated. Here we show that the M1 muscarinic receptor agonist Xanomeline accelerates the 2-sec IPI spike timing. The acceleration is not observed in the 2-sec IPI control. We also found that the muscarinic receptor dependent spike acceleration was significantly reduced by a KCNQ channel opener Retigabine and protein kinase C(PKC) inhibitor Calphostin-C. These observations indicate that the muscarinic spike acceleration depends on KCNQ channel current modulation via the PKC pathway. COI:No

2P-066

Cav1.2 channel regulation by PKA phosphorylation involves modulation of calmodulin binding to the channels

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Cav1.2-type Ca^{2+} channel is widely expressed in nervous system, cardiac muscle and smooth muscle, regulating many processes including cell excitation, muscle contraction and gene expression. Calmodulin (CaM) binding to CT1 (proximal part of α subunit C-terminal), is essential in Ca^{2+} channel activity regulation. Recently, our study (Lyu et al., J Pharmacol Sci 133, 2017) showed that the CT3 (distal part of α subunit C-terminus) bound to CT1 and inhibited the interaction between CaM and CT1 to regulated the channel activity. In this study, we examined the effect of PKA phosphorylation on binding of CaM and CT3 to CT1. We found that, at low [Ca^{2+}], PKA phosphorylation increased CaM binding to CT1 significantly. In addition, CT3 binding to CT1 was significantly decreased. These results have suggested that phosphorylation of CT1 by PKA act by two pathways. Firstly, phosphorylation of CT1 by PKA directly promotes binding of CaM to CT1, and secondly, phosphorylation of CT1 by PKA suppresses CT3 binding to CT1 to indirectly promotes the binding of CaM to CT1. On the other hand, at high [Ca^{2+}], PKA inhibited the binding of CaM to CT1. This suggests that PKA phosphorylation also affects Ca^{2+}-dependent inactivation. COI:No

2P-067

Effects of the disruption of the disulfide bonds in the extracellular domain of the FMRFamide-gated Na^+ channel

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FMRFamide-gated Na^+ channel (FaNaC) is a homo-trimeric peptide-gated sodium channel, which is activated by a molluscan cardioactive peptide, FMRFamide. In the extracellular domain of a FaNaC subunit as well as other subunits in DEG/ENaC family, there are seven disulfide bonds (SS bond). Because the SS bond is usually stabilize a structure of protein, some or all of the SS bonds are likely to be important for the channel function. To investigate the importance of the SS bonds, we mutated cysteines involved in SS bonds to alanine to disrupt the formation of SS bonds. Here, we call the mutants by the position of SS bond, i.e., SS1, SS2 and etc. We compared the steady state activation of the mutant channels by dose-response analysis in *Xenopus* oocytes. In a standard solution (ND96), EC50 of the wild type FaNaC (WT) was usually 4-5 μM. SS3, SS6 and SS7 did not express the measurable currents in response to 10 μM FMRFamide in ND96. By contrast, EC50s of SS2, SS4 and SS5 in ND96 were 3.9, 2.7 and 3.1 μM, respectively. Although SS1 was slightly activated by 10 μM FMRFamide, this mutant was much less responsive to FMRFamide (EC50 was not obtained, but estimated to be more than 100 μM). Taken together, these results suggest that some but not all of the SS bonds are required to maintain the functionality of FaNaC, and that SS1 may be involved in a structure which determines the affinity of FaNaC. COI:No

2P-068

Pain and itch reductions by 4-isopropylcyclohexanol targeting TRP channels and ANO1

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Transient receptor potential vanilloid 1 (TRPV1) channels and anoctamin 1 (ANO1) interaction enhances nociceptive signals in primary sensory neurons. Through the investigation to search other TRP-ANO1 interactions, we found that menthol strongly inhibits ANO1 currents. Moreover, isopropylcyclohexane was identified as a core chemical structure to inhibit ANO1 currents. However, the action of ANO1 current inhibition by isopropylcyclohexane was slower than that of menthol. To overcome the problem, we focused on hydrophilicity of the chemical and found that a more hydrophilic compound, 4-isopropylcyclohexanol, rapidly inhibits ANO1 currents similar to menthol. 4-isopropylcyclohexanol also inhibited capsaicin-induced TRPV1 currents. Interestingly, the inhibitory mechanism was not channel pore blocking, but the chemical reduced only open time of single channels. In addition, 4-isopropylcyclohexanol strongly inhibited capsaicin-induced depolarization and action potential generation in small dorsal root ganglia (DRG) neurons of mice. Furthermore, 4-isopropylcyclohexanol reduced capsaicin-evoked pain-related behaviors and histamine-dependent itch-related behavior in mice. These results suggest that multiple inhibitory effects by one chemical are also important to reduce pain and itch sensations. Thus, 4-isopropylcyclohexanol could be an important chemical compound to develop novel analgesic or antipruritic agents. COI:No

2P-069

Cell-specific Precise Mathematical Modeling of hiPSC-CMs Revealed Two Opposite APD Reaction in I_{Kr} Blocking Test

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BACKGROUND: Human induced pluripotent stem cells (hiPSCs) derived cardiomyocytes (-CMs) exhibit varying action potential (AP) morphologies, and their electrophysiological features are considerably different from those of adult CMs. Therefore, development of accurate in-silico model of hiPSC-CMs is of enormous importance in facilitating their application for drug safety tests. METHODS: We developed new hiPSC-CMs mathematical models based on HuVEC model (Asakura et al, 2014), adopting experimental data of ion-channels, and considering the features of absence of T-tubles, scattered Ca2+ releasing units on cell membrane, and automaticity of AP firing. We recorded APs from 50 hiPSC-CMs, and recapitulated all AP morphologies in simulation model. After that, IKr-blocking test was performed.RESULTS: We successfully recapitulated all 50 AP morphologies of hiPSC-CMs from multiple cell lines. In simulational IKr-blocking test, AP duration (APD) prolongation was observed in 34 cells (64%). In 16 cells, APD shortening and rising of maximum diastolic potential (MDP) was observed. Most of APD-shortening-cells (n = 14) have character of MDP < -63.0mV.CONCLUSION: Cell-specific mathematical modeling of hiPSC-CMs revealed two opposite APD reaction in IKr-blocking test. Cell-specific simulation enables prediction of drug reaction of hiPSC-CMs and To interpret the results of drug testing of hiPSC-CMs appropriate interpretation the results of drug testing of hiPSC-CMs. COI:No

2P-070

The molecular basis of SKF-96365 and efonidipine block of human cardiac Kv1.5 channels.

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The voltage-gated Kv1.5 channel is encoded by mammalian KCNA5 gene and expresses atrial myocytes functionally and is absent in ventricular myocytes in human. The present study investigated the blocking effects of SKF-96365, which is a transient receptor potential canonical (TRPC) channel blocker, and efonidipine, which is an L-type and T-type calcium channel blocker, on human Kv1.5 (hKv1.5) channels, using site-directed mutagenesis combined with whole-cell patch-clamp technique, as well as computer docking simulations. SKF-96365 and efonidipine inhibited hKv1.5 channel current in a concentration-dependent manner. The channel block time constant during depolarization was much shorter in 10 μM SKF-96365 of 1.7 ms than in 3 μM efonidipine of 80.8 ms. The patch-clamp experiments with wild type or mutant hKv1.5 channel showed that SKF-96365 interacted with Thr479, Thr480, Arg487, Ile502, Val505, Ile508, Ler510, Val512 and Val516. The docking simulation study showed that SKF-96365 had interactions with Met478, Thr479, Thr480, Val505, Ile508, Ala509, Val512, Pro513 and Val516 in hKv1.5 channels. From the docking simulation study, efonidipine also interacted with these amino acid residues, but docking conformation with hKv1.5 channel was different from SKF-96365. These results suggest that the specific amino acid residues in hKv1.5 channels and the structure of compounds have key roles in the channel blocking effects. COI:No

2P-071

Mechanisms of regulation of Na-Pi transporter activity

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Inorganic phosphate (Pi) is an important constituent for bone and many organic molecules. Pi homeostasis is regulated by kidney in which Na-Pi transporters act as the main molecule for reabsorption of Pi. These transporters are localized at apical brush border membrane in the renal proximal tubule. One of the regulation mechanisms of these transporters activity in the tubule is to control the counts by vesicular transport system through exocytosis and endocytosis, but other mechanisms, especially in regard to the regulation of its activity on plasma membrane, is not clear.It has been reported that the activity of many ion channels expressed on plasma membrane is regulated by phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) which is abundant in the membrane. Because Na-Pi transporters function on plasma membrane, we hypothesized that the transporter activity also depends on PI(4,5)P2. To test the hypothesis, we are examining PI(4,5)P2 dependency of mouse Na-Pi transporter (mSLC34A1) by using voltage-sensing phosphatase (VSP) that has phosphatase activity for phosphoinositide depending on membrane potential. COI:No

2P-072

Activation of ciliary beating by Sei-hai-to (TJ-90) in airway ciliary cell of mice

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Sei-hai-to (TJ-90, Qing Fei Tang), a Chinese traditional medicine, increases ciliary beat frequency (CBF) and ciliary bend angle (CBA) mediated via cAMP accumulation controlled by Ca^{2+}-activated PDE1A. A high concentration of TJ-90 (higher than 40 μg/mL) induced two types of the CBF increase, a transient CBF increase and a sustained CBF increase, while it only induced a sustained increase in CBA. Under the condition with blockade of $[Ca^{2+}]_i$ increase by 10 μM BAPTA-AM (Ca^{2+} chelator) or inhibition of PDE1 by 8MmIBMX (an inhibitor of PDE1), TJ-90 (400 μg/mL) induced the sustained increase in CBF without showing any transient CBF increase. The both types of transient and sustained increases in CBF induced by TJ-90 (> 40 μg/mL) were mimicked by application of procaterol of a low concentration, such as 100 pM, and ionomycin, such as 500 nM. Thus, TJ-90 induces finite increases in cAMP and $[Ca^{2+}]_i$ leading to transient or sustained CBF increase in airway ciliary cells depending on the balance of increases in cAMP and $[Ca^{2+}]_i$. The CBF and CBA in the chronic administration of TJ-90 in airway ciliary cells and ependymal cells (1 g/kg/day for 6 months) were larger than those in mice administered with only water similar to the acute TJ-90 administration. COI:No

2P-073

Effects of chloride ion channel blocker on the adipogenic differentiation of rabbit ASCs

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Adipose tissue-derived stem cells (ASCs) is known to have the potential into many kinds of functional cells. However, there are few studies about Cl^- channels of rabbit ASCs.
In this study, We isolated adipose tissue from rabbits and confirmed gene expressions of ASCs markers (CD29, CD90) and adipocyte markers with RT-PCR.
Further, we visualized and quantitated "lipid droplets (LDs)" in differentiated cells with fluorescent dyes, to evaluate this adipogenic differentiation. Large lipid droplets were found and cell proliferation was suppressed with adipogenic medium. Cl^- channel blockers (NPPB and DIDS) suppressed the cell proliferation. However, Cl^- channel blocker treatment inhibited LDs accumulation significantly in dose-dependent manner with adipogenic medium.
NPPB treatment diminished mitochondrial membrane potential and enhanced intracellular H_2O_2 production. This treatment suppressed LDs accumulation. However, IAA-94, that is known as CLIC channel blocker has not suppressed it. These results imply Cl^- channel play a important roles in adipogenic differentiation of rabbit ASCs. COI:No

2P-074

Effect of carbocistein via Cl^- on ciliary bend angle in mouse airway ciliary cells

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Carbocistein (CCis), a mucoactive agent, stimulates airway ciliary beating in mice by increasing the ciliary bend angle (amplitude, CBA) and the ciliary beat frequency (CBF). The increases in CBA (30%) and CBF (10 %) were mediated via a decrease in intracellular Cl^- concentration ($[Cl^-]_i$) and an elevation of intracellular pH (pH_i) in airway ciliary cells of mice. The $[Cl^-]_i$ decrease (Cl^- pathway) increased CBA by 20 %, not CBF, independent of the presence of CO_2/HCO_3^- in the extracellular solution. The $[Cl^-]_i$ decrease was induced by the stimulation of Cl^- release via NPPB-sensitive Cl^- channels. Reduction of extracellular Cl^- concentration using a NO_3^- solution under the CO_2/HCO_3^- -free condition, which diminishes $[Cl^-]_i$, increased CBA, but not CBF and the further addition of CCis did not increase CBA and CBF. The pH_i elevation (pH_i pathway) increased both CBA and CBF by 10 % in a manner dependent on the presence of CO_2/HCO_3^-. The CCis-induced elevation of pH_i was induced by the stimulation of HCO_3^- entry via the DIDS-sensitive Na^+/HCO_3^- cotransport (NBC). Thus, CCis decreases $[Cl^-]_i$ via stimulation of Cl^- release through Cl^- channels, elevating CBA by 20 %, and also increases pH_i via stimulation of the NBC-mediated HCO_3^- entry, increasing both CBF and CBA by 10 % in airway ciliary cells of mice. (COI:No) COI:No

2P-075

Adenosine triphosphate regulates translocation of glucose transporter 1 to the plasma membrane

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Adenosine triphosphate (ATP) is critical determinant for vascular endothelial growth factor signaling in endothelial cells. Under hypoxia, expression of glucose transporters (GLUTs) was induced in endothelial cells to increase glucose uptake to generate ATP. However, regulation of trafficking of GLUTs to the plasma membrane is not well understood. Here, we examined a role of ATP in translocation of GLUT1 to the plasma membrane in endothelial cells during hypoxia. Human umbilical vein endothelial cells (HUVECs) were exposed to 12 h of hypoxia (1% O_2). Hypoxia significantly increased GLUT1 expression in HUVECs and stimulated translocation of GLUT1 to the plasma membrane, which was associated with decrease of intracellular ATP concentration (67.35 ± 7.86% of normoxia, means ± SE, $P < 0.01$). Antimycin-A or 2-deoxyglucose decreased cellular ATP in normoxia, and those reagents induced translocation of GLUT1. Cobalt chloride, which increased HIF1-alpha expression in normoxia, did not influence cellular ATP and cell surface expression of GLUT1. Interestingly, hypoxia induced translocation of GLUT1 was inhibited by KATP channel inhibitor glybenclamide, while mitochondrial KATP channel inhibitor 5-HD did not influence translocation of GLUT1 during hypoxia. These observations indicated a decrease of intracellular ATP triggered translocation of GLUT1 to the plasma membrane, that would contribute to glucose uptake in HUVECs during hypoxia. COI:No

2P-076

Effects of LED irradiation on human dermal fibroblasts

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We have reported that 405 nm, 532 nm, and 808 nm low-power laser irradiation (LLI) have cell death induction, proliferation enhancement, and proliferation suppression of human-derived glioblastoma (A-172), respectively. In this study, LED irradiation effects on human dermal fibroblasts were examined. The human dermal fibroblasts were purchased from JCRB (No.0075). The cells were cultured in 10 cm dish and 8-well chamber slide. The selected wells was irradiated with 405±5 nm (10 mW), 532±5 nm (10 mW) and 660±5 nm (10 mW) LEDs for 20, 40 and 60 min. 48 hours after, cells were stained with the mitochondrial probes (Mitotracker Orange) for fluorescent observation. The number of cells as well as their morphology were analyzed by fluorescence microscope (KEYENCE BZ9000). Blue LED irradiation significantly reduced both cell number and cell surface area at 24 and 48 h after irradiation. In addition, B-LED induced the reduction of filopodia as well as mitochondria. Green LED irradiation showed no-effect on cell number, but significantly increased cell surface area, filopodia and mitochondria. In contrast, red LED increased cell number whereas showed no-effect on cell surface area, filopodia and mitochondria. It is suggested that B-LED induces cell death like 405 nm LLI, G-LED does cell activation also like 532 nm LLI, but effects of R-LED irradiation are inconsistent with those of 808 nm LLI. COI:No

2P-077

Effects of 405 nm LED light on cultured HeLa cells

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We tested effects of 405 nm wavelength light irradiation on cultured HeLa cells. Cells were plated in plastic dish (3cm diameter) and were maintained for 24-48 hours. The cells were irradiated with the light at 146 mW/cm^2 on both normal and lower glutathione (GSH) cells. The lower GSH cells were obtained by preincubation with 1-Chloro-2,4 dinitrobenzene (CDNB) or buthionine sulfoximine (BSO). Reactive oxygen species (ROS) were monitored by fluorescent probe, 2',7'dichlorodihydrofluorescein diacetate (H_2DCF-DA). We tried to measure the intracellular ROS accumulation after 385, 395 and 405 nm lights irradiation for 15 min. The ROS accumulation by these lights irradiation on the normal cells indicated a similar tendency and 405nm light showed relatively high ROS accumulation compared to the other 2 lights. Then, we also measured the intracellular ROS accumulation in the lower glutathione (GSH) cells. The 405 nm light irradiation for more than 2 hr induced cell death in BSO-loaded cells. These results suggest that the intracellular ROS induced by 405 nm light are containing singlet oxygen ($^1O_2$) and hydroxyradical ($^{\cdot}OH$), and the intracellular glutathione plays an important role of scavenging these ROS. COI:No

2P-078

Buffering cAMP in olfactory receptor neurons

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Olfactory receptor neurons utilize cAMP in spatiotemporally diverse aspects: odor signal transduction and formation of axonal network called the olfactory map.In response to odor stimulation, the odorant receptors activate the G-protein to produce the cAMP surges, which switch on the cyclic nucleotide gated channels for generating receptor potentials. Meanwhile, the spontaneous activation of G-protein coupled receptors sets the basal cAMP level, which in turn regulates the spontaneous firing at rest via cAMP-gated ion channels and determines the location of axonal targeting during development and neural regeneration via A-kinase. The mammalian olfactory receptor neurons are so tiny that cAMP dynamics should be safely stabilized to avoid excessive effects under the influence of continued external stimuli. Here, assuming the presence of cAMP buffers in the cytoplasm, we show the experimental and simulated results to narrate the biophysical contribution of cAMP-binding proteins localized in the membrane and the cytoplasm to the buffering regulation of phasic and tonic cAMP signaling. COI:No

2P-079

Hypotonic cell swelling was affect by intracellular condition

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We reported that the initial cell swelling ratio (CSR_{ini}) after hypotonic challenge differs in an extracellular pH condition (Fujii et.al. 2016 in the Annual Meeting of the Physiological Society of Japan), CSR_{ini} which measured by flowcytometry decreased in a lower extracellular pH condition (pH 6.4) in comparison with a physiological pH condition (pH 7.4). We assumed that the lower extracellular pH caused activation of sodium proton exchanger and subsequently sodium ion gradient was smaller than physiological extracellular pH, such as pH 7.4. To confirm this assumption, we measured intracellular pH and intracellular sodium concentration ($[Na^+]_i$) by using fluorescence indicator BCECF-AM and sodium green, respectively. In an extracellular pH 6.4 condition, intracellular pH was lower than control condition, such as pH 7.4, and $[Na^+]_i$ was higher than control condition. In a lower pH condition, water movement from extracellular fluid was caused by higher $[Na^+]_i$, and subsequently cell volume was increase. We assumed that a decrease of initial cell swelling ratio after hypotonic challenge was caused by the results of increased initial cell volume in a lower pH condition. COI:No

2P-080

Cellular model of ischemic heart disease using human induced pluripotent stem cells

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Ischemic heart disease is the number one cause of death globally. However, there is no effective treatment for the disease at present. Although many studies about pharmacological cardioprotection and stem cell therapy for ischemic heart disease using animal models have been advanced, it is difficult to extrapolate results from animal models to human due to different cardiovascular physiology. To produce a model of ischemic heart disease using human cells, we explored experimental parameters using a rat cardiomyocyte cell line H9c2 at first. When subjected to ischemic condition (2% oxygen and glucose free) for 24 h, cellular viability of H9c2 cells measured by MTT assay and flow cytometry were significantly decreased. Based on this observation, we used cardiomyocytes derived from human induced pluripotent stem cells (hiPS-CM) to produce a cellular model of ischemic heart disease. In response to the ischemic condition, cellular viability and contractile function of the hiPSC-CMs decreased. These results suggest that a model of ischemic heart disease was successfully produced using hiPSC. It is expected that unnecessary animal experiments can be avoided using our hiPSC ischemic heart disease model. COI:No

2P-081

KwikPrep: a 3-minutes genomic DNA preparation for PCR genotyping of mouse models

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For physiological studies using genetically-engineered mouse models, PCR genotyping is essential. However, mouse genotyping demands much labor and budget, because preparation of genomic DNA (gDNA) involves complex procedures. Here, I present a simple and affordable technique for making PCR-ready gDNA preparations. Tiny drops of mouse blood (1-2 μL) were spotted on No.2 qualitative filter paper, and were stored as dried blood spots (DBS). The DBS was processed in 3 simple steps: 1) From the DBS, a small disc (1.5 mm diameter) was punched out, and was immersed in 50 mM-NaOH (20 μL) in a PCR tube. 2) The tube was heated at 95° C for 1 minute on the PCR machine, or alternatively, it was stood at room temperature for >15 minutes. 3) The tube was neutralized to pH 8.4 with 6-times volume (120 μL) of 10 mM-Tris-HCl (pH 5.0), while the pH was verified as olive green color of thymol blue dye included in the solutions at 0.004%. In 3 minutes, the DBS filter paper disc became a PCR-ready gDNA preparation, which consistently provided dense and clear bands of the PCR products. Further analysis of this gDNA preparation revealed the followings: a) PCR inhibition by blood proteins was successfully avoided by alkaline denaturation and simple dilution. b) The gDNA was highly concentrated on filter paper because of adsorption of DNA by cellulose. c) The DBS could be preserved at room temperature for >5 years, while maintaining clear PCR bands. d) Excess heating in NaOH solution rapidly degraded gDNA, leading to complete loss of PCR bands. This novel technique provides a smart way for preparing and preserving gDNA samples for PCR. COI:No

2P-082

Development of the reconfigurable maze

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Several shapes of maze have been used for elucidating the neuronal mechanism for cognitive functions such as spatial navigation, working memory and decision-making. However, the position of paths, walls, gates and feeders in the conventional maze cannot be easily changed after construction. Here we developed a novel maze, called reconfigurable maze, in which the paths, treadmills, sensors, feeders, movable/fixed gates can be systematically reconfigured. In addition, we made a controller for the sensors and actuators in the maze on an Arduino microcontroller and a user interface with Matlab program. Using the maze, we could reproduce various types of conventional maze such as figure-eight maze and plus maze in a single environment. In exchange for the flexibility, there are spatial gaps between the paths. To evaluate whether animals' cognition is affected by those gaps, we recorded multiple single-unit activities from the hippocampus while rats navigate the maze, and compared the place fields of place cells on the gaps with that on the paths in the maze. We discuss about the efficiency of our reconfigurable maze for elucidating the neuronal mechanism for spatial navigation.

This work was supported by MIC SCOPE (152107008) and JSPS KAKENHI (16H06543, 16H02840, 16K13115) . COI:No

2P-083

ET-1-L-PGDS-PPARγ regulates hypoxia-induced ANP secretion in beating rat atria

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Lipocalin-type prostaglandin D synthase (L-PGDS) and peroxisome proliferator activated receptor γ (PPARγ) play important roles in cardiovascular diseases. Nevertheless, effects of endothelin-1 (ET-1) on L-PGDS and PPARγ protein levels and its role in hypoxia-induced atrial natriuretic peptide (ANP) secretion are unclear. In perfused beating rat atria, we observed that hypoxia significantly increased ET-1 release and stimulated ANP secretion, while upregulating L-PGDS. Hypoxia-induced ANP secretion was clearly attenuated by antogonists of ET-1 receptor type A and type B, BQ123 and BQ788, downregulating L-PGDS protein levels. It was also attenuated by L-PGDS antagonists, AT-56 and HQL-49, downregulating L-PGDS protein levels. In addition, hypoxia-induced ANP secretion was accompanied by increased PPARγ protein levels and was strongly attenuated by PPARγ antagonist GW9662. Hypoxia-induced increase in atrial PPARγ protein levels were dramatically inhibited by BQ123, BQ788 and AT-56. These results indicated that hypoxia promotes ANP secretion, at least in part, by activating ET-1-L-PGDS-PPARγ signaling in beating rat atria. COI:No

2P-084

Cardioprotective Effects of Alamandine via MrgD receptor by Anti-apoptosis, Anti-oxidant and ANP system in Rats

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The aim of this study is to investigate whether alamandine protects the heart against ischemia-reperfusion (I/R) injury. Pretreatment with alamandine (0.1mg/kg) for 2hr before ischemia improved an increased post-ischemic left ventricular end-diastolic pressure (LVEDP) and a decreased post-ischemic left ventricular developed pressure (LVDP) induced by reperfusion compared to untreated hearts. Alamandine markedly decreased infarct size and lactate dehydrogenase levels in effluent during reperfusion. Pretreatment with MrgD receptor blocker and Ang II type 2 receptor (AT2R) antagonist but not with Ang II type 1 receptor (AT1R) antagonist attenuated the improvement of LVEDP, LVDP, and +-dP/dt induced by alamandine. Alamandine treatment increased Mn-superoxide dismutase, catalase, and heme oxygenase-1 protein levels, which was attenuated by pretreatment with MrgD receptor blocker and AT2R antagonist. Alamandine treatment also decreased Bax, caspase-3 and caspase-9 protein levels, and increased Bcl-2 protein level, which were attenuated by pretreatment with MrgD receptor blocker and AT2R antagonist. Alamandine also caused increases in ANP secretion. These results suggest that the cardioprotective effects of alamandine against I/R injury may be partly related to activating anti-oxidant and anti-apoptotic enzymes via MrgD receptor and ANP system. Supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (NO 2017-R1A2B-4002214 and 2016R1A6A3A11930515). COI:Properly Declared

2P-085

Deletion of calcineurin B homologous protein 3 (CHP3) exacerbates chronic hypoxia-induced cardiac hypertrophy in mice

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Pathological cardiac hypertrophy is a major risk factor for development of heart failure. A Ca^{2+}-binding protein, Calcineurin B Homologous Protein 3 (CHP3, also called tescalcin) which is predominantly expressed in the heart, was suggested to function as a negative regulator for cardiac hypertrophy from in vitro study. We then generated a CHP3-deficient mice and analyzed the CHP3 function in vivo. Survival rate, cardiac function and heart weight of CHP3-deficient mice were not different from those of the wild type mice. We further investigated the effect of chronic hypoxia on CHP3-deficient mice. In the wild-type mice, chronic hypoxia induced right ventricular hypertrophy without apparent phenotypic change in the left ventricle. In contrast, in addition to the right ventricle it caused significant left ventricular hypertrophy in the CHP3-deficient mice. The expression of CHP3 in the heart of the wild type mice was markedly decreased by hypoxia. These results suggest that CHP3 is an important molecule that negatively regulates hypoxia-induced cardiac hypertrophy by controlling its expression level. COI:No

2P-086

Effects of stretch-induced reactive oxygen species on calcium handling in mouse ventricular cardiomyocytes

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Myocardial diastolic stretch activates NADPH oxidase (NOX) 2 to increase reactive oxygen species (ROS) production. Although it has been reported that the stretch-induced ROS stimulates ryanodine receptors to increase calcium spark rate during diastolic phase, its effects on calcium handling during contraction is not clear. In the present study, we investigated the effects of NOX2-derived stretch-induced ROS on calcium transient shape. Ventricular cells were enzymatically isolated from either 8 to 12-week-old mice (WT) or NOX2 knockout (KO) mice hearts. The cells were loaded with Fura-4F AM and superfused at room temperature under 1 Hz electrical stimulation. After obtaining a stable beating state, 5-10% axial stretch was applied using computer-controlled piezo-manipulated carbon fibers, attached to both cell ends. Calcium transient curves were obtained before and immediately after applying the stretch. The maximum uprising rate of the transient curve after stretch tended to increase in WT group, while it did not change in NOX2 KO group. The decaying time constant of the transient significantly prolonged after stretch in WT group, while it did not change in NOX2 KO group. The results suggested that stretch-induced ROS affects calcium handling during not only diastole but also systole to modulate excitation-contraction coupling. COI:No

2P-087

A potential link between L-type $Ca_V1.3$ channel and TRPM4 Ca^{2+}-activated nonselective cation channel in cardiac pacemaker cells

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The dihydropyridine-sensitive sustained inward Na^+ current (I_{st}) has long been suggested to play a crucial role in pacemaker activity in sinoatrial node cells, yet the molecular mechanism underlying this current remains unknown. We have recently reported that L-type $Ca_V1.3$ channel is required for the generation of I_{st} in mouse sinoatrial node cells (Toyoda *et al.*, Sci Rep 7:7869. 2017). However currently available recombinant $Ca_V1.3$ channels exhibit selectivity to Ca^{2+} and no detectable permeability to Na^+ in the presence of physiological external Ca^{2+}. Here we found that I_{st} was strongly inhibited by 9-phenathrol and flufenamic acid, both are known to block TRPM4 Ca^{2+}-activated nonselective cation channel. In addition, significant reduction in I_{st} was also observed either by intracellular BAPTA (10 mM) loadings or by srcoplasmic reticulum block after combined treatments with ryanodine (10 μM) and thapsigargin (3 μM). These pharmacological assessments suggest that I_{st} is a Ca^{2+}-activated Na^+ current through TRPM4 channels. Confocal measurements of $[Ca^{2+}]_i$ in sinoatrial node cells loaded with Fluo-4 revealed a nifedipine-sensitive biphasic increase in the $[Ca^{2+}]i$ composed of a rapid transient increase followed by a sustained $[Ca^{2+}]_i$ elevation in response to membrane depolarizations. Our data support emerging evidence for the functional coupling of $Ca_V1.3$ and TRPM4 channels via intracellular Ca^{2+} to generate the dihydropyridine-sensitive voltage-dependent Na^+ current, I_{st}, in cardiac pacemaker cells. COI:No

2P-088

Analysis of transmural energy consumption distribution under various transmural residual stress distribution attributed to end-diastolic myocardial tissue and sarcomere length using ring shape LV model

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It is known that residual stress exists in the left ventricular(LV) wall, and there is a report that the tissue is compressive at the endocardium and tensile at the epicardium on the unloaded state, however, there is also an opposite report. Residual stress has two meanings, resting passive elastic force caused by collagen fibers and cytoskeleton, and resting sarcomere length(SL) that determines the active contraction force. It is reported that, the transmural distribution of residual passive elastic force has a positive correlation with the resting SL(Rodriguez et al, 1993). In this study, we evaluated the influence of the transmural distribution of residual passive elastic force and resting SL on cardiac function using ring shape LV model(Amano et al, 2008). The ring shape LV model is the model approximating the short-axis cross-sectional shape of the LV and consists of 5 layers in the transmural direction and 80 finite elements in the circumferential direction. The force-length area(FLA) is known to be an index of the energy consumption of cardiac myocytes. Thus in this study, we evaluated energy consumption of each layer by assuming that FLA of each layer corresponds to energy consumption of each layer. By changing the transmural distribution of residual passive elastic force and resting SL independently, it was suggested that former had small influence while latter had a great influence on the distribution of the energy consumption in the LV wall. COI:No

2P-089

Electrophysiological effects on cardiac Na/Ca exchanger (NCX1) inhibitors and NCX1 stimulators

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We have been examined effects of several agents on cardiac Na^+/Ca^{2+} exchange current (I_{NCX}). So far we found agents which inhibit or stimulate the function of cardiac Na/Ca exchanger (NCX1). Several antiarrhythmic drugs inhibited I_{NCX} in a concentration-dependent manner. 2,3-buanedione monoxim (BDM), benzyloxyphenyl derivatives also inhibited I_{NCX} in a concentration-dependent manner. The properties of the NCX1 blockers are as follows; In the presence of trypsin in the pipette solution, the inhibitory effects of amiodarone, bepridil and BDM on I_{NCX} were attenuated, suggesting that these drugs inhibit NCX1 from the intracellular side. In contrast, aprindine, azimilide, dronedarone, cibenzoline, carvedilol, benzyloxyphenyl derivatives were trypsin-insensitive NCX1 inhibitors. Benzyloxyphenyl derivatives inhibit NCX in an intracellular Na^+ concentration-dependent manner. Four benzyloxyphenyl derivatives suppressed the unidirectional outward I_{NCX} more potently than unidirectional inward I_{NCX}. The mechanism of this mode-dependency is unknown but may be related to intracellular Na^+ concentration. Nicorandil and flecainide stimulated I_{NCX} in a concentration dependent manner. The enhancing effect of NCX1 by nicorandil may be contributed to bind intracellular cGMP increasing to regulatory sites of intracellular large loop between 5th and 6th transmembrane segments of NCX1. However, it is unknown about the enhancing mechanism of flecainide on NCX1 function. COI:No

2P-090

Distinct dynamical mechanisms underlying the initiation of early afterdepolarizations in a human ventricular myocyte model

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Early afterdepolarization (EAD) is known as a cause of ventricular arrhythmias in long QT syndrome (LQTS). We investigated dynamical mechanisms of EAD formation in LQTS by bifurcation analyses of the ten Tusscher-Panfilov model (Am J Physiol, 2006) for human ventricular myocytes (HVMs). Effects of modulating the delayed-rectifier K^+ channel currents (I_{Kr}, I_{Ks}), L-type Ca^{2+} channel current (I_{CaL}), Na^+/Ca^{2+} exchanger current (I_{NCX}) and intracellular Ca^{2+} dynamics were examined by constructing bifurcation diagrams; equilibrium points, limit cycles, and bifurcation points were plotted as functions of parameters. A modified model cell could reproduce bradycardia-related EADs in LQT2-type HVMs and β-AS-induced EADs in LQT1-type HVMs. The model cell reproduced two types of EADs with different initiation mechanisms: I_{CaL} reactivation-dependent EAD and spontaneous SR Ca^{2+} release-mediated EAD. Slow-fast decomposition analysis demonstrated the distinct mechanisms of EAD initiation in the two types of EADs. With normal SR Ca^{2+} uptake rate (P_{up}), EAD generation depended strongly on reactivation of I_{CaL}; however, spontaneous SR Ca^{2+} release and resultant EAD formation, attributable to instability of intracellular Ca^{2+} concentrations, became prominent when P_{up} increased. This model would be useful for systematically investigating dynamical mechanisms of EAD-related arrhythmias in LQTS. COI:No

2P-091

3D reconstruction of rabbit pacemaker cells using serial block face scanning electron microscopy

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The cardiac conduction system is composed of pacemaker cells specialized for generation and propagation of the electrical impulse. Despite its well-known electrophysiological function, *in situ* cellular morphology of the pacemaker tissue is yet to be elucidated. Here we analyze 3D ultrastructure of the sinoatrial node (SAN) cells and Purkinje fibers (PFs) of the rabbit conduction system using Gatan 3View, a novel electron microscopy system. The SAN contained isolated and clustered SAN cells alongside an abundance of undulating collagen and elastin fibers and nerve fiber network. SAN cells exhibited small spindle or spider-like shapes with various length and width of processes. They also contained disorganized mitochondria and myofilaments of various size and density. SAN cells communicated with neighboring cells via intercalated discs or invaginated plasma membrane. PFs were spindle-shaped and longitudinally arranged, forming a bundle enveloped with connective tissue. Mitochondria and myofilaments were not packed but longitudinally aligned. The PF-ventricular junction was made up of thin layers containing two different cell types that were flat-shaped with more organized mitochondria and myofilaments, and contained t-tubules. The junctional cells contacted some ventricular myocytes situated in the deeper layer. These findings correspond with the unique functional features of the SAN and PFs. COI:No

2P-092

Effects of bilateral central amygdala lesions on spontaneous cardiac baroreceptor reflex

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Previously, we showed that the amygdala plays important roles in cardiovascular regulation not only in alerting situation but also in resting state because bilateral lesions of the central nucleus of the amygdala (CeA) caused chronic arterial pressure (AP) increase. In this study, we aimed to determine the cause of this chronic pressor effect induced by CeA lesions. We hypothesized that this effect may be due to a decrease in the baroreceptor reflex gain (BRG) and/or shift in the set point. We measured AP in rats (n=5) via telemetry for 24 hours. Mean AP (MAP), heart rate (HR), spontaneous cardiac BRG, and parasympathetic activity (HF power of pulse interval) from pressure pulse waves were calculated using Hey-Presto software. We compared these parameters before and after bilateral CeA lesions. Following CeA lesions, we observed an increase in MAP (pre:98.1±0.7mmHg, post:104.0±0.8mmHg, $p<0.01$), a decrease in HR (pre:414±4bpm, post:380±6bpm, $p<0.01$), an increase in spontaneous cardiac BRG (pre:0.38±0.01ms mmHg $^{-1}$, post:0.52±0.02ms mmHg $^{-1}$, $p<0.01$), and an increase in HF (pre:9.04±0.19ms ^{2}, post:10.11±0.19ms ^{2}, $p<0.01$). These results suggest that the chronic pressor effect of CeA lesions may be due to a shift in set point, rather than a decrease in the BRG. The decreased HR, and increased HF we observed after CeA lesions, may be the result of secondary compensatory mechanisms associated with pressor responses. It is also possible that the set point shift involves additional nuclei such as sympathetic premotor neurons of the rostral ventrolateral medulla. COI:No

2P-093

Elastic structures of connectin shorten in coronary circulation hearts

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Mammalian and bird hearts have coronary circulation system that supplies oxygen and nutrition into heart muscles via blood vessels. It enables the strong pump hearts with compacta myocardium in highly active and energy-consuming animals. On the other hand, amphibian and some reptile hearts directly take oxygen and nutrition into spongiosa myocardium from heart lumen (sinusoidal circulation). Blood flow of coronary circulation occurs mostly during diastole because vascular compression limits flow during systole. Therefore, the coronary circulation hearts should have stiffer mechanical property to prevent excessive extensions of the heart during diastole resulting in a reduction of blood flow. The expandability of the heart is mainly determined by an elastic protein connectin, which is the largest protein that generates passive tension during diastole. To understand the extension restriction of coronary circulation hearts, we investigated the elastic region of connectin in chicken, crocodile, turtle and frog hearts and compared them to that in human heart. We found the elastic regions of connectin were shorter in coronary circulation hearts with comparison to sinusoidal circulation hearts. We also found that the elastic regions of connectin were shortened by different way between mammal hearts and bird hearts. These results indicated that the shortening of elastic regions of connectin may relate to the extension restriction of coronary circulation hearts and the shortening occurred independently in mammals and bird hearts. COI:No

2P-094

Is morphology of intracellular organelle altered just after initiation of cardiac contraction?

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Backgrounds: We have demonstrated that the beginning of calcium transient precedes an initiation of contraction in the developing embryonic cardiac crescent in rodent animals. However, mechanisms of the time lag between the calcium transient and contraction remain be unknown. Therefore, in the present study, we examined structure of organelles in rat cardiac crescent assessed by transmission electron microscope (TEM) because the structure might be changed after initiation of regular cardiac cycle. Methods and Results: Embryo in Wister rat were removed from embryonic day 10.0 (E 10.0), around cardiac crescent begins to contract. The embryos were divided into two groups by the cardiac crescent before or after the initiation of contraction. Although TEM image showed that slight increase in cytoplasmic area relative to nucleus area was seen after the initiation of contraction, there was no apparent morphological difference in shape of sarcoplasmic reticulum, mitochondria, golgi apparatus, lysosome, and cell-cell adhesion among the two groups. Notably, typical structure of the sarcomere with the Z-Line and M-Band, observed in cardiac crescent at E11.0, was not observed in cardiac crescent even after contraction at E10.0. Conclusions: These findings suggest that the morphological changes in intracellular organelles does not contribute, at least mainly, to the gap between calcium transient and contraction at the initiation of cardiac contraction. COI:No

2P-095

Mathematical modeling of noradrenaline-induced automaticity in rat pulmonary vein cardiomyocyte

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Pulmonary veins (PVs) are considered to trigger atrial fibrillations by inducing ectopic activities. In experiment, noradrenaline (NA) stimulation was shown to induce arrhythmogenetic automaticity in rat PV cells (PVCs). Although involvement of Ca^{2+} release from sarcoplasmic reticulum (SR) was suggested to play an important role in inducing the spontaneous rhythm by activating NCX, detailed ionic mechanisms underlying the automaticity remain to be clarified. For quantitative understanding of the mechanisms, it is inevitable to develop a mathematical model. Therefore, we firstly developed an intracellular Ca^{2+} dynamic model and applied bifurcation analysis. It was revealed that within a certain range of total intracellular Ca^{2+} amount, the model had unstable equilibrium points accompanied by stable limit cycles. It was suggested that this Ca^{2+} dynamic model had a potency generate a rhythmic Ca^{2+}-induced Ca^{2+} release (CICR) in itself. Then a mathematical model of the PVC was developed by integrating experimental data published previously. It was revealed that whether action potentials (APs) are triggered by delayed after depolarizations (DAD) induced by CICR via NCX or not depended on the balance between the amplitude of DAD and the whole cell membrane conductance. Lastly, the effects of NA stimulation on generation of automaticity were investigated using the full model. Functional roles of individual ionic channels and transporters in generating NA-induced automaticity in the PVC model will be discussed. COI:No

2P-096

Relationship of ATP consumption between the cell model and the Laplace heart model in a strongly-coupled simulation

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We aim at comparing ATP consumption estimated from the volume-pressure diagram in a simplified blood circulation model and that estimated directly from the cell model, which is used in a simple circulation model. The comprehensive human ventricular myocyte (HuVEC) model was used to drive the blood circulation in the heart model of the Laplace-type. Namely, the ATP consumption by the Na/K pump and the sarcoplasmic Ca uptake (SERCA) were estimated during the excitation-contraction coupling. The contraction module of the HuVEC model was improved by using the Hinch et al. (2004) model, which can satisfy the local control theory of the membrane excitation-contraction coupling. In addition, we used the cell contraction model (Muangkram et al., 2017) which is able to calculate the ATP consumption of the contraction by the myosin ATPase. The Laplace heart model is composed by a pair of atrial and ventricular compartments, and is connected to the preload and afterload to achieve blood circulation. The half sarcomere length was determined at every time point of numerical integration to give a common rate of sarcomere length change both in the cell model and in the Laplace ventricular heart model (strong-coupling simulation). We could confirm the linear relationship between these two estimates of ATP consumption. COI:No

2P-097

Ionic mechanisms in the electrical activity of hiPS-CMs analyzed by developing a mathematical model

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Mathematical models have been well established for various functional units of the myocardial cells; individual ion channels, ion transporters, sarcoplasmic reticulum to control cytosolic Ca^{2+} concentration and excitation-contraction coupling. By integrating these elementary models into a cell model, quantitative and comprehensive analysis have been conducted to demonstrate the unique configuration of each action potential configuration. In hiPS-CMs, a growing number of reports are now available for ionic channels. Based on the common model structure of the human ventricular myocyte (HuVEC model), we constructed a model of hiPS-CM using ion channel models adjusted to their experimental data. The ventricular, atrial and nodal-types of action potentials could be approximately reconstructed by varying the expression level of individual ion channels. However, differentiating experimental action potential configuration into the three types was statistically insignificant. We could revealed contribution of individual ionic currents to a specific phase of the AP configuration by driving the gating of Vm-sentive channels using the action potential time course recorded in various hiPS-CMs. In spontaneously beating cells, the hyperpolarization-activated channel was activated during the diastolic potential in the ventricular and atrial-type cells, but not in the nodal type cells. The delayed rectifier K current (I_{Kr}) was responsible for the repolarization at about -50 mV in all hiPS-CMs. COI:No

2P-098

Functional coupling between TRPC3 and Nox2 mediates pathological cardiac remodeling

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Chronic stresses induces pathological cardiac remodeling in which production of reactive oxygen species (ROS) plays a critical role. We have revealed that those ROS were produced by NADPH oxidase 2 (Nox2), despite low Nox2 expression levels in the normal heart. Therefore, positive-regulatory mechanism should exist in the transition of the heart from adaptation to maladaptation, but the underlying mechanism is obscure. We demonstrate that transient receptor potential canonical 3 (TRPC3) Ca2+-permeable channel acts as a positive regulator of ROS (PRROS) in cardiomyocytes and cardiac fibroblasts, and specifically regulates pressure overload-induced maladaptive cardiac fibrosis in mice. TRPC3 physically interacts with Nox2 through TRPC3 carboxyl-terminal regions, escaping Nox2 from proteasomal degradation, resulting in amplification of Ca2+-dependent Nox2 activation. The TRPC3-regulated ROS production mediates Rho-dependent cardiac fibrosis through microtubule-associated Rho guanine nucleotide exchange factor, GEF-H1. Furthermore, the TRPC3-Nox2 coupling mediates cardiac atrophy in mice treated with doxorubicin, a chemotherapy drug which is known to have sever cardiotoxicity. These results suggest that functional and physical coupling of TRPC3 and Nox2 mediates various stress-induced cardiac remodeling and inhibition of TRPC3-Nox2 coupling will be a promising therapeutic target for the treatment of heart failure. COI:No

2P-099

Therapeutic effects of voluntary wheel running on cardiac dysfunction associated with cancer cachexia

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Cardiovascular disorders in cancer patients with cachexia have recently become a great concern. However, the relationship between cancer cachexia and cardiac dysfunction remains unclear, due to lack of suitable models. We established a novel mice model of cancer cachexia by implantation of human stomach cancer cell line 85As2, which shows anorexia, body weight loss, and low fat-free mass with a loss of skeletal muscle mass similar to those observed in patients, from 2 weeks after implantation. Plasma levels of leukemia inhibitory factor, known as a cachexia-related factor, increased with the cancer stage progression. Additionally, left ventricular ejection fraction significantly reduced with decreasing heart weight. Thus, our cancer cachexia mice model is suitable for studying cancer cachexia with cardiac dysfunction. To date, the exercise is considered to be one of therapy for chronic heart failure. In the present study, we sought to determine the effects of voluntary wheel running on cancer cachexia-induced cardiac dysfunction using this model. COI:No

2P-100

The role of Cdk4-Cyclin D3 complex in the enucleation of mouse erythroblasts

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During the late stage of mammalian erythropoiesis, an erythroblast divides from five to six times. The erythroblast gradually decreases its size as it divides. Finally, its nucleus moves to one cytoplasmic direction and the erythroblast divides into a reticulocyte and a condensed nucleus covered by cell membrane.Cyclin D is one of the core members of the mammalian cell cycle machinery. Once induced, cyclin D binds and activates the Cdk4 or Cdk6, and promotes progression from the G1 to S phase of the cell cycle. Here, we studyed about the role of D-type cyclin and Cdk4 or Cdk6 in the enucleation of mouse erythroblasts. Only cyclin D3 was expressed in erythroblasts as D-type cyclin. Cyclin D3 formed a complex mainly with Cdk4. The Cdk4-Cyclin D3 complex was degraded during the enucleation, and the degradation of Cdk4-Cyclin D3 the complex was inhibited by the addition of proteasome inhibitors. The proteasome inhibitors also suppressed the enucleation of erythroblasts. When erythroblasts were stimulated again with the second addition of erythropoietin, the enucleation was delayed and the degradation of Cdk4-Cyclin D3 complex was suppressed. Furthermore, addition of Cdk4 inhibitor upon the second erythropoietin stimulation suppressed the delay of enucleation.These results suggest that the degradation of Cdk4-Cyclin D3 complex is required for enucleation of mouse erythroblasts. COI:No

2P-101

Transferrin-Transferrin receptor 1 signaling is required for mouse erythroblast enucleation through the mechanism independent of iron uptake.

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During the late stage of mammalian erythropoiesis, the nucleus of erythroblast undergoes extrusion from the cytoplasm, while being surrounded by a segment of the plasma membrane. However, the signaling pathway involved in this step of erythropoiesis remains obscure.In this study, we used erythroblasts from spleens of anemia-induced adult mice and erythroblasts from E14.5 fetal mouse livers, to unveil the mechanisms of erythroblast enucleation. The enucleation was assessed by using the cell-permeable DNA staining dye SYTO16 and flow cytometer.As results, both types of mouse erythroblasts were neither survived nor enucleated in the medium which does not contain holo-transferrin (holo-Tf), but were survived and enucleated in the medium containing holo-Tf. Furthermore, anti-Transferrin receptor 1 (TfR1) monoclonal antibody blocked the survival and enucleation. A small-molecule natural product, hinokitiol, which can restore iron transport into cells without transferrin molecules, compensated the effect of holo-Tf for survival of mouse erythroblasts but not for enucleation.Our results suggest that Tf-TfR1 signaling has a crucial role in enucleation of mouse erythroblasts, through the mechanism independent of iron uptake. COI:No

2P-102

TRPM8 channel is involved in the ventilatory response to CO_2 mediating hypercapnic Ca^{2+} responses

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Central chemoreceptor in the medulla detects the change of CO_2 or pH in order to regulate respiration for maintaining blood gas and pH within a normal physiological range. We previously reported that transient receptor potential (TRP) channel broad-spectrum blockers Ni^{2+} and ruthenium red, and a TRPV1 and TRPM8 specific blocker BCTC attenuated the hypercapnia-induced Ca^{2+} response in primary culture of medullary cells. We also found the mRNA expression of TRP channels (TRPV1 and TRPM8) in the medullary cells (Cell Calcium 2010). In this study, the role of TRP channels for the ventilatory response to CO_2 was investigated in vivo. To this end, respiration of unrestrained adult TRPV1-, and TRPM8-channel knockout mice were measured by whole body plethysmography. Under control conditions (0% CO_2) and hypercapnia (8% CO_2), no difference in respiratory parameters was observed between adult wild-type and TRPV1-channel knockout mice. However, TRPM8-channel knockout mice showed a decrease in respiratory frequency (fR) and tidal volume (VT) leading to a decrease in minute volume under the hypercapnia. Furthermore, we measured intracellular Ca^{2+} responses of TRPM8-overexpressing HEK cells to hypercapnic acidosis. Subpopulations of cells that exhibited the hypercapnic acidosis-induced Ca^{2+} response also responded to the application of menthol (TRPM8 agonist). These results suggest that the TRPM8 partially mediates the ventilatory response to CO_2 via changes in intracellular Ca^{2+} and is a candidate of central chemosensing proteins. COI:No

2P-103

Effects of blockade of glycine receptors in the thoracic spinal cord on the amplitude of the inspiratory thoracic motor activity

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The inspiratory outputs are larger in the intercostal muscles positioned at more rostral segments. Such rostrocaudal gradient is kept in the *in vitro* preparation from neonatal rat. It is known that there are many propriospinal respiratory neurons in the thoracic spinal cord, and anatomical studies showed no evidence that the bulbospinal inspiratory neurons have systematic patterns of connections to different segments. Therefore, such gradients should be generated by the propriospinal interneurons. To clarify the involvement of glycinergic inhibitory interneurons, a glycine receptor antagonist, strychnine, was locally applied to the thoracic spinal cord, and effects on the thoracic inspiratory motor activity were examined. The electrical activity was obtained from third and eleventh thoracic ventral root (T3VR, T11VR). Under 10 μM strychnine, the seizure-like activity often occurred. The respiratory activities were discriminated by their shape of discharge. The ratio of the amplitude of the inspiratory activity under strychnine to that under control was about 124 % for T3VR and 175 % for T11VR. The ratio of the amplitude of the inspiratory activity under strychnine to that of the seizure-like activity was 63 % for T3VR and 32 % for T11VR. These results suggest that both glycinergic and other excitatory interneuron would involve in the rostrocaudal gradient of the inspiratory motor output. COI:No

2P-104

Spontaneously breathing neonatal rat and respiratory sinus arrhythmia

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Objective: We examined respiratory sinus arrhythmia (RSA, the acceleration and deceleration of heart rate (HR) during inspiration and expiration, respectively) in spontaneously breathing neonatal rat. Methods: Neonatal rat (2 to 4-day-old) under 1-3 % isoflurane anesthesia was inserted a tube into the abdominal cavity and a temperature probe into the rectum, and placed fine ECG electrodes subcutaneously. The rat was then placed in a small double-parted chamber to measure respiratory rate (fR) and HR in normoxia. Recording was started once fR and HR had stabilized and the animal's body temperature had recovered to the level before anesthesia. After control recordings without anesthesia were obtained, rat was received dexmedetomidine, a potent alpha2-adrenoceptor agonist (n=28), intraperitoneally. Data were analyzed breath-by-breath and averaged for the 10 consecutive breaths selected toward the end of 5-min-long data collection. For each breath, RSA corresponded to the difference in HR between peak and trough (delta HR, beats/min) in percent of mean HR (RSA = delta HR/mean HR %). Results: As fR and HR were decreased by dexmedetomidine, RSA tended to increase. By including all data, results of regression analysis suggested that 1/fR and HR/fR influence RSA in neonatal rats. Conclusion: Our results suggest that RSA, a physiological phenomenon, may track the cardiorespiratory changes to match pulmonary blood flow and breath-by-breath ventilation in immature animals. COI:No

2P-105

Cognitive function and hippocampus volume in chronic obstructive pulmonary disease.

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Cognitive function and the structural alteration of the brain are relatively unexplored in patients with chronic obstructive pulmonary disease (COPD). In this study, we sought to examine the relationship between MRI-measured brain volumes in the hippocampus (HI) and amygdala (AMG) and cognitive function measured with the Mini Mental State Examination (MMSE) in COPD and age-matched healthy control subjects. MRI was performed at Ebara Hospital (3T Magentom Prisma, Siemens). MRI volumetric measurements of the HI and AMG were made by manual tracing with ANALYZE software (Mayo Clinic). There was no significant difference in MMSE scores between the two groups ($P = 0.31$). However, MRI volume analysis showed that the left HI was smaller in COPD subjects than controls ($P=0.008$), but there were no differences in the AMG or right HI between the groups (all $P > 0.05$). There was also a negative correlation between the volume of the left HI and pack-year smoking history in COPD subjects ($r = -0.9$). We found that COPD subjects had a smaller hipocampal volume depending their smoking history, the clinical significance of which remains uncertain. COI:No

2P-106

Reconstructed postsynaptic excitatory and inhibitory conductance profiles of respiratory interneurons in rat brainstem in situ reveal network organization of respiratory CPG

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The pre-Bötzinger Complex (pre-BötC) and the Bötzinger complex (BötC) are the essential core components of the mammalian brainstem respiratory central pattern generator (CPG), in which pre-inspiratory/inspiratory, early-inspiratory, post-inspiratory and augmenting-expiratory interneuron populations are functionally interacting to generate a normal three-phase pattern of respiratory neural activity. However, functional connectivity and synaptic interactions among these interneuron populations remain largely unknown. We obtained the membrane potential trajectories of pre-BötC and BötC respiratory interneurons by current-clamp recordings with sharp-electrodes in arterially perfused juvenile rat brainstem-spinal cord preparations in situ, and the dynamical changes of phasic excitatory and inhibitory conductances of these neurons by using the analytical techniques we developed that allow retrieval of postsynaptic excitatory and inhibitory conductances at high temporal resolution. The reconstructed excitatory and inhibitory synaptic conductance profiles are consistent with the local microcircuit organization of the respiratory CPG we previously proposed to account for the rhythmic alternation of inspiratory and expiratory phasic activity. COI:No

2P-107

Respiratory rhythm generation under the reduced mutual excitatory synaptic connections

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The mutual excitatory synaptic connections between respiratory neurons in the medulla are thought to be important in respiratory rhythm and pattern generation. Pacemaker property of respiratory neurons has been also suggested to contribute to respiratory rhythm generation. We previously reported inhibitory effects of eugenol on respiratory burst generation. The experiments were performed in the brainstem-spinal cord preparation that were isolated from newborn rats (P0-P3) and superfused by modified Krebs solution at 25-26oC. The inspiratory C4 ventral root activity was monitored. Bath application of eugenol (0.5-1 mM) induced depression of respiratory activity. After washout of eugenol (20 min application), typically, depression of the respiratory rhythm was reversed in association with shortening of the burst duration of C4 inspiratory activity (approximately 10 % of control). The decrease in the burst duration continued for more than 1 hr after washout. The burst duration of pre-inspiratory and inspiratory neurons was also shortened and only one action potential appeared during each burst phase. Therefore, we hypothesized that mutual excitatory synaptic connections were not involved in the rhythm generation with the shortened burst activity after eugenol treatment. This rhythm was dose-dependently inhibited by treatment with 0.01-0.1 mM riluzole (a persistent sodium channel blocker). The results suggested a contribution of pacemaker type neurons in the rhythm generation without mutual excitatory synaptic connections after eugenol treatment. COI:No

2P-108

Low body weight agreement attenuates the equivalent of dual energy X-ray absorptiometry and multi-frequency bioelectrical impedance analysis in body composition assessment in Korean adults.

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Low agreement of body composition analysis (BCA) between dual-energy X-ray absorptiometry (DXA) and multi-frequency bioelectrical impedance analysis (MF-BIA) has been reported. We examine whether this discrepancy is influenced by the precision of DXA in body weight (BW) measurement. This cross-sectional study enrolled 1353 participants aged 53-83. Whole body scan of DXA and eight-polar tactile-electrodes impedance-meter using four electronic frequencies of 5, 50, 250, and 500 kHz were employed for BCA. Agreement level between BW estimated by DXA and actual BW (WgtA) was calculated. Agreement of BCA by DXA versus MF-BIA across WgtA groups was assessed. DXA substantially wrongly estimated BW, especially in men. 13.5%, 5.1%, and 5.6% participants accompanied with BW bias levels as 2%, 3%, and 4% and higher, respectively. Correlation of BCA by DXA versus by MF-BIA in body fat mass, percent body fat, and lean body mass (LBM) was gradually reduced, whereas root mean squared error was increased in concordance with the reduction of WgtA. DXA provided a lower LBM amount compared to that of MF-BIA and this difference increased significantly across groups of poor WgtA. Lower WgtA contributed remarkably to the difference in BCA measured by DXA and MF-BIA. This work was funded by 2014-E71003-00, 10068076, and 2014M3A9D7034366 COI:No

2P-109

Relationship between oral function and motor ability in top Japanese junior badminton players

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The aim of this study was to examine the relationship between oral function and motor ability in top Japanese junior badminton players. Subjects were 50 badminton players (U16: 24 girls; U19: 26 girls), who were representative candidates of junior athletes in Japan. Sports-tests prescribed by the Nippon Badminton Association were carried out. Sports-tests were consist of sit-up, side steps, double jumps (1 min, 2 min, and 3 min), and sprints (50 m and 100 m). Occlusal force was analyzed using the Dental Prescale. Differences in occlusal force and sports-test results between age groups were assessed using Student's t-test or Mann-Whitney test. And correlations between occlusal force and sports-test results were analyzed. Occlusal force was higher in the U19 group than in the U16. The following sports-test results were superior in the U19 compared with the U16: sit-ups, side steps, and double jumps (1 min, 2 min, and 3 min). In the U16, no significant correlation was observed between occlusal force and sports-test results. In the U19, positive correlations were observed between occlusal force and side steps and between occlusal force and double jumps (2 min and 3 min). These findings suggest that occlusal force and sports-test results in top Japanese junior badminton player tend to improve with age. In the U19, occlusal force was associated with sit-ups and double jumps, which evaluate agility, dexterity, and endurance. Higher occlusal force was shown to be linked to better sports-test results in these athletes. COI:No

2P-110

Influence of inspiratory muscle training on pulmonary functions with postural changes in obese men

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The purpose of this study was to investigate the influence of inspiratory muscle training (IMT) on the vital capacity (VC) in obese men with a focus on the change of posture.
Methods: We enrolled 20 obese men (IMT group, 10; control group, 10) with a body mass index of >25. As a training, all participants breathed for 15 min at a respiratory rate of 15 breaths/min three times per week for 6 weeks. During the training, the inspiratory loads were adjusted to 30% of the maximum inspiratory pressure (PImax) in the IMT group and to the minimum load in the control group using an inspiratory loading device. We evaluated PImax, maximum expiratory pressure (PEmax), and VC, before the training and 2, 4, and 6 weeks after the training. In addition, the rate of change in VC when the posture was changed from the sitting to the supine position was examined.
Results: Although PImax and PEmax significantly increased after 4 weeks of training in the IMT group,($p< 0.05$), there was no significant difference in the control group. VC was significantly lower in the supine position than in the sitting before and after the training($p< 0.05$). However, after 6 weeks of training in the IMT group, the rate of decrease in VC was smaller and lower than in the control group significantly($p< 0.05$).
Conclusions: IMT for obese men showed the less rate of decrease in VC with posture change from the sitting to the supine position. COI:No

2P-111

Effect of exercise training on gut microbiota in healthy elderly women

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This study aimed to examine whether exercise intervention modifies the composition of intestinal microbiota in healthy elderly women. Twenty-two sedentary elderly women participated in a non-randomized comparative trial. Subjects underwent either of the following two programs: trunk muscle training program (CT group) or aerobic exercise program (AE group), which included brisk walking at an intensity of >3 METs. The composition of intestinal microbiota extracted from the stool samples was determined using PCR before and after the 12-week intervention. The assessment of daily physical activity by accelerometer and the 6-minute walk test (6MWT) were also performed. Thirteen participants in the AE group and six in the CT group completed the study. The ratio of intestinal Bacteroides significantly increased only in the AE group (40.5% ± 13.5% to 47.1% ± 10.2%, $p = 0.023$), particularly in the subjects who could increase the time spent in brisk walking. The post-intervention distance in 6MWT was positively correlated with the increase in the ratio of intestinal Bacteroides with intervention in all subjects ($r = 0.59$, $p = 0.008$). Aerobic exercise can increase intestinal Bacteroides in association with an improvement in the cardiorespiratory fitness in healthy elderly women. COI:No

2P-112

Vocalization during the upper and lower body exercise changes the ventilation and the peripheral circulation states

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We reported that vocalization during exercise suppressed the value of minute ventilation (VE) and carbon dioxide output, increased the value of $FetCO_2$ (=$PaCO_2$). Vocalization during exercise may change the peripheral circulation state, because $PaCO_2$ is one of the factors that change the circulatory system. In previous study, the upper body exercise increased the value of VE, so we conjectured the upper and lower body exercise (UL-Ex) with vocalization (Voc) might increase $PaCO_2$ at lower workload. In this study, we investigated whether UL-Ex with Voc increased the value of $FetCO_2$ and change the peripheral circulation state. 6 subjects (4 males and 2 females) performed the continuous exercise using a cycle ergometer for 3-min at 40%, 60% and 80%VO_2peak with or without the upper body exercise (using dumbbell at 0.5-2.0 kg) and Voc (reading aloud about 500 characters per min). We measured ventilatory variables (VE, $FetCO_2$, etc) at rest and during exercise for 1-min between the 2nd and 3rd min in each workload and Venous Oxygenation Index (VOI) at rest and just after each workload. The value of VE tended to increase in UL-Ex at all workloads, and tended to suppress in Voc. The value of $FetCO_2$ tended to increase in UL-Ex with Voc at all workloads, especially, significantly increased at 60%VO_2peak. The %change of VOI tended to increase in UL-Ex with Voc at 40%VO_2peak, but tended to decrease at 80%VO_2peak. The ventilation states caused by vocalization during the continuous UL-Ex may enhance the peripheral circulation at lower workload, and may suppress at higher workload. COI:No

2P-113

Skeletal muscle pathology of patients with CKD and their muscle function before and after renal transplantation

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Muscle atrophy and muscle weakness are serious complications in patients with chronic kidney disease (CKD). Reduced muscle mass correlates with the progression of CKD and the risk of mortality. Renal transplantation (RT) has become a choice of treatment for patients with CKD; however, whether there is an improvement in muscle mass and function after RT has not been clarified. Therefore, in this study, we aimed to clarify (1) internal oblique muscle pathology of male patients with CKD (before RT), and (2) differences in muscle mass and physical function before and after RT. Muscle atrophy was observed in most of the patients before RT. Mitochondrial abnormalities and myofibrillar disorganization were also frequently observed (in approximately 50% of patients); however, these histological changes were minor. Muscle strength before RT was substantially reduced compared with that of age-matched healthy subjects. Most CKD patients before RT had a low skeletal muscle mass index (SMI), which was considered to be a result of sarcopenia and presarcopenia. No correlation was observed between SMI and dialysis history. At 6 months after RT, there was a trend of increase in muscle mass and strength in the upper and lower extremities. We further analyze the association between muscle pathology and muscle function and the predictive factors of muscle weakness of CKD patients. COI:No

2P-114

Identification of mechanically-insensitive muscular afferents and their activation in rats

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Mechanically-insensitive afferents (MIAs), also called as "silent" or "sleeping" nociceptors, do not respond to mechanical stimulation in normal condition, but they become active to noxious stimulation to play roles in hyperalgesia. The existence of MIAs in the skeletal muscle and their activation mechanisms have not been clarified. In this study we tried to identify muscular MIAs using *in vivo* single-fiber recording technique, and investigate their axonal properties and alterations in the responsiveness to noxious stimulation. One hundred sixty-four C-fibers were electrically identified, and seventeen of them (10.3%) were presumed to be MIAs based on their axonal properties. Three of the 17 MIAs have acquired the responsiveness to mechanical stimulation after a cocktail injection of inflammatory substances (bradykinin, serotonin, prostaglandin E_2, and histamine). Our data demonstrated the existence of MIAs in the rat skeletal muscle, and the modal shift of MIAs from silent to active may contribute to the peripheral mechanisms of muscular mechanical hyperalgesia. This work was supported by JSPS KAKENHI (JP26860161, JP16H03202 and JP16K15338), and partly by the Japan Agency for Medical Research and Development (AMED) Grant 17gm0810010h0502. There were no conflicts of interest in this study. COI:No

2P-115

Spin-spin relaxation of 1H NMR signals from highly ordered myosin filaments suspension

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The dynamic structure change of water molecules surrounding contractile proteins might play an important role in cross-bridge cycling during contraction. The spin-spin relaxation process of 1H-NMR signals from suspension of myofibrils prepared from rabbit could be well represented by the summation of several exponentials indicating that water molecules in the suspension could be conveniently grouped into several components based on the relaxation time constant (T2). In the M or MT state, myofibril affects water molecules within 500 nm from its surface differently from water molecules in the bulk solution, and releases many water molecules in the MDPi or MD state. This may suggest that the potential of the water molecules that surround myofibril proteins are dynamically changed during cross-bridge cycling. COI:No

2P-116

Deletion of Egr3 decreases Pax7 protein level in mouse myoblast

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The function of muscle satellite cells is regulated by numerous transcription factors. Paired-box transcription factor seven (Pax7) is critically important to determine the fate of the muscle satellite cell such as quiescence, proliferation or differentiation. We have previously demonstrated that early growth response 3 (Egr3) is involved in proliferation of myoblasts (i.e. activated satellite cells). However, the relationship between Pax7 and Egr3 is currently unknown. The purpose of the present study is to clarify the effect of Egr3 deletion on the Pax7 protein level in myoblasts. The satellite cells were harvested from Egr3-floxed mice that conditionally expressed Cre-ERT2 protein driven by Pax7-promotor. The cells as an activated myoblast were expanded in culture with several pre-plating purifications. For deletion of Egr3, the cultured cells were treated with 4-hydroxytamoxifen (4-OH-TMX; 50 nM) for 4 consecutive days and grown further for 3 days without 4-OH-TMX. Then the cells were harvested to measure the mRNA levels of Egr3 and Pax7 by quantitative RT-PCR. The Pax7 protein levels were examined by Western blotting. The data was analyzed by unpaired t-test. Egr3 mRNA levels were significantly ($p < 0.05$) decreased (< 1/20) by 4-OH-TMX treatment. Pax7 mRNA levels were not changed by 4-OH-TMX treatment. However, the Pax7 protein level was significantly ($p < 0.05$) decreased by 4-OH-TMX treatment. These results suggest that Egr3 is associated with post-translational modification of Pax7 protein such as protein degradation in myoblasts. COI:No

2P-117

Application of CGRP upregulates MyHC I mRNA through IL-6 independent manner in C2C12 cells

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Our previous study using differentiated C2C12 cells indicated that myosin heavy chain type I (MyHC I) and interleukin-6 (IL-6) mRNA expression levels were significantly increased by the application of calcineurin (CN) activators, such as chlorogenic acid and oleic acid. The effects of these CN activators on the MyHC I mRNA were attenuated by the co-administration of anti-IL-6 receptor antibody. Thus, the effects of CN activators on the upregulation of MyHC I mRNA were considered as a result of increase in IL-6 production. In this study, we examined the effects of calcitonin gene-related peptide (CGRP) which is known to secrete from motor nerve-endings on mRNA levels of MyHC I and IL-6 in C2C12 cells. C2C12 cells were induced to differentiate to myotubes by medium exchange to D-MEM containing 2%FBS. The cells were incubated in D-MEM containing 2%FBS with chemical compounds at the beginning of differentiation and removed after 24hr, and were maintained in differentiation medium for 3 days. MyHC I and IL-6 mRNA expression levels were measured by the real-time PCR method. Administration of CGRP to the culture medium did not affect to the IL-6 mRNA level in the differentiated C2C12 cells. However, MyHC I mRNA level was significantly increased by the administration of CGRP. These results indicate that the effect of CGRP on upregulation of MyHC I mRNA does not depend on CN-IL-6-mediated processes. Further experiments need to be done to clarify the CGRP-mediated intracellular signaling in the upregulation of MyHC I mRNA. COI:No

2P-118

Study of junctophilins in a zebrafish larva

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Junctophilins (JPHs) are key proteins that associate t-tubules and sarcoplastic reticulum (SR) membranes in muscles. The mammalian genome contains four JPH genes; JPH1, JPH2, JPH3 and JPH4. In mice, skeletal muscles express JPH1 and 2, cardiac muscles express JPH1, while JPH3 and 4 are reported to be expressed in non-muscle cells. JPH2 is the most intensively-studied junctiophilin, which plays significant roles in excitation-contraction coupling in skeletal and cardiac muscles. KO mice die before birth due to the defect of cardiac activity.
Zebrafish larva is transparent and develops outside of the mother's body. This is an advantage for studying roles of JPHs in living organisms. However, there have been no report on zebrafish JPHs. On zebrafish genome, there are two JPH1s (JPH1a and 1b), JPH2 and JPH3, but we did not find a gene homologous to mouse JPH4. We performed in situ hybridization and revealed that JPH1a was expressed mainly in the skeletal muscle at 24 hours post fertilization (24hpf), while it was expressed only in the heart at 3 days post fertilization (3dpf). The expression pattern of JPH1b was similar to that of JPH1a. JPH2 was expressed in the skeletal muscle at both 24hpf and 3dpf, which is the same to mice JPH2. We also found the signal in the gill and the heart in 3dpf fish. The expression pattern of JPH2 in zebrafish was similar to that in mice. We will discuss the roles of JPHs including some additional data in the meeting. COI:No

2P-119

The psychoactive drug 25D-NBOMe induces rhabdomyolysis in zebrafish

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Zebrafish are good animal models for human diseases due to their high genetic homology to humans, visibility of organ structure, easy genetic manipulation and high-throughput analyses. NBOMes are known as a psychoactive drug and potent agonist of 5-hydroxytryptamine (serotonin) HT2A receptor and induces serotonin syndrome with rhabdomyolysis. To create animal models of rhabdomyolysis, zebrafish were treated with NBOMes and examine the surviving rate and muscle structures.Zebrafish larvae were treated with 0-20 μg/mL of 25D-NBOMe or vehicle for one day. Their survival rate and muscle birefringence after the treatment with 25D-NBOMe were monitored using a dissection microscope. They were co-treated with a 5-HT2A receptor antagonist aripiprazole or a 5-HT2C antagonist SB242084 with 25D-NBOMe to rescue their survival rate and muscle abnormalities. Treatment with 25D-NBOMe reduced muscle birefringence of zebrafish larvae dose-dependently, and their survival rate to about 40% after treatment with 15 μg/mL. Immunofluorescence microscopy using antibodies against myosin and β-dystroglycan demonstrated the disrupted muscle structures, which might recapitulate NBOMe-induced rhabdomyolysis. A 5-HT2A receptor antagonist aripiprazole or a 5-HT2C antagonist SB242084 improved the 25D-NBOMe-induced rhabdomyolysis. We developed a new animal model of serotonin-receptor-dependent rhabdomyolysis in zebrafish, which could recapitulate NBOMe-induced rhabdomyolysis. COI:No

2P-120

Effects of beta escin skinning on X-ray diffraction pattern of taenia cecum smooth muscle from guinea pig

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Beta escin skinning (cell membrane permeabilization) is known to well keep cellular function compared with Triton X-100 or saponin skinning in smooth muscle preparations. To evaluate the effects of beta escin skinning on structure of contractile filaments of the taenia cecum from guinea pig, contractile filament lattice and periodical arrangement of myosin filaments were measured by X-ray diffraction using an intense X-ray beam from a synchrotron radiation source. On equator, combination of three diffractions at Bragg spacings of 10.4(Rh3), 14.1(Rh2), and 22.4(Rh1) nm showed broad diffraction in the intact taenia preparation. Also, the third, fifth and higher order collagen meridional reflections, a meridional reflection from the 14.4 nm thick filaments, and the 5.9 nm actin layer-line were observed. When the taenia preparation was skinned with 50 micro M beta escin for 60 min at 30 ºC, intensities of all diffractions described above were decreased by 10-20%, although relative intensities among diffractions were not significantly changed. Also skinning increased lattice spacings of the three equatorial diffractions by 5-10 %. These results indicate that organization of contractile filaments of the taenia cecum is relatively well preserved after beta escin skinning, even though with a small change in filament lattice presumably due to some experimental factors such as a difference in osmotic pressure. COI:No

2P-121

Improvement of motor function by electrical muscle stimulation after spinal cord injury in rats.

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The aim of this study was to identify the effects of electrical muscle stimulation after spinal cord injury in rat model. Eight weeks old SD male rats were used for spinal cord injury model. 20g rod with 2mm apex diameter was dropped from the height of 25mm on the exposed spinal cord of T9 level. Electrical stimulation (ES) was applied by 10mA, 2Hz, 10 minutes for both anterior tibial muscle, 5 days a week for 4 weeks. Motor function were assessed by BBB score, inclined plane and rotarod. For histological assessment, BDNF producing cells, and cavity volume were calculated. Quantitative analysis of BDNF was also done by ELISA. Comparison was made among three groups: sham, spinal cord injury (SCI) and spinal cord injury with electrical muscle stimulation (SCI+ES). Data are shown as mean±SEM. For statistical analysis, Mann-Whitney U-test was used for cavity volume, and Kruskal-Wallis (Steel Dwass) for the rest. Four weeks after the injury, the BBB score and inclined plane significantly improved in the SCI+ES compared with SCI, although the difference was not seen in rotarod. The cavity volume had a tendency to decrease in SCI+ES. One week after the injury, the density of BDNF positive cells was higher in SCI+ES compared with SCI, and they were also NeuN positive. ELISA demonstrated a higher tendency of BDNF in SCI+ES. Percutaneous electrical stimulation accompanied with muscle contraction was effective for functional recovery in spinal cord injury rats. COI:No

2P-122

Calcium imaging of the cerebellar mossy fiber activities

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Cerebellar mossy fibers are one of the two major inputs to the cerebellum, providing excitatory inputs to Purkinje cells via granule cell-parallel fiber pathway. Previous studies using whole-cell recordings from granule cells and mossy fiber boutons revealed that mossy fiber activities correlate with sensory inputs and motor activities. However, how multiple mossy fibers activities are spatially and temporally distributed still unknown. Calcium imaging from the mossy fibers is necessary to understand the encoding of sensorimotor activities by population of mossy fibers. Therefore, we measured the activity of cerebellar mossy fibers by calcium imaging in Thy1-G-CaMP7 transgenic mice. We first confirmed that G-CaMP7 localized in the mossy fiber terminals with immunocytochemistry. We also confirmed that some G-CaMP7 positive mossy fibers originate from the pontine and cuneate nuclei by retrograde labelling. We observed sensory response of the mossy fibers by stimulation of forelimb in anesthetized mouse with wide-field calcium imaging. To monitor the mossy fiber activity during movements, we used forelimb movement tasks and observed mossy fiber activities correlating with forelimb movements. Activities of single mossy fiber terminals during forelimb movements can also be observed by two-photon calcium imaging, and we will consider the spatiotemporal pattern of the mossy fiber activity related to forelimb movements. COI:Properly Declared

2P-123

Correlation between FBS test and bilateral lower limb muscle strength, agility, root mean square in patients with femoral neck fracture

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The purpose of this study is to search the relationship between Functional Balance Test (FBS) and lower limb muscular strength (LLMS), agility, Root Mean Square(RMS) and to examine factors affecting balance. The subjects were seven femoral neck fracture patients who were hospitalized in our hospital, and the average age was 81±7.5 years old. Measurement of LLMS adopted the maximum value measured by handheld dynamometer. Measurement was made on the gluteus medius(GM), quadriceps femoris(QF), gastrocnemius(GS) and tibialis anterior(TA) muscles. In the agility test, the number of opening and closing motions of the lower limbs for 20 seconds was measured at the chair position. Using a three axis accelerometer, the sagittal, the frontal, the horizontal plane, and the three axis synthetic RMS at walking were calculated. The standing RMS was measured using the center of gravity swing meter. In order to search the relation with FBS, Spearman's relation coefficient was calculated. Multiple regression analysis was performed with dependent variables as FBS and independent variables as measurement items(the statistical significance level was 5%). Significantly different relations were found in the affected GM, the affected GS, the normal QF, synthetic RMS, standing RMS, 10 m walking. The item with a significant difference in multiple regression analysis was the affected GS, agility test ($P<0.05$). COI:No

2P-124

A double knockout zebrafish revealed distinctive regulations of nicotinic acetylcholine receptors (nAChRs) in slow and fast muscles.

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Nicotinic acetylcholine receptors (nAChRs) expressed in the neuromuscular junction are pentamers, composed of α, β, δ, and ε (or γ) subunits. However, recent studies suggested that the subunit composition of nAChRs in zebrafish slow muscles lack ε / γ subunit. To investigate the distinctive compositions and functions of nAChRs in slow and fast muscles further, we generated a double knockout zebrafish line that lacked both ε and γ subunits (ε / γ -DKO). Zebrafish is an ideal model for analyzing functional difference between slow and fast muscles, because the two types of muscles are spatially segregated and can easily be distinguished by their location. We found that the ε / γ -DKO has the ability to swim, and we also found that nAChR expression was limited to slow muscles. Moreover, patch clump recordings showed that fast muscle cells in the ε / γ -DKO failed to exhibit mEPSC, whereas slow muscle cells generated normal mEPSC. Congruently, in vivo Ca2+ imaging in the ε / γ -DKO showed that slow muscles exhibited Ca2+ transients, while fast muscles did not generate calcium response. These results strongly support the idea that nAChRs lacking ε / γ subunit function in vivo and are specifically expressed in slow muscles. Interestingly, nAChRs lacking ε / γ subunit cannot be expressed in fast muscle. Thus, distinctive mechanisms may regulate nAChR subunit compositions in slow and fast muscles. COI:No

2P-125

The chemogenetic suppression of the primate subthalamic nucleus induces abnormal involuntary movements

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The "rate model" of the basal ganglia (BG) functions proposes that the neuronal firing rate changes in the BG impair voluntary movements as seen in hemiballism, which is caused by lesions in the subthalamic nucleus (STN). The STN sends strong excitatory outputs to the internal segment of the globus pallidus (GPi), the output nucleus of the BG; the hemiballism is presumably induced by activity decrease in the GPi. To elucidate the neural mechanisms of this movement disorder, the Designer Receptors Exclusively Activated by Designer Drugs (DREADD) was utilized to reversibly inhibit the STN activity.

We injected an adeno-associated viral vector expressing the inhibitory DREADD receptor, hM4Di, to the motor area in the STN of a Japanese monkey (*Macaca fuscata*). More than 3 weeks later, clozapine N-oxide (1.0 mg/kg BW) was intravenously administrated; the suppression of the STN activity induced abnormal involuntary movements on the contralateral upper limb and disturbed reaching motion.

The GPi neurons exhibited the excitation, inhibition, or mixed activity changes during the reaching movement. With the STN suppression, the pause durations were prolonged and the Fano factors, or the trial-to-trial variance, increased in the GPi; however, the average firing rates were not affected. These results suggest that the dynamic neuronal activity changes in the BG impair voluntary movements, contrary to the prediction by the "rate model." COI:No

2P-126

Involvement of satellite cell activation underlying nitric oxide mechanisms in ectopic orofacial pain

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Background Some of the recent studies have reported the involvement of nitric oxide (NO) in the enhancement of neuronal activities resulting in persistent pain. To evaluate ectopic orofacial pain mechanisms, we examined if NO was involved in satellite glial cell (SGC) activation in the trigeminal ganglion (TG) following trigeminal nerve injury. MethodsInferior alveolar nerve transection (IANX) was performed in anesthetized rats. Head-withdrawal threshold (HWT) to mechanical stimulation of the whisker pad skin was assessed. Glial fibrillary acidic protein (GFAP) and neuronal NO synthase (nNOS) immunohistochemistries were conducted in IANX rats, and GFAP inhibitor, Fluorocitrate (FC) and nNOS inhibitor, NLPA were administrated into TG. HWT was measured, and the number of GFAP- and nNOS-immunoreactive (IR) cells in the TG was counted on day 3. ResultsThe HWT was significantly reduced in IANX rats on days 1, 3, 5 and 7 compared with sham rats. The number of neurons encircled with GFAP-IR cells and nNOS-IR neurons was significantly increased in the TG on day 3 following IANX. After nNOS inhibitor administration into the TG in IANX rats, the number of neurons encircled with GFAP-IR cells significantly reduced, and reduced HWTs were recovered. ConclusionsThe present findings suggest that the NO expression was enhanced following IANX and released from injured TG neurons, resulting in SGC activation. Activated SGCs are further involved in the hyperactivation of TG neurons innervating in the whisker pad skin, causing ectopic orofacial pain. COI:No

2P-127

Peptidylarginine deiminase is involved in maintaining structure of stratified oral mucosa of rats

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In skin, profilaggrin (proFLG) is known to be cleaved into FLG, a monomeric form (~48 kDa) that is involved in generating the physical barrier made of keratins. Subsequently, the monomer is citrullinated by peptidylarginine deiminases (PAD) and finally degradated. In this study, we examined the role of PAD in maintaining structure of stratified oral mucosa of rats. We performed immunofluorescence, immunoblot and PAD assay, and constructed organotypic culture models. In the cornified layer of skin, FLG and keratin 1 (K1) were co-localized with PAD1. Beside no co-expression of FLG or K1 on the palatal surface, punctuated co-expression of (pro)FLG, PAD1 and citrullinated proteins were found in granular layer of palate. Immunoblot revealed a considerable amount of FLG monomer in the skin, but only traces in the palate. PAD1 expression was the highest in the palate among the three epithelia, while PAD2 and PAD3 were in skin, suggesting a site-specific expression of PAD isozyme with different Ca2+-dependency. Next, we examined the effect of a PAD inhibitor using a organotypic model derived from palate. Cl-amidine, which inhibited protein citrullination, augmented the level of FLG monomer and co-expression with K1, and reduced the thickness of the cornified layer. These results suggested that PAD-dependent (pro)FLG degradation is likely to be involved in maintaining structure of stratified oral mucosa. COI:No

2P-128

Effect of S-PRG filler eluate on MAP kinase-family phosphorylation of human gingival fibroblasts

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The S-PRG filler is known to have a high cariostatic effect and to release 6 ions (Na^+, F^-, Al^{3+}, BO_3^{3-}, Sr^{2+} and SiO_3^{2-}). We examined the effect of S-PRG eluate on human gingival fibroblasts (HGF) and assessed the effect of various concentrations of S-PRG eluate (1, 1/2, 1/5, 1/10, 1/100, 1/200, 1/500, 1/1000, 1/2000, 1/5000 and 1/10000) on the proliferation of HGF. Solutions diluted to 1/100 or more showed a cell proliferation similar to the control group. We detected MMP-1 and MMP-3 secreted into the culture supernatant by western blotting when S-PRG eluate (1/100, 1/500, 1/1000, 1/5000 or 1/10000) was added to HGF. We found that production of MMP-1 and MMP-3 were enhanced by S-PRG eluate. We used immunoblotting to assess the effect of S-PRG eluate (1/1000 and 1/10000) on the induction of phosphorylation of p38, ERK 1/2 and JNK in HGF. A time course study revealed that phosphorylation of p38 and ERK 1/2 occurred within 1 minute. However, phosphorylation of JNK was not enhanced by S-PRG eluate. These results suggest that phosphorylation of p38 and ERK 1/2 may be involved in the production of MMP-1 and MMP-3 by S-PRG eluate. COI:No

2P-129

Effects of Imidapril on swallowing activity in *in situ* rat preparations

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Imidapril is a long-acting inhibitor of angiotensin converting enzyme (ACE) used in the treatment of hypertension, congestive heart failure and diabetic nephropathy. ACE inhibitors have been also suggested to prevent aspiration and improve the impaired swallowing processes as a result of increases in release of substance P from the vagus nerve. In the present study, we examined the effects of imidapril on swallowing activity in motor nerves innervating the pharyngeal muscles. Experiments were performed on *in situ* perfused brainstem preparation of juvenile rats aged between postnatal days 21-33. We recorded activity from the vagus (VN), hypoglossal (HGN) and phrenic nerves. The spontaneous swallowing activity intermittently occurred in the VN and HGN, which were synchronized with each other. Injection of 0.8 ml distilled water into the oral cavity consistently evoked swallowing burst activity in the VN and HGN. Application of imidapril (6 μg/ml) to the perfusate increased the peak amplitude of the burst discharge in the VN during orally-evoked swallowing activity (119 $\pm$ 5.4% of control, $n = 5$, $P = 0.012$), although the frequency and burst duration of the swallowing activity were not significantly changed. In contrast, imidapril did not alter the peak amplitude, the frequency and burst duration of the swallowing activity in the VN during spontaneous swallowing activity. These results suggest that imidapril may improve the impaired swallowing by increases in activity of the pharyngeal muscles. COI:No

2P-130

Japanese Rice Wine (Sake) reduced enhanced masseter muscle nociception under psychophysical stress conditions in rats

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Sake intake with an appropriate quantity promotes one's healthy life such as reduction of psychological distress. The aim of this study was to test if Sake had inhibitory roles on enhanced masseter muscle (MM) nociception under psychological stress conditions in rats. Repeated forced swim (FST) or sham treatments were conducted for 3 days. Sake, 15% ethanol alone or saline was given systemically after each stress and sham sessions from Day 1 to 3. At Day 4 rats were sacrificed after MM injection of formalin to perform Fos immunohistochemistry. The number of Fos positive cells was quantified at trigeminal caudalis (Vc) region. Sake and ethanol reduced immobility time during FST, indicating the reduction of depression-like behavior. FST enhanced Fos response by formalin at Vc region compared to sham rats, indicating facilitatory effect of psychophysical stress conditionings on MM nociception at Vc region. Daily administration of ethanol or Sake for 3 days reduced Fos response in FST but not in sham rats. Inhibitory effect on Fos response by Sake was greater than that by ethanol alone. These data indicated that Sake has inhibitory roles on FST-induced increases in MM nociception and this inhibitory effect could be due to the action of both ethanol and non-ethanol constituents in Sake. COI:No

2P-131

Analysis of local renin-angiotensin system in the geniculate ganglion and the rostral nucleus of the solitary tract in rats.

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<Introduction> Angiotensin II, the end-product in the renin-angiotensin system (RAS), is well known to control blood pressure and fluid/electrolyte balance through activation of angiotensin II type-1 receptor (AT1). Recently, angiotensin II has been known to modulate neuronal excitability, neurite elongation and neuronal migration through activation of angiotensin II type-2 receptor (AT2) in neuronal cells. We hypothesized that if AT2 is expressed in the gustatory neural pathway, angiotensin II may regulate plasticity of the neural circuitry via AT2. In this study, we investigated expression of RAS component genes in the geniculate ganglion (GG) and the rostral nucleus of the solitary tract (rNST) in rats. <Materials & Methods> GG or rNST tissue punches were obtained from rats under anesthesia. Total RNA was extracted from GG or rNST tissue punches and cDNA was synthesized from the RNA template by reverse transcription. Levels of RAS component genes were determined by real-time PCR. <Result & Discussion> Expression of angiotensinogen, renin, angiotensin-converting enzyme, angiotensin II type-1a, -1b and type-2 receptor mRNAs were evident in the GG and the rNST. This result suggests that angiotensin II produced in local RAS may regulate gustatory neural circuitry in the medulla through AT2 receptor activation. COI:No

2P-132

Odontoblasts express plasma membrane Ca^{2+}-ATPase 1 and 4

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Odontoblasts are involved in physiological dentin formation and mineralization as well as sensory transduction following various stimuli to dentin surface. In odontoblasts, the regulation of intracellular Ca^{2+} is crucial for the cellular function. Intracellular Ca^{2+} level is mediated by Ca^{2+} influx, mobilization and extrusion mechanism. Ca^{2+} influx from extracellular medium and/or Ca^{2+} release from intracellular Ca^{2+} store increase intracellular Ca^{2+} concentration. At the same time, the increased intracellular Ca^{2+} is extruded by Ca^{2+}-ATPase (PMCA) and/or Na^+- Ca^{2+} exchanger (NCX) in the plasma membrane. In previous study, we reported the functional expression of NCX1 and 3 and coupling between TRP channels and NCX in odontoblasts. On the other hand, Linde et al have reported the expression of PMCA in odontoblasts, however its detailed expression patterns and pharmacological properties remain unclear. In this study, we investigated the mRNA expression of PMCA1-4 in human dental pulp cells with odontoblastic differentiation (HOB cells), exhibiting positive staining for dentin sialoplotein, dentin matrix protein-1 and nestin by real-time reverse transcription polymerase chain reaction (RT-PCR). HOB cells expressed PMCA1 and 4. The results suggested that PMCA 1 and 4 play a role in maintaining intracellular Ca^{2+} concentration in odontoblasts. COI:No. COI:No

2P-133

Mechanisms of hypometabolism in daily torpor in inbred mice

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Some mammals enter a hypometabolic state either daily torpor (minutes to hours in length) or hibernation (days to weeks), when reducing metabolism would benefit survival. If we can induce such active hypometabolism in human, the significance to clinical scenes will be enormous. In 2016, we developed a method to induce daily torpor stably in mice (Sunagawa GA and Takahashi M, *Sci Rep*, 2016). Applying this methods to various inbred strains, recently, we have found that two inbred strains C57BL/6J (B6J) and C57BL/6N (B6N) have distinct torpor phenotypes. When the ambient temperature (T_A) is 20 ° C, the 89% highest posterior density interval (HPDI) of body temperature (T_B) (° C) were [31.4, 34.2] and [27.3, 30.5], and 89% HPDI of oxygen consumption rate (VO_2) (ml/g/h) were [1.75, 2.57] and [1.01, 1.45], respectively for B6N and B6J. Because the genome of B6N and B6J have no more than 10,000 base pair differences, we hypothesized that the torpor phenotype is regulated by relatively few genes or gene loci. We, therefore, intercrossed B6N with B6J and tested the torpor phenotypes in their siblings (B6NJ-F1: female B6N crossed to male B6J). When the T_A is 20 ° C, the 89% HPDI of T_B (° C) were [27.3, 30.9] and [27.2, 30.8], and the 89% HPDI of VO_2 (ml/g/hr) were [0.89, 1.49] and [0.98, 1.88], respectively for B6NJ-F1 and B6JN-F1. This is clearly showing that in the F1 generation mice, the torpor phenotype of B6J dominates the B6N phenotype. Given these results, we are currently processing CAGE (Cap Analysis of Gene Expression) of hypometabolic tissues in torpid mice to identify the responsible gene locus for the torpor phenotype. COI:No

2P-134

Estrogen replacement enhances insulin-stimulated AS160 activation and improves insulin sensitivity in ovariectomized rats

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Menopause is associated with insulin resistance, but the role of estrogen remains unclear. In the present study, we examined in vivo effects of estrogen on insulin sensitivity and insulin signaling in ovariectomized rats. Female Wistar rats at 9 wk of age were ovariectomized and treated with placebo (Pla) or 17 β-estradiol (E2) pellets (1.5 mg/60-day release, sc) 4 wk after ovariectomy. After 4 wk of replacement therapy, the Pla group showed greater intra-abdominal fat accumulation than the E2 group did. Intravenous glucose tolerance test revealed that insulin sensitivity was significantly lower in the Pla group. In addition, insulin injection (10-5 mol/L in 1 ml/100 g body weight of normal saline) into the portal vein induced phosphorylation of Akt2 in the gastrocnemius which was enhanced in the E2 group compared with the Pla group. Similarly, insulin-stimulated AS 160 phosphorylation in the muscle was increased in the E2 group with elevation in the protein expressions. However, AMPK expression and its phosphorylation in the muscle were not different between the Pla and E2 groups. These results suggest that estrogen replacement restored insulin tolerance by improving insulin-stimulated AS160 activations in the muscle. COI:No

2P-135

Effect of irreversible anhidrosis due to long-term skin application of aluminum chloride for generalized hyperhidrosis on human thermoregulation: association with structural changes in eccrine sweat glands

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Topical aluminum chloride ($AlCl_3$) application can reduce perspiration in localized hyperhidrosis. We studied the effect of whole-body, long-term $AlCl_3$ application on human thermoregulation and sweat gland structure in a 44-year-old man with irreversible anhidrosis, and no other abnormalities, due to long-term $AlCl_3$ therapy for generalized hyperhidrosis. His sweat glands were histologically normal. $AlCl_3$ solution (20%) was applied on the whole body once daily. A thermoregulatory sweat test was conducted at 40° C in an artificial climate chamber. Panhidrosis distribution was observed by Minor's method; tympanic temperature (Tty) was measured continuously to determine core temperature. Eccrine sweat glands were studied via back skin biopsies (Tyler technique and serial section for samples). After 7 years of therapy, heat exposure-induced Tty elevation was high, resembling that in men with acquired idiopathic generalized anhidrosis (AIGA). Tty lowered as sweating revived after stopping $AlCl_3$ application. Although $AlCl_3$ application was stopped after 14 years, sweating has not revived and Tty elevation remained high, similar to findings in AIGA. Anhidrosis became irreversible when coria eccrine sweat ducts disappeared after lumens enlarged and vacuoles appeared in acinar cells. This led to acinar and secretory cell atrophy and sweat gland loss. Sweating can only be revived before sweat ducts disappear. COI:No

2P-136

Establishment of a method for inducing hibernation-like hypothermia by cooling under anesthesia in non-hibernators rats

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Hypothermia can be applicable for treatment for ischemia reperfusion injury. However, it is usually difficult to induce hypothermia in homeothermic animals because body temperature is strictly maintained by thermoregulatory center in the brain. On the other hand, body temperature of mammalian hibernators decreases extremely during hibernation. To establish a method to induce hibernation-like hypothermia in non-hibernators, we attempted to decrease body temperature in rats by cooling following inhalation of isoflurane that is easy to control depth of anesthesia. Male SD rats were anesthetized by isoflurane inhalation and placed in ambient temperature at 4° C for cooling. Isoflurane concentration was controlled depending on body temperature. During inducing hypothermia, rectal temperature and electrocardiogram (ECG) were measured. When the inhalation of isoflurane was stopped at rectal temperature of 25° C, the rats showed shivering and rectal temperature increased. When the inhalation of isoflurane was stopped at rectal temperature of 22.5° C, the rats did not show shivering and rectal temperature continued to decrease, eventually reaching to 15° C. Abnormal ECG did not occur during hypothermia. The obtained results indicate that thermoregulatory center might have low temperature threshold and hibernation-like hypothermia can be induced by reducing body temperature less than low temperature threshold of thermoregulatory center. COI:No

2P-137

Promotion of lipid excretion and improvement of intestinal flora by wx/ae rice which is rich in resistant starch

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Wx/ae rice is a consequence of double mutant of amylose-free waxy (wx) mutant and amylose-extender (ae) genes in Kinmaze rice (wild type :WT). Wx/ae brown rice contains abundant resistant starch and γ-oryzanol which reduces the blood lipid. For purpose of examining improvement of lifestyle diseases by wx/ae rice , after having 8 week old male mice were fed a high-fat diet(HFD) , 1)chow diet(CD), 2)HFD + wx/ae brown rice, 3) HFD+ resistant starch derived from wx / ae rice, 4) HFD+ WT brown rice was switched to feed for 4 weeks. In the groups of mice fed CD, wx/ae brown rice or wx/ae derived resistant starch , blood neutral fat concentration was significantly lower than before change of diet . The amount of neutral fat excretion per 1 g of faeces was significantly increased in the groups of mice fed wx/ae brown rice or wx/ae-derived resistant starch. Switchig to wx/ae brown rice, the amount of feces also increased significantly. As a result of studies on intestinal flora, the profile of Bacteroidetes increased markedly and the % of Firmicutes decreased only in the indigestible starch group derived from wx/ae brown rice and wx/ae. These results suggested that resistant starch derived from wx/ae rice and wx/ae brown rice may improve lifestyle diseases by improvement in the intestinal flora and excretion of lipid into feces. COI:No

2P-138

Inhibitory effect of peripheral warming on efferent sympathetic tone to the rat epididymal white adipose tissue

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We have been clarifying that non-invasive and thermo-local cutaneous (NTLC) stimuli make the rat autonomic nerve to cause almost opposite responses, that is 'sustained decrease' in renal sympathetic nerve activity (RSNA) and 'transient increase' in gastric vagus nerve activity. Here, changing the viewpoint to a mechanism on body temperature regulation, we focused on the influence of NTLC stimuli on sympathetic nerve activity (SNA) to epididymal white adipose tissue (EWAT), to clarify the causality between NTLC stimuli and lipid metabolism. Method was nearly the same as the previous reports, except for using the sympathetic nerve innervating EWAT. The result and interpretation are as follows: peripheral warming on rat hind paws induced the same but weak sustained decrease in a magnitude of SNA comparing with the control as observed in RSNA. Thus, NTLC stimuli might seem to induce a common operation on sympathetic nerves toward visceral organs such as EWAT and kidney. The present 'warming-induced SNA inhibition' might lead to reduce lipolysis and/or to promote lipogenesis in EWAT. Namely, lipid metabolism is said to be regulated by not hormones but SNA. We concluded that NTLC stimuli applied to drive the sequential events of somatosensory activation, central nervous process, SNA and lipid metabolism might be effective as with thermal stimuli to the whole body. (COI: NO.) COI:No

2P-139

Rhythm of Electrogastrograms

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[Objective] The present study represents an attempt to explore the rhythm of digestive functions by electrogastrography in healthy young male adults. [Method] Ten healthy young male adults participated in the study. Twenty-four hour electrogastrograms were recorded using the Portable Data Recorder DL2000, with the sampling time set at 10 msec, via surface electrodes applied to 2 sites on the abdominal wall separated by the stomach underneath. The subjects followed their usual patterns of eating and sleeping during the 24-hour recording period, and their dietary details and meal times were recorded. The sleep and daytime activities of each subject were recorded with the Actigraph. The electrogastrographic waveform data were subjected to frequency analysis using software originally developed by us utilizing the Microsoft Windows Network. [Results] The electrogastrographic spectrograms revealed peaks at 3 cpm (MLF) and 6 cpm (MHF) during sleep confirmed with an Actigraph. The frequency power ratio (MHF/MLF) showed regular, approximately half-hourly, variation. These periodic fluctuations disappeared during meal times. [Conclusion] We thought that these periodic fluctuations could be an indicator of the digestive functions. COI:No

2P-140

Evaluation of Deglutitive Function by 2-Channel Surface Electromyography

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[Objective] In a previous study, we demonstrated prolongation of the peak latency, reduction in the peak amplitude on single-channel EMG in elderly patients. In this report, we present the results of evaluation of swallowing dysfunction by 2-channel EMG. [Method] Ten healthy young adult volunteers (aged 21.3±0.4 years) participated in the study. Surface EMG was recorded using Neuropack μ (Nihon Kohden), with the sampling time set at 1msec, via electrodes applied on to the ventral aspects of the geniohyoid muscle (Ch1) and thyrohyoid muscle (Ch2). Five mL of a liquid sports drink and 5 mL of a jelly-form sports drink were used for the deglutitive loading. The recorded EMG waveforms were smoothed by the moving-average method with 500 data points. The peak latency and peak amplitude of the smoothed waveforms were measured for each channel. [Results] The peak latency (msec) was 370.2±113.4 in Ch1 and 510.1±183.7 in Ch2 during liquid swallowing, with corresponding values of 523.3±259.4 and 617.5±333.3, respectively, during jelly swallowing. Thus, there was a significant difference in the peak latency during liquid swallowing between Ch1 and Ch2 ($p = 0.016$), whereas no such difference was noted on jelly swallowing. [Conclusion] Assessment by 2-channel surface EMG revealed that inter-muscle difference in the time of onset of muscle contraction was less during jelly swallowing. COI:No

2P-141

Regulation of sperm hyperactivation by progesterone in rats

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Capacitation is an essential process for mammalian sperm in order to be fertilized. Capacitated sperm exhibits a specialized flagellar movement that refers to "HYPERACTIVATION". In the present study, we show that rat sperm hyperactivation is regulated by progesterone. Progesterone enhanced rat sperm hyperactivation through a membrane progesterone receptor in a dose dependent manner. In addition, regulation of rat sperm hyperactivation by progesterone was associated with transmembrane adenylate cyclase and cAMP-dependent protein kinase. Although it is known that progesterone stimulates calcium signals, calcium signals were not a main regulation of rat sperm hyperactivation by progesterone. In hamsters and humans, regulations of sperm functions by progesterone are suppressed by estradiol. In rats, regulation of sperm hyperactivation by progesterone was suppressed by estradiol through a membrane estrogen receptor in a dose dependent manner. In conclusion, rat sperm hyperactivation was regulated by progesterone and estradiol through membrane steroid receptors, and its regulation was associated with cAMP signals. COI:No

2P-142

Investigation of gene expressions related to aggression in quail diencephalon

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In National Institute for Environmental Studies (NIES), Japan, a strain of Japanese quail (*Coturnix japonica*) called NIES-L has been established by rotation breeding in a closed colony over 35 years and keeps highly inbred-like characteristics. Another strain called NIES-Brn has been maintained by random breeding in a closed colony and keeps outbred-like characteristics. By comparison of behaviors between two strains, we have already detected that NIES-Brn strain quails showed significantly higher number of aggressive behaviors such as grabbing, mounting and cloacal contact-like actions compared to NIES-L strain quails. In this study, we examined gene expressions in the diencephalon of both strains by DNA microarray in order to find genes related higher aggression in NIES-Brn strain. We detected 418 genes showing over 2 fold increased expression score in NIES-Brn strain. Among 418 NIES-Brn-biased genes, we found that the mRNA level of mesotocin (oxytocin homologue) in the diencephalon was significantly higher in NIES-Brn strain using realtime RT-PCR. In addition, the population of cells having large cellular size and expressing mesotocin was significantly higher in NIES-Brn compared to NIES-L, suggesting that the cells with larger size promote more production of mesotocin mRNA. Elevated mesotocin levels might be associated with higher aggression in NIES-Brn strain. COI:No

2P-143

Membrane potential of oocytes obtained from mice at different age and its effects on embryogenesis

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Morphological inspection is the most commonly used technique to pick quality oocytes and embryos for artifical fertilization. To raise reproductive ratio, a new selection method from new point of view is needed. The membrane potential reflects expression of ion channels and completeness of cell membrane, it may evaluate oocyte quality. We previously showed that there was wide dispersion of membrane potential among eggs without morphological difference, using single and 4-cell stage bovine eggs. It implied this technique couldbe applied for quality selection of oocytes. In this study, we analyzed theeffect of female age on membrane potential of mouse oocytes. Four-week (before sexual maturation), 9-week (after maturation), and 30-week (aged) old female mice were treated with CARD HyperOva and hCG to stimulate hyperovaluation. And we measured membrane potential with voltage-sensitive fluorescent dye after direct recording using single electrode. Most of oocytes which have good morphological characteristics indicated positive membrane potential, but some of them performed potential near zero voltage. Because it is possibleto scratch oocytes during separation from cumulus cell-oocyte complex. Though it effects quality of oocytes, morphological analysis may not be able to detect mechanical damage. The effect of mechanical damage and age-related damage on membrane potential will be discussed. COI:No

2P-144

Temporally genetic expression of KCC2 in GnRH neurons *in vivo* causes impairment of fertility

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Gonadotropin-releasing hormone (GnRH) secreting neurons are the final output of the central nervous system driving fertility. While GABA is generally an inhibitory neurotransmitter in the adult brain, we previously reported that GABA evoked excitatory responses in adult GnRH neurons *in vitro*. However, the precise physiological role of the excitatory action of GABA on GnRH neurons remains elusive. GABA acts excitatory when $[Cl^-]_i$ is high, due to the low expression of the K^+-Cl^- cotransporter (KCC2), which excludes Cl^- from neurons and maintain GABAergic synaptic inhibition. To investigate the functional role of the excitatory action of GABA in GnRH neurons *in vivo*, we generated new transgenic mice (GnRH-tTA::KCC2-tetO) in which KCC2 can be induced restricted in GnRH neurons using tetracycline controlled gene expression system. Using this mice, GABA action to GnRH neurons could be modulated *in vivo* restricted in GnRH neurons reversibly in specific time point. GnRH-tTA::KCC2-tetO mice failed to exhibit estrous cyclicity, ovulation and pregnancy. Ovarian histology revealed an abundance of immature follicles. Furthermore, GnRH-tTA::KCC2-tetO mice showed advanced vaginal opening. GABA inhibited spontaneous firing of GnRH neurons in GnRH-tTA::KCC2 tetO mice. GnRH-tTA::KCC2-tetO male mice showed normal fertility. These results suggest that the excitatory action of GABA on GnRH neurons has an important role in the female reproduction. COI:No

2P-145
Paternal psychological stress causes an impairment of emotional behavior in offspring mice

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Psychological stress influences metabolic and brain functions. Many researches show parental psychological stress affect brain functions in next generation, however, these mechanisms are still unclear. In this study, we investigated whether acute paternal psychological stress just before mating would affect a formation of offspring's higher brain functions including emotional behavior in offspring mice. Male C57/BL6 mice (aged 8-9 weeks) were subjected to restraint stress for 2 hours by using 50-mL conical centrifuge tube. Restraint stress was continued for 2 weeks. Thereafter, each male mouse was mated with virgin female for 2 days. Pregnancy was confirmed in vaginal plug formation. We performed a behavioral analysis to evaluate emotional behaviors in these offspring mice (10 weeks of old), and compared with non-stress offspring mice (control mice). In this research, prenatally-stressed offspring mice showed markedly increased depression-like and anxiety-like behaviors. Furthermore, we found that prenatally-stressed offspring mice had an excess risk avoidance behavior in electrical stimulation. These findings indicate that acute enhancement of paternal psychological stress before mating may influence a formation of offspring's high brain function. COI:No

2P-146
Analysis of learning induced insulin signaling in the hippocampal synapses of diabetic model mice.

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Learning dependent synaptic plasticity in the hippocampus is required for cognitive functions. Synaptic dysfunction is a distinctive neuronal alternation in the early stages of dementia and there is a correlation between the severity of the synaptic dysfunction and the progression of clinical symptoms. Epidemiological studies have shown that Diabetes Mellitus (DM) is a risk factor for dementia, and the progression of cognitive impairment is accelerated by DM. Alteration in neural insulin signaling may be associated with pathogenesis of DM-related cognitive impairment. However, the mechanism of the interaction between neural insulin signaling and synaptic dysfunction is largely unknown. Our recent works show that activated hippocampal cytoplasmic insulin signaling correlates with cognitive impairment in the physiological type 2 diabetes model (DIO: Diet Induced Obesity) mice. Here, we found thatinsulin signaling components are localized not only in cytoplasm but also in the postsynaptic density (PSD), and synaptic insulin signaling is upregulated in the hippocampus of DIO mice. Furthermore, we found thatlearning induced synaptic insulin signaling activation was disrupted in the hippocampus of DIO mice. Thus, these results suggested that excessive activation of synaptic insulin signaling facilitates DM-related cognitive impairment correlated with inhibition of hippocampal synaptic plasticity. COI:No

2P-147
Influence of hypoxia and dehydration on thermoregulatory responses during exposure to low ambient temperatures

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The mechanism of acute mountain sickness (AMS) is thought to be complicatedly intertwined with many factors; thereby, the precise mechanisms are still unclear. In our previous questionnaire survey of Mt. Fuji climbers, the thermal comfort while ascending and staying on top was significantly lower in climbers with AMS than in those without AMS. Moreover, concerning the thermal sensation while staying on top, those with AMS tended to feel colder. In addition, the feeling of thirst while ascending was significantly greater in climbers with AMS than in those without. It was considered that the fluctuation of body temperature and the dehydration state accompanying the decrease in ambient temperature during altitude climbing may be related to the onset of AMS caused by hypoxic exposure. The aim of the present study was to investigate the influence of a cold environment, hypoxic exposure, and dehydration status on thermoregulatory responses in an animal model. Male Wistar rats were exposed to oxygen constantly maintained at about 12% concentration, corresponding to an altitude of around 4,000 m. The ambient temperature conditions were set at 24° C, 15° C, and 10° C. Body temperature, activity, and heart rate variability were measured under free-moving conditions. The dehydrated animals showed further reduction in body temperature by exposure to a hypoxic environment. The hematological properties and metabolic responses were compared under each environmental temperature condition and dehydration status. COI:No

2P-148
Thermoregulatory behavior in ovariectomized mice using a new evaluation system

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Background We previously found that ovariectomy alters autonomic responses to cold and heat in rats and mice. However, it remains unclear if ovariectomy also affect behavioral responses such as cold or heat escape behavior. Aims First, we develop a new system to estimate the escape behaviors for mice, and evaluate the usefulness. Second, we evaluate the influence of ovariectomy on the escape behavior. Methods Twelve ICR mice were used in the present study. At the age of 11 wks, they were divided to two groups. One group were bilaterally ovariectomized under inhalation anesthesia with 2% enflurane, the other group was sham operated. The escape behaviors were assessed around 2-wk and 4-wk after the surgery. The system evaluating the escape behaviors had five Peltier boards at the bottom, which were place in a cross-shape. The temperature of each board could be controlled with a PC program (Labview). In a heat-escape test, 4 boards were set at 43° C and 1 board 32° C. The setting was changed every 5 min, and lasted for 90 min. The central board was always at 43° C. In case of a cold escape behavior, 4 boards were set at 18° C and 1 board 32° C. The location of a mouse was identified with a photodetector. Results The all mice showed the escape behavior in the new behavior system. Moreover, the ovariectmized mice showed smaller time-duration, staying in boards set at 18° C. Conclusion We develop a new system to evaluate heat/cold escape behavior for mice. Ovariectomy may affect cold escape behavior, which may be resulted from peripheral mechanisms such as thermal sensation of the skin. COI:No

2P-149
Seasonality of clock gene expressions in obese and non-obese men

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Obesity is a significant health problem in the world and it has been rapidly increasing over the past two decades. Obese people have an increased risk factors for developing cardiovascular, renal, and hormonal diseases and sleep disorders. These incidences were increased in winter season in cold environmental temperature and during short days. Several clock genes, including Bmal1 and Rev-erb-α (Nr1d1), were reported to be involved in adipocyte differentiation and in lipogenesis. In the present study, we investigated the seasonal differences of clock gene expressions in obese men. We hypothesized that the clock gene expressions in winter season were decreased more in obese men than in non-obese men. Six of non-obese men (BMI 21.5 ± 1.8; mean ± SD) and 5 of obese men (BMI 31.4 ± 6.2) participated in summer and winter seasons. Total RNA was isolated from buccal epithelial cells in saliva samples. We measured the clock gene expression of Clock, Bmal1, Per1, Cry2, Rev-erb-α and Rev-erb-β by real-time PCR. In the results, Bmal1, Per1 and Rev-erb-β were significantly decreased more in obese men than in non-obese men. Bmal1 and Rev-erb-β were also significantly decreased more in winter than in summer in obese and non-obese men. These results suggest that seasonal change of some circadian clock gene expression might be reduced in winter in obese men. COI:No

2P-150
Effects of the transient ischemia caused by sustained high +Gz acceleration on the hippocampal pyramidal cells

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Pilots of high-performance aircraft are exposed to high head-to-foot acceleration (+Gz) during the short turn, which may induce temporary severe brain ischemia in the absence of a proper anti-G protection. We previously reported that sustained +Gz acceleration for 3 min caused myocardial injuries in rats, however effects on the brain tissue have not been clarified. In this study, to investigate the effects on hippocampal pyramidal cells in the rats exposed to sustained +Gz acceleration, we intracerebroventricularly (i.c.v.) infused with propidium iodide (PI), which labels necrotic cells. Male Sprague-Dawley (SD) rats were placed a catheter in the left femoral artery to measure arterial pressure at the brain level (APLB). One group was exposed to an acceleration of 4.0 to 9.0 +Gz for 3 min in a centrifuge, which was manually calibrated to achieve an APLB of 0 mmHg (+Gz group). The other group without acceleration was used as control. 4 days after +Gz acceleration, both groups were infused with PI (0.66 μg/2 μl) by i.c.v. for 30 min and perfused with 4% paraformaldehyde. The fixed brains were sliced at 6 μm and hypothalamic slices were spread onto glass slid and coverslipped. We observed PI fluorescent labeling pyramidal cells in +Gz group, but not in a control group, although no difference was found in Nissl staining between the two groups. These results indicated that the transient ischemia caused by sustained +Gz acceleration may lead the pyramidal cells to necrosis-like cell death. COI:No

2P-151

Effects of estradiol on energy intake under chronic psychosocial stress in ovariectomized rats.

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The occurrence of adverse metabolic consequences due to chronic exposure to psychosocial stressors is more frequent in women than men, implicating a role for ovarian hormones in modulating stress-induced changes in appetite. In fact, estrogen has suppressive effects on appetite and thus body weight. However, it remains unclear whether estrogen modulates the food intake under stressful conditions. We examined the effects of estradiol replacement on food intake and fat palatability under chronic psychosocial stress (CPS) in ovariectomized rats. Female rats aged 10 wk were ovariectomized and assigned either to a placebo (Pla) group or a group treated with 17 β-estradiol (E2) subcutaneously implanted with E2 pellet. At 14 wk of age, rats were given a high-fat diet (HFD) in addition to standard diet. Food intake and body weight of the rats were measured every day for 3 weeks from the day when free-choice HFD started. Half of the rats in each group aged 15 wk were daily exposed to CPS for 2 weeks. Daily energy intake of Pla-CPS group was significantly reduced in the second half of CPS period, compared to Pla-Control group, but not in the E2 group. CPS has no effects on fat palatability in both groups. These results suggest that estrogen protects chronic psychosocial stress-induced decrease in energy intake under CPS condition without mediating fat palatability. COI:No

2P-152

Echinochrome A increase the mass and function of the mitochondria by upregulation of mitochondria biogenesis genes

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Echinochrome A (Ech A) is a substance extracted from sea urchin, and is known to have antioxidant and cardioprotective effects against ischemia reperfusion injury. In this study, we investigated whether mitochondrial biogenesis and oxidative phosphorylation in cardiomyoblast H9C2 cells are due to Ech A. To study the effects of Ech A on mitochondrial biogenesis, we measured mitochondrial mass, level of oxidative phosphorylation, and mitochondrial biogenesis regulatory gene expressions. As a result, it has been shown that Ech A does not cause cytotoxicity. However, it enhanced oxygen consumption rate and mitochondrial ATP level. Likewise, treatment with Ech A increased mitochondrial content in h9c2 cells. Furthermore, treatment with Ech A upregulated biogenesis of regulatory transcription genes, including proliferator-activated receptor gamma co-activator (PGC)-1α, estrogen-related receptor (ERR)-α, peroxisome proliferator-activator receptor (PPAR)-γ, and nuclear respiratory factor (NRF)-1 and such mitochondrial transcription regulatory genes as mitochondrial transcriptional factor A (TFAM), single strand binding protein (SSBP). In conclusion, these data suggest that Ech A is a potentiate marine drug which enhances mitochondrial biogenesis COI:No

2P-153

Effect of Naringin on vascular dysfunction in hypercholesterolemic rats

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Hypercholesterolemia is known to cause endothelial dysfunction and contributes to the development of atherosclerosis. The aim of the present study was to investigate the effect of naringin on oxidative stress and endothelial dysfunction in aortae from hypercholesterolemic rats. Hypercholesterolemic rats were induced by feeding high cholesterol diet for 8 weeks. Naringin (100 mg per kg BW) or simvastatin (40 mg per kg BW) was administered orally for 4 weeks (from 5th to 8th week of diet treatment). At the end of experiment, vascular reactivity and aortic superoxide anion generation were determined by organ bath techniques and dihydroethidium fluorescent dye, respectively. Eight weeks after high cholesterol diet consumption, serum TC, triglyceride and LDL levels were elevated but HDL was reduced when compared to normal diet rats (control). In aortic rings from hypercholesterolemic rats, endothelium-dependent relaxation to acetylcholine was impaired whereas endothelium-independent relaxation to sodium nitroprusside was unaffected. The treatment with naringin or simvastatin lowered the elevated blood TC, triglyceride and LDL levels in hypercholesterolemic rats. In addition, the endothelium-mediated aortic relaxation in rats fed with high cholesterol diet was restored after naringin and simvastatin consumption. Naringin or simvastatin treatment also reduced supoeroxide generation in hypercholesterolemic aortae. These results show that the naringin treatment decreases oxidative stress and improve endothelium-dependent relaxation in aortae from hypercholesterolemic rats. COI:No

2P-154

Coffee aroma controls allergic rhinitis and behavioral preferences in mice

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Various stresses are reported to affect the symptomatic manifestations of allergic diseases, such as atopic dermatitis and allergic rhinitis. To clarify the effects of coffee aroma on allergic rhinitis, we employed ovalbumin- and histamine-induced rhinitis models. In addition, to verify whether coffee aroma acts as a stressor in mice, we conducted a behavioral preference test. Brazilian and Guatemalan coffee powder were used. Female BALB/c mice aged 6 weeks were exposed to coffee aroma in a plastic container for 15 min. Just after the exposure, 2 μL of ovalbumin or histamine solution was administered into the nasal cavities using a micropipette, and the frequency of sneezing and nasal rubbing was quantified for 10 min. Pretreatment with Brazilian coffee aroma dose-dependently decreased the amount of sneezing and nasal rubbing in both rhinitis models. However, the Guatemalan coffee had no effect. The preference test revealed that mice avoided the Brazilian coffee aroma, but not the Guatemalan aroma. These results suggest that Brazilian coffee acts as a stressor in mice and that suppression of rhinitis symptoms is linked to aroma aversion. Stress hormones, such as adrenaline and noradrenaline, may be related to the anti-rhinitis effects of coffee aroma. COI:No

2P-155

Cardiac Ca^{2+} transients effects due to deferiprone and efonidipine treatment in iron-overloaded thalassemic mice

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Iron-mediated Ca^{2+} dysregulation plays a role in pathophysiology of iron-overload cardiomyopathy. However, the mechanism of iron-induced cardiac Ca^{2+} dysregulation and roles of the iron chelator deferiprone (DFP) and the T-type Ca^{2+} channel blocker efonidipine (EFO) on cardiac Ca^{2+} transients in thalassemic (HT) mice are still unknown. We tested the hypothesis that iron overload led to impaired Ca^{2+} transients, resulting in left ventricular (LV) dysfunction, and DFP and EFO attenuated these effects, thus improving LV function in iron-overloaded HT mice. HT mice were fed with iron diet for 90 days, followed by either DFP or EFO treatment for 30 days. Chronic iron feeding led to iron overload condition, indicated by increased plasma non-transferrin bound iron (NTBI), leading to decreased cardiac intracellular Ca^{2+} transients amplitude, rising rate and decay rate, thus contributing to decreased %LV ejection fraction in HT mice. Treatment with DFP and EFO shared similar efficacy in decreasing plasma NTBI, and improving LV function, without attenuating Ca^{2+} dysregulation in iron-overloaded HT mice. Since both drugs improved LV function without altering Ca^{2+} homeostasis, these findings suggest that a new drug that can remove cardiac iron deposits and improve cardiac Ca^{2+} regulation may provide better treatment efficacy under iron overload condition. COI:No

2P-156

Aloe vera: an alternative medication to attenuate non-alcoholic steatohepatitis

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Objective: To examine the effects of Aloe vera in alleviating non-alcoholic steatohepatitis (NASH) and demonstrating the mechanism pathway.Materials and Methods: Male Sprague-Dawley rats were randomly into 3 groups (n=18). Control group, rats were fed with standard diet. NASH group, rats were fed with high fat high fructose (HFHF) diet consisted of 55% fat, 35% carbohydrate (20% is fructose), and 10% albumen. Aloe group, rats were fed with HFHF plus 50 mg/kg of Aloe vera by gavage once daily. After 8 weeks, liver samples were collected for H&E, malondialdehyde (MDA), glutathione (GSH), interleukin-18 (IL18), nuclear factor kappa B (NF-κB), peroxisome proliferators-activated receptor gamma (PPARγ), and apoptosis expression by immunohistochemistry.Results: HFHF diet induced NASH caused the increasing of MDA, IL-18, NF-κB, apoptosis and decreasing hepatic GSH, PPARγ expression when compared with control. Aloe vera treatment showed significantly decreased MDA, IL-18, NF-κB, apoptosis and restoring hepatic GSH, PPARγ expression when compared with NASH group. The liver histopathology of NASH group showed moderate to severe liver steatosis, hepatocellular ballooning and necro-inflammation. In Aloe group, liver histopathology was improved to mild and near normal. Conclusion: Aloe vera improved liver damage of NASH via mechanism of alleviating oxidative stress, inflammation, and restoring GSH and PPARγ expression. Aloe vera may be an alternative medication to attenuate non-alcoholic steatohepatitis. COI:No

2P-157

Ghrelin administration reduces the oxidative stress and end-organ damage during cardiopulmonary bypass in a rat model

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Cardiopulmonary bypass (CPB) induced inflammation significantly contributes to the development of postoperative complications, including respiratory failure, myocardial, renal and neurological dysfunction. We investigated the protective effects of ghrelin against the CPB-induced inflammatory reactions, oxidative stress, and acute organ damage. Adult male SD rats were subjected to CPB for 4h and received saline (n=6) or a bolus of ghrelin (150 μg/kg, sc). Blood samples were taken before starting, after 2h and 4h of CPB. To examine organ failure after post-operative recovery, in additional rats, we collected blood samples & organs at 48h post recovery from CPB, with saline or ghrelin treatment at the end of CPB. We measured the plasma cytokine levels of (TNF-α, IL-6), catecholamines (norepinephrine, epinephrine, and dopamine) and organ damage markers (LDH, AST, ALT). Nitrosative stress markers (3-NT) were measured by western blot. Ghrelin treatment significantly reduced organ damage markers and protein levels of 3-NT, particularly in the lung and liver, and dopamine levels. However, ghrelin treatment only partly attenuated inflammatory cell invasion, the increase in cytokines, epinephrine and to a lesser extent norepinephrine when compared to the CPB saline. These results suggest that ghrelin partially inhibits the inflammation and oxidative stress in the short term after CPB. However, it remains to be established if these benefits can be extended after recovery from CPB. COI:No

2P-158

Upregulation of hypothalamic arginine vasopressin after bilateral nephrectomy in transgenic rats expressing arginine vasopressin-enhanced green fluorescent protein

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The paraventricular nucleus (PVN) of the hypothalamus is known to be an integrative site of the neuroendocrine system and autonomic nervous system. Our previous study demonstrated that upregulation of the corticotropin-releasing hormone (CRH) gene in the parvocellular division of the PVN (pPVN) after bilateral nephrectomy as a model for acute kidney injury (AKI) in rats. CRH-expressing neurons in the pPVN also produce arginine vasopressin (AVP) under stressful condition. Hypothalamic AVP plays an important role in stress-induced activation of the hypothalamic-pituitary adrenal axis. We previously generated transgenic rats that express the AVP-enhanced green fluorescent protein (eGFP) fusion gene, which is a quantitative indicator of AVP.

In this study, we studied the effects of bilateral nephrectomy on hypothalamic AVP by using the AVP- eGFP transgenic rats. The eGFP fluorescent intensities in the pPVN were significantly increased 12 and 20 hours after bilateral nephrectomy. Immunohistochemistry for Fos revealed that several brain areas were activated after bilateral nephrectomy. We were able to visualize and quantitatively evaluate AVP-eGFP synthesis and neuronal activations after bilateral nephrectomy. Neural and humoral factors to activate central nervous system after bilateral nephrectomy should be determined by further study. COI:No

2P-159

Artificial blood substitutes control the pathophysiology of hemorrahgic shock with coagulopathy

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Background: We developed Hemoglobin vesicle (HbV) and fibrinogen γ-chain (HHLGGAKQAGDV, H12)-coated, ADP-encapsulated liposomes [H12-(ADP)-LIPO], which are artificial substitute for Red blood cells (RBCs) and platelet.Objective: To evaluate the efficacy of HbV and H12-(ADP)-LIPO for alternative treatment instead of massive transfusion in rabbits with hemorrhagic shock and trauma-induced coagulopathy.Methods: Thrombocytopenia was induced in rabbits by repeated blood withdrawal and isovolemic transfusion of autologous RBCs. Subsequent penetrating liver injury caused uncontrolled hemorrhage, resulting in exacerbation of thrombocytopenia and hemorrhagic shock. H12-(ADP)-LIPO with platelet-poor plasma (PPP) (n=20) or platelet-rich plasma (PRP, n=16) was administered to the thrombocytopenic rabbits during liver hemorrhage. After achieving hemostasis, the subject animals receiving H12-(ADP)-LIPO/PPP were administered HbV (n=10) or RBCs transfusion (n=10). Also, the subjects receiving PRP were administered RBC (n=10) or 5% albumin (n=6).Results: In anemic and thrombocytopenic rabbits (Hb < 6.0 g/dl, platelet count < 40,000 / μ L), administration with HbV andH12-(ADP)-LIPO as well as transfusion with RBCs concentrate and PRP rescued 60-70 % animals from liver hemorrhage because of potent hemostasis in the liver bleeding site and improvement of severe anemia, although rabbits receiving 5% albumin showed no survival in the first 24 hours.Conclusions: HbV andH12-(ADP)-LIPO may be effective instead of standard transfusion with RBCs, platelets and plasma for acute hemorrhagic shock and trauma-induced coagulopathy. COI:No

2P-160

Involvement of endothelin in tongue cancer pain in rats.

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In many clinical cases, oral cancer patients do not complain of obvious pain at early stage. Due to the lack of pain at early stage, detection of oral cancer is sometimes delayed. It is necessary to detect oral cancer at early stage in order to treat cancer patients appropriately. To evaluate the mechanisms underlying tongue cancer pain, we examined the involvement of endothelin in tongue pain associated with tongue cancer in rats.Squamous cell carcinoma cells (SCCcs) were inoculated into the tongue. After SCC cells inoculation, tumor growth progressed over time. Head-withdrawal reflex threshold (HWRT) to mechanical but not heat stimulation of the tongue was significantly decreased on day 11 after SCCcs inoculation. HWRT to mechanical and heat stimulation of the tongue was not changed in control group. After SCCcs inoculation, the amount of β-endorphin and erndothelin-1 in tongue tissue is significantly increased in SCCcs-inoculated rats compared with PBS-inoculated rats on day 6. We also observed endothelin A (ET-A) receptor expression in SCCcs in vitro. The decrement of HWRT is significantly recovered in SCCcs-inoculated rats following ET-A receptor antagonist or μ-opioid receptor antagonist into the tongue.These results indicate that β-endorphin released from SCC cells by endothelin-1 signaling may be involved in depress of tongue cancer pain at early stage. COI:No

2P-161

The effect of dopamine on IPSCs of layer V pyramidal cells in the ACC of ADHD model rats

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The prefrontal cortex (PFC) is a putative brain region responsible for attention-deficit/hyperactivity disorder (ADHD). It has been hypothesized that, dopamine D1 receptor (D1R)-mediated neurotransmission may be altered at the PFC including anterior cingulate cortex (ACC) in pathophysiological state of ADHD, since D1R gene DRD1 haplotype is associated with the inattentive symptoms of ADHD. Spontaneously hypertensive rat (SHR) has been widely studied as an animal model of ADHD. In order to elucidate dopamine (DA)-mediated modulatory actions on GABAergic transmissions, we recorded inhibitory postsynaptic currents (IPSCs) from the layer V pyramidal cells in ACC slices obtained from SHR and their control rats Wistar-Kyoto (WKY). We found that the bath application of DA increased both of miniature and spontaneous IPSCs frequency in WKY but not those in SHR. Evoked IPSCs (eIPSCs) amplitude was more enhanced by DA in WKY than in SHR. Amplitude of unitary IPSCs from fast-spiking interneurons were potentiated by DA in WKY but not in SHR. Pharmacological experiments revealed that a D1R agonist increased the amplitude of eIPSCs in both WKY and SHR and the increase was larger in WKY than in SHR. These results suggest that hypofunction of the D1R-mediated inhibitory synaptic transmission onto layer V pyramidal cells in the ACC may contribute to the pathophysiology in ADHD. COI:No

2P-162

Characterization of rats with neonatal dopamine depletion in development of body weight and motor control

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The dopaminergic neuronal system plays a crucial role in voluntary movement including anxiety-related and feeding behaviors. Rats with neonatal dopamine (DA) depletion exhibit motor hyperactivity during the juvenile period. Recently we have shown that the rats with neonatal DA depletion exhibit anxiolytics behavior during adulthood as well as motor hyperactivity. In the present study, to elucidate detail of characterization of rats with neonatal DA depletion, we measured weekly food intake and body weight, and then performed open field (OF) tests under two different stress conditions depending of the intensities of room illumination, namely low- (mild stress) and high- (strong stress) light condition. The adult rats with a neonatal treatment of 6-hydroxydopamine (6-OHDA: catecholamine neurotoxin) postnatal 4 days showed significant decreases in the food intake and body weight during the developmental period compared with vehicle-treated rats. In high-light condition of the OF test, although vehicle-treated rats showed significant decrease in distance traveled, and increases in moving speed and anxiety-related behavior compared with those in low-light condition, 6-OHDA-treated rats showed no changes in these behavioral indexes. We also examined effects of continuous infusion of atomoxetine (noradrenaline reuptake inhibitor) on the 6-OHDA-induced alteration. The treatment of atomoxetine ameliorated only the motor hyperactivity. These data suggest that neonatal DA depletion causes various disorders which different neural systems participate in. (COI: No) COI:No

2P-163

Efficacy of Acupuncture for Chemotherapy-induced Peripheral Neuropathy in Rat

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Paclitaxel (PTX) is a mitotic inhibitor used in cancer chemotherapy, however, it causes chemotherapy-induced peripheral neuropathy (CIPN). It is known that the pathogenic basis of CIPN involves peripheral microvascular damage. However, there is limited evidence of the effect of these alternative therapies on CIPN. Therefore, we tested the influence of electro-ACU with PTX-CIPN model rats.The SD rats were divided into 3 groups: PTX group, ACU of PTX-treatment (ACU-PTX), and control group. All rats were injected intraperitoneally on 4 alternate days with vehicle or 2.0 mg/kg PTX. Electro-ACU, 1Hz, 20 min., 3-5V, was applied to ZuSanli acupoint (ST36) in the right side limbs every other day. Behavioral assays were carried out by mechanical allodynia von Frey test in the feet. The lumbosacral spinal cord was collected for microscopy examination. In addition, dorsal root ganglions (DRG) were collected for the purpose of determining the factor in the neuro-vascular wiring.In the PTX group, occurrence of mechanical allodynia and appearance of astrocytes were recognized, but ACU-PTX group did not show these changes. Additionally, the DRG of PTX group showed decreased mRNA expression of vascular endothelial growth factor and neuronal NOS in comparison with the control and ACU-PTX group.Our study indicates that applying ACU relieves PTX-CIPN by suppressing the astrocytes in the dorsal horn of spinal cord. These results suggest that acupuncture stimulation prevents CIPN through the neuroprotection on improvement peripheral microvascular. COI:No

2P-164

Effect of Juzentaihoto on a Mouse Model of Chronic Fatigue Syndrome

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Chronic fatigue syndrome (CFS) is characterized by the sensation of abnormally prolonged fatigue, pain, impaired thinking, depression, and sleep disturbance. In Oriental medicine (Kampo), juzentaihoto (JTT) has been traditionally used against anemia, anorexia, extreme exhaustion, fatigue, and general weakness, particularly after illness. Polyriboinosinic:polyribocytidylic acid (poly I:C) is frequently applied in CFS animal experiments to mimic a condition of viral infection. Therefore, the purpose of this study was to examine the effect of JTT on a mouse model of CFS induced by intraperitoneal injection of poly I:C. 5 weeks ages male ICR mice were randomly divided into four groups: control, poly I:C, JTT, and poly I:C+JTT. Mice were administered JTT (1%) in their food (chow). All mice were injected intraperitoneally on 2 alternate days (days 1 and 3) with vehicle (saline) or 20 mg/kg Poly I:C. Subsequently, the locomotor activity was measured once a day over 5 days. Furthermore, the expression of interleukin (IL)-1β and IL-1ra mRNAs in the hypothalamus was measured using real-time polymerase chain reaction. The results indicated a significantly decreased locomotor activity and increased mRNA expression of IL-1β in the hypothalamus of the Poly I:C group compared with controls. No significant differences were observed in the JTT and JTT+poly I:C groups. These results indicated that JTT reduced the poly I:C-induced fatigue, plausibly through the anti-inflammatory effect of JTT. COI:No

2P-165

Compensative sprouting of the cortico-brainstem projections induced by an intensive rehabilitation in internal capsule hemorrhage rats

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Reorganization of the descending tracts is often shown after stroke, which could be induced by rehabilitation. We previously reported that forced impaired limb use (FLU) promoted the sprouting of the cortico-rubral tract (CRT) and it was closely tied with functional recovery in internal capsule hemorrhage (ICH) rats. In this study, we challenged to investigate the sprouting of the CRT and the cortico-reticular tract (CReT) after ICH in detail and its contribution for recovery. Wistar rats were injected with collagenase into the internal capsule. Biotin dextran amine was injected into the ipsi-lesional motor cortex at post-ICH day 1. Then, the rats were forced to use their impaired forelimb for days 1-8. We found abundant sprouting fibers around ipsilateral red nucleus and bilateral reticular formation in ICH-FLU group on day 12 and 28. To block the CRT or the CReT selectively, we next injected lentivirus vectors into the red nucleus (NeuRet-TRE-EGFP.eTeNT) and reticular formation (NeuRet-MSCV-Cre) and then injected AAV vectors (AAVdj-CaMKII-rtTAV16/AAVdj-Flex-DIO-hM4D-mcherry) into the motor cortex. We revealed that the recovered forelimb function was impaired in ICH-FLU group in case of the CRT blockade during post-ICH days 13-20. The data suggest that the CRT is an important pathway that contributes to rehab-induced recovery after ICH. COI:No

2P-166

Lipopolysaccharide administration possibly causes partial hypoxia in the rat brain regulating the stress response.

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By continuous or repeated exposure to stressors, many lines of the study demonstrate neurons in the hippocampus are damaged to apoptotic degeneration. However, initial patho-physiological events which causes neuronal damage of the hippocampal neurons are poorly understood. Therefore, we focused on oxygenation around the neurons under the stressful condition in the brain. In this study, we used pimonidazole, a probe developed for detecting hypoxia in the tissue, and qualitatively analyzed if there are hypoxic area or cells in the rat brain. Male Wistar-Kyoto rats (180-220 g) received administration of lipopolysaccharide (LPS; 1 mg/kg, i.p). LPS administration significantly increased the plasma corticosterone level. Besides, immunohistochemical analysis revealed that there were the pimonidazole-positive regions and cells in the amygdala, hippocampus and paraventricular nucleus of the brain after the LPS injection. The pimonidazole-positive cells in the hippocampus may be more vulnerable than other cells to coticosterone exposure. The LPS-induced hypoxic events may lead to abnormal cellular activities regulating the function of the hypthalamus-pituitary -adrenal (HPA) axis in the rat brain. Further investigations need to reveal the relation between the LPS-induced hypoxia and neuronal damage in the controlling system of the HPA axis. COI:No

2P-167

Emerin deficient mouse and anti-senescence

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Emerin is an inner nuclear membrane protein and its deficiency cause X-linked Emery-Dreifuss muscular dystrophy (EDMD). EDMD is clinically characterized as progressive muscular dystrophy, early joint contractures, and cardiomyopathy with conduction defects. To elucidate the roles of emerin, we produced a mouse model of emerin deficiency (Emd). Unlike human patients, Emd mice show only minimal muscle involvement and mild conduction delay in later stage. Surprisingly, Emd mice had strong reproductive ability even after 40 weeks of age. We performed microarray analysis using atrium from Emd and wild-type mice at 7 weeks of age. Interestingly, enhanced mitochondrial function and increased stress responsive genes were observed in Emd mice. These results are quite similar to those reported in the gene expression patterns of muscle satellite cells form younger mice. From these results, emerin deficiency might be associated with anti-senescence. COI:No

Poster Presentations

Day 3

March 30 (Fri), 12:30 – 14:00

3P-001－3P-008	CNS Function (2)
3P-009－3P-018	Behavior Science･Biorhythm (3)
3P-019－3P-035	Neuron･Synapse (3)
3P-036－3P-047	Sensory Function (3)
3P-048－3P-058	Autonomic Nervous Systems (2)
3P-059－3P-076	Ionic Channel・Receptor (3)
3P-077－3P-086	Cell Physiology・Molecular Physiology (3)
3P-087－3P-092	Education
3P-093－3P-109	Heart・Circulation (3)
3P-110－3P-113	Kidney・Urination
3P-114－3P-118	Physical Fitness・Sports Medicine (2)
3P-119－3P-126	Muscle Physiology (2)
3P-127－3P-131	Motor Function (2)
3P-132－3P-139	Nutrition・Metabolism・Thermoregulation (3)
3P-140－3P-147	Endocrinology
3P-148－3P-155	Environmental Physiology (3)
3P-156－3P-165	Pathophysiology (3)
3P-166	Others (2)

3P-001

Motoric electrocorticography signal in primate primary somatosensory cortex during voluntary movement.

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During execution of body movement, the sensorimotor system simultaneously predicts subsequent sensory feedback that is compared with the actual feedback. When the actual sensory feedback is comparable to that prediction, the sensory feedback is subjectively perceived weaker than the physical strength (sensory attenuation).
To investigate the neuronal correlate of the sensorimotor comparison process underlying sensory attenuation during voluntary movement, we recorded single cell activity from primate primary somatosensory cortex (SI) during a tactile self-stimulation task. In this task, the monkey moved a lever manipulandum back and forth with the right hand. At the same time, a brush moved on the left palmar and digit skin synchronously with the lever movement, delivering tactile stimulation. Compared with passive brush stimulation, the tactile response of right SI neurons (contralateral to the brush) was diminished when brush stimulation was delivered by the monkey's own action. We also recorded electrocorticography (ECoG) from monkey's left primary motor cortex (MI) and SI (contralateral to the lever). We found ECoG activities related to the movement of the right hand in both MI and SI. These results indicate that, during tactile self-stimulation with a brush controlled by the monkey's own action, a motoric signal from the MI propagates to the ipsilateral SI, and this might modulate the tactile response of contralateral SI neurons through callosal SI-SI connection. COI:No

3P-002

Relationship between the Functional Connectivity during resting-state fMRI and Cognitive Assessment Battery in elderly subjects

Yoshikawa Akira[1], Masaoka Yuri[1], Yoshida Masaki[2], Koiwa Nobuyoshi[3], Kubota Satomi[1], Manabe Ryo[1], Ida Masahiro[4], Izumizaki Masahiko[1]

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Our aim of this research is to investigate the relationship between functional connectivity during resting-state fMRI (rs-fMRI) and cognitive function measured with Mini-Mental State Examination (MMSE).
Twenty-five healthy elderly subjects (74.4y, male: 13, female: 12) with no history of brain diseases participated in this study, and an informed consent were obtained for all subjects. Subjects were divided into two groups: subjects with normal MMSE scores (H-MMSE) and subjects with low MMSE scores (L-MMSE). Four minutes and thirty seconds rs-fMRI was recorded by a clinical 3T scanner (MAGNETOM Trio A Tim System, Siemens). Functional connectivities between 116 nodes defined by Automated Anatomical Labeling (AAL) during rs-MRI time-series were tested. Temporal correlations between nodes were measured to create connectivity matrix, and we performed the network based statistics to exploit significant connections comprising effect of interest.
H-MMSE group had higher connection in left parahippocampal-both side of amygdala.
These areas of connection may important role for cognitive function, and could be reflected as sign of cognitive impairment. COI:No

3P-003

Gamma oscillation in ventral hippocampus and basolateral amygdala coincided with hippocampal high frequency oscillation controls fear memory consolidation

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The direct pathway from ventral hippocampus (vHPC) to basolateral amygdala (BLA) is involved in forming contextual fear memory. It is known that a process of spatial memory consolidation requires sharp wave-ripple complex (SWR) that occurs in the CA1 region of the hippocampus during slow-wave sleep. However, for fear memory consolidation, the relationship between SWR in vHPC and BLA neuronal activities is still elusive. To understand this issue, we investigated the relationship between freezing behavior of rats, hippocampal high frequency oscillation (HFO; 140-250 Hz) including SWR, and BLA activities during a resting period after contextual fear conditioning. HFO in vHPC was observed during a resting period at the home cage after foot shock. The occurrence of HFO was modulated by the phase of gamma oscillation (30-80 Hz) generated in hippocampus. The peak of cross-correlation (CC) between the gamma oscillations of vHPC and BLA was observed around the vHPC HFO. Modulation index (MI) that represents the depth of modulation between HFO occurrence and the phase of gamma oscillation in vHPC, showed significant correlation with the peak value of CC between vHPC and BLA. The peak value of CC was also correlated with the power of BLA gamma oscillation. The power of BLA gamma oscillation showed significant correlation with a freezing period 24 hours after conditioning. These results suggests that gamma oscillation in vHPC and BLA coincided with vHPC HFO controls fear memory consolidation. COI:No

3P-004

Neuronal activity involved in temporal classification in the monkey medial premotor areas

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To elucidate neuronal mechanisms of interval timing, neuronal activity was recorded from the medial premotor areas (MPA) while two monkeys performed a duration classification task. In the task, the subjects were presented a visual cue on the center of the monitor for variable duration from 0.8 to 4.8 sec., they were required to press the proper key according to the classification result of cue durations. The right key was for long (3.2 to 4.8 sec), and the center and left keys were for middle (1.6 to 2.4 sec) and short (0.8 to 1.2 sec) cues, respectively. For the spatial control of key selection and movements, the subjects also performed a spatially cued delayed response task. In this control task, a visual cue was presented on the right, center, or left of the monitor for the variable duration. The subjects pressed the proper key according to the cue position following a 1 sec delay period. In the temporal task MPA neurons showed phasic activity with constant peak times after the cue onset. Peak times of this cue activity were distributed with a peak of 1.1 sec after the cue onset. During the delay period MPA neurons exhibited phasic activity selective to one, or two duration categories of the cue presented just before. MPA neurons also showed gradually increasing activity during the latter part of the cue period. These results suggest that MPA could be involved in timing and estimating visual signal durations and representing judgement results. COI:No

3P-005

Amygdala reduction could be an early indication for cognitive impairment in elderly subjects.

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Aim of this study is to investigate a relationship between cognitive function and structural volume changes in hippocampus(HI)-amygdala(AMG) in elderly subjects. Elderly subjects with self-reported non-complain about their memory and mild cognitive deficits (age from 60 to 83 years old) were participated in this study, and informed consents were obtained from all subjects. Subjects were tested Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MOCA-J), and olfactory test, and underwent magnetic resonance imaging (MRI) to measure whole anatomical brain. HI and AMG were manually traced via the software ANALYZE (Mayo Clinic). There was a positive correlation between bilateral AMG and MMSE scores ($r=0.8<$), and between right AMG and olfactory scores($r=0.8$), indicating that subjects with low cognitive and olfactory function showed smaller AMG. These subjects were likely to be categorized as Mild Cognitive Impairment (MCI). MCI has been reported progressively developing to dementia. Measurement of voxel-based morphometry of AMG might be used for an index of early diagnosis of MCI. COI:No

3P-006

C-Fos expressions in the cerebral cortex during rubber tail task in mice

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By an analogy from a rubber hand illusion in human, we found that mice responded as if their own tails were being touched when the rubber tails were grasped after synchronous stroking to rubber tails and their tails (Rubber tail task) (Wada et al., 2016). In this study, we investigated the c-Fos expression during the task to elucidate related neural circuits following our qualitative trial.We perfused mice just after delivering to the synchronous or asynchronous stroking to both the rubber tails and actual tails more than 20 minutes ($n = 12$). After perfusions, each section of each level (approximately 2, 1, -1, -2 and -3 mm from Bregma) was immunostained with anti c-Fos antibody, and immune-positive cell densities at each 100 μ m square were calculated in the whole sections. Then, we compared c-Fos positive cell densities among the groups. We found that c-Fos positive cell densities were significantly high in the posterior parietal cortex, primary somatosensory cortex and insular cortex in the mice who experienced the synchronous stroking, compared with the controls ($p < 0.01$). The regions were generally comparable to human imaging studies of the rubber hand illusion. And we thus speculate that the prominent activation in the posterior parietal cortex and insular cortex might reflect visuotactile integrations and illusory ownership of the rubber tails during the synchronous stroking. COI:No

3P-007

Neural basis of extraordinary higher temporal resolution in a case of autism spectrum disorders.

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We have reported that a person (19 y.o. male) with autism spectrum disorders (ASD) had extraordinary higher temporal resolution of vibrotactile stimuli in temporal order judgment (TOJ) task; he could judge the order even if the temporal lag of two vibrations was 7.6 ms (mean = 64.6 ms in controls). Thus, we elucidate underlying neural basis of his idiosyncratic tactile temporal processing by using fMRI. We used a non-magnetic 8-pin braille stimulator. Two successive stimulation were delivered to the ventral surface of each index finger. We asked to judge which stimulus was delivered latter by pushing corresponding button. Numerosity judgement (NJ) task, in which the participant was required to answer which stimulator delivered more number of pins was also performed. Correct responses in TOJ task by the patient in MRI scanner were 100 % for short (1-15 ms) and 75 % for long (25-100 ms) stimulus onset asynchrony (SOA) conditions (8 controls: 68 % and 51 %, respectively). We compared the brain activity between the patient and controls in TOJ task by the Crawford's modified T-test, after subtracting the brain activity in NJ from TOJ. We found strong activation in the right medial frontal cortex (MFC) and the left posterior medial frontal cortex (pMFC) in the patient. These areas are known to contribute to the monitor and adjustment in task performances. The results may mean that the MFC activation may underlying of extremely high temporal resolution in the ASD patient. COI:No

3P-008

Factors influencing motor learning and behavioral flexibility: the endocannabinoid system and theobromine

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We have studied motor learning and behavioral flexibility, using 3-lever operant task. We found that the performance of this task was impaired in the mice lacking the CB1 cannabinoid receptor (CB1-KO mice) or the endocannabinoid 2-AG synthesizing enzyme DGLα (DGLα-KO mice), and improved in the mice fed theobromine (TB-mice), which is the primary methylxanthine produced by Theobroma cacao and reported to augment cAMP/CREB/BDNF pathways. These results indicate that inhibition of the endocannabinoid system and oral administration of theobromine have opposite effects on motor learning. In this study, we further analyzed lever-press patterns. We used the operant box containing three levers. The mice were trained to press any active lever, as shaping, for a food reward (1-lever task). The number of active levers was initially set to three, then decreased to two, and finally to one. In the next step, they were trained to press three levers in a given sequence (3-lever task), and then in a reversed order (reversal). On the whole, the lever-press patterns were similar between CB1-KO and DGLα-KO mice and contrary to those of TB mice. For example, in 1-lever task the ratio of lose-stay (pressing the same lever after pressing an inactive lever) was higher in CB1-KO and DGLα-KO mice and lower in TB-mice than control mice. Our data suggest that the endocannabinoid system is involved in motor learning and its flexibility, which are also influenced by orally administered theobromine. COI:No

3P-009

Sharp Wave Ripples Modulate Memory Consolidation in Sleep via Activation of the cAMP Signaling Pathway

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Hippocampal EEG patterns known as sharp wave-ripples (SPWr) are observed during quiet wakefulness and slow wave sleep. SPWr are essential for hippocampus-dependent memory consolidation - during post learning sleep there is an increase in the number of SPWr, while interrupting SPWr leads to memory impairments. However, the mechanisms underlying SPWr in memory have not been well explored. We investigated whether SPWr in sleep affect memory consolidation through up-regulating cAMP dependent protein kinase A (PKA) and exchange protein (Epac) in the hippocampus. In the first set of experiments, during sleep following contextual fear conditioning (CFC), rats received stimulation to the ventral hippocampal commissure upon (1) detecting SPWr (to suppress them), (2) 250 ms later (stimulation control), or (3) no stimulation (controls). 4h later, rats were sacrificed and their hippocampi dissected into dorsal and medial CA1, CA3 and DG regions for Western blot analysis of PKA and Epac. SPWr suppression produced a significant decrease in (1) PKA in dorsal CA1 and CA3, and (2) Epac in dorsal CA3 and DG as well as medial CA1. To further validate the hypothesis that SPWr affect memory via PKA/Epac activation, in a second set of experiments, CFC rats were infused into the hippocampus with the cAMP analog Sp-cAMPs under SPWr suppression or stimulation control conditions and we tested fear memory 24h later. Rescue of the memory by the cAMP analog in SPWr surpassed rats would be direct evidence for an interaction between SPWr and cAMP for memory consolidation in sleep. (Supported by 26285161 to CP.) COI:No

3P-010

Role of the circadian transcriptional repressor, *REV-ERBα*, in the regulation of food intake under stress

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Daily rhythms in behavioral and physiological processes, including the sleep/wake and fasting/feeding cycles, are programmed by the circadian clock system. We have recently reported that mice without *Rev-erb*α, a circadian clock gene, show altered mood-related behavior and are sensitive to stress. Therefore, we have tested the hypothesis that the daily feeding rhythm might be altered in *Rev-erb*αdeficient mice when exposed to stress. To examine the effects of stress on feeding behavior, we exposed both wild-type and *Rev-erb*αdeficient mice to 2 days of stress (tilting the animal's cage at 30 degrees) and examined feeding behavior as well as expression levels of appetite-control neuropeptides in the hypothalamus. Here we show that, when exposed to stress, 24 hr food intake is deceased in both wild-type and *Rev-erb*αdeficient mice although the feeding rhythm is not altered. Interestingly, however, the degree of stress-induced reduction in food intake is more striking in *Rev-erb*αdeficient mice than wild-type animals. Importantly, expression levels of genes encoding AgRP and NPY, powerful orexigenic neuropeptides, are elevated in *Rev-erb*αdeficient mice even under basal condition and are no longer altered by stress while stress significantly increases levels of these orexigenic neuropeptides in wild-type animals. These findings suggest that loss of *Rev-erb*αcauses dysregulation of the AgRP/NPY pathway which is associated with the regulation of both feeding behavior and mood. COI:No

3P-011

Impaired dopamine-prolactin signaling in pregnant mothers causes neglect-like behavior in the offspring

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Maternal child neglect is an increasingly prevalent public health issue, however, our knowledge of the biological underpinnings of these behaviors is incomplete. Here we show that the factors determining a mother's motivation to take part in maternal behaviors are present during her own fetal development. We found that Cin85-deficient mother mice had reduced prolactin secretion from the anterior pituitary glands due to excessive dopamine signaling in the hypothalamus. Their offspring matured normally and produced their own pups; however, as adults, maternal behaviors such as pup retrieval and breastfeeding were strongly inhibited. Interestingly, when wild-type fertilized eggs were transplanted into the fallopian tubes of Cin85-deficient mothers, they demonstrated inhibited maternal behaviors as adults. By contrast, when Cin85-deficient fertilized eggs were transplanted into the fallopian tubes of wild-type mice, the pups exhibited normal maternal behaviors as adults. Moreover, when prolactin was administered to Cin85-deficient mice during late pregnancy, a higher proportion of the resultant pups exhibited maternal behaviors. On the other hand, placental lactogen, another lactogenic hormone, did not contribute to neglect behavior of Cin85-deficient mice. These results suggest that maternal prolactin secreted perinatally is extremely important in determining the expression of maternal behaviors in the next generation. COI:No

3P-012

Maternity effect of Lateral Septal Oxytocin Receptors

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Studies with mice deficient in oxytocin (Oxt) or oxytocin receptor (Oxtr) genes have suggested that Oxt / Oxtr system has essential functions of maternal behavior. Both postpartum and virgin females lacking the Oxtr gene (Oxtr -/-) showed impaired maternal behavior. OXTR-Venus knockin (Oxtr Venus/+) mice were generated in order to identify OXTR-expressing neurons. They showed several nuclei with high densities of OXTR expressing neurons, including the lateral septal nucleus (LS) and medial preoptic area (MPOA), which have been implicated in the regulation of maternal behavior. To determine whether OXTR in the LS played a critical role in regulating maternal behavior, we restored Oxtr expression in the LS of Oxtr -/- female mice using adeno-associated virus (AAV)-Oxtr and observed partial recovery of maternal behavior after parturition. Recently we established Oxtr -Cre knock-in mice expressing Cre recombinase under the control of endogenous Oxtr gene promoter. Eliminating OXTR expressing neuron in the LS of Oxtr -Cre mice using AAV-FLEX-dtA showed impaired maternal behavior.The number of OXTR neurons expressing Fos was increased in the LS of Oxtr Venus/+ mice during parturition and when they engaged in maternal behavior compared to Oxtr Venus/Venus (Oxtr -/-) . These data suggest that OXTR signaling in the LS is important to regulate maternal behavior. COI:No

3P-013

Interruption of brain masculinization by perinatal hypothyroxinemia in the male rat

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Although it has been demonstrated that thyroid hormone plays an important role in normal development of brain, little is known how thyroid hormone influences on sexual differentiation of the mammalian brain. We examined effects of Thiamazole (MMI), a thyroid hormone synthesis inhibitor, during the critical period of brain sexual differentiation on adulthood sexual behavior in male rats. MMI was treated to ED18-PD6 male rat pups via drinking water of their dams at doses of 0 (CONT), 0.002% (LOW) and 0.02% (HIGH). In Experiment I, they were orchidectomied and simultaneously implanted with a Silastic capsule containing testosterone at 4 weeks old. After maturation, they were weekly subjected to preference tests for conspecific odors (male vs. receptive female) and copulation tests, indicating that HIGH males showed mild impairment of male-type preference and significantly decreased number of intromissions in copulation test. In Experiment II, they were orchidectomied at 4 weeks old, and implanted with a Silastic capsule containing estradiol at 8 weeks old followed by weekly olfactory preference tests and female sexual behavior tests with stud males. In the olfactory preference test, CONT but not LOW and HIGH males preferred male odor to receptive female odor. In sexual behavior tests, HIGH males showed significantly high lordosis quotients than CONT males. These results indicate a possible involvement of thyroid hormone in sexual differentiation of mammalian brain. Thyroid hormone may promote masculinization and/or defeminization brought by sex steroids during the perinatal critical period. COI:No

3P-014

Withdraw

3P-015

Sleep parameters and autonomic nervous response in menopausal women

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The present study aimed to investigate changes in sleep parameters in the daily lives of 6 healthy women aged 45-55 years who participated in this study on working days (WD) and on holidays (HD), and provided consent before the study. The experiment was performed in each participant's home. RR interval was measured by heart rate monitor (Union tool Co), and sleep parameters were measured using a Nemuri Scan mat (Paramount Bed Co). Before and after the night of the experiment, participants underwent salivary tests to analyse salivary cortisol and melatonin concentration. On the following morning, the participants reported their subjective perceptions of the parameters and sleep quality using the OSA questionnaire. Changes in autonomic function were estimated using the time domain for RR intervals or Lorenz plot method for 150 min after sleep onset. The mean total sleep time on WD and HD was 346.0 ± 81.6 and 368.1 ± 9.4 min, respectively. The RR interval before sleep on a HD tended to be higher than that on a WD. However, the time course of the RR interval for 150 min after sleep onset followed the same progress on WD and HD. No significant difference was found on the total sleep quality score of OSA between WD and HD. The mean melatonin concentration on HD after waking decreased compared with that before sleep onset. We concluded that either of the autonomic nervous response and salivary hormones may be affected by WD and HD. This work was supported in part by a Japanese grant aid for scientific research (15k11896). COI:No

3P-016

Social defeat stress elevates FGF21 in blood and liver in mouse

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The energy metabolism is affected by various stressor, such as cold exposure, exercise and social living. However, it is unclear whether the energy metabolism aggravated by stressor change the stress-like behavior.Fibroblast growth factor 21 (FGF21) is a member of FGF family and stimulates energy metabolism. Previous studies demonstrated that FGF21 induced the expression of corticotropin releasing hormone (CRH) which is one of the stress hormone, and is expressed by corticosterone.Previous our study using the social-defeat stress paradigm (SDS) showed that the mice exposed SDS elevated blood corticosterone level, and energy metabolism. Thus we speculate that FGF21 may be involved in stress-like behavior and stress-induced energy metabolism after SDSWe therefore investigated whether SDS induced FGF21.We discovered that the mice exposed SDS for 3 days showed higher FGF21 level in blood, and this is immediately effect after physical contact in SDS according to higher corticosterone level in blood. In addition, the FGF21 level in blood repeated to increase after each physical contact during SDS. To identify which organ produce the FGF21 induced by SDS, we measured gene expression of FGF21 in several organs which contribute to the production of FGF21. Our data showed that the mice exposed SDS for 3 days significantly increased expression of FGF21 only in liver.These data suggest that FGF21 induced by SDS that might be produced by liver has the role to regulate stress condition in common with corticosterone. COI:No

3P-017

Effect of GABA on circadian rhythm in cell and slice cultures of rat SCN

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The suprachiasmatic nuclei (SCN) of the hypothalamus contain a major pacemaker for the generation of circadian rhythms in mammals. Isolated rat suprachiasmatic cells cultured in vitro express circadian oscillation of vasopressin (AVP) release for weeks. Since GABA has been postulated as a coupling agent for the SCN cells, we examined the effect of GABA on the rhythm of AVP release. When GABA was added to the culture medium, AVP release from the cells was markedly reduced. The GABA-induced inhibition occurred at all phases of the circadian cycle. While GABA disturbed the circadian oscillation of AVP release, it was gradually restored. After the recovery of the rhythm, phase-shift was observed. Magnitude and direction of the shift depended on timing of the application, maximum phase delay at early subjective day, and the maximum phase advance at late subjective day. In a few cases where GABA was applied at early subjective day, AVP rhythm was disturbed over 2 days. Phase response curve is similar to that induced by TTX. GABA also markedly reduced AVP release in slice cultures of the SCN. However, no phase shift was observed in the slice cultures. These results suggest that GABA would induce phase shift of AVP rhythm but the neural communication preserved in slice cultures would restore the phase of the rhythm to its original state. COI:No

3P-018

Castrated males attract intact males: dose elevated gonadotropin produce chemical attractants?

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When presenting odors of intact and castrated males simultaneously, male rats showed longer exploration to odor of castrates than that of intacts, indicating that castrates may produce chemical attractants for males. It is, however, unlikely that a lack of androgen caused by castration produce chemicals of which intact males have less. Then, we hypothesized that elevated gonadotropin resulting from a loss of negative feedback control provokes attractiveness of castrated males. Twenty-four Long-Evans male rats were given mating experience, and screened by olfactory preference tests for an odor-pair of intact males and receptive females. Eight males with good performance in olfactory preference tests were sham-operated and served as probe males and simultaneously gonadally intact males (SHAM). Remaining 16 males were orchidectomized (Gdx) under isoflurane anesthesia. Three weeks later, a half of Gdx males were injected with a gonadotropin releasing hormone antagonist, degarelix acetate (30mg/kg, ip, Gonax, Astellas Pharma Inc., Tokyo), and the remaining half and probe males were injected with saline, ip. From a week later, probe males were weekly subjected to 3 olfactory preference tests using different stimulus pairs: 1) SHAM vs. Gdx, 2) SHAM vs. Gonax, and 3) Gdx vs. Gonax. Probe males showed significant preference for Gdx to SHAM and for Gdx to Gonax males, and no preference in the SHAM and Gonax pair. These results demonstrate that the attractiveness of castrated male rats for intact males were produced by elevated gonadotropin following orchidectomy. COI:No

3P-019

Local electrical stimulation activates dendritic compartment-specific inputs in layer 5 pyramidal neurons of primary visual cortex

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In layer 5 pyramidal neurons of the primary sensory cortex, perisomatic dendritic area including basal dendrites receives sensory feedforward inputs from the thalamus via layer 4 and 5 whereas the distal apical dendrite in layer 1 receives feedback associative inputs from higher brain areas. To study the balance of information flow between these two inputs under the different brain state exerted by neuromodulators, it is a prerequisite to stimulate each pathway. Since optogenetic approach using channelrhodopsin-2 is laborious and time-consuming, we studied whether simple local electrical stimulation activates the specific dendritic compartments, using FM1-43 dye unloading in the primary visual cortex of the rat. After loading FM1-43 dye into synaptic vesicles, electric stimulation (5 Hz) was delivered to either layer 1 or layer 5 with extracellular stimulus electrodes to unload FM1-43 dye. Unloading of FM1-43 in layer 1 was occurred only by electrical stimulation of layer 1 but not by layer 5. Likewise, unloading of FM1-43 at the layer 5 was detected with electric stimulation of layer 5 but not of layer 1. Thus, these results indicate that local electrical stimulation of layer 1 and layer 5 specifically activates inputs in distal apical dendritic and perisomatic basal dendritic compartments, respectively. Supported by Basic Science Research Program through the NRF funded by the Ministry of Education, Science and Technology (2016R1A2B2016533). COI:No

3P-020

Effect of Botulinum Toxin Type A on the Activation of Trigeminovascular Nociceptive System

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Effects of Botulinum toxin type A (BTXA) as a preventive treatment on the trigeminovascular nociceptive system activated by cortical spreading depression (CSD) were investigated and compared with those of sumatriptan, an abortive treatment medication.Adult male Wistar rats were pretreated with normal saline solution, BTXA or sumatriptan before CSD induction with KCl or no CSD induction with NaCl. Changes in cerebral blood flow at parietal cortex was measured for 90 min after the induction. Parietal cortex, trigeminal ganglion (TG) and trigeminal nucleus caudalis (TNC) were then collected for c-Fos, nNOS and CGRP measurement.BTXA significantly decreased the cumulative blood flow and number of hyperemic peaks but unlike sumatriptan did not change the averaged peak-to-peak duration of peaks induced by CSD. Numbers of CGRP positive cells at TG and c-Fos positive cells at TNC were reduced by BTXA. At parietal cortex and TNC, BTXA was effective in reducing c-Fos and nNOS expression. Sumatriptan had similar effects as those of BTXA except at TG where it could reduce basal c-Fos expression.In conclusion, BTXA and sumatriptan decreased CGRP and NO production, thus lessened the persistent activation of peripheral and central neurons involved in the sensitization of the trigeminovascular nociceptive system. COI:No

3P-021

Modulatory mechanism of release probability at the presynaptic site by Netrin-G2

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Netrin-G2 is a trans-synaptic adhesion molecule which is expressed at the presynaptic terminal of medial perforant path (mPP) /granule cell synapse in dentate gyrus (DG). In previous study, release probability(*Pr*) of neurotransmitter from the axonterminal of mPP was suggested to be reduced in *Ntng2* knocked out mice. Here, we estimated release probability and its Ca-dependency in *Ntng2* KO mice. Using a slice preparation of mice DG, field EPSC was recorded in picrotoxin, APV and CTZ containing ACSF. Release probability was estimated from cumulative amplitude curve of field EPSC responding to high frequency stimulation (80Hz). Under basal condition in normal ACSF containing 2 mM Ca^{2+}, *Pr* of mPP axon terminal in KO mice was significantly smaller (0.38 ± 0.03, n = 3) than that in WT (0.54 ± 0.14, n = 3). However, *Pr* of KO mice increased up to 0.5 after repetition of high frequency stimulation, and remained for several minutes. This increase of *Pr* in KO mice was supposed to be attributable to enhancement of Ca-dependent vesicle priming in the presynaptic terminal. Actually, increase in external Ca ion concentration to 3 mM enhanced *Pr* up to 0.53 ± 0.02 (n = 3), which was comparable to that of WT in normal ACSF (2 mM Ca). Thus, we concluded that Netrin-G2 is involved in modulatory system to keep Ca- sensitivity of the priming process high in the presynaptic terminal of mPP axon. COI:No

3P-022

Synaptic plasticity induced by androgen in the brain area related to extinction memory acquisition after conditioned taste aversion in mice

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We have reported that pubertal exposure to testosterone enhances the extinction memory retention after acquisition of the conditioned taste aversion (CTA) memory. We double-labeled the axon terminals of projection neuron from ventromedial prefrontal cortex (vmPFC, a pivotal brain site of extinction) to amygdala (an association center of CTA), by BDA and SNAP25 or GAD65, and we compared the number of the double-labeled terminals. The result revealed that there are differences between sexes as well as between gonadectomized and sham groups in SNAP25-expressed axon terminals, but no difference in GAD65-expressed axon terminals. Our previous study using Golgi staining method, demonstrated that the exposure of testosterone at the sexual pre-maturation period enhanced dendritic spine density (DSD) in amygdala, suggesting that androgen induces not only to increase the number of axon terminals of glutamatergic neurons projecting from vmPFC to amygdala, but also to increase the DSD in amygdala neurons. To confirm this hypothesis, we are exploring to visualize dendritic spines of GABAergic interneuron in amygdala and the corresponding axon terminals of glutamatergic neurons projecting from vmPFC simultaneously. COI:No

3P-023

Alterations of MAP2 immunostainability in drebrin knockout neurons

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Drebrin has critical functions in synaptic plasticity; therefore it is thought to be in a responsible position of learning and memory. It has been reported that NMDAR activation suppresses microtubule entry in dendritic spines (Kapitein et al., 2011) and drebrin acts as a positive regulator of microtubules entry into spines (Merriam et al., 2013). To investigate further relationship between drebrin and microtubules, we used primary cultured hippocampal neurons prepared from drebrin knockout (DXKO) mice. Drebrin has two isoforms, embryonic (E) and adult (A) isoforms, and both isoforms were genetically deleted in the DXKO mice. First, microtubules associated protein 2 (MAP2) was analyzed immunocytochemically using 21 days in vitro (DIV) neurons. We detected less MAP2 positive neurons in DXKO neurons than in wild-type (WT) neurons. We have reported that drebrin A is required for the NMDAR activity-dependent up-regulation of the NR2A subunits within spines and at synapses (Aoki et al., 2009). Therefore we examined the accumulation of NMDAR subunits at dendritic spines immunocytochemically. Our results indicated that the labeled NR2A were less in DXKO mice. Furthermore we investigated the effect of NMDAR antagonist on MAP2 immunoreactivity, and found that an NMDAR antagonist attenuated immunoreactivity of MAP2 in WT neurons. Taken together, it is suggested that activations of NMDAR in DXKO mice is weaker and it may cause less immunoreactivity of MAP2. Further study is needed to reveal the function of NMDAR in DXKO mice. Supported by KAKENHI (16K18376) and AMED (17bk0104077h0001). COI:No

3P-024

Light-evoked synaptic responses obtained from the insular cortex in the trigeminal spinal subnucleus caudalis

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It is well known that the trigeminal spinal subnucleus caudalis (Sp5C) receives orofacial noxious information from the trigeminal primary afferents, and sends the information to the higher central nervous system. The insular cortex (IC) plays a principal role in processing noxious inputs, and the direct descending projections from IC to Sp5C have been reported. However, little information is available in terms of the modalities of the descending projections to excitatory and inhibitory Sp5C neurons. Here, we investigated electrophysiological characteristics of glutamatergic and GABAergic/glycinergic Sp5C neurons using VGAT-Venus transgenic rats, and examined how IC projections modulate the activities of Sp5C neurons by an optogenetic technique. Sp5C lamina I/II neurons were classified into five subtypes: fast-spiking, single-spiking, long afterhyperpolarization, late-spiking, and bursting neurons. Most excitatory neurons belonged to the single-spiking and late-spiking subtypes. Selective stimulation of the IC axons in the slice preparations obtained from rats that received injection of AAV-ChR2-mCherry into IC induced EPSCs both in excitatory and inhibitory Sp5C neurons. These findings suggest that IC neurons induced facilitative effects on Sp5C neurons. COI:No

3P-025

Forskolin or dideoxyadenosine mimic the dopamine-dependent synaptic plasticity in IPSC of Substantia Nigra pars reticulate (SNr) GABA neurons in an acute Parkinson model rat brain slice.

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I had already reported that a high frequency electrical stimulation on subthalamic nucleus (STN-HFS), which imitated the deep brain stimulation (DBS) on STN for a progressed patient of Parkinson disease, induced the dopamine-dependent synaptic plasticity in the IPSC at SNr GABA neurons evoked by an electrical stimulation onto a putative "direct pathway" in the slices from a reserpinized rat in the ACSF with 100 μM α-methyl-*L*-tyrosine (AMPT), a dopamine synthesis inhibitor. IPSC-LTP is dependent on the D_1-dopamine receptor activation. On the other hand, IPSC-LTD is dependent on the D_2-dopamine receptor activation. Forskolin (an adenylate cyclase activator, 3 to 7 μM) with STN-HFS induced IPSC-LTP in the half of neurons tested (3 of 6 neurons). The relative amplitude of IPSC was 1.861 (n = 3) at 120 min after STN-HFS. In the rest of neurons, IPSC gradually potentiated for about 1 hour, and then depressed. The potentiation was accompanied with the increase in sIPSC frequency. The depression was accompanied with the decrease in sIPSC frequency. These decreases in IPSC amplitude and sIPSC frequency might be due to the depletion of synaptic vesicles. Dideoxyadenosine (an adenylate cyclase inhibitor, 5 μM) with STN-HFS induced IPSC-LTD in two neurons tested. The relative amplitude of IPSC was 0.709. This decrease in IPSC amplitude was accompanied the decrease in sIPSC frequency. Thus, it is suggested that the activation and inhibition of adenylate cyclase with STN-HFS resulted in IPSC-LTP and -LTD, respectively. COI:No

3P-026

Effect of very early exercise on motor functional recovery and brain damage after hemorrhage in rats

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This study focused on the effect of very early exercise on motor functional recovery and brain damage following intracerebral hemorrhage (ICH) in rats. ICH was induced in rats by an injecting collagenase into the left striatum. Subjects were randomly assigned to no training ICH (ICH), no training placebo surgery (SHAM), very early treadmill exercise (ICH + VET), early treadmill exercise (ICH + ET), and late treadmill exercise (ICH + LT) groups. The ICH + VET group trained for 7 days in the period from 24 hours to the 6th day post-surgery. The ICH + ET group trained for 7 days in the period from the 2nd to 8th day post-surgery. The ICH + LT group trained for 7 days in the period from the 9th to 15th day post-surgery. The ICH + ET group showed significantly improved sensorimotor function compared with the ICH, ICH + VET, and ICH + LT group. The cortical thickness and neuronal number of the ICH + ET group was significantly higher than that of ICH, ICH + VET, and ICH + LT groups. TGF-b1mRNA expression of ICH group was significantly higher than that of SHAM and ICH + VET group. Very early exercise may not recover sensorimotor function to inhibit the expression of anti-inflammatory factor after ICH. These results suggest that after cerebral hemorrhage, early treadmill exercise may promote sensorimotor functional recovery by inhibition of cortical atrophy compared with late treadmill exercise. COI:No

3P-027

Temporal patterns of multi-pathway signals in the cerebellar cortex

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Somatosensory signals from the facial area are conveyed to the cerebellar cortex directly via trigeminocerebellar pathway as well as indirectly via cortico-ponto-cerebellar pathway. Both of these pathways form mossy fibers, projecting to the granule cells in the cerebellar cortex. Besides these fibers, climbing fibers from the inferior olive also transmit somatosensory signals to the Purkinje cells. To reveal the temporal patterns of these multiple types of signals, we made whole-cell recordings from granule cells and extracellular unit recording from Purkinje cells in anesthetized mice that express channelrhodopsin 2 in inhibitory neurons. The activity of somatosensory cortex of these mice can be suppressed by light illumination in a temporarily and spatially restricted manner. In response to tactile stimulation to the upper lip, synaptic currents appeared with two distinct (early and late) latencies in the granule cells. The early response was not affected but the late response was eliminated by suppression of somatosensory cortex, suggesting that the early response was direct trigeminal signal and the late one was indirect signal via somatosensory cortex. In Purkinje cells, the early increase and decrease of simple spikes were not affected, but the late increase and decrease of simple spikes as well as complex spikes are suppressed, by cortical suppression. These results illustrate the distinct temporal patterns of direct and indirect sensory signals in the cerebellar cortex. COI:No

3P-028

Composition of nicotinic acetylcholine receptors in murine muscle fibers

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The nicotinic acetylcholine receptors (AChRs) are composed of five subunits in vertebrate neuromuscular junctions (NMJs). The difference of AChR subunit compositions between muscle fiber types have been revealed in zebrafish: the AChRs in slow muscle fibers contain the α, β and δ subunits and lack the ε / γ subunits. Based on this finding in zebrafish, we extended our focus to study the expression of the ε subunit in adult mice, at the level of mRNA and protein. The mRNA expression of ε subunit of AChRs were detected in both soleus and extensor digital longus (EDL) in adult mice. Western blot analysis using the anti- ε subunit antibody detected specific bands at 49 kDa in both muscles. Interestingly, immunohistochemistry using the ε antibody stained NMJs, that only partially co-localized with α-bungarotoxin (α-BTX). These results indicate that mice may also have muscle fibers that are composed of subunits without ε / γ subunits, congruent to findings in zebrafish. We will discuss the relationships between the muscle fiber type and the composition of AChR subunits in mice. COI:No

3P-029

NPY mediates fasting-induced excitatory synaptic depression onto oxytocin neurons in the hypothalamic paraventricular nucleus

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Oxytocin (Oxt) neurons in the paraventricular nucleus of the hypothalamus (PVN) are implicated in satiety and energy expenditure. We previously reported that excitatory synaptic transmission onto Oxt neurons was depressed by fasting in a NMDA-type glutamate receptor (NMDAR) dependent manner. However, signal molecules mediating fasting-induced depression of the synaptic transmission remain unknown. The miniature excitatory postsynaptic current (mEPSC) and AMPA-NMDA ratio onto PVN-Oxt neurons in acute slice from male oxytocin-mRFP transgenic rats were measured under voltage clamp.Application of neuropeptide Y (NPY), similarly to fasting, decreased mEPSC amplitude and increased AMPA-NMDA ratio, suggesting that NPY mediated fasting induced NMDAR dependent depression of excitatory synaptic transmission onto PVN-Oxt neurons.In the presence of Y1 receptor antagonist andby increasing intracellular cAMP with IBMX, NPY failed to alter mEPSC onto PVN-Oxt neurons.In summary, NPY, which is released by fasting, decreases NMDAR via Y1 receptor-mediated suppression of cAMP, leading to decrease AMPAR at postsynapses of PVN-Oxt neurons. COI:No

3P-030

Pruning of collateral from corticospinal tract axons is necessary for the motor recovery after spinal cord injury

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After spinal cord injury (SCI), the reorganization of corticospinal tract (CST) can lead to the motor recovery. In the course of the reorganization, axotomized CST fibers form collaterals and connect with propriospinal neurons, and then excess collaterals are pruned. Although the pruning is thought to elaborate the neural pathway, its molecular mechanism and functional role are still unknown. Here we employed incomplete SCI model mice undergoing dorsal hemisection at T8 level. We found that Neuropilin-1 (Nrp-1) mRNA was upregulated in layer 5 neurons in the motor cortex 14 days after SCI, when the pruning occurred. Then we demonstrated that Nrp-1 knockdown resulted in the increased number of collaterals 28 days after SCI. Using retrograde tracer and immunohistochemistry we revealed that propriospinal neurons in the cervical cord expressed Smaphorin3A. We further uncovered that the genetic deletion of Nrp-1 specifically in the hindlimb motor area delayed the motor recovery after SCI. Moreover, the rehabilitative training by using Rotarod enhanced pruning of collaterals and motor recovery. Therefore, these results suggest that the pruning of collaterals is necessary for the motor recovery after SCI. COI:No

3P-031

Whole brain activity mapping with quantitative activation-induced manganese-enhanced MRI in dopamine D1 receptor conditional knockdown mice.

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Dopamine (DA) is one of the most important neurotransmitter in the central nervous system, especially in basal ganglia, and is believed to modulate the neuronal activities. In the striatum, direct pathway neurons express dopamine D1 receptors (D1R), whereas indirect pathway neurons express dopamine D2 receptors (D2R). It is thought that this dichotomy contributes to the distinct roles for direct and indirect pathway neurons of striatum. However, how DA signal modulates the whole brain activities has not been well explored. In this study, to reveal how DA signal via D1R modulates the neuronal activities, we conducted the whole brain activity mapping in D1R conditional knockdown mice with a novel MRI method, quantitative activation-induced manganese enhanced MRI (qAIM-MRI; Kikuta et al., 2015). The mouse exhibited impaired motor ability when D1R expression was suppressed. The neuronal activities in the substantia nigra pars reticulata were decreased compared to normal conditions. These results could not be simply explained by the current model of the basal ganglia circuitry. COI:No

3P-032

The α_{2A} adrenoceptor suppresses excitatory synaptic transmission to both excitatory and inhibitory neurons in layer 4 barrel cortex

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The mammalian neocortex is widely innervated by noradrenergic (NA) fibres from the locus coeruleus. To determine the effects of NA on vertical synaptic inputs to L4 cells from the ventrobasal thalamus and L2/3, thalamocortical slices were prepared and whole-cell recordings were made from L4 cells. Excitatory synaptic responses were evoked by electrical stimulation of the thalamus or L2/3 immediately above. NA suppressed (about 50% of control) excitatory vertical inputs to all cell types (RS, RSNP, FS) in a dose-dependent manner. The presynaptic site of action of NA was suggested by three independent studies. First, responses caused by iontophoretically applied glutamate were not suppressed by NA. Second, paired pulse ratio was increased during NA suppression. Finally, a CV^{-2} (coefficient of variation) analysis was performed, which suggests again a presynaptic mechanism for the suppression. Experiments with phenylephrine (α1 agonist), prazosin (α1 antagonist), yohimbine (α2 antagonist) and propranolol (β antagonist) indicated that suppression was mediated by α2 adrenoceptor. To determine whether the α2A adrenoceptor subtype was involved, α2A adrenoceptor knockout mice were used. NA failed to suppress EPSCs in all cell types, suggesting an involvement of α2A adrenoceptor. Altogether, we concluded that NA suppresses vertical excitatory synaptic connections in L4 excitatory and inhibitory cells through presynaptic α2A adrenoceptor. COI:No

3P-033

Accumulation of AMPA receptor in functional recovery after cortical injury

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Acute damage to central nervous system such as stroke is a leading cause of serious functional disability. Restoration of functional disability is considered to be the result of compensative neural plasticity in the intact brain regions. Synaptic AMPA receptor (AMPAR) delivery is a fundamental mechanism underlying behaviors that requires neural plasticity. Facilitation of experience-dependent synaptic AMPAR delivery could result in rehabilitative training-dependent motor cortical reorganization after brain damage. However, due to the lack of molecular imaging techniques in vivo, the molecular mechanisms underlying cortical reorganization during functional recovery remain poorly understood. To this end, we developed a novel PET probe to detect the AMPARs in the living brain. Here, we demonstrated that accumulation of AMPAR in the peri-injured region of cortical cryogenic injury after rehabilitative training of forelimb reaching in "recovered" rats, but not "non-recovered" rats. Furthermore, pharmacologically blockade of AMPAR in the AMPAR accumulated region inhibited forelimb reaching performance in "recovered" rats. These results indicate that AMPAR accumulation in the peri-injured region contribute functional recovery after cortical damage. PET imaging of AMPAR will provide the evaluation of degree of recovery in living human brain. COI:No

3P-034

pH imaging in the brain using high-resolution ion image sensor

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Our brain functions (e.g. memory, learning, and perception) are regulated by precise control of neurotransmitters in neural circuit. Recent studies have shown that proton functions as neurotransmitters (Du et al, 2014), and localized pH fluctuations occur in the brain (Magnotta et al, 2012). However, pH change in the brain have not been elucidated by neural circuit level. Thus, the development of non-labeling ion imaging has been expected. In this study, we developed a needle type pH image sensor with 32x128 pixels for in vivo application. The pixel pitch of the sensor is 23 μm and the time resolution is about 20msec (50frame/sec). To insert in the murine brain, the geometry was modified to needle structure with 1.76x11.46 mm and 100 μm thickness. The needle type pH image sensor was covered by parylene C with a thickness of 4 μm without pH sensing area for waterproofing. We inserted the sensor to visualize pH condition in the barrel cortex by single cell resolution. Interestingly, we found the depth dependency of pH change after insertion of the sensor. High-frequency responses were synchronously induced by administering SR95531, GABAA receptor antagonist. Furthermore, the synchronous responses were reduced by tetrodotoxin administration. Finally, the frequency was also increased by sensory stimulation after administering SR95531. COI:No

3P-035

Identification of substances affect the activity of corticotropin releasing factor (CRF) neurons in the hypothalamus

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In the modern stressful society, functional disorder like insomnia caused by stress is becoming more and more serious social problem. It is reported that the corticotropin releasing factor (CRF) neurons in the hypothalamus are the beginning of hypothalamic-pituitary-adrenal (HPA) axis and play a central role of stress response. On the other hand, orexin neurons in the hypothalamus are implicated in sleep/wakefulness system. These two neural systems are thought to be interact to regulate stress and sleep/wakefulness. The regulatory mechanism of the CRF neurons is not revealed in detail. Since the activity of the CRF neurons is predicted to be controlled by various neurotransmitters and modulators, we focused on the bioactive substances which affect the activity. To identify the factors, we performed calcium imaging in acute mouse brain slices. We generated adeno-associated virus vector which specifically express calcium indicator Yellow Cameleon (YC)-Nano50 in the CRF neurons. We screened various bioactive substances such as hormones, prostaglandins, interleukins and cytokines, particularly related to sleep, food intake and stress response. In this study, we newly identified substances which affect CRF neurons. To reveal the role of these substances in vivo, we will perform the behavioral experiments. COI:No

3P-036

Hemokinin-1 (1-5) elicits the inhibitory effect on pruritic processing in the rat spinal cord

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Hemokinin-1 (HK-1) is a peptide, and shares the hydrophobic carboxyl-terminal (C-terminal) region common to mammalian tachykinin peptides, such as substance P (SP). It is generally believed that C-terminal fragments of SP have an excitatory effect, while the pretreatment with amino-terminal (N-terminal) fragments of SP inhibits the function of SP; however, there is no available information on HK-1. Therefore, to clarify the characteristics of N-terminal fragments of HK-1, HK-1 was divided into HK-1 (1-5) as the N-terminal fragment and HK-1 (6-11) as the C-terminal fragment. Intrathecal administration of HK-1 (6-11) induced scratching behavior similar to HK-1, while HK-1 (1-5) hardly induced scratching. Furthermore, intrathecal administration of HK-1 (1-5) and SP (1-5) markedly attenuated the induction of flinching and enhancement of c-Fos expression in the spinal cord following intradermal administration of formalin, a noxious stimulant, while the pretreatment with HK-1 (1-5), but not SP (1-5), markedly attenuated the induction of scratching behavior by subcutaneous administration of pruritic agents, such as serotonin or histamine. Taken together, these findings indicate that HK-1 (1-5) suppresses pruritic and nociceptive processing, while SP (1-5) suppresses nociceptive processing, and suggest that HK-1 (1-5) may be a useful tool for revealing pruritic processing and HK-1 may play a crucial role in pruritic processing. COI:No

3P-037

Functional connectivity on olfactory processing neuronal circuits

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Functional magnetic resonance imaging (fMRI) has been used to investigate central mechanism of olfaction in humans, yet little is known about olfactory processing brain network. In order to investigate brain network for odor perception in human, we utilized ultra high field 7 Tesla (7T) MRI for fMRI. Nineteen healthy participants were scanned by fMRI. The olfactory task consisted of 12 alternating 6s odorants sniffing (isovaleric acid, peppermint, coffee and odorless air) and 24s rest block design. By sniffing of odorants and odorless air, BOLD signal activation was detected in the piriform cortex. ROI analysis revealed that sniffing of odorants induced activation in both anterior and posterior regions of piriform cortex, whereas sniffing of odorless air induced activation in the posterior region only. Functional connectivity from the piriform cortex was examined by the psychophysiological interaction (PPI) analysis. PPIs and showed that anterior piriform cortex connected with amygdala, medial orbitofrontal cortex, nucleus accumbens and posterior insula. On the other hands, posterior piriform cortex connected with the lateral and medial orbitofrontal cortex, amygdala, thalamus and anterior insula. These results suggested that information of odor and odorless air are functionally dissociable in the piriform cortex, and odor and odorless air sensory information could be processed through different pathway. Furthermore, we propose odor-modulating neuronal network model assessed by dynamic causal modeling. COI:No

3P-038

In vivo sensory neuronal responses in the anterior cingulate cortex

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Anterior cingulate cortex (ACC) is known to be an important brain region in the perception of pain. The precise mechanisms of pain perception in the ACC, however, are not fully understood. Rats were ventilated under urethane and isoflurane anesthesia, and then a small hole was made on the skull to insert the recording electrodes. In vivo extracellular and patch clamp recordings were made from ACC neurons. AAC neurons exhibited up- and down-states and fired spontaneously during up-states. Mechanical stimulation applied to the skin of the hind paw elicited action potentials in ACC neurons. But the mechanical response and spontaneous firings were inhibited when the anesthetic depth was increased. To classify recorded neurons based on their firing properties, current injections were applied through the recording pipette under current-clamp conditions. The recorded neurons were classified into three types, bursting, intermediate and regular spiking neurons. Although most of the three types of ACC neurons did not respond by cutaneous innocuous mechanical stimulation, intrinsic bursting neurons increased firing frequency in response to mechanical noxious stimulation by increasing the duration of up-state potentials. Under voltage-clamp conditions, the duration of inward currents was also increased by noxious stimulation. The present results suggest that ACC neurons in particular IB type pyramidal neurons may have an important role on the perception of pain. COI:No

3P-039

Spinal sensory processing from the lower urinary tract

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The lower urinary tract is composed of the bladder and urethra, and the afferent sensory information through small myelinated A delta and unmyelinated C fibers to the spinal cord has an important role on the precise coordination of voiding reflex. However, it is still unclear how spinal dorsal horn receives sensory information from the lower urinary tract. We have classified spinal dorsal horn neurons into two types based on their sensory responsiveness during voiding in our previous study. In this study, we further analyze physiological roles of the spinal neurons in particular the neurons receiving sensory information from the urethra. During voiding reflex, the spinal sensory neurons receiving from the urethra elicited firings at the peak of intravesical pressure. When capsaicin was perfused into the urinary bladder, the neurons increased the firing frequency at the peak pressure, and followed by sustained firings even after voiding reflex. Capsaicin applied separately into the urethra decreased voiding reflex intervals. These results suggest that sensory information through TRPV1-expressing afferent fibers from the urethra controls voiding cycle and may induce urethral pain. COI:No

3P-040

The modulation of the sensory responses of rat S1 by cortical direct current stimulation

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Transcranial direct current stimulation, a non-invasive brain stimulation technique, has been used in the clinical treatment to improve cognitive and motor functions. However, physiological details of cortical direct current stimulation (DCS) are not well understood. To investigate the effects of the DCS to the cortex, we recorded the sensory responses in the rat forelimb sensory area (S1) by using the voltage-sensitive dye optical imaging and electrophysiological recording (LFP). The sensory stimulus was presented to the forelimb, and forelimb-evoked responses were compared between with or without DCS to the forelimb S1.When anodal DCS was applied to the forelimb S1, forelimb-evoked sensory response was significantly increased immediately after the stimulus onset. Spatial extent of sensory response recorded by the optical imaging was increased corresponding to the intensity of the DCS. When cathodal DCS was applied to the forelimb S1, sensory response was significantly decreased and disappeared by 10 minutes after stimulus onset. These results suggest that the forelimb-evoked sensory response is modulated by the DCS with the polarity specific manner.Finally, we investigated whether above modulation effects relate to the isoflurane anesthesia concentration. Here, anodal DCS and sensory input to the forelimb were applied with 1.0% or 1.5% isoflurane anesthetized condition. We found that the effect of enhancement induced by the anodal DCS is significantly diminished in 1.5% anesthetized rats, suggesting that the modulation effect of DCS can relate to the degree of awakening of the subjects. COI:No

3P-041

Contribution of thalamic relay for "blindsight"

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If the primary visual cortex (V1) was damaged, our visual awareness was impaired. However, some patients can perform manual response or saccadic eye movement to visual target in the lesion affected visual field. This phenomenon is called "blindsight". Leopold and his colleague reported that lateral geniculate nucleus (LGN) plays a key role in "blindsight" as a visual input relay (Schmid et al., 2010). On the other hand, our previous study revealed that monkeys with the V1 lesion cannot perform saccadic eye movement after inactivation of neural input from the superior colliculus (SC) to the pulvinar (Pul) (in preparation). Thus, the thalamic relay for the blindsight is still controversial. These two nuclei, LGN and Pul, independently projects to extrastriate cortices. To solve such contradiction, a double dissociation experiments in the same animal are needed. For this purpose, we made microinjection of muscimol, a $GABA_A$ receptor agonist, into the LGN and Pul, respectively in the same monkey with unilateral V1 lesion performing visually guided saccadic eye movement task. Furthermore, to obtain neuroanatomical evidence for the visual pathway in the blindsight, we injected AAV with red anid green fluorescent protein expressing gene into the SC on ipsilesional and contralesional side, respectively. Results of these experiments suggested the involvement of both the Pul and LGN in "blindsight". COI:No

3P-042

Theta band brain activity during checking the unmatched olfactory-taste information

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Taste and smell are the most fundamental sensory information. When we eat food, and if smell is different from the original smell of the food, recognition mechanism of the food may be disturbed. In the present study, we investigated influences of olfactory stimulation on taste perception. Each olfactory stimulation was delivered for one minutes with a tastant in the mouth. During each session, EEGs were measured from the frontal region of the heads of normal subjects, and frequency analysis was done. Chocolate and plum gummi candy were used for tastants. Chocolate and plum were used for match olfactory stimulation, respectively, and garlic and lavender oil were used for mismatch olfactory stimulation. After each recording session, the participants was asked to rate feeling of taste of foods. In the case of delivering mismatch olfactory stimulation with respective tastant, the occupancy rate of theta frequency band increased, as compared with in the case of delivering match olfactory stimulation. In addition, there was the tendency that when feeling of taste intensity increased, occupancy rate of alpha frequency band also increased. The present findings suggest that when we receive unmatched sensory inputs against the object, subjective feeling is disturbed, and theta band brain activity emerge during checking the unmatched information. COI:No

3P-043

Effects of Ca^{2+} and 2-APB on the transduction channels of microvillus olfactory receptor neurons of goldfish

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Teleost olfactory epithelium (OE) has different types of olfactory receptor neurons (ORNs) such as ciliated ORNs using cAMP pathway and microvillus ORNs (mORNs) that appear to involve inositol phospholipid pathway for the olfactory transduction. In the present study, we recorded whole-cell current responses of ORNs from the slice preparation of the goldfish OE. To reveal the channel properties, we study the effects of Ca^{2+} and 2-APB on the odorant-induced currents of mORNs. When Ca^{2+} in the control bath solution with 1 mM Ca^{2+} and 1 mM Mg^{2+} was replaced with equimolar Mg^{2+} by using the solution containing 2 mM Mg^{2+} and 0.2 mM EGTA, the peak of the amino-acids response was decreased to 45%, indicating external Ca^{2+} enhances the current response. On the other hand, in the same treatment, the single-channel current amplitude measured by noise analysis was increased from 0.7 to 1.3 pA at a holding potential of -66 mV, showing external Ca^{2+} suppresses the channel. When the control solution was replaced to the solution containing 0 mM Ca^{2+}, 1 mM Mg^{2+} and 0.2 mM EGTA, the channel size was enlarged to 2.4 pA, suggesting external Mg^{2+} also has the suppressive effect on the channel. We tested the effect of 2-APB that has been reported to block TRPC2 channels of mouse vomeronasal sensory neurons. Bath-applied 200 μM 2-APB suppressed 90% of the control response, suggesting that TRPC2 channels of teleost mORNs are sensitive to 2-APB. These channel properties found here would be useful to analyze functional properties of teleost mORNs. COI:No

3P-044

The electrophysiological study on the effect of novel mutations of *SCN11A* on pain pathway neuronal excitability

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Intro:We previously performed genetic analysis in families with infantile limb pain episodes characterized by cold-induced deterioration and mitigation in adolescence, and reported two mutations:R222H/S in *SCN11A*. This disease was named "Infantile episodic limb pain". Since then, more cases who were clinically suspected of this disease underwent genetic analysis and other novel mutations were identified:F814C and F1146S. In this study, we conducted electrophysiological studies of the two mutations. **Method**:Two knock-in mouse models were generated using CRISPR/CAS9, each harboring F802C (FC) and F1125S (FS) (the orthologs of the human F814C and F1146S). Dorsal root ganglion (DRG) neurons, which transmit pain to the spinal cord, were isolated from L4 to L6 sections of 6-8-week-old wild type (WT), FC, and FS mice and subjected to patch clamp method. **Result**:In preliminary analysis, the resting membrane potential was elevated in FC mice compared to WT (FC,-31.8±3.61mV,n=2; WT,-60.0±1.38mV,n=9). The firing frequency of evoked action potentials was also increased in FC mice compared to WT. **Discussion**:The preliminary data suggest higher excitability in FC mice than WT. We proceed with further analysis to assess the effect of FC and FS on DRG neuronal excitability. COI:No

3P-045

Electrical properties of cells from human olfactory epithelium

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Electrical properties of olfactory receptor cells (ORCs) are generally examined using animals as newt, mouse and frog, while fewer electrophysiological study using human ORCs have been reported. In the present study, Olfactory epithelium collected from patients during endoscopic sinus surgery without any clinical troubles were treated with collagenase and cells obtained from olfactory epithelium (COE) were identified by a double-label staining using DAPI and anti-olfactory marker protein antibody. In the recording membrane currents with the whole-cell patch clamp technique, while animal ORCs are reported to express a remarkable transient inward current at the depolarizing voltage step, however, our present study showed that only one sample of 20 human COEs expressed it. The inward current was activated by depolarization beyond -40 mV, and reached a peak at -30 mV. Delayed and sustained outward currents were observed (444±106 pA at 40 mV pulse: n=20), and suppressed by TEA (n= 3). These properties are similar to those of newt ORCs reported previously. In conclusion, our results showed that the most of human COEs did not show the transient inward current, which may contribute to future studies dealing with human olfactory signal transduction in the olfactory epithelium. COI:No

3P-046

Single-unit responses of by prosthetic retinal stimulation : double pulse analysis

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We have been developed the novel retinal prosthetic system, Suprachoroidal Transretinal Stimulation (STS), for photoreceptor degenerating diseases. In STS, the stimulating electrode array is implanted in sclera and do not directly contact retinal tissue to avoid physical damage of retina. Previously we investigated the response properties within 100 msec after STS by single-unit recording from lateral geniculate nucleus (LGN) relay cells in cat, and reported that single pulse stimulation elicited the burstic discharges, which occurred alternately on ON and OFF cells (PSJ meeting, 2017). Here we applied the double pulse stimulation with various intervals to investigate the interaction of the STS responses.

The size of single electrode in the implanted array was 0.5 mm in diameter and 0.3 mm in height, which was the same as the device for clinical use. More than 10 days after surgery, single unit activities of LGN relay neurons were recorded under general anesthesia. The stimulation parameter was biphasic, 500 or 1000 uA, and 0.5 ms/phase duration. The interval of double pulse was changed from 5 to 50 msec.

The first burst by STS was remained when the second burst by preceding STS was overlapped. On the other hand, it was suppressed when the silent period between first and second burst by preceding STS was overlapped. These results suggest that the burstic response by STS was made by the inhibition between the bursts. COI:Properly Declared

3P-047

A mechanism of action of vasopressin V1a receptors on the recurrent inhibition between mitral cells and granule cells in the mouse accessory olfactory bulb

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Central vasopressin (AVP) facilitates social recognition and modulates numerous complex social behaviors in mammals. Recently, vasopressin neurons were reported to exist in the accessory olfactory bulb (AOB). The AOB has been demonstrated to be a critical site for mating-induced mate recognition in female mice. The effect of AVP, however, on the synaptic transmission between dendrites in the AOB of female mice is largely unknown.

To address this issue, we previously measured synaptic currents (IPSCs) from mitral cells in the AOB. We have demonstrated that AVP significantly reduced the IPSCs through AVP V1a receptors. The reciprocal transmission, however, contains both glutamatergic transmission from mitral to granule cells and GABAergic transmission from granule to mitral cells. Thus, it is unclear whether AVP acts on the excitatory and/or the inhibitory transmission.

In the present study, we have given attention to the effect of V1a receptor activation on GABAergic trasmission. AOB slices were prepared from 23- to 35-day-old Balb/c mice. Using the patch-clamp technique in whole-cell configuration, the current responses of mitral cells were recorded in the presence of antagonists for glutamatergic transmission, CNQX (10 μM) and AP5 (50 μM). An extracellular application of vasopressin did not affect the magnitude of the response of mitral cells to GABA, excluding the possibility for V1a receptors to modulate the synaptic transmission from granule to mitral cells through a postsynaptic mechanism. COI:No

3P-048

Are PVN-RVLM neurons involved in sympathetic dysfunction in heart failure?

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In heart failure (HF), sympathetic nerve activity becomes hyperactive. In this study, we tested the hypothesis that neurons in the paraventricular hypothalamic nucleus (PVN) that send projections to the rostral ventrolateral medulla (RVLM) are involved in sympathetic hyperactivity in HF. HF was induced in rats by left coronary artery ligation. More than eleven days after the ligation or sham surgery, adeno-associated virus vector that encodes the light-activated inhibitory opsin Archaerhodopsin-T was microinjected into the rat PVN bilaterally. More than three weeks later, intermittent bouts (1- to 4-s stimulation to non-stimulation) of photostimulation (532 nm wavelength, 2.5 or 5 mW) for 1 min were provided to the RVLM bilaterally of the anesthetized rats in order to inhibit PVN-RVLM neuronal activity. In rats with HF [N=12, 38 ± 5% of infarct size (IS), 8 ± 4 mmHg of left ventricular end-diastolic pressure(LVEDP)], photostimulation at 5 mW significantly ($P < 0.05$) changed renal sympathetic nerve activity (RSNA) while this stimulation had no effect in sham-operated rats (N=6, 0 ± 0% of IS, 2 ± 0 mmHg of LVEDP). In either HF (N=8) or sham (N=4) rats, photostimulation at 2.5 mW had no significant effect on RSNA. The index of decreased RSNA during 12 bouts of 1-s photostimulation at 5 mW, as assessed by the area under the curve, was significantly enhanced in HF rats than that in sham rats (-55 ± 11 vs. -20 ± 5 arbitrary unit). We suggest that dysfunction of PVN-RVLM neurons may mediate sympathetic hyperactivity in HF. COI:No

3P-049

Age-related changes in inhibitory regulation of bladder micturition contractions induced by skin stimulation

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We examined effect of aging on function of skin afferent fibers that inhibit bladder micturition contractions. We used anesthetized male rats in three different age groups: young adult (4-5 months old), middle aged (6-9 months old), and aged (27-30 months old). The bladder was expanded to produce rhythmic micturition contractions. Skin afferent fibers were activated for 1 min either by electrical stimulation (0.1-10 Hz) of the cutaneous branch of the pudendal nerve or by gentle mechanical stimulation to the perineal skin with an elastomer roller. When skin afferent nerves were activated electrically, micturition contractions were inhibited in a similar manner in all age groups, with long latency inhibition induced by excitation of Aβ fibers and short latency inhibition by additional Aδ and C fiber excitation (at 1-10 Hz). On the contrary, when skin afferent nerves were activated mechanically by rolling, latency of inhibition following rolling stimulation was prolonged in aged rats. Single unitary afferent nerve activity of low-threshold mechanoreceptors (LTMs) from the cutaneous nerve was recorded. The discharge rate during rolling was not significantly reduced in Aβ units but was much lower in Aδ and C units in aged rats than in young adult rats. These results suggest that the neural mechanism that inhibits bladder micturition contractions by skin afferent input is well maintained in old age, but the early inhibition by gentle skin stimulation is decreased because of reduced responses of Aδ - and C-LTMs. COI:No

3P-050

Nodding-off behavior observed before sleep onset in mice

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Introduction: We previously reported a new noninvasive ECG method with multi-stripe-electrode ECG (msECG) sensor, which allowed mice free roaming but required sweating on their soles for ECG detection due to the use of dry electrodes without electro-conductive gel.
Methods: A mouse cage with msECG sensor was placed in a Faraday cage. Fifteen msECG-sensor signals were stored in a PC via an amplifier and A/D converter by Clampex7 software. Mouse behavior was also recorded by a DVD recorder. Intact, freely-behaving C57BL/6J mice were put on the msECG sensor for ECG recording.
Results: While the msECG sensor detected ECG only occasionally during active period, ECG continuously appeared from around sleep onset for a maximum of ~15 minutes of sleep period and disappeared upon awakening (n = 7). One mouse allowed ECG recording from ~7 minutes before the sleep onset, probably drowsy (quiet waking) state, and showed a nodding-off behavior synchronized with pronounced HR fluctuation (between ~250 and ~600 bpm) for ~130 s with an interval of 4.0 ± 1.2 s. Thereafter, the head movement stopped but HR fluctuation further continued for 23 s until the mouse fell asleep. The lowest HR during the nodding-off was substantially lower than the lowest HR during sleep.
Conclusion: We first demonstrated the synchronized nodding-off and HR fluctuation in drowsy mice. The present ECG protocol may contribute to investigate the altering interactions between cardiac, autonomic and central nervous systems toward sleep initiation. COI:No

3P-051

Colokinetic effect of somatostatin on the spinal cord in anesthetized rats.

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Defecation is regulated by the enteric nervous system and two defecation centers in the central nervous system, the pontomedullary brainstem and lumbosacral spinal cord. However, it remains unclear that underlying mechanisms of the central nervous system, especially about the lumbosacral defecation center, in regulating colorectal motility. We previously showed that ghrelin activates defecation reflexes in the spinal defecation center. It is well known that somatostatin exerts an opposite effect to that of ghrelin in the central nervous system. In this study, therefore, we examined the effect of somatostatin in the spinal defecation center in anesthetized rats. Intrathecal application of somatostatin into the lumbosacral cord caused propulsive contractions of the colorectum, although somatostatin administered intravenously or intrathecally to the thoracic cord failed to enhance colorectal motility. Transection of the thoracic cord had no significant impact on the colokinetic action of somatostatin. The somatostatin-induced colorectal contractions were abolished by bilateral severing the pelvic nerves. Our results demonstrate that somatostatin acting on the spinal defecation center causes propulsive motility of the colorectum in rats. Considering that somatostatin is involved in nociceptive signal transmission in the spinal cord, nociceptive stimulation on the colorectum might activate defecation reflexes. COI:No

3P-052

Hypothalamic GLP-1 acts on the hypothalamic paraventricular nucleus and causes neural activation of the catecholamine neurons in the medulla and renal sympathoexcitation

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Previous our experiment examined effects of intracerebroventricular (ICV) injection of GLP-1 on autonomic nerve outflows in anesthetized mice, and found that GLP-1 dose-dependently increased sympathetic nerve activity to the kidney, and that ICV injection of GLP-1 also elevated renal sympathetic discharge and blood pressure in anesthetized rats. Here, to investigate area of hypothalamic action by GLP-1, c-fos immunoreactive experiment and microinjection study were performed. ICV GLP-1 increased c-fos positive cells in the PVN and ARC in rats. GLP-1 injection in the paraventricular nucleus (PVN) not but the arcuate nucleus caused renal sympathoexcitation in rats. Moreover, in rats injected GLP-1, c-fos was expressed in the catecholaminergic neurons of the both rostral and caudal ventrolateral medulla of the medulla oblongata. We suggest that hypothalamic GLP-1 acts on the PVN and caused sympathetic and cardiovascular change through the medullary catecholaminergic system. COI:No

3P-053

Responses of central amygdala neuronal and sympathetic nerve activity to odor-cue fear conditioning in rats.

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The amygdala plays a central role in acquisition of fear memories. Fear exerts tonic effects on behavior and autonomic function. However, there has been lack of information on responses of amygdala neuronal activity and autonomic nervous system activity to fear. In the present study, we measured central amygdala neuronal and sympathetic nerve activity simultaneously in response to conditioned fear induced by an odor paired with foot shocks in conscious rats. Male Wistar rats were chronically implanted with electrodes for measurement of central amygdala neuronal activity (CeANA), renal (RSNA) and lumbar sympathetic nerve activity (LSNA), and electroencephalogram (EEG), electromyogram (EMG), and electrocardiograms (ECG), and with a catheter for measurement of arterial pressure (AP). For rats that received fear conditioning, the trials consisted of an odor conditioned stimulus (CS; anisole) and a fear-producing shock unconditioned stimulus (US; 5 mA, 1 sec). For control experiments, rats received sham trials consisting of either a different odor (eugenol) without foot-shock or scent-free paper without foot-shock. Contextual- and anisole-cue fear conditioning caused increases in freezing behavior, LSNA, and AP, a decrease in heart rate, and no changes in RSNA. Eugenol and scent-free paper did not cause significant changes in CeANA, RSNA, or LSNA. These data suggest that the amygdala contributes significantly to the acquisition of fear memories induced by odor conditioned stimuli, causing differential changes in sympathetic nerve activity. COI:No

3P-054

Effects of stimulation of cervical sympathetic nerve and head-down postural rotation on the cerebral blood flow

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This study was undertaken to elucidate the neural mechanisms controlling cerebral blood flow (CBF) during head-down postural alterations, using anesthetized rats and rabbits. We examined first effects of electrical stimulation of the cervical sympathetic nerve (CSN) on CBF in the parietal cortex. Electrical stimulation at 30 Hz of the CSN for 30 seconds induced a transient increase of CBF which was followed by a decrease of the flow, and then the CBF returned toward the pre-stimulation baseline level. The decrease in flow was eliminated by venous injection of phenoxybenzamine, suggesting that it probably is elicited by vasoconstrictive response of arteries to noradrenaline. Next, changes in CBF and activity of the CSN and renal sympathetic nerve (RSN) during head-down rotation (HDR) were studied. The animal was mounted on a table, tilted to a 45 degree head-down rotation in 5 seconds and kept at the posture for 2 minutes. HDR induced a transient decrease of CBF which was followed by an increase in the flow and then recovered to the pre-HDR level within a minute. Activity of CSN did not decrease during HDR, although activity of RSN decreased during HDR. These results suggest that CSN activity might be associated with controlling CBF transiently during HDR. COI:No

3P-055

Effects of restraint water-immersion stress on hippocampal CA1 neuronal and sympathetic nerve activities in conscious rats.

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The hippocampus has been thought to play a significant role in adaptation to chronic stress; however, there has been lack of direct evidence for changes in hippocampal neuronal activity during the adaptation process. The aim of the present study was to record hippocampal CA1 neuronal and sympathetic nerve activities during chronic stress. Wistar rats were chronically implanted with a combined probe made with multiple electrodes, for measurement of hippocampal CA1 neuronal activity and renal (RSNA) and lumbar (LSNA) sympathetic nerve activity. Rats were exposed repeatedly to restraint water-immersion stress (RWIS) for 90 min/day over 5 days. Hippocampal CA1 neuronal activity, RSNA, and LSNA were measured continuously and simultaneously before, during, and after RWIS. Hippocampal CA1 neuronal activity decreased immediately after onset of RWIS and the decreased level was maintained throughout the 90 min of RWIS on the first day of stress exposure. On day 5 of RWIS, hippocampal CA1 neuronal activity decreased immediately after onset of RWIS, however it gradually recovered to the control level during the RWIS period. Thus, hippocampal CA1 neurons gradually adapted to the repeated RWIS exposure. In contrast, RSNA and LSNA increased immediately after the onset of RWIS. The increased levels were maintained throughout the RWIS period and showed no adaptive response during repeated exposure to RWIS. COI:No

3P-056

Fatigability closely associates with discoordination between Heart Rate Variability and Physical Activity during free-moving days in younger Women

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Fatigability relates to numerous diseases and also to autonomic nervous system. We investigated whether or not fatigability is associated with the coordination between the physical activity (PA) and heart rate variability (HRV). Ninety-five adult women were divided into younger and older. The younger group comprised 50 women aged 22-59 years, and the older group comprised 45 women aged 60 years and more. HRV and PA data were simultaneously obtained every minute for 24 hours during the free-moving day. The ratio of low frequency/high frequency and HFnu were used as HRV indices. We defined %Lag0 as the % ratio of the lag = 0 min between HRV and PA in 1 hour. Cornel Medical Index was used to determine the degree of physical and psychological symptoms. In younger group, subjects with high fatigability scores had significantly lower %Lag0 between HRV and PA in the hour before sleep, compared with those with low fatigability ($p<0.05$), but not significant in the hour after wake-up. In older group, there were no significant differences in %Lag0 between high and low fatigability score groups both inth hour before sleep and after wake-up. These results suggest that the coordination between HRV and PA diminished by fatigability in younger, particularly in the hour before sleep during the free-moving days. COI:No

3P-057

Short response latency of hypothalamic waking-specific neurons to auditory arousal stimulus

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Many studies have strongly suggested that hypothalamic orexin neurons are involved not in the initiation but in the maintenance of wakefulness. We have, however, reported that the identified orexin neurons started their firing almost simultaneously at the onset of EEG changes in spontaneous awakening in mice. Furthermore, we have found that orexin neurons very quickly responded to the stimuli, not only in relation to spontaneous awakening, but also to the evoked awakening by external stimuli (Takahashi et al. 2008). However, the precise latency of the responses remains undetermined. In the current study, we recorded spontaneous neuronal activity across sleep-wake cycles in the rat perifornical area, where orexin neurons exist, and measured the latency of their firing after the onset of changes in local field potentials in the ventral cochlear nucleus (VCN) in response to hand-clapping during slow-wave sleep. Waking-specific neurons, which fired only during wakefulness and possibly included orexin neurons, showed a very short latency of 9.5 $\pm$ 2.4 ms (n = 23) after VCN activation, while the other types of waking-active neurons responded with a latency longer than 100 ms. The latency of the startle reflex observed in the neck muscle activity was 7.3 $\pm$ 1.8 ms. These results indicate that waking-specific orexin and non-orexin neuron receive ascending inputs originating from auditory arousal stimuli as early as the muscular responses to the stimuli via the startle reflex pathway, suggesting that these neurons may play some supplementary roles in the start of wakefulness. COI:No

3P-058

Do Mozart's and Bach's music have a relaxation effect?

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It has been commonly proposed that listening to Mozart's music has beneficial effects on physical and mental health. Moreover, it has been reported that Bach's music induces relaxation, similar to Mozart. Despite these widespread claims, few scientific or clinical studies have shown compelling evidence demonstrating relaxation effects of both composers' music. We aimed to evaluate the effects of Mozart's (K448) and Bach's (BWV1049) music on heart rate (HR), blood pressure (BP), and HR variability in young adults. After 10 min of rest, 17 healthy volunteers underwent three tasks in a randomized order: an examination to induce a mental workload and two 8-min periods of listening to K448 and BWV1049, separately. A control group underwent 8-min periods of silence. An electrocardiogram continuously measured HR parameters from the start of each task to completion. There were significant decreases in HR and diastolic BP when listening to both composers and during silence in controls, with no significant change in systolic BP in any conditions. High frequency power (HF) did not significantly change in BWV1049 and control; however, HF significantly decreased in K448. The low frequency (LF)/HF ratio was not significantly lower after the examination compared to other tasks. We did not identify significant changes in HR, BP, or LF/HF in young adults while listening to music from both composers; however, HF decreased after listening to K448. Our findings suggest that Mozart's and Bach's music yields little relaxation effect, indicated by unchanged HR, BP, and autonomic nervous activity. COI:No

3P-059

Identification of critical amino acids in the C-terminal of TREK-2 K^+ channel for ATP- and pH_i-sensitive regulation

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TWIK-Related two-pore domain K+ channels (TREKs) are regulated by intracellular pH (pHi) and PI(4,5)P2. Previously, Glu306 in proximal c-terminal (pCt) of mouse TREK-1 was identified to sense the acidic pHi activation. However, the direction of PI(4,5)P2 sensitivity is controversial between research groups. Here we investigate the residues of Ct for the pHi and ATP-sensitivity in human TREK-2 (hTREK-2). In inside-out patch clamp recordings ($I_{TREK-2,i-o}$), acidic pHi-induced activation was absent in E332A, and partly attenuated in E335A. Neutralization of cationic Lys (K330A) also eliminated the acidic pHi-sensitivity. Unlike the inhibition of wild type (WT) $I_{TREK-2,i-o}$ by intracellular ATP, neither E332A nor K330A was inhibited by ATP. Neutralization of triple Arg (R355-7A) suppressed the basal activity of $I_{TREK-2,i-o}$. R355-7A could still be activated by acidic pHi. In whole-cell recordings ($I_{TREK-2,w-c}$), K330A and E332A showed higher basal activity. $I_{TREK-2,w-c}$ of R355-7A was markedly lower than wild type, while showing prominent activation by arachidonic acid. The results suggest concerted roles of the charged residues Lys330 and Glu332 for the inhibition by physiological PI(4,5)P2 and activation by acidic pHi. The more distal triple Arg355-7 might play as a pivotal role for the spontaneous activation of TREK-2 under the ATP-free condition, i.e. disinhibition from the intrinsic PI(4,5)P2. COI:No

3P-060

Disruption of negative feedback regulation by Ca^{2+}-calmodulin is a cause of gain-of-function in renal channelopathy

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Focal segmental glomerulosclerosis (FSGS) is an important cause for the end-stage kidney diseases, some of which are linked to TRPC6 gain-of-function mutations. Therefore, inhibitory regulation of this channel could have a profound influence on the progression of FSGS. Ca^{2+}-dependent inactivation (CDI) mediated by calmodulin (CaM) is critical for regulating TRPC6 channel activity, however, its underlying mechanisms and contribution to FSGS are yet unveiled. CaM lobe mutations in both N- and C-lobes of CaM equally impaired CDI. Stoichiometric experiments concluded a 1:2 binding of CaM to CBDs of TRPC6. These results indicated that CDI could be explained by the close proximity of two CBDs due to a 'bridge' mechanism via each CaM lobe binding. Furthermore, we found that the coiled-coil segment adjacent to the CBD of TRPC6 contributes to maintaining the proximal distance of two CBD's by its self-assembly and deletion of the coiled-coil segment caused a marked delay of CDI. Coiled-coil mutations found in some FSGS mutants also delayed CDI, thus their excessive activities may be explainable by the disruption of negative gating modulation via CaM. Our results provide a mechanistic insight into CDI which is orchestrated by the assembly of CaM-CBD complex and coiled-coil segment of TRCP6 channels. COI:No

3P-061

WNK3 kinase regulates inwardly rectifying potassium current in layer V pyramidal neurons of the mouse prefrontal cortex

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WNK kinases are an essential element of the signaling cascade regulating internal Cl^- concentrations in pyramidal neurons. WNK3 kinase also regulates RBFOX1, an RNA binding protein generating splice variants of ion channels and synaptic proteins. Therefore WNK3 kinase may affect the functioning of pyramidal neurons.Using WNK3 knockout mice we examined electrophysiological properties of layer V prefrontal pyramidal neurons at P21-27 in brain slice preparations. Our results indicate that loss of WNK3 activity caused a hyperpolarized resting membrane potential(RMP) and changes in membrane excitability. Both single and repetitive AP firing suggest alterations in multiple channel properties. Changes in synaptic inputs are also indicated.Voltage clamp recordings to ascertain the contributors to the hyperpolarized RMP, indicate a key regulation of the inward rectifying potassium (IRK) conductance. However ambient GABA mediated $GABA_B$ receptor coupled Girk channel currents were not affected indicating involvement of classical inward rectifier channels. This enhancement of IRK conductance in WNK3 KO mice was phosphorylation dependent as demonstrated by a rescue experiment with an active WNK3 fragment which reversed the K^+ currents to control values. We further analyzed the phosphorylation changes in the $K_{ir}2.X$ channel subunits to confirm their identities. COI:No

3P-062

Optimization of experimental conditions in intracellular calcium mobilization measurement at the single cell level

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Introduction Orexin is a hypothalamic neuropeptide regulating sleep/wakefulness. We have discovered a nonpeptide orexin type 2 receptor (OX2R)-selective agonist, YNT-185, using CHO cells stably expressing human OX1R or OX2R for evaluation of intracellular Ca mobilization activity. However, making stably transfected cells takes a long time. There are also a number of reports of heterodimeric interactions between co-expressed pairs of GPCR and it changed the response to its ligand. The purpose of this study is to establish a robust measurement of intracellular Ca mobilization in single cells transfected with two GPCRs using Functional Imaging Cell-sorting System (IMACS, Hamamatsu Photonics).

Methods We constructed 2 types of vectors, pEF1α-mOX1R-mCherry and pEF1α-mOX2R-eGFP. We transfected pEF1α-mOX1R-mCherry or pEF1α-mOX2R-eGFP, or co-transfected both, in HEK293 cells or CHO cells. To evaluate the intracellular Ca mobilization in single cells using Fura-2 indicator, we optimized the density, size and intensity of cells by Gate function of IMACS.

Results We confirmed that these constructs were expressed and localized on the plasma membrane, whether alone or in combination of the two vectors. We established the density, size and intensity of cells for intracellular Ca measurement. Now we are estimating the effect of two co-expressed receptors on the intracellular Ca mobilization activity in CHO cells. COI:No

3P-063

Electrophysiological analysis of transmembrane channel-like protein (TMC) 4

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Transmembrane channel-like (TMC) family consists of eight known isoform (TMC1 to TMC8). The TMC1 and TMC2 have been proposed to form a mechanoelectrical transducer (MET) channel of outer hair cells and mutations of these proteins contribute to deafness. TMC6 and TMC8 mutations are known to increase the risk of skin carcinoma. Although it is possible that the TMC proteins play important physiological roles, the function of TMC proteins is poorly known. In the present study, we could clone TMC4 from cDNA library of human colonic cancer KM12-L4 cells. Then, we investigated the electrophysiological properties of the TMC4 protein using the whole-cell patch-clamp recordings. Under physiological experimental conditions, the TMC4-expressing HEK293T cells exhibited larger voltage-independent currents with the reversal potential near 0 mV compared with mock-transfected cells. The ion selectivity of TMC4 channels was $K^+ = Cs^+ > Na^+$. The TMC4-dependent currents were inhibited by an application of Gd^{3+}, a non-selective cation channel blocker. In addition, cell poking in patch-clamping cells induced the transient TMC4-dependent current. These results suggest that TMC4 is a non-selective cation channels sensitive to mechanical stimuli. COI:No

3P-064

TRPA1 channel contributes to oxygen sensing in placenta

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Molecular oxygen (O_2) is a prerequisite for cellular respiration in aerobic organisms. It is therefore fundamental that aerobic organisms sense and respond to hypoxia, thus allowing them to adapt to variable habitats and physiological situations. Recently, our group has shown that the TRPA1 channel in vagal nerves acts as a novel hypoxia sensor responsible for acute responses. These findings suggest that there are different O_2-signaling mechanisms that respond to varying degrees of hypoxic stimulus. The placenta is the highly specialized organ of pregnancy that supports the normal growth and development of the fetus, and acts to provide oxygen and other nutrients to the fetus. Here, we studied O_2-sensing by TRPA1 in placenta and/or umbilical cord. Immunostaining and RT-PCR showed that TRPA1 is expressed in human umbilical vein endothelial cells (HUVECs). In addition, TRPA1 shows hypoxia-induced Ca^{2+} entry in HUVECs. This Ca^{2+} response was inhibited by the TPRA1-specific blocker HC-030031. Furthermore, all of pregnant *Trpa1* knockout mice are died when they induce hemolytic anemia from 9.5 days post-coitum (dpc) to 11.5 dpc, which time is known as the important day to form umbilical cord. This phenomenon is completely different from pregnant wild-type mice. Thus, acute hypoxia disables pregnant *Trpa1* knockout mice not only from continuing the pregnancy but also from surviving. Taken together, these findings suggest that TRPA1 play an important role in O_2 sensing at placenta and/or umbilical cord. COI:No

3P-065

SLCO2A1 is involved in a pathway for ATP release from cultured cells and Langendorff-perfused hearts

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Cellular release of ATP is a key event in powerful purinergic signaling in animal tissues. Released ATP is known to play a protective role in ischemia/reperfusion heart injury. We showed that ATP release is mediated by Maxi-Cl channels in many cell types including cardiac myocytes. Since we have recently identified SLCO2A1, which is known to be a prostaglandin transporter (PGT), as the core component of Maxi-Cl channel, we examined here whether SLCO2A1 is involved in the machinery of ATP release. Swelling-induced ATP release from Maxi-Cl activity-rich C127 cells was inhibited by a known PGT antagonist and *Slco2a1*-targeting siRNA. Heterologous expression of SLCO2A1 in Maxi-Cl activity-deficient HEK293T cells elicited Maxi-Cl channel activity and augmented swelling-induced ATP release. When Langendorff-perfused mouse hearts were subjected to oxygen-glucose deprivation (OGD), ATP was, in a manner sensitive to a PGT antagonist, released from the hearts to the coronary effluent upon reoxygenation with glucose-containing perfusate. This OGD-induced cardiac ATP release was suppressed when mice were pre-injected in vivo with *Slco2a1*-targeting siRNA. Thus, it is concluded that SLCO2A1 is essentially involved in a pathway for ATP release from cultured cells and Langendorff-perfused hearts. (COI:NO) COI:No

3P-066

Screening of oxidative stress sensors in TRPM7 channels

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We have previously shown that ion channel activity of TRPM7 is inhibited by oxidative stress induced by an application of H_2O_2. The TRPM7 current is inhibited by intracellular Mg^{2+}, and the oxidative stress increases its Mg^{2+} sensitivity. The present study aimed to identify the oxidative stress sensor in TRPM7 by site-directed mutagenesis. Since a cysteine modulating reagent, N-methyl maleimide inhibited TRPM7 current in a similar way to H_2O_2, cysteine residues are likely targets for oxidation to inhibit the channel activity. Among 36 cysteines contained in mouse TRPM7, one or more cysteines were either deleted or substituted with alanine or serine. Each of 30 mutant channels was expressed in HEK293 cells, and the effect of H_2O_2 on its current was tested. Whole-cell patch clamp recordings revealed that mutation of cysteines other than C1809 and C1813 (both located in a zinc finger motif at the carboxyl terminal) had no effect on inhibition of the channel activity by H_2O_2. Mutation of either C1809 or C1813, as well as deletion of whole zinc finger motif, caused marked reduction of the channel activity, hence the inhibitory effect of H_2O_2 could not be tested. We conclude that the zinc finger motif is important for TRPM7 channel activity and is the most plausible candidate as the oxidative stress sensor, through which H_2O_2 induces conformational changes of the channel to increase its intracellular Mg^{2+} sensitivity that results in the current inhibition. COI:No

3P-067

Structural and electrophysiological analysis of the ion selectivity of prokaryotic sodium channel

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The selective permeation of sodium ion by voltage-gated sodium channel (Nav) plays the main role in the transition of the action potential in the neural cells.

We evaluated the electron density in the selectivity filter of the crystal structure of NavAb, which is homologue of prokaryotic Nav (NavBac), and mutants under various cationic conditions. Depends on the ionic radius or hydrogen exchange ratio of each cation, the different election density was observed. Especially, the side chain of extracellular serine and the main chain of intracellular leucine residues of the selectivity filter seem to play dehydration checkpoint of the permeation of the hydrated cations.

The smaller-residue mutations of the serine residue increased the lithium ion selectivity. Lithium ion has smaller ionic radius but more slowly exchanges hydrated water than sodium ion. Hydroxyl group of serine side chain is thought to concern with the dehydration of the permeating ion. Additional water was observed above the selectivity filter of serine-to-glycine mutant. The larger-side chain mutation of leucine residue lost the ionic current and rendered narrow the radius of ion pore in the crystal structure.

These results suggested that the two residues of the selectivity filter discriminate the ionic radius and have a dehydrating function the hydrated cations. COI:No

3P-068

Properties and roles of flufenamate-sensitive ion channels stimulated by hyperosmolality in vasopressin neurons

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Brain osmosensory magnocellular neurons producing arginine vasopressin (AVP) respond with enhanced secretion of AVP to increased osmolality of body fluid. It was previously reported that a type of cation channel, called stretch-inactivated cation channel (SICC), is activated in these cells by hyperosmotic stress. The SICC currents were insensitive to capsaicin but sensitive to ruthenium red. Since we also observed hypertonicity-induced activation of ion channel currents in AVP neurons isolated from the supraoptic nucleus (SON) of AVP-eGFP-TG rats, we here first studied the properties of hypertonicity-activated ion channel (HAIC) currents. The HAIC activity was found to be sensitive to flufenamic acid (FFA) as well as to capsazepine but not to ruthenium red. At the previous PSJ meeting (in 2014), we reported that AVP neurons possess the ability of regulatory volume increase (RVI) after shrinkage under sustained hypertonic conditions. We thus next studied the roles of HAIC in the RVI, because FFA unmasked the RVI process. The HAIC activation was found to bring about cell depolarization and stimulation of tetanus toxin-sensitive AVP secretion. Thus, it is concluded that FFA-sensitive ion channels, distinct from SICC, are activated by hypertonicity to stimulate the exocytosis of AVP, which results in additional shrinkage, thereby apparently masking the RVI process. COI:No

3P-069

A Landscape for Structural Stabilities during Gating Transitions in the KcsA Potassium Channel

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We have measured twisting conformational changes of the KcsA potassium channel upon gating by the diffracted X-ray tracking method. In this method, single-molecule conformational changes were tracked by the motions of the X-ray diffraction spot from a gold nanocrystal attached to the channel as a probe. Previously we reported the twisting angles showed wide distributions, which seemed to have some discrepancy with two states, an open and a closed states, model. To examine the reason for the wide angle distributions, we fabricated new probes for recordings to change the load for the motions. Here we report the stepwise twisting motions of the channel recorded with the new probes. The residence time for each twisting angle differed significantly, which reflected the structural stability of the angle. This residence time map provided a landscape for structural stabilities during gating. The previously recorded wide twisting angle distributions can be explained by the existence of these multiple structural stable states. COI:No

3P-070

Functional expression of inwardly rectifying K^+ channel in glioblastoma stem-like cells

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Introduction: Glioblastoma multiforme (GBM) is the most fatal malignant primary brain tumor. GBM contains functional subsets of cells called glioblastoma stem-like cells, which are radioresistant and chemoresistant and eventually lead to tumor recurrence. However, the identity of ion channels in glioblastoma stem-like cells has not been extensively investigated. **Objectives:** The present study aimed to identify functional ion channels in glioblastoma stem-like cells. **Methods:** We established the stem-like cells from human GBM using three-dimensional cell culture and measured whole-cell currents using patch-clamp techniques. **Results:** We found inwardly rectifying K^+ (Kir) and voltage-gated K^+ currents in the glioblastoma stem-like cells. The Kir current was observed in 19 out of 25 cells. An inward conductance of the Kir currents was proportional to $[K^+]_o^{0.48}$. The selectivity sequence based on conductance ratios was K^+ (1.00) > Rb^+ (0.76) >> Cs^+ (0.10) > Na^+ (0.05). The currents were blocked by extracellular Ba^{2+} and Cs^+ in a voltage- and a concentration-dependent manner, with K_d values at 0 mV of 95 μM and 93 mM, respectively. **Conclusion:** These results indicated that Kir channels contribute to setting membrane potential in glioblastoma stem-like cells and have the potential to be therapeutic targets in GBM. COI:No

3P-071

Regulation of CALHM1 channel gating and association with lipid microdomains by protein S-palmitoylation

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Emerging roles of CALHM1, a novel voltage-gated ion channel, include neurotransmission of tastes in taste buds and memory formation in the brain, highlighting its physiological importance. However, the regulatory mechanisms of the CALHM1 remain entirely unexplored, hindering full understanding of its contribution in vivo. In fact, the different gating properties of CALHM1 in vivo and in vitro suggest undiscovered regulatory mechanisms. Here, we discovered the regulation of CALHM1 gating and association with lipid rafts via S-palmitoylation. CALHM1 is palmitoylated at two intracellular cysteines. Enzymes that catalyze CALHM1 palmitoylation are identified by screening 23 members of the DHHC protein acyltransferase family. Epitope-tagging of endogenous CALHM1 proteins in mice reveals that CALHM1 is basally palmitoylated in taste buds in vivo. Functionally, palmitoylation downregulates CALHM1 without effects on its synthesis, degradation, and cell surface expression. Mutation of the palmitoylation sites has profound impact on CALHM1 gating, shifting the conductance-voltage relationship to more negative voltages and accelerating the activation kinetics. The same mutation also reduces CALHM1 association with detergent-resistant membranes. Our results comprehensively uncover a post-translational regulation of the voltage-dependent gating of CALHM1 by palmitoylation. COI:No

3P-072

Simulation Analysis of GLP-1-RegulatedMembrane Excitability And $[Ca^{2+}]_i$ Dynamics In Pancreatic β-Cells

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Pancreatic β-cells generate bursts of action potentials in response to glucose, thereby inducing cyclic changes of $[Ca^{2+}]_i$ that drive pulsatile insulin release. The incretin hormone GLP-1 potentiates this insulin secretion at least in part by regulating membrane excitability and Ca^{2+} dynamics. However, it remains to be determined exactly how cellular factors interact in a dynamic manner to contribute to the effects. Here, we apply simulation analysis to provide the first quantitative explanation for the established ability of GLP-1 to promote bursting activities in β-cells. Building on our prior model, we expand the analysis to include the ability of GLP-1 to exert stimulatory effects at IP_3 receptors (IP_3R) and the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) located in the endoplasmic reticulum (ER), while also modeling its effects at plasma membrane voltage-gatedCa^{2+} channels (VGCC), delayed rectifier K^+ channels, ATP-sensitive K^+ channels, and nonselective cation channels (NSCCs). Unexpectedly, simulation analysis reveals that a stimulatory action of GLP-1 at NSCCs underlies its ability to shorten the time interval separating bursts of action potentials. Simultaneously, GLP-1 prolongs the action potential burst duration not only from its stimulatory effect at VGCCs, but also from its ability to promote IP_3R-dependent release of Ca^{2+} from ER Ca^{2+} stores. COI:No

3P-073

Comparison of electrophysiological properties of P2X7 receptors between neurons and glial cells in rat trigeminal ganglion

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P2X7 receptor, which is one of the ionotropic P2X receptors, has been suggested to contribute to regulating and/or generating neuropathic pain. The aim of this study was to investigate biophysical properties of P2X7 receptor in each neuron and glial cell that are obtained from rat trigeminal ganglions. Under whole-cell patch-clamp recordings, applications of 100uM ATP and 100 uM Bz-ATP induced biphasic inward currents in both the neurons and glial cells. The current density (pA/pF) of the Bz-ATP-induced current in glial cells was significantly larger compared to that recorded from the neurons. Repeated application of these agonists caused desensitizing effect on the currents in both the neurons and glial cells. In both the neurons and glial cells, 100 uM Bz-ATP-induced inward currents were significantly suppressed by selective antagonist for P2X7 receptor (6 uM A-740003). Current decay time constant of the inward currents was significantly larger in glial cells, suggesting that there are biophysical differences in the P2X7 receptor-mediated currents between glial cells and neurons of the trigeminal ganglion. COI:No

3P-074

Divergent distribution of D1 or D2 dopamine receptor-expressing astrocytes in substantia nigra, striatum, and visual cortex of adult mouse

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Dopaminergic neurons in substantia nigra pars compacta (SNc) release dopamine (DA) from their dendrites (dendritic release) extending deeply into the adjacent nucleus SN pars reticulata (SNr), which consists mostly of GABAergic projection neurons. Although extremely dense immunoreactivity for D1 dopamine receptor (D1R) is found in SNr, no change in firing was detected in most (46/48) GABAergic SNr neurons when DA was administered in acutely dissociated condition. Interestingly, we found strong D1R immunoreactivity on fine processes of GFAP-expressing astrocytes, and not on neurons, in the SNr of adult mice, suggesting a role of astrocytes in DA-mediated information processing in this nucleus (Nagatomo K et al, ***Front Neuroanat***, 2017). No such D1R immunoreactivity was detectable in visual cortical astrocytes and neurons. In striatum, in contrast, not only D1R-positive but also D1R-negative astrocytes were detected as known well for neurons there. In addition, triple immunostainings for D1R, D2R, and GFAP suggest marked heterogeneity in D1R and D2R expression in astrocytes in the striatum as known for neurons. COI:No

3P-075

Effects of lipid bilayer fluidity on clustering-dispersion of the K+ channel KcsA

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Ion channels often form self-assembled cluster in cell membranes. However, the little is known about the molecular mechanisms of the self-assembly. Recently we have revealed the gating-associated clustering-dispersion of pH-dependent potassium channel KcsA by atomic force microscopy (AFM). The channels were clustered when the gate was closed at neutral pH, whereas they were dispersed when the gate was opened at acidic pH. Here, to examine the effect of bilayer fluidity on the channel dispersion, the KcsA channels were reconstituted in several types of the membrane having different fluidity. AFM observation of the reconstituted membranes demonstrated that the fluidic membrane remarkably facilitated the dispersion at acidic pH. In the cholesterol-containing bilayer, the channels induce phase-separated structure of the membrane with different height and the channels were localized in thinner phase. The lipid might modulate the clustering, localization and function of the channels in membranes. COI:No

3P-076

Roles of channels and transporters in epithelial-mesenchymal transition on head and neck epithelial cell lines.

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The epithelial-mesenchymal transition (EMT) in head and neck tissue is reported as an important deriver of tumor progression and organ fibrosis. Transforming growth factor- β 1 (TGF- β 1) and some substance are known as trigger factors of EMT, but the whole system and detail of EMT are still unknown. Channels and transporters of epithelium involves cell volume regulation by transporting ions and driving water molecule. Therefore, epithelial channels and transporters may be important factors for morphological change such as cell differentiation and transdifferentiation. The aim of this study is to investigate the roles of channels and transporters in EMT of head and neck epithelial cell line using those blockers and activators.

OSC-20 cell line (human oral squamous cell carcinoma) treated with some ion channel blockers obviously showed mesenchymal morphology. Furthermore, the expression levels of mesenchymal marker vimentin in OSC-20 cell line treated with ion channel blockers were 20-fold higher than that of non-treated OSC-20 cell line.

These findings suggest that some ion channels expressed in OSC-20 cell line are closely related to EMT. COI:No

3P-077

Expression of Olig2 in neuronal cell division

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Olig2, a basic helix-loop-helix transcription factor, is expressed in neuronal progenitor cells and oligodendrocytes and plays a role in the neurogenesis in brain. Recently, it is revealed that not only neuronal progenitor cells but also neurons show cell division. The present study investigated the expression of Olig2 in newly divided neuronal cells. Neuronal cells were cultured and their cell division was observed under time-lapse microscopy. Then, cells were fixed with 4% paraformaldehyde and stained with neuronal and glial cell markers. All of newly divided neuronal cells were stained with the mature neuronal cell marker MAP2 but not the astrocyte marker GFAP or the oligodendrocyte marker O4. Twenty four percent of newly divided MAP2-positive neuronal cells expressed Olig2. Interestingly, just after cell division (within 30 min), no neuronal cells expressed Olig2. Non-divided differentiated neurons were not stained with Olig2. Thus, neuronal cells having division potential may express Olig2 after cell division and until differentiation and play a role in neurongenesis. COI:No

3P-078

Microscopic observation analysis of cold-stress damaged HeLa cells

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HeLa cells suffered severe damage during 24 hr incubation at 4° C. The cell was swollen and intracellular small vesicles had been almost disappeared; however, the plasma membrane looks like still intact. The aim of this study is to evaluate the effect of extracellular ion condition on cold-stress viability by microscopic observation. We attempted to incubate the cell in several media, which substituted cation and anion instead of NaCl in basal medium. Na substitution by the other alkali metals did not improved cold-stress damage and the cells were swollen. In the case of Cl substitution by gluconate, the cells were not swollen. In this study, we performed microscopic observation to evaluate intercellular organelle (lysosome and mitochondria) condition. COI:No

3P-079

Re-evaliation of cell swelling mechanism in cold responses of HeLa cells

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HeLa cells show cell swelling during 24 hr incubation at 4° C. The cell swelling in cold stress has been considered as the result of Na^+/K^+ pump inhibition. We compered the cell swelling in the cold stress and the application of Na^+/K^+ pump inhibitor. The cell swelling in cold stress was inhibited by Cl- channel inhibitor DIDS. Propidium iodide did not enter into the cell in 24 hr incubation at 4° C. Ouabain treated cells at 37° C showed also cell swelling, however the blleb structure was observed in several hours. The Cl^- channel inhibitor could no inhibit quabain-induced cell swelling and the membrane integrity was damaged. The difference of these two conditions may be explained by the enzymatic activities induced by cell swelling in different temperature, however the further experiments should be done for the conclusion. COI:No

3P-080

Effect of Cs+ ion on the cellular metabolism

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We have reported that cesium ion inhibits the growth of HeLa cell by the inhibition of glucose metabolic pathway. In this meeting, we will show more detail effect of cesium ion. By addition of 10 mM Cs, mRNA levels of glycolysis genes, such as GAPDH, PKM2, LDHA and LDHB decreased in HeLa cells. We also observed the same inhibition pattern of each protein by western blot analysis. The activity of glycolysis enzymes in the crode extract from HeLa cells was examined. We observed the inhibition of activities of each enzymes by 10 mM cesium by spectrophotometric assay. Those data indicate that Cs ion inhibits cell growth via both inhibiton of gycolysis enzyme expression and function of each enzyme. COI:No

3P-081

Analysis of Molecular and Cellular Roles of the GON domain in Calcium homeostasis

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ADAMTS9 is a metalloprotease that cleaves components of the extracellular matrix and is also implicated in intracellular protein transport. We found that the downregulation of human ADAMTS9 and its *C. elegans* orthologue GON-1 results in the inhibition of protein transport from the endoplasmic reticulum (ER) to the Golgi apparatus. ADAMTS9 has a unique C-terminal domain called the "GON domain". The function of intracellular protein transport is dependent on the GON domain but independent of protease activity. However, molecular mechanisms of ADAMTS9 in cells remain unknown.

To investigate the role of ADAMTS9/GON-1 in the secretory pathway, we searched for genes whose depletion suppressed the *gon-1* phenotype. We identified several suppressor genes. To determine whether the GON domain interacts with the suppressor genes, we performed immunoprecipitation experiments in HEK293 cells transfected with the Flag-tagged GON domain and Myc-tagged candidate proteins. We found that the GON domain interacts with several suppressor gene products.

Previous reports indicated that calcium is important for the transport of secretory proteins from the ER. We investigated whether GON-1 is involved in calcium homeostasis. We found that calcium homeostasis is compromised by GON-1 depletion. Now, we are investigating whether the above-mentioned suppressor genes have a role to play in calcium homeostasis. COI:No

3P-082

Ambroxol-stimulated increases in CBA and CBF via pH_i increase and $[Cl^-]_i$ decrease in airway ciliary cells of mice.

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Ambroxol (ABX), a secretolytic agent, regulates many cellular events in airway ciliary cells: e.g., ABX increases ciliary beat frequency (CBF) and ciliary bend angle (CBA). However, the regulatory mechanism of ABX on CBF or CBA is unknown. We found that ABX induced an increase in the intracellular pH (pH_i) and a decrease in the intracellular Cl^- concentration ($[Cl^-]_i$), the role of which in the regulation of CBF and CBA was clarified using mice in the present study. The ABX-induced increase in pH_i was abolished by DIDS (a blocker of Na^+-HCO_3^- cotransporter (NBC) and Cl^-/HCO_3^- exchanger (anion exchanger: AE)), while it was enhanced by Cl^- removal from the extracellular solution (blockade of AE). These observations suggest that NBC and AE play respectively positive and negative roles in the ABX-induced increase in pH_i. DIDS abolished the ABX-induced increase in CBF, but the Cl^- removal enhanced CBF similar to those on pH_i, suggesting ABX elevates CBF via an increase in pH_i. The Cl^- removal elevated CBA independent of the pH_i associated with a decrease in $[Cl^-]_i$, which was associated with a decrease in cell volume and abolished by blocked of the cell volume decrease. This observation taken together with the observation of the ABX-induced decrease in $[Cl^-]_i$ suggests that the decrease in $[Cl^-]_i$ elevates CBA. In conclusion, ABX inducing pH_i elevation and $[Cl^-]_i$ decrease increased CBA and CBF in airway ciliary cells of mice. COI:No

3P-083

The spatiotemporal control of Ras-PI3K signaling and endocytosis

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Ras, a member of small GTPases, regulates various functions through spatiotemporal control of effectors. We have previously clarified that the Ras- phosphoinositide 3-kinase (PI3K) complex is preferentially translocated from the plasma membrane to the endosomes upon growth factor stimulation and Ras-PI3K signaling thereby promotes endocytosis. However, the molecular mechanism of the Ras-PI3K translocation remains unclear. By comparison of the amino acid sequences of effector molecules, we found a 28 amino-acid-length sequence specific to PI3K that was named Ras-PI3K endosomal localization (RAPEL) sequence. PI3K deficient in RAPEL sequence bound to Ras as did the wild type, but failed to be translocated to the endosomes. Moreover, overexpression of RAPEL sequence suppressed translocation of the Ras-PI3K complex to the endosomes, indicating the presence of a regulator(s) for Ras-PI3K signaling through the interaction with RAPEL. Therefore, we screened the interacting molecules using a proteomics approach, and identified a subunit of P-ATPases as a candidate. The binding of the subunit of P-ATPase to RAPEL was confirmed by immunoprecipitation. When the protein was expressed as a GFP-tagged form in mammalian cells, it was localized in the endosomes in addition to the endoplasmic reticulum, suggesting that this molecule plays a role in endocytosis. Indeed, the overexpression of the protein promoted endocytosis, implicating it in the control of endocytosis through spatiotemporal regulation of Ras-PI3K signaling. COI:No

3P-084

Auto-regulation of osteoblast mechanosensitivity via $P2Y_2$ receptor

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We previously demonstrated that mechanical stimulation (MS) of osteoblast (OB) elicited Ca^{2+}-dependent afferent signal transmissions from OB to sensory neurons in co-culture system. This transmission is found to be elicited by opening stretch activated calcium channel of OB and followed by release of ATP. The aim of the present study was to reveal the mechanotransduction mechanism for the bone homeostasis. We focused on the contribution of $P2Y_2$ auto-receptor and its downstream signaling mechanism mediated via Rho kinase (ROCK) to mechanosensitivity of OB. We used OB isolated from calvaria of neonatal mice and cultured for 7 days and examined $[Ca^{2+}]i$ increasing response to repeated MS to compare the intensity ratio of the 2nd response to the 1st response. In control group, the 2nd one decreased to approximately 40%. However, in $P2Y_2$ receptor antagonist-treated group the 2nd one was significantly larger than that in control group. Furthermore, we obtained similar results in ROCK antagonist-treated group. These results suggested that $P2Y_2$ auto-receptor and its downstream signaling mechanism mediated via ROCK regulates mechanosensitivity of OB by release of ATP. COI:No

3P-085

Exocytic mechanism analyzed with fluorescence and two-photon microscopy.

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We have investigated the SNARE assembly and PKA activity in the pancreatic beta cells using two-photon excitation microscopy and Forster resonance energy transfer (FRET) with fluorescent lifetime imaging. We also compared the data with cases of presynaptic terminals of cortical neurons. SNAP25 is one of the SNARE proteins at the target plasma membrane, and it carries two α helixes, SN1 and SN2. We measured intermolecular FRET ratio between mtq-SNAP25 and SN1-Venus-SN2. High binding fraction was observed at the presynaptic terminals. In contrast, in the pancreatic islet preparations, no significant assembly was detected. The high FRET signals in the synaptic boutons indicated the domain-swapped model; where SN1 bound to the SN2 of the other SNAP25 molecule, and formed oligomer. We further characterized synaptic transmissions rescued by several FRET probes of SNAP25 linker mutants in the cultured cortical neurons from SNAP25-deleted mice. Our data suggested that the domain swapping of SNAP25 with the linker of appropriate length was necessary for the ultrafast exocytosis in presynaptic terminals. We further prepared the FRET probe reflecting PKA activities, AKAR. We transfected the cDNA of AKAR into the pancreatic islets, and found heterogeneity among the cells. The region near the plasma membrane showed higher FRET ratio, and it was not correlated with the expression levels of fluorescent probes. We further analyzed the changes in PKA activities during the glucose stimulation, and studied the correlation with exocytosis. COI:No

3P-086

Identification of LOXL2 in the exosomal fraction upregulated prior to lymph node metastasis of a head and neck squamous cell carcinoma cell.

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Premetastatic niche is believed as one of the most important step of initiation of cancer metastasis, and elucidation of the molecular mechanism of this event is in an urgent need for cancer therapy. In the case of lung metastasis of breast cancer cell, lysyl oxidase (LOX) had been reported to be secreted from breast cancer cells and modulate construction of lung tissue extracellular matrix suitable for bone marrow derived cells (BMDC) to adhere, resulting in acceptance of metastasized cancer cells. We had established lymph node metastasis mouse model of human head and neck squamous cell carcinoma (HNSCC), and found premetastatic niche-like event in submandibular lymph node on tongue cancer metastasis with candidate factors responsible for the event. Lysyl oxidase like factor 2 (LOXL2) mRNA was found in this study as upregulated factor in primary head and neck squamous cell carcinoma supposed to induce CD31 positive structure constructed by BMDC. In the present study, we identified this enzyme in the exosome fraction derived from the conditioned medium of metastatic HNSCC but not in that of a non-metastatic cancer cell. Moreover, LOXL2 knockdown metastatic HNSCC cell exhibited decreased metastasis to lymph node. We would like to discuss as to how LOXL2 can act in lymph node metastasis of HNSCC. COI:No

3P-087

Partial Exposure of Frog Heart to High-potassium Solution: An Easily Reproducible Model Mimicking ST Segment Changes in Ischemic Heart Disease

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Ischemic heart disease, including myocardial infarction and angina pectoris, is a leading cause of morbidity and mortality in the world. In our previous study, by simply inducing burn injuries on the bullfrog heart, we reproduced abnormal ST segment changes in the electrocardiogram (ECG), mimicking those observed in ischemic heart disease. As for the physiological mechanisms, cellular damage caused by the injuries makes the extracellular concentration of potassium ions higher around the cells, making their resting membrane potential significantly higher than that of the adjacent intact cells. In the present study, instead of inducing burn injuries, we directly exposed the surface of the frog heart to high-potassium solution to create a concentration gradient of the extracellular potassium within myocardium. By simultaneously recording the cardiac action potential, we noted significant elevation of the resting membrane potential. The currents of injury, generated by the voltage gradient between the myocardium with high and normal potassium concentrations, negatively deflected the ECG vector during the diastolic phase and made the ST segment appear elevated during the systolic phase. In addition to subepicardial burn injuries, the partial exposure to high-potassium solution is also an easily reproducible model of heart injury. These frog heart models would be suitable to learn the mechanisms of ST segment changes observed in ischemic heart disease. COI:No

3P-088

A microprocessor-based general purpose closed loop data recording system for behavioral neuroscience

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Behavioral neuroscience study requires carefully designed and precisely implemented behavioral task to elicit neuronal activity of interest. Flexible designing and temporally precise execution of such a task, however, requires either high level of engineering skill of software and hardware or costly commercial toolkit. Therefore, I developed a microprocessor based task control system that allows users to freely design various behavioral tasks and simultaneously record physiological signals. The system consists of a master program that runs on a Windows based PC and a firmware running on a microprocessor that serves as a slave. These software communicate with each other via USB interface. The master program sends the slave firmware the structure of the behavioral task that users prescribe in an ASCII text file, then prompts the initiation of a trial. The firmware presents the stimuli prescribed by user, records subject's response as well as physiological data, and sends them back to the master at the end of a trial. As a practical application of this system, I built a toolkit to record electroencephalography (EEG) for student education. It consists of the above task-control system, and a custom-built amplifier for EEG recording. Taking advantage of the task control system's feature to flexibly design trials, I succeeded in building a versatile recording device that measures diverse kind of EEG signals and behavioral events with temporal precision of sub-millisecond. This task control system is expected to serve both in student education and in research project by faculty members. COI:No

3P-089

High-Calcium Exposure to Frog Heart: A Simple Model Representing Hypercalcemia-induced ECG Abnormalities

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Clinical studies have shown that hypercalcemia induces various abnormalities in electrocardiogram (ECG), including prolongation of the PR interval, increased amplitude of the QRS complex, Osborn waves, shortening of the QT interval and elevation of the ST segment. Using cardiac muscles isolated from canine hearts, previous in vitro studies revealed the morphological changes in the action potential induced by hypercalcemia. In the present study, by simply adding a high concentration of calcium solution to the surface of the bullfrog heart, we reproduced ECG abnormalities representing those observed in hypercalcemia, such as the Osborn waves and shortening of the QT interval. The rise in the extracellular calcium concentration may have activated the outward potassium currents during phase 3 in the action potential, and thus decreased its duration. In addition to the known decrease in the phase 2 duration, such changes in phase 3 were also likely to contribute to the shortening of the QT interval. The dual recordings of the action potential in cardiomyocytes and the ECG waves enabled us to demonstrate the mechanisms of the ECG abnormalities induced by hypercalcemia. COI:No

3P-090

Trial of physiology group study using 'Step-by-Step Study of Human Life Sciences'

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"Step-by-step study of human life sciences" is an innovative digital educational material with straight-forward illustrations/animations and simple multi-choice questions presented in very small steps for beginners. In the 2nd-year physiology course at a health-related junior college, the material was used in active learning; members of each group of 4 students explained representative illustrations of that lecture to each other. Understanding was assessed by having all students take a very short oral exam and an online test (consisting of questions randomly chosen from the question pool in the steps discussed in that lecture) at the end of the lecture. Opportunities for extra credit were given to the group in which all members passed both oral and online examinations. An anonymous survey (n=142) showed that 90.8% thought "the step-by-step material was more appropriate for group study than other materials", and that 88.7% thought "such learning makes it easier to memorize". This suggests the possibility that "step-by-step" is beneficial for group discussion and memorization. Overall 62.7% thought that "group discussion would be easier to understand than by listening to a review by the instructor". Further study is needed to initiate active learning more effectively at health-related colleges. COI:No

3P-091

Efficient and effective assessment of reports in laboratory-course in physiology using e-learning system

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Laboratory-course in physiology is an effective tool for understanding real physiology. Because it requires a lot of resources such as animals and equipments, we set three weeks intensive program in which students go through 6 subjects by rotation. However, writing reports in a short period tend to cause poor quality and copy-and-paste. To remove negative factors by reducing efforts of handling reports, we employed an e-learning system (WebClass, ver. 10.00c, DATA PACIFIC JAPAN).Methods: Using WebClass, we set (1) pre-course self-study quiz, (2) submission, assessment, correction of reports (3) post-questionnaire. Evaluation of the system by students were collected with registered questionnaire, and opinions of teachers by interview.Results: Vast majority of students (>84%) highly evaluated the on-line submission system, because of its laborsaving features, uploadable at any time from anywhere and re-uploadable until deadline. Teachers also received benefits from the PC-based correction system. Marking up on PC greatly reduced handling effort of reports, in turn, it raised the quality and quantity of comments. On the other hand, the self-rating by students on the quality of their outputs was not high (<30% satisfied). They felt difficulty on some specific task of PC-based writing, such as drawing figures, handling many data files, scanning paper-based data. The deadline date seemed to have a large influence on it. COI:No

3P-092

Improvement of students' self-evaluation through repeated practice with rubric-based assessments in the orthoptics department of Niigata University of Health and Welfare

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In orthoptic education, there are acute requirements for improving practical training because orthoptics covers a wide range of ophthalmological examinations, and supports for visually disabled persons. One of the problems is inadequate students' self-evaluation resulted from less-standardized evaluations assessed by multiple teachers. In the U.S., rubric assessments have been used widely from elementary to higher educationanl levels since the late 1970's. Because a rubric is a set of the explicit and descriptive criteria for scoring, assessments with rubric should help both students and instructors to consistently assess the students' performance. In this study, to investigate long-term effects of rubric-based training, we repeated practice-assessment-feedback cycles for nine week with a rubric, and analyzed time-course of disagreements between self- and teacher-evaluated scores throughout the whole in-house practice course. We found that the disagreements between both scores decreased significantly, even with seven teachers participated in, suggesting that students' self-evaluation improve through repeated rubric assessments. The result indicates advantages of repeating rubric-based formative assessments in orthoptic practices. COI:No

3P-093

Activation of the ATP-sensitive K^+ current simulated during the burst of EAD in the HuVEC model

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It is well established that the ATP-sensitive K^+ current (I_{KATP}) is evoked by a decrease in cytosolic [ATP] $_i$, and thereby the plateau of the ventricular action potential is largely depressed. However, it is usually difficult to record I_{KATP} activation except under a serious anoxic conditions. We simulated a few numbers of the early-after-depolarizations (EADs) evoked by delaying further the slow inactivation of the late mode I_{Na} (I_{NaL}). The rising phase of EAD is mediated by the Ca^{2+} current (I_{CaL}) and thereby twitch contraction is activated. When this cell model was electrically connected via gap junction in a one-dimensional array of 1200 models, repetitive EAD discharge occurred and propagated in a random manner within the array. To examine the balance between production-consumption of ATP, the contraction model detailed for the ATP consumption was used in the single HuVEC model. For simplicity, the repetitive and maintained discharge of EAD was achieved by freezing the slow inactivation of I_{NaL}. At the physiological O_2 tension of ~35 mmHg within the mitochondria matrix, [ATP] $_i$ gradually decreased to a steady level of ~1 mM and the burst of EAD continued showing enlarged amplitude. Further decrease in O_2 tension to ~1 mmHg, quitted the burst to reveal the stable resting potential. The [ATP] $_i$ quickly recovered to the physiological level. We will further examine the hypoxic effect in the model of cell array. COI:No

3P-094

Comparison of extensibility of rat compact left ventricle and turtle spongious ventricle

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Cardiac tissues in extant vertebrates are classified into two structural types: compact and spongy myocardium, which are associated with blood supply systems i.e. coronary and sinusoidal circulation, respectively. However, functional differences in the structure of these myocardial tissues are not well understood. Here, we compared passive mechanical properties of the spongious ventricle of turtles with compact left ventricle (LV) of Wister rats. To compare hearts of different sizes, we examined the end-diastolic pressure-volume relationship of diastolic-arrested ventricles normalized by myocardial weight, and found that rat LVs were significantly stiffer than turtle ventricles. To evaluate cellular extensibility, we performed tensile tests of isolated cardiomyocytes of turtles and rats. The obtained force-sarcomere strain curves showed that the extension of rat cells was restricted compared with turtle. Because an elastic protein connectin generates passive tension during diastole, we determined the primary structure of connectin in turtle hearts. The length of elastic PEVK segment of connectin in rat hearts was shorter than that in turtle hearts, indicating that connectin in rat hearts was stiffer than that in turtle hearts. These results suggested that rat compact LV was restricted in extension at the molecular/cellular level compared with turtle spongious ventricle. This may be evolutionary adaptation to prevent coronary flow cessation due to excessive tissue extension. COI:No

3P-095

Endothelial regulation of synchronous spontaneous Ca^{2+} transients in the mural cells of rectal arterioles.

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[Background] The mural cells (vascular smooth muscle cells and pericytes) of microvessels in hollow organs, e.g. bladder, stomach and intestines, exhibit rhythmic spontaneous Ca^{2+} transients underlying spontaneous constrictions that may maintain microcirculation blood flow during sustained organ distension. Here, we examined the roles of endothelium in the regulation of arteriolar mural cell spontaneous Ca^{2+} transients, focusing on endothelial NO and small (SK) and intermediate (IK) Ca^{2+}-activated K^+ channels. **[Methods]** Cal-520 intracellular Ca^{2+} imaging was conducted to record Ca^{2+} dynamics in arteriolar mural cells, using the rat rectal submucosa. **[Results]** In submucosal arterioles about 20 μm in diameter, mural cells with a round cell body showed synchronous spontaneous Ca^{2+} transients. L-nitroarginine (100 μM), an inhibitor of nitric oxide synthase (NOS), increased the frequency of spontaneous Ca^{2+} transients, decreased their amplitude and area under curve (AUC) and slightly disrupted their synchrony among mural cells. The endothelium showed eNOS immunoreactivity. NS309 (3 μM), an opener of SK/IK channels, attenuated spontaneous Ca^{2+} transient amplitude and AUC. **[Conclusion]** 1) In rectal small arterioles, NO endogenously released from endothelium decreases the frequency of spontaneous Ca^{2+} transients, increases their amplitude and stabilises Ca^{2+} transient synchrony among mural cells. 2) Hyperpolarisation by endothelial SK/IK channel opening may be conducted to mural cells via heterocellular gap junctions to inhibit their spontaneous Ca^{2+} transients. COI:No

3P-096

A simulation method of estimating intact action potentials in a beating heart from experimental data recorded by suction electrode technique

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Monophasic action potentials can be promptly recorded by applying the suction electrode (SE) at the surface of beating ventricle in situ (Hoffman et al., 1959). Action potentials (AP) thus recorded show a plateau potential configuration quite similar to real AP, although the AP amplitude is usually less than a half of real AP. On closer inspection, a number of modulations emerge in its configuration. If these changes in AP are explained in terms of methodological reasoning, application of the SE technique might be widely expanded. Approximately, the mechanism of AP recording by SE might be comparable to the 'injury potential'. The electrode tip has a relatively large pore (~1 mm in diameter) to pull in a part of muscular tissue into the pore by applying a negative pressure. The amplitude of recorded AP is increased by decreasing the leak conductance to the ground and by increasing the access conductance to the intracellular potential. In the present study, we developed an equivalent electrical circuit of SE applied to the middle of an array of 200 guinea pig ventricular cell models. The experimental AP recorded through SE could be best reproduced by adjusting parameters of the model circuit connected to the cell array (i.e. both the leak and access conductances at the electrode tip and the number of cell models sucked into the electrode). By improving the reproducibility, the real AP might be safely estimated by the SE method. COI:No

3P-097

The effect of lubiprostone on iPS cell-derived cardiomyocytes

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[Backgrounds] ClC-2 channel is thought to be involved in the inwardly rectifying chloride ion current. When ClC-2 in cardiomyocytes is activated, it is possible that strong inward-rectification activation and weak outward-rectification activation cause depolarization and accelerate repolarization, which may increase automaticity and trigger arrhythmia. However, detail about functional role of ClC-2 on cardiomyocytes is largely unknown.[Methods and Results] We have generated cardiomyocytes from mouse iPS cells and examined the characterization of the iPS cell-derived cardiomyocytes (iPS-CM) in terms of gene expression, immunoreactivity, and spontaneous beating activity. Furthermore, the role of ClC-2 on spontaneous beating-rhythm was analyzed. The expression of cardiomyocyte-related genes was detected. By immunostaining, cardiac troponin I positive-cells were confirmed and connexin 43 was obviously localized at the intercellular junctions. Addition of isoproterenol increased beating rate of iPS-CM. In contrast, lubiprostone, an activator of ClC-2, decreased beating rate.[Conclusion] Cardiomyocytes were successfully generated from mouse iPS cells, judging from gene expression, immunoreactivity, and spontaneous beating activity. Moreover, lubiprostone weakened spontaneous beating rate of iPS-CM suggesting a new function of ClC-2 on cardiomyocyte beating. COI:No

3P-098

Hypokalemia-induced ventricular arrhythmogenicity is increased in transgenic mice overexpressing HCN2 specifically in the heart

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Hypokalemia is a frequent complication in heart failure patients treated with diuretics, and potentially increase vulnerability to arrhythmia. In the failing heart, reactivation of fetal cardiac genes including HCN channel has been suggested to underlie arrhythmogenicity. Theoretically, hypokalemia should hyperpolarize resting membrane potential (RMP), thereby activate HCN channels. We therefore examined the effects of hypokalemia in transgenic mice overexpressing HCN2 specifically in their hearts (HCN2-Tg). In 5 mM K^+ solution, RMP was not significantly different between WT and HCN2-Tg myocytes. In 3 mM K^+ solution, RMP of HCN2-Tg (-91.2 $\pm$ 3.3 mV) was significantly more depolarized than that of WT (-95.0 $\pm$ 2.2 mV). 3 μM ivabradine hyperpolarized RMP of HCN2-Tg (-93.4 $\pm$ 2.9 mV), suggesting that HCN2 had been activated in hypokalemia: Furthermore, spontaneous action potential was induced in 57% of HCN2-Tg myocytes, but in none of WT myocytes. We next recorded ECGs in WT and HCN2-Tg mice after 6 weeks feeding K-free diet. The incidence of arrhythmia was significantly increased in HCN2-Tg than in WT. These findings suggested overexpression of HCN2 might increase the vulnerability to arrhythmia in hypokalemia (K.O. and Y.K. equally contributed to this work). COI:No

3P-099

Sympathetic control of the left ventricular function during dynamic exercise in conscious rats

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We have previously examined the role of cardiac sympathetic nerve activity (CSNA) on the heart rate control during dynamic exercise in conscious cats. The rapid activation of CSNA was followed by an exercise tachycardia at the onset of treadmill exercise (Tsuchimochi H et al, Am J Physiol 2002). On the other hand, it remains to be elucidated how sympathetic nervous system controls the left ventricular (LV) functions at the onset of dynamic exercise. The aim of this study was therefore to measure LV pressure and LV dP/dt changes during treadmill exercise in conscious rats. To address this, we used a telemetry device for direct measurements of LV pressure. A high-fidelity, solid-state pressure-sensor catheter tip was inserted into the LV chamber through the apex of the heart, and the transmitter body of the telemetry device (TRM54P, Millar) was implanted in the abdomen. In some rats, stroke volume and cardiac output were also measured by implanting a transit time flow probe around the ascending aorta. A minimum of 1 week was allowed for recovery. LV parameters were recorded at rest and during a graded exercise test before and after a β-adrenergic blockade. We developed integrated analyses of the LV functions in conscious rats in this study. A technique for the measurement of right ventricular functions should also be established. COI:No

3P-100

Functional evaluation of myocardium mitochondria in magnesium deficient rat

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Magnesium (Mg) deficiency has been reported to be associated with the development of cardiovascular diseases. We have reported that the cardiac functions of Mg-deficient (Mg-def) rat hearts were less than that of normal rat hearts, and mitochondrial functions were impaired in Mg-def rat hearts. The aims of this study were evaluation of mitochondrial function in Mg-def rat hearts by observation of mitochondria structures, measuring mitochondrial permeability transition pore (mPTP) and mitochondrial membrane potential. Although there was no difference of mitochondria structures between Mg-def and normal rat groups, the amount of intact mitochondria in Mg-def group was less than that of normal group. Opening of mPTP was estimated from Ca^{2+} retention capacity to rupture the mitochondria membrane. Mg-def group required less amount of Ca^{2+} retention capacity than that of normal rat group. We next evaluated mitochondria membrane potential by JC-1 fluorescent dye using flow cytometry analysis. The proportion of mitochondria with green fluorescence was higher in Mg-def group than that in normal rat group, suggesting that hearts in Mg-def rats have unhealthy or weak mitochondria. These results suggest that Mg deficiency induces the mitochondria membrane fragility. We considered that depression of cardiac function in Mg-def rat was associated with mitochondria membrane fragility. COI:No

3P-101

Carrier-mediated serotonin efflux from platelets in the heart

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Increased serotonin (5-HT) in the myocardial ischemic region has been reported to induce the receptor-dependent deleterious effects and the monoamine oxidase-dependent production of reactive oxygen species, and promote cardiac cell injury. Thus, to reduce the cardiac cell injury during myocardial ischemia, it is important to know the mechanisms responsible for 5-HT increase during myocardial ischemia. It has been considered that 5-HT is released from dense granule of activated platelets during myocardial ischemia. Recently, however, we have found in the experiment of anesthetized rats and rabbits that the ischemia-induced 5-HT release is suppressed by fluoxetine, a selective 5-HT reuptake inhibitor. This finding has suggested 5-HT release from platelets via fluoxetine-sensitive 5-HT reuptake transporter, i.e. carrier-mediated 5-HT efflux. In this study, applying microdialysis technique to the heart of anesthetized rats, we created a pharmacological ischemic condition by local administration of sodium cyanide through dialysis probe and investigated myocardial interstitial 5-HT and 5-HIAA (5-hydroxyindole acetic acid, a metabolite of 5-HT by monoamine oxidase) levels in the presence or absence of local administration of fluoxetine. Sodium cyanide increased myocardial interstitial 5-HT and 5-HIAA levels. In the presence of fluoxetine, sodium cyanide did not change myocardial interstitial 5-HT and 5-HIAA levels. Our results suggest that inhibition of vesicle transport by ischemia increases cytosolic 5-HT of platelets and induces carrier-mediated 5-HT release. COI:No

3P-102

Regulation of the spontaneous contractile activity of the guinea pig mesenteric lymphatic vessels by TRPV4 and endothelium

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Lymphatic vessels have spontaneous contractile activity, which is activated according to the increase of the intraluminal pressure. A stretch activated cation channel, TRPV4 is expressed on many types of cells including endothelial cells of arterioles. The endothelial cells of lymphatic vessels express the nitric oxide synthetase that controls the spontaneous activity. In the present study, the hypothesis that the TRPV4 on the lymphatic vessels and the endothelium may regulate the lymphatic spontaneous activity. The guinea pig mesenteric lymphatic vessels were isolated and pinned on the silicone sheet and perfused by Krebs solution. The diameter was measured with Diamtrak system. The membrane potential of the lymphatic vessels were recorded using the conventional glass microelectrode method. Application of GSK1016790A, a TRPV4 agonist increased the frequency of the spontaneous contractility of some lymphatic vessels but not all vessels. HC067047, a TRPV4 antagonist, inhibited the activation by the GSK1016790A. In presence of Nitro-L-arginine (LNA), GSK1016790A increased the frequency on all preparations. The resting membrane potential of the lymphatic vessels were -45 mV, and spontaneous action potentials were observed. Application of GSK1016790A initially inhibited the spontaneous electrical activity followed by the increasing the frequency. In presence of LNA, GSK1016790A expressed only the enhancing effect on the electrical activity. These results suggest that the activation of TRPV4 is regulated by the nitric oxide which may be released from the endothelium. COI:No

3P-103

Inhibition of Cyclooxygenase Contracts Chicken Ductus Arteriosus

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Background: Ductus arteriosus (DA) is an essential fetal artery that connects the main pulmonary artery and the descending aorta. Mammalian DA closes right after birth through vasoconstriction via a decrease of circulating prostaglandin E_2 (PGE_2). Avian DA also closes after birth although avian has no placenta that is a source of PGE_2 in mammals. Previous study has demonstrated that PGE_2 signal pathway is not involved in constriction of isolated chicken DA. However, the *in vivo* effect of PGE_2 in avian DA has been little investigated.Aim: To elucidate the effect of PGE_2 in chicken DA functional closureMethod and Results: First, we measured blood concentration of PGE_2 in chicken at embryonic day 19 (e19) by enzyme immunoassay. Blood concentration of PGE_2 in chicken DA was higher than that of chicken aorta at e19. Next, we determined the expression of prostaglandin E receptors in chicken DA. EP2, EP3, and EP4 receptors in fetal chicken DA were higher than that of fetal chicken aorta. EP2 receptor was significantly down-regulated after hatching. These data suggest that PGE_2 works on fetal chicken DA. Finally, we performed a rapid whole-body freezing method to evaluate DA closure *in vivo*. We measured internal diameter of DA two hours after *in ovo* injection of indomethacin, which is a nonselective cyclooxygenase inhibitor. Indomethacin constricted chicken DA at e19, but did not constrict chicken aorta. These data suggest that PGE_2 acts as a vasodilative factor on DA closure in avian.Conclusion: Inhibition of cyclooxygenase contracts chicken DA. PGE_2 signal may play an important role in an acute vasodilatation of chicken DA closure. COI:No

3P-104

Endogenous ATP inhibitorily modulates hypoxia-induced excitation of the sympathetic premotor neurons in the RVLM in the *in situ* arterially-perfused preparation of rats.

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Neurons in the rostral ventrolateral medulla (RVLM neurons) generate the activity of the cardiovascular sympathetic nerve (SNA). We have reported that the RVLM neurons are excited by the central hypoxia. However, the hypoxia-sensing mechanism is still unclear. In this study, we tried to examine if endogenous ATP is related with the hypoxia-sensing mechanism of the RVLM neurons in the *in situ* arterially perfused preparation of rats. We systemically applied a P1 or a P2 purinergic receptor antagonist, PPADS (100 μM) or caffeine (100 μM), and observed the effects on responses of the SNA to the application of NaCN into the RVLM (5 mM, 30 nl). As a result, administration of PPADS or caffeine did not affect the basal SNA. On the other hand, administration of PPADS significantly enhanced the excitation of SNA which was caused by injection of NaCN into the RVLM but caffeine did not change. The PPADS-induced enhancement was suppressed in the presence of a glycinergic receptor blocker, strychnine (0.5 μM, 50 nl) but not changed in the presence of a GABAergic receptor blocker, bicuculline (5 μM, 50 nl). These results may indicate that hypoxia-induced activation of P2 purinergic receptors by endogenous ATP facilitates glycine release from neurons which innervates the RVLM neurons, and inhibitorily modulates the excitation of the RVLM neurons to hypoxia. COI:No

3P-105

Differential oxygenation responses of the prefrontal cortex and forearm skeletal muscle during supraventricular tachycardia

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Supraventricular tachycardia (SVT) causes reductions of arterial blood pressure (AP) and cerebral perfusion which sometimes lead to syncope. It is unknown how the cerebral and peripheral vascular systems operate to maintain the cerebral oxygenation against the SVT-induced hypoperfusion. We examined the cerebral and skeletal muscle oxygenation responses during SVT. Near-infrared spectroscopy (NIRS) was used to measure the prefrontal and forearm tissue oxygen index (TOI) and normalized tissue hemoglobin index (nTHI). The NIRS and hemodynamic data were collected during electrophysiological study (EPS) in 13 patients (55±5 years old, 8 men) with SVT. AP decreased (-18±6 mmHg, $P<0.01$) during 20 s of EPS-induced SVT (151±6 bpm). The prefrontal TOI and nTHI decreased transiently from 6 s to 14 s of SVT and then returned to the baseline level. In contrast, the forearm TOI and nTHI decreased continuously from 11-13 s. The AP and prefrontal NIRS responses suggest that SVT causes reductions of cerebral oxygenation and blood volume, which may be recovered by cerebral vasodilatation. The continuous reduction of forearm TOI and nTHI suggest that muscle blood flow was restricted by vasoconstriction during SVT, which may contribute to redistribution of the blood to the brain. COI:Properly Declared

3P-106

Abnormal respiration and metabolism in the HCM knock-in model mouse

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The S179F knock-in model mouse expresses mutant cardiac troponin T from human patients diagnosed with hypertrophic cardiomyopathy (HCM). This mouse recapitulates much of the human phenotype including sudden cardiac death, hypertrophy, fibrosis, myocyte disarray and higher Ca2+ sensitivity. We now report on respiration and aerobic metabolism in this model mouse using the Oxymax-CLAMS system for 3 consecutive days. In HCM mice, food and water intake, O2 consumption, CO2 production and heat production were the same as wild type (WT) mice at 6 months of age. However, at 1 month of age, food and water intake was more than 70% greater, while energy production was only 20% greater compared to WT mice. Based on the respiratory quotient, the main energy source in the inactive phase was carbohydrates in HCM mice, but lipids in WT mice. Whereas, the main energy source in the active phase was carbohydrates in both HCM and WT mice. In conclusion, the aerobic respiration and metabolism of HCM mice was found to be abnormal in the early postnatal period, but normalized as young adults. COI:No

3P-107

Comparison of PWV and Beta in the elastic and muscular arteries in response to hemorrhage in rabbits

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Cardio-ankle vascular index (CAVI) is a blood pressure-independent arterial wall stiffness parameter. We calculated Beta by applying the theory of stiffness parameter β to the aorta and peripheral artery. We compared change in vascular stiffness between the elastic (aorta) and muscular (common iliac-femoral) arteries using pulse wave velocity (PWV) and Beta in response to 15% hemorrhage of total blood volume in 12 rabbits aged 10-12 months under pentobarbital anesthesia. Pulse waves at the ascending aorta (AA), distal abdominal aorta (dAbd) and distal end of left femoral artery (Fem) and flow waves at AA were simultaneously recorded under regular cardiac pacing before and after the hemorrhage at 4 ml/kg/min for 180 s from the inferior vena cava. PWV in the aorta (aPWV), from dAbd to Fem (ifPWV) and from AA to Fem (afPWV) was determined by the difference in the rising time of two pulse waves and distance of two pressure sensors. Beta was determined as $\mathrm{Beta}=2\rho/\mathrm{PP}\times\ln(\mathrm{SBP}/\mathrm{DBP})\times\mathrm{PWV}^2$ (ρ: blood density, SBP, DBP and PP: systolic, diastolic and pulse pressures). aPWV, ifPWV and afPWV decreased significantly due to the hemorrhage. aBeta and afBeta increased significantly while ifBeta decreased significantly due to the hemorrhage. These indices returned to the prehemorrhage level due to reinfusion of the withdrawn blood. We conclude that the response of PWV and Beta to the hemorrhage was different in the elastic and muscular arteries. COI:No

3P-108

Cardiac contraction affects cerebral oxygenation during hypotension with supraventricular and ventricular tachycardia

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Cerebral vasoconstriction during hypotension with ventricular tachycardia has been reported, but the underling mechanism was unclear. We tested a hypothesis that the cardiac contraction, i.e. heart rate and/or contractility, irrespective of hypotension affects cerebral oxygenation during supraventricular tachycardia (SVT) and ventricular tachycardia (VT). Heart rate (HR), arterial blood pressure (AP), and the prefrontal tissue oxygen index (TOI) with near-infrared spectroscopy were measured during electrophysiological study in 8 patients. We selected similar hypotension trials ranging from -30 to -50 mmHg in SVT (10 trials, 4 patients) and VT (9 trials, 4 patients), and then compared HR and TOI responses. Although the decreases in AP were similar in SVT and VT (-37 ± 2 vs. -38 ± 2 mmHg), the prefrontal TOI decreased greater in SVT than VT (-5.2 ± 0.4 vs. -3.3 ± 0.6%, $P < 0.05$). HR increased greater in SVT than VT (85 ± 3 vs. 74 ± 8 beats/min, $P < 0.05$), and the increase in HR correlated with the TOI fall ($r = -0.80$, $P < 0.05$). The ejection fraction in SVT and VT were 70 ± 2 and 44 ± 14%. These results suggest that a higher cardiac contraction with tachycardia may lead to decrease in cerebral oxygenation, which is irrespective of hypotension. COI:Properly Declared

3P-109

Construction of LVAD model using rat excised heart

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[Introduction]Recent years, Clinical trials are being conducted to expand the application of LVAD insurance.In Japan, the waiting period for transplantation is prolonged year by year, and research on the influence of DT is necessary.Studies in large animals, because there is a need for devices of prices and large-scale surgery, can not be performed easily.With small animals, heart failure models by gene modification, medication, surgery are becoming easier to make.By conducting experiments with small animals it is thought that research can progress more.[Purpose]We construct an LVAD model using rat excised heart.[Result]We made LVAD model as shown in the schematic diagram.By gradually performing LVAD assist in the heart in the LVP lowering state, we showed stepwise increase in AoP, decrease in ESP and decrease in SV.[Discussion]AoF is decreasing than expected, and an increase in CoF is occurring.When the auxiliary flow rate is gradually changed, the area per heartbeat of the P-Vloop decreases.This indicates that the cardiac Stroke Work is decreasing.From the above, the effectiveness of this LVAD model was suggested. COI:No

3P-110

Expression and Localization of Doc2B in podocytes in rat kidney glomeruli

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Although neuron-like glutamatergic signaling between podocytes seems to contribute to glomerular function, it remains unknown how glutamate is secreted from the cells. Neurons release glutamate by SNARE-mediated exocytosis that is partly regulated by Doc2B. To explore the possible involvement of Doc2B in renal function, we have studied the expression and localization of Doc2B in rodent kidneys in the current study. In immunoblotting experiments, a rabbit anti-Doc2B IgG recognized a major band of ~50 kDa in homogenates of rat and mouse kidneys as well as brain, indicating the expression of Doc2B in the kidney. The localization of Doc2B in podocytes was revealed by double immunofluorescence microscopy for synaptododin (podocyte marker) and immunoelectron microscopy. To pave the way for further studying the role of Doc2B in podocytes, its expression in a cultured cell line was examined. Immunoblotting demonstrated that differentiation under specific conditions increased the expression of Doc2B in cultured human podocytes. In differentiated podocytes, synaptopodin was localized along the stress fiber-like structure, while Doc2B was diffusely scattered in synaptopodin-rich regions. Finally, neural SNAREs (syntaxin1, SNAP-25, synaptobrevin2) were detected in cultured podocytes in immunoblotting. These results suggest that Doc2B may be involved in membrane trafficking such as vesicle exocytosis in podocytes. COI:No

3P-111

Biphasic increase of fludrocortisone-induced plasma erythropoietin concentrations in mice.

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Aldosterone and its analogue may rescue anemia shown in patients with hypoadrenocorticism (Addison's disease) through interaction with the conventional mineralocorticoid receptor (MR). Methods: Male mice (C57BL/6J, 10 weeks) were injected with fludrocortisone (Fld), a MR agonist (2.5 mg/100 g BW, i.p.) at time 0. In the kidney, Fld-induced expression of erythropoietin (Epo), hypoxia-inducible factor 2α (HIF2α) and prolylhydroxylase 2 (PHD2) mRNAs were investigated by using in situ hybridization technique at time 2, 4, 6 and 72. Results: Plasma erythropoietin concentrations ([Epo]) appropriately and significantly increased from < 0.6 (control; time 0) to 2 mIU/ml at 2, 4, and 6 hrs after Fld injection, and returned to < 0.6 mIU/ml at 72 hr. Fld-induced expression of Epo mRNA was moderate in medullary thick ascending limb of Henle's loop and strong in collecting ducts (CDs), although it was negligible in the peritubular interstitial cells (PTI). On the other hand, expression of HIF2α and PHD2 mRNAs significantly increased in both glomerulus and proximal/distal nephrons including CDs. Interestingly, HIF2α also increased in the PTI. Conclusion: Fld-induced expression of Epo and related mRNAs in the kidney may account for homeostatic control of plasma [Epo] in human and animals. COI:No

3P-112

Physiological role of Ca-sensing receptor expressed in type B intercalated cells of the mouse kidney cortical collecting duct

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Background: Phosphate (Pi)-deprived mice showed hypercalcemia and hypercalciuria as well as paradoxical metabolic acidosis with alkalinuria (Yasuoka et al., 2015). In this study, we investigated whether Ca-sensing receptor (CaSR) expressed in the basolateral membrane of cortical collecting duct (CCD) type B intercalated cells (IC-B) is involved in urinary alkalinization. Methods: CCD and outer medullary collecting ducts (OMCD) were microdissected without collagenase. Intracellular [Ca] ($[Ca^{2+}]_i$) was estimated time-dependently in each tubule cell loaded with Fluo4-AM. Some C57Bl/6J mice (10 weeks, male) were fed with low Pi diet (1% Ca, 0.02% Pi) for 1 week. Their urine was hourly collected on the day of experiments after treatment of NPS2143, CaSR antagonist, or control vehicle. Results: Application of NPS2143 significantly decreased urinary excretion of Ca compared with control after 2 hr [1.18 ± 0.09, 2.51 ± 0.22, respectively], normalized by urinary creatinine. Urine pH also decreased [6.0 ± 0.15 (NPS2143), 7.6 ± 0.08 (control)]. More importantly, basolateral application of R568 (CaSR agonist) transiently increased $[Ca^{2+}]_i$ only in a few cells of the CCD, but not in the OMCD. Conclusion: Present studies revealed that NPS2143 reduce hypercalciuria and reverse alkalinuria in Pi-deprived mice. The basolateral CaSR in IC-B may respond to the increase of plasma $[Ca^{2+}]$ and cause the paradoxical alkalinuria with acidosis. COI:No

3P-113

Development of a mathematical model of trans-epithelial ion transport

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As the glomerular filtrate pass through the renal tubules, the epithelial cell layer reabsorbs most of solutes into the interstitial fluid. We aim at developing a mathematical model of this mechanism. We made use of the reduced model of cardiac myocyte model, in which all ion movements across the membrane are represented by background ionic currents; INab, IKb,ICl b, and these passive ion fluxes were balanced by Na/K pump and the Na, K, 2 Cl cotransporter (NKCC) ('VolumeRegulation' in the e-Heart educational materials). The membrane water flux was also calculated from the osmotic balance across the cell membrane with the experimental measurements of water permeability. This model well reconstructs steady state of the cell volume as well as the resting membrane potential. These cells were held together by tight junctions and ion channels localized on the luminal side, and the ion transporters on the basolateral side to satisfy the functional polarization of the epithelial cell membrane. A net transcellular flux of Na+ was obtained from the luminal side to interstitial side and smaller fluxes of K+ and Cl- in the reverse direction. The membrane potential was ~-85 mV. The passive backward diffusion of ions through the paracellular route generated the Donnan's potential of a few mV. We are trying to add the capillary model to complete this basic model of tubular reabsorption. COI:No

3P-114

The correlation of mitochondrial metabolic adaptation with calcium uniporter expression in rat skeletal muscle

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Purpose: Recently, mitochondrial calcium uniporter (MCU), a channel protein for Ca^{2+} entry into mitochondria, is suggested to play a key role in metabolic adaptation. Here, we studied whether MCU expression correlates with mitochondrial metabolic adaptation of skeletal muscle induced by exercise and diet restriction. Methods: F344 female rats voluntarily ran on a rotary wheel ergometer with a load of 30% body weight for 8 weeks from 6 weeks of age with/without diet restriction (n=8, 7, 6 for sedentary control, exercise, exercise+diet-restriction). Plantaris and soleus muscles were analyzed for the expression of proteins associated with fission (DRP1) and fusion (OPA1) of mitochondria and of MCU. Metabolic function was evaluated by activities of citrate synthase and β-hydroxyacyl CoA dehydrogenase. Results: The activities of citrate synthase and β-hydroxyacyl CoA dehydrogenase indicated that each of the present exercise condition and the additional diet restriction successfully induced metabolic adaptation in both plantaris and soleus muscles. However, the expressions of DRP1, OPA1 and MCU showed exercise-induced explicit increase only in plantaris muscle, and diet restriction had no appreciable additional effect on their expressions in both muscles. Conclusion: MCU expression would not be a general key regulator of mitochondrial metabolic adaptation in rat skeletal muscle, although MCU expression may partly contribute in the exercise induced adaptation process in some particular muscles. COI:No

3P-115

Effects of estradiol infusion on dopamine-related gene expression and voluntary physical activity in ovariectomized spontaneously hypertensive rats

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It has been reported that dopamine (DA) neurons of the ventral tegmental area (VTA) and DA receptor-expressing neurons in the nucleus accumbens (NAcc) are involved in regulating motivation to voluntary exercise. It has been also suggested that female hormones affect motivation to exercise. In this study, we investigated whether estradiol regulates physical activities and if it did, whether altered expression profiles of DA-related genes would be involved in underlying mechanisms. Female spontaneously hypertensive rats (SHRs) were used in this study. Both ovaries were dissected out and one week after the operation, estradiol (OVX+Est) or vehicle (Cont) was infused by osmotic mini-pump (s.c.). After two weeks of estradiol / vehicle infusion, running distance of voluntary wheel exercise was measured for 6 days. Gene expression levels of DA receptors (D1 and D2) in the NAcc were measured after one month of estradiol / vehicle infusion. The total running distance was significantly higher in the OVX+Est compared to Cont, whereas gene expression levels of DA receptors in NAcc were not affected by estradiol infusion. Our findings suggest that estradiol may affect motivation to exercise, although underlying mechanisms have not been elucidated yet. Gene expression profiles of tyrosine hydroxylase and dopa decarboxylase in the VTA are currently under examination. COI:No

3P-116

Walking evaluation with acceleration sensor - Effects of difference in heel height on gait -

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[Background and purposes] It's necessary to keep balance during walk with high-heeled shoes, and which influences the walking posture. In this study we examined the effects of intervention of advices and training of stylish walk (Posture Walking; PW) on steps etc. during the walk. [Methods] Subjects were divided into two groups of with or without PW training program. Subjects of training group received three weeks of PW program with high-heeled shoes and sneakers. An acceleration sensor was attached on the hips and subjects walked 10 m of return trip on the flat floor. To examine the degree of stress before and after the intervention, amylase activity was measured with an analyzer saliva amylase monitor. The acceleration wave form was analyzed and parameters of the body weight movement etc. were calculated. [Results and discussion] By the intervention of walking guidance to pay attention to posture by PW, there was a tendency that the balance between the front and the rear at the time of walking improved after the PW intervention. In addition, there was a tendency that the movement especially decreased immediately after intervention. It was suggested that by practicing PW which pay attention to posture and acquiring a method of walking beautifully, it leads to a reduction of the burden on the body especially when walking with high-heeled shoes. Then, the fluctuation of the saliva amylase value before and after intervention was different every subject. COI:No

3P-117

Effects of difference in the range from sea level to moderate altitude at field on physiological response during endurance exercise.

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Ventilatory response and lactate metabolism are reported to be enhanced in endurance exercise under acute/transient exposure to hypobaric hypoxia (Koistinen P. et al.1995). It is unclear how acute hypobaric hypoxia at moderate altitude, up to 1500 m, affects physiological responses during exercise. This study defines the cardiorespiratory response during a 20 m shuttle run (Shuttle test) as maximal exercise and submaximal step tests from sea level to 2000 m and to each 500 m altitude in the field. The subjects were 8 healthy men who were not acclimatized to altitude (aged 20. 8±1.4 years (mean±SD), $V0_{2max}$ 58.4±3.6 ml/kg/min). The subjects performed the step test (height: 40cm, step rate: 30 steps/min) for 5 min at 0, 500, 1000, 1500 and 2000 m. Also, they conducted the Shuttle test at 0 and 1500 m. The arterial oxygen saturation ($Sp0_2$), ventilator volume (V_E), oxygen uptake ($V0_2$), heart rate (HR), blood lactate concentrations (La), and rating of perceived exertion (RPE) were measured. At 1500 m and 2000 m, V_E, La and RPE significantly increased, and $Sp0_2$ decreased during the step test ($P<0.01$). The performance of the Shuttle test was significantly lower at 1500 m than at 0 m ($P<0.01$). HR_{peak} showed no significant difference between 0 and 1500 m. When non-acclimatized men were exposed acutely to moderate altitude, cardiorespiratory response at rest, and also the performance and lactate metabolism, were affected during endurance exercise from the altitude of 1500 m. COI:No

3P-118

Relationships between physical fitness and body composition with growth in urban adolescent

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Physical fitness of adolescent has been decreased compared with previous-generations, especially in metropolitan area. In this study, we investigated the relationships between body composition and physical fitness in urban middle and high school students from the data of three years as cross-section study. The first grade of middle (81 boys, 144 girls) and high school students (86 boys, 136 girls) were recruited. They performed the new physical fitness test of MEXT. We evaluated their body composition and calculated whole body and limbs skeletal muscle mass using bioelectrical impedance method.We found that the index of agility (repeated side steps), speed (50m sprint), muscle strength (standing broad jump distance), and endurance (long distance running time) were correlated with whole body or quadriceps muscle mass in high school students (all, $p<0.001$). In contrast, in middle school students, these correlations were observed but not between the index of muscle strength and whole body or quadriceps muscle mass.Our results indicate that functional development of physical fitness might be delayed compared with physical growth somatotype in urban middle school students, indicating the necessity of physical activity to improve physical fitness in urban adolescent. COI:No

3P-119

The effect of calcitonin gene-related peptide (CGRP) on mRNA levels of myosin heavy chain class II (MyHC II) and interleukin (IL) -6 in mouse myocytes

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CGRP is a neuropeptide secreted by motor neuron in skeletal muscle. However, contribution of CGRP in mRNA expression of MyHc II in skeletal muscle were not determined.We recently reported that calcineurin activation enhanced IL-6 mRNA and that IL-6 induced by calcineurin activation might increase MyHC II_b mRNA in murine myocytes.In the present study, we exmamined that the effects of CGRP on expression levels of MyHC II_b, MyHC II_a and IL-6 mRNAs.C2C12 cells were cultured by medium containing with or without agent at the beginning of differentiation. The mRNA expression levels were measured by quantitative RT-PCR method using Taqman probes.We demonstrated the effect of CGRP on mRNA expression levels of MyHC II_b and MyHC II_a in C2C12 cells. These mRNA levels were decreased by medium supplemented with CGRP.Then, we examined the effect of CGRP on IL-6 mRNA level. The expression level of IL-6 mRNA was also not affected by medium supplemented with CGRP.These results indicated that CGRP pathway is not participated in mRNA expression of IL-6, MyHC II_b and MyHC II_a in C2C12 cells. COI:No

3P-120

Influence on light reflex of TRPC knockout on mouse intraocular smooth muscle

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Since vision is an important sensory organ for mammals, mechanisms to properly capture visual information are developed very well. The iris has a role of adjusting the size of the pupil to adjust the amount of light incident on the retina, the pupillary sphincter contained therein is only involved in the miosis and is dominated by parasympathetic nerves It is a special smooth muscle. To maintain the light intensity properly, the iris needs to rapidly change the diameter of the pupil (rapid phase) and maintain the pupil for a long time (sustained phase). Extracellular calcium influx is required for intraocular smooth muscle, but there are some molecular entities and mechanism of action, but details are unknown. So far, we have shown that calcium influx in sustained phase is mediated by two nonselective cation channels (NSCC) with different unit conductance by experiments in bovine ciliary muscle. Expression of TRPC1, TRPC3, TRPC4, TRPC6, Orai1, etc. has been confirmed as a molecule candidate, but it is difficult to apply gene knockdown etc. to bovine material, details of the relationship with NSCC have been clarified absent. Therefore, experiments were carried out using TRPC 3 and TRPC 6 knockout mice and double knockout mice based on these, as experimental materials, using mice which are relatively easy to genetically modify. We report the results of the miosis analysis by light reflex which can measure the contraction of intraocular smooth muscle non-invasively. COI:No

3P-121

Analysis of aggregated proteins in HSPB8 myopathy using zebrafish models

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Heat shock protein B8 (HSPB8), a member of the small heat shock protein family, is known to have chaperone activity and be involved in protein quality control. Previous studies reported that mutations in *HSPB8* cause hereditary motor neuropathy and myopathy. Recently, two novel candidate mutations of *HSPB8* were identified in families with protein-aggregated myopathy. However, the pathogenic mechanisms of HSPB8 myopathy remains to be elucidated. In this study, we firstly establish zebrafish models of HSPB8 myopathy to confirm the pathogenicity of these novel *HSPB8* mutations. We also tried to identify abnormal aggregated proteins for the purpose of clarifying the pathological mechanisms of HSPB8 myopathy. We carried out microinjection of wild-type or mutant human *HSPB8* mRNA in zebrafish embryos at 1-2 cell stage. Then we analyzed phenotype of these fish at 5 days post-fertilization. Overexpression of mutant *HSPB8* mRNA resulted in morphological abnormalities at higher rate compared to expressing wild-type *HSPB8* mRNA-injected and uninjected fish. Furthermore, it revealed that these abnormal fish had severe muscle degeneration and protein aggregation. Our data suggest that the novel mutations of *HSPB8* may cause myopathy with protein aggregation. COI:No

3P-122

In vivo analysis of individual sarcomere dynamics in the beating mouse heart.

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Sarcomeric contraction in cardiomyocytes serves as the basis for pump functions of the heart. Although sarcomeres play a pivotal role in the circulatory system, myocardial sarcomere length (SL) changes have not been systematically investigated *in vivo*. In the present study, we developed a high-speed (100 fps), high-resolution (20 nm) spinning disc confocal-imaging system for the beating mouse heart *in vivo*. Based on the expression of α-actinin-AcGFP under this optics system, we simultaneously analyzed both the physiological sarcomere dynamics in a single myofibril consisting of ~30 sarcomeres (i.e., with a near entire length) in a ventricular myocyte, and the hemodynamic parameters (i.e., ECG, LVP and PV loop). The findings were as follows: First, the SL values were 1.88 ± 0.29 and 1.66 ± 0.19 μm, respectively, in diastole and systole, and the individual SL values varied markedly throughout the cardiac cycle even in the same myofibril. Second, the dynamic behavior of each sarcomere was not consistently synchronized with that of the whole myofibril. Third, the correlation (R) between the dynamics of an individual sarcomere and that of the whole myofibril varied markedly, i.e., from -0.2 to 0.8, throughout six cardiac cycles. At the meeting, we will discuss how myofibrillar contractions are organized in the beating heart *in vivo*. COI:No

3P-123

Complexes of calcium waves in colonic musculatures of mice

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In the colon, coordinated motions are required for smooth passage of contents, and unique electrical complexes consisting of rapid and slow oscillations occur. In mechanical aspect, colonic muscle should generate strong propulsive force, compared to the small intestine where semi-liquid contents are moderately agitated and slowly transferred. It is hypothesized that colonic smooth muscle tissue tightly regulates intracellular Ca^{2+} for powerful constrictions. However, little is known about spatio-temporal Ca^{2+} dynamics in the colon.

In this study, we performed Ca2+ imaging in transgenic mice of tetO-YC -Nano50::Parvalbumin-tetracycline transactivator, expressing a FRET-based highly sensitive Ca2+ indicator in muscle. Musculatures were isolated from a mid part of the colon, and mounted on a heating glass (at 35° C). Excitation light of 435 nm was applied to the samples on an inverted microscope, and a pair of emission images of CFP and YFP were simultaneously acquired at ~100 ms intervals in a single CCD camera through an image splitting optics. Changes in intracellular Ca^{2+} level were estimated by the ratio of CFP and YFP emission images. Degrees of sample contraction were estimated by solving the two equations for CFP and YFP emission.

We found that colonic musculatures generate Ca^{2+} wave complexes, presumably corresponding to slow and rapid oscillations in the electrical complexes COI:No

3P-124

High Hydrostatic Pressure induces Mouse Cardiomyocyte Contraction

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Cardiomyocytes are subject to several types of mechanical stress such as stretch and pressure. The mechanical stress affects the morphology and physiological function of cardiomyocytes such as Frank-Starling mechanism and our previously investigated slow force response to stretch. However, the effect of pressure in cardiomyocytes has never been observed due to the lack of quantitative measurement system. Therefore, we investigated the influence of hydrostatic pressure on cardiomyocytes. In this study, we used our developed hydrostatic pressure system and high hydrostatic pressure microscope to observe the morphology and alteration of $[Ca^{2+}]_i$ in mouse cardiomyocytes under the high-pressure conditions. The high-pressure (5, 10, and 20 MPa) significantly reduced the sarcomere length as an index of morphological change, while the release of the high pressure recovered the contraction. High pressure microscope showed that $[Ca^{2+}]_i$ in cardiomyocytes did not induce the contraction. Electron microscope indicated that the high pressure did not collapse the organization of cardiomyocytes. Furthermore, 2, 3-Butanedione monoxime (50 mM) inhibited the high-pressure-induced contraction of cardiomyocytes. Our results suggested that high hydrostatic pressure directly regulates actomyosin interaction and leads to the contraction of cardiomyocytes. COI:No

3P-125

Sarcomeric auto-oscillations in single myofibrils from the heart of patients with dilated cardiomyopathy

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In the present study, we analyzed the mechanical properties of single sarcomere dynamics during sarcomeric auto-oscillations (Ca-SPOC) that occurred at partial activation under the isometric condition in myofibrils from donor hearts and from patients with severe dilated cardiomyopathy (DCM: New York Heart Association classification III - IV). Ca-SPOC reproducibly occurred in the presence of 1 μmol/L free Ca^{2+} in both non-failing (NF) and DCM myofibrils, and sarcomeres exhibited a saw-tooth waveform along single myofibrils, composed of quick lengthening and slow shortening. The period of Ca-SPOC was longer in DCM myofibrils than in NF myofibrils, in association with prolonged shortening time. Lengthening time was similar in both groups. Then, we performed troponin (Tn) exchange in myofibrils with a DCM-causing homozygous mutation (K36Q) in cardiac TnI. Upon exchange with the Tn complex from healthy porcine ventricles, the period, shortening time and shortening velocity in K36Q myofibrils became similar to those in Tn-reconstituted NF myofibrils. Protein kinase A abbreviated the period in both Tn-reconstituted NF and K36Q myofibrils, demonstrating acceleration of cross-bridge kinetics. To conclude, 1) sarcomere dynamics is depressed under loaded conditions in DCM myofibrils due to impairment of thick-thin filament sliding. 2) Microscopic analysis of Ca-SPOC in human cardiac myofibrils is beneficial to systematically unveil the kinetic properties of single sarcomeres in various types of heart disease. COI:No

3P-126

Analysis of changes in expression level of sarcolenmmal proteins after exercise in a rat model for delayed onset muscle sorness (DOMS).

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DOMS is commonly experienced after unaccustomed exercise, especially after lengthening contraction (LC), and characterized by tenderness and movement related pain (mechanical hyperalgesia). Using a rat model, we previously reported that nerve growth factor (NGF) and glia cell-line derived neurotrophic factor were upregulated after some delay in a muscle after a bout of exercise, and then caused sensitization of muscle nociceptors to the mechanical stimuli. We also observed adaptive phenomenon in DOMS called a repeated bout effect, in which NGF production and mechanical hyperalgesia were reduced after 2nd bout. To understand the mechanism for this adaptation, we examined expression levels of several membrane proteins that have functions in membrane stabilization and/or maintenance of sarcolemma. LC was applied only to a left hind leg and a right leg was used as a control. Five days after LC, the extensor digitorum longus muscles of both sides were isolated, and the membrane proteins and their transcripts were analyzed by western-blot and qRT-PCR, respectively. Five days after the first bout, mitsugumin 53 (MG53), which has a role in membrane repair, was significantly upregulated at both protein and mRNA levels compared with the control. The results proposed that the increased ability of membrane repair is one of the possible causes of repeated bout effect. COI:No

3P-127

Gait change in normal subjects under optokinetic stimulation using virtual reality

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This study investigated the effect of optokinetic stimulation (OKS) using virtual reality (VR) on postural stability and gait in normal subjects. Five subjects wearing a head-mounted display (HMD, Oculus Rift) were asked to walk along a 4-m straight line at normal gait speed. OKS was provided using a pattern of random dots in a virtual three dimensional space that was moved continuously at 40° /s in the vertical (VOKS), rightward horizontal (HOKS), or clockwise torsional (TOKS) directions. Following 15 s of OKS, subjects were asked to start walking. Gait parameters, including speed, cycle, gait path (GP), and the mean foot sole pressure (FPS) were measured for each OKS direction. Gait speed during VOKS, TOKS, and HOKS decreased by 10 - 25% compared to the static OKS (the same visual pattern, but without movement). During TOKS, the stance phase duration on the right limb increased compared to the static OKS, and GP shifted clearly to the right. The mean FPS on the right limb was higher during both TOKS and HOKS compared to that during static OKS. Previous studies have reported that the center of gravity in post-stroke patients often deviates toward the non-paretic side, and that shifting their weight-bearing back onto the paretic side is critical for the recovery of balance control. Our results indicated that OKS, especially TOKS, can shift weight-bearing toward into the stimulated direction, suggesting that OKS with VR might be useful for gait training in stroke patients. COI:No

3P-128

Interaction between Water and Myoproteins Evaluated with Scanning Calorimetry

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MRI reflect not only water content, but also water states in biological tissue. Details of tissue water state are, however, not clarified yet, but interaction between water and macromolecules is considered to restrict molecular motional freedom similarly to the freezing of water. In DSC of skinned fibers prepared from sartorius muscle of *Rana Catesbeiana* at rigor condition, extra heat absorption with temperature was observed at -24, -21, 0, 46, 65° C. We consider the peak at 46° C to represent myosin denaturation because 1)TEM of the fibers showed selective deterioration of A-band structure after the scan up to 60° C, 2)selective removal of myosin filaments from the fiber selectively diminished the peak, and 3)denaturation temperature of rabbit psoas myosin was reported to be around 50° C (Dergez et. al.). We consider the peak at 65° C to represent actin denaturation because 1)TEM showed I-band deterioration after the scan up to 80° C, 2)selective removal of actin filaments from the fiber selectively diminished the peak, and 3)denaturation temperature of actin was reported to be around 65° C (Dergez et. al.). The denaturation and removal of myosin and actin filaments differentially affected the peaks at -24, -21° C as well as integrated heat absorption from -80 to +20° C. These results suggested 1)the native actin and myosin filaments independently restrict considerable volume of surrounding water at the physiological temperature range and 2)actin filaments and their restricting water have higher heat capacity than the rest of fiber components. COI:No

3P-129

Relationship between complex spike response to optokinetic stimulation and simple spike response to vestibular stimulation in the Purkinje cells of the cerebellar nodulus and uvula

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We examined the relationship between the firing pattern of complex spike (CS) during sinusoidal optokinetic stimulation (OKS) and that of simple spike (SS) during sinusoidal head rotation (HR). For OKS, a random dot pattern, presented on a monitor in front of the animal, was rotated sinusoidally in the vertical, torsional, or horizontal plane. For HR, sinusoidal vertical HR in four stimulus planes of pitch, roll, and two diagonal planes were used. CS activity showed direction-specific responses to OKS in one of the three stimulus directions. According to the maximal CS response during OKS, cells responding to torsional OKS tended to localize to areas up to 1.0 mm from the midline, while those responding to vertical OKS tended to be located more laterally. SS activity also showed rotational plane-specific responses. Pitch-type cells tended to localize to areas up to 1.0 mm from the midline, while roll-type cells tended to be located more laterally. Since the rostro-caudal zonal organization of CS and SS activities seemed to be similarly arranged, we tested firing responses to combined stimulation of optimal OKS for CS activity and optimal HR for SS activity, to correspond their on-directions in the same phase. We found in some cells that SS activity showed clear decreases in the response amplitude, suggesting phase-dependent modulation of SS activity by the optokinetic response of CS activity. COI:No

3P-130

Transformation of locomotor patterns from quadrupedal to bipedal in Japanese monkeys on a treadmill: kinematic analysis

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Japanese monkeys can learn to walk bipedally and to transform its locomotor patterns from quadrupedal to bipedal without interruption of on-going movements. To investigate how monkeys walking quadrupedally superimposed upright posture on hind-limb stepping movements for accomplishing bipedal locomotion (BpL), we videotaped the animals exerting quadrupedal locomotion (QpL) and BpL alternately, and analyzed the transforming behavior kinematically. We found that, during QpL, the posture transformation was initiated by a hind limb in the swing phase, being swung slower and making the step length (distance between two feet on the belt) shorter than conventional QpL. Next, the contralateral hind limb was swiftly moved further forward and placed on the belt around where the vertical line passing through the imaginary center of mass projected on. Then, the hip joint was rapidly extended and the trunk was righted up, being fully supported by the one hind limb. A few quick steps followed this righting action, in which the step length was shorter than conventional BpL. Finally, cyclic trunk tilts appeared to accompany the rhythmic hind-limb stepping in a well-coordinated manner, that is stable BpL. These results identify behavioral processes of seamlessly switching over from QpL to BpL, and they open the way to our understanding of how on-going activities in the subcortical locomotor circuits are accommodated to volitional motor commands from the cerebral cortex. COI:No

3P-131

The effect of voluntary exercise on motor recovery in Intracerebral Hemorrhage rat Models

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The motor dysfunction after stroke causes an enormous burden such as deterioration of quality of life in surviving patients. The psychological factors such as the patient's motivation promotes motor recovery, but it is still unclear the mechanism of this effect. The purpose of this study was to compare the effects of forced and voluntary exercise for motor function recovery in intracerebral hemorrhage (ICH) rat. Male SD rats were injected with collagenase (200U/mL) into striatum to induce ICH. ICH rats were divided randomly into three groups: forced exercise (F-Ex., n=7), voluntary exercise (V-Ex., n=6) and no exercise (Non-Ex., n=7). The F-Ex. group was trained with treadmill exercise and the V-Ex. group was trained with wheel running. The period of rehabilitation was 4 to 14 days after the surgery. Motor functions were assessed by motor deficit score (MDS) and beam walk test (2.4 cm, 1.0 cm wide) in all groups. Both of the trained groups had greater scores than the non-Ex. group. There were no differences of the recovery between the F-Ex. and V-Ex. groups in MDS and 2.4 cm wide beam walking tests. The score of the 1.0 cm beam walking test was greater in the V-Ex. than the F-Ex. group. These data suggested that the difference of the recovery of the motor function by two kinds of exercise may be involved in motivation and stress. COI:No

3P-132

Energy substrates appearance and disappearance rate calculation from tracer experiments considering gluconeogenesis

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For quantitative understanding of the dynamics of energy substrates, it is necessary to construct a mathematical model. To construct the model, the metabolic substrate fluxes between several compartments should be experimentally obtained in various exercise intensities. Such information was reported by using the tracer experiments. In the experiment, [3-^{13}C] lactate and [6-6-^{2}H] glucose were infused into the blood and changes in those concentrations were measured [Bergman et al., 2000]. From these concentrations, fluxes were estimated by using the Steele equation. In the equation, it was assumed that the substances flowed only in one direction; from tissue to blood and subsequently to another tissue. However, in reality, glucose and lactate transform each other in the muscle and liver. Therefore, we constructed a three-compartment model in which the labeled glucose and lactate were exchanged among hepatic cells, blood, and muscular cells, considering gluconeogenesis and glycolysis. As a result, we could quantitatively estimate appearance and disappearance fluxes of glucose and lactate at rest, and at the low and the high exercise intensities. It was revealed that the lactate fluxes in the steady state calculated in our model were greater only by several percent than those calculated in the Steele equation. This result suggested that the effect of lactate recycling by gluconeogenesis on net lactate flux was small. COI:No

3P-133

EID1 inhibits lipid accumulation through suppression of GPDH expression

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Obesity is a condition characterized by excess adipose tissue and is the most common metabolic disorder. Adipocytes are derived from mesenchymal stem cells. A recent study has shown that EP300-interacting inhibitor of differentiation 1 (EID1) reduces the accumulation of triglycerides, a kind of neutral fat, in mouse pre-adipocyte 3T3-L1 cells. Although the suppression of fat accumulation is extremely important, our knowledge of the biological underpinnings of the mechanism of inhibitory effect of EID1 is insufficient. Here we report that over-expressed EID1 suppresses fat accumulation of preadipocytes 3T3-L1 cells through downregulation of glycerol-3-phosphate dehydrogenase (GPDH), a marker of adipocyte differentiation. EID1 expression vector was transfected to cultured 3T3-L1. Then, transfected cells were stimulated with IBMX, dexamethasone, insulin, and rosiglitazone for induction to differentiated adipocytes. After 9 days of stimulation, almost all 3T3-L1 cells without EID1 transfection were observed the accumulation of lipid droplets. In contrast, 70% of 3T3-L1 cells transfected with EID1 did not differentiate and the activity of GPDH was significantly reduced. Furthermore, this inhibitory effect was keep during the expression of EID1. These findings indicate an important function of EID1 in the regulation of adipocyte differentiation through downregulation of GPDH. COI:No

3P-134

Effect of *coriandrum sativum* on invasion abilities of cancer cells in *vitro*

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Coriandrum sativum is an annual herb in the Apiaceae, and this leaf and seed are used for cooking. However, the anti-cancer activity of *Coriandrum sativum* has not yet been fully elucidated. In the present study, the effects of *Coriandrum sativum* on cancer cells were investigated by using HepG2, Caco2 and B16 cells. The extract of *Coriandrum sativum* was prepared with methanol after lyophilization for 24 hours. Then methanol was removed by using rotary evaporator. The proliferative effects of *Coriandrum sativum* on these cells were assessed by WST-1 assay. The trans-well migration assay was used to examine migration abilities and trans-well invasion assay was used to examine invasion abilities of cancer cells. After treatment of each cell with the extract of *Coriandrum sativum*, the conditioned medium was analyzed by using gelatin or fibrin zymography. In the concentration of *Coriandrum sativum* at which the proliferations of HepG2, Caco2 and B16 cells were not influenced, the migration abilities and invasion abilities of these cells were impaired. Gelatin and fibrin zymography showed that the extract of *Coriandrum sativum* reduced MMP-2 or u-PA activity in the conditioned medium. These findings suggest that *Coriandrum sativum* inhibits invasion activity of each cancer cell through reduction of protease activity involved in degradation of extra cellular matrix. COI:No

3P-135

Voluntary exercise enhances pilocarpine-induced saliva secretion and aquaporin 1 expression in rat submandibular glands

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We have reported upregulation of the expression levels of aquaporin (AQP) 1 and 5 in the submandibular glands (SMGs) in heat-acclimated rats. In this study, we examined pilocarpine-induced saliva secretion and AQP expressions in rats after voluntary exercise. Male Wistar rats, 10-week-old, were kept for 40 days in cages either with a running wheel (exercise rats, n = 6) or with a locked wheel (control rats, n = 6). After the training period, the rats were anesthetized and pilocarpine was intraperitoneally injected (0.5 mg/kg) to stimulate saliva secretion. Saliva was collected, and the SMGs were sampled and subjected to Western blot, reverse transcriptase polymerase chain reaction, and immunohistochemical analyses. Pilocarpine induced a greater amount of saliva in the exercise rats than in the control rats. Expression levels of AQP1 mRNA and protein were significantly higher in the exercise rats' SMGs than those of the control rats, but the expression of AQP5 was not affected by voluntary exercise. Voluntary exercise increased the expression of vascular endothelial growth factor (VEGF) and cluster of differentiation 31 (CD31), a marker for endothelial cells, in the SMGs. Voluntary exercise promoted pilocarpine-induced saliva secretion, probably via an increase in the expression level of AQP1 due to VEGF-induced CD31-positive angiogenesis in the SMGs. COI:No

3P-136

Molecular mechanisms of neurogenic fever: the involvement of cyclooxygenase-1 and microsomal prostaglandin E synthase-1

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Neurogenic fever (NF) often occurs after acute brain injury, such as brain hemorrhage. NF is deleterious to the injured brain and needs to be suppressed. However, its precise control is hampered by the lack of information about the molecular mechanism of NF. In this study, we aimed to examine whether NF involves the central production of prostaglandin E2 (PGE2), as in the case of fever under infectious/inflammatory conditions. We used a murine model of NF, in which hemorrhage was induced by microinjection of collagenase into the preoptic area, the thermoregulatory center. Diclofenac and ketoprofen, non-selective cyclooxygenase (COX) inhibitors, significantly, but partially, suppressed NF suggesting a partial involvement of PG system. SC560, a COX-1 specific inhibitor, was effective in reducing NF and PGE2 levels in hemorrhaged region. To further confirm the involvement of PGE2 in NF, we examined NF and levels of PGE2 in the hemorrhaged region both in wild mice and mice deficient for microsomal PGE synthase1 (mPGES1), an enzyme crucial to the synthesis of PGE2. In mPGES1-deficient mice, PGE2 production was almost completely suppressed whereas the suppression of NF was partial. There was a linear relationship between the size of hemorrhage and the degree of NF both in wild mice and mPGES1-deficient mice. However, the regression line for mPGES1-deficient mice was shifted downwards compared to that for wild mice. These results indicate that NF in the current murine model is brought about by both PGE2-dependent and PGE2-independet mechanisms. COI:No

3P-137

Effects of hyperbaric treatment at 1.3 atmospheres absolute on hyperglycemia and hyperinsulinemia in type 2 diabetes

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The purpose of the present study was to investigate the effect of hyperbaric treatment at 1.3 atmospheres absolute (ATA) on hyperglycemia and hyperinsulinemia in type 2 diabetes. Otsuka Long-Evans Tokushima fatty (OLETF) and Long-Evans Tokushima Otsuka (LETO) rats were used as diabetes with hyperinsulinemia and non-diabetes without hyperinsulinemia models, respectively. All rats were devided into two groups; non-treatment or treatment with hyperbaric chamber groups. The rats with the treatment were exposed to hyperbaric chamber at 1.3 ATA for 8 h in a day from 24 to 40 weeks old. The fasting glucose level at 40 weeks old was significantly higher in OLETF rats than those of LETO rats. However, the value was significantly lower in OLETF rats by the hyperbaric treatment than those of non-treatment rats. The fasting insulin level was also significantly higher in OLETF rats than those of LETO rats, while increased insulin level of OLETF rats were divided into two groups, high and low insulin level. These results suggest that hyperbaric treatment at 1.3 ATA is appropriate as complementary treatment for type 2 diabetes.There are no conflicts of interest to declare. COI:No

3P-138

Expression of platelet-derived growth factor receptor α (PDGFRα)-immunoreactivity (ir) is suppressed by refeeding after 48-h fasting in the hypothalamus of male mice

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We previously showed that fasting significantly increased PDGFRα mRNA and its protein of the hypothalamus in male mice fed with chow diet but these changes were impaired with high-fat (HF) diet. Next, we revealed that using the tyrosine kinase inhibitor, HF diet-induced obesity and glucose tolerance were partially prevented by imatinib intraperitoneal treatment with no effect in mice fed cow diet. Further, we found that imatinib intraventriclar treatment itself increased body weight regardless of diet. These results suggest that PDGFRα system in the hypothalamus is differently involved in the regulation of feeding behavior compared with peripheral PDGFRα system. In the present study, mice were refed after fasting. Control mice were fasted for 48-h. They were sacrificed, perfused with 4% paraformaldehyde, and processed for immunocytochemistry. Numerous PDGFRα-ir cells were evident through the brain after fasting. Refeeding after fasting decreased these PDGFRα-ir cells. We semi-quantified these changes by Image J. Compared with fasted mice, PDGFRα-ir cells in the cortex were decreased by refeeding. However, this decrease was much more prompt in the area of the arcuate nucleus (15 times, compared to the cortex) and the dorsomedial hypothalamus (10 times, compared to the cortex) where feeding related areas. We speculate that PDGFRα system in the hypothalamus is involved in the regulation of feeding behavior under normal feeding condition. COI:No

3P-139

Comparison of different hands and feet cooling techniques for attenuating core temperature elevation during exercise in the heat

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Pre-cooling is a popular strategy for alleviating heat strain. Because whole body water immersion that most commonly used to pre-cool in experimental studies is not always possible in all field settings, we have shown that hands and feet water immersion reduced heat strain. In the present study, we aimed to compare the effectiveness of three different cooling techniques for improving the practical utility. Eight males engaged in 60 min of walking at a moderate speed in a hot environment (37° C) while wearing protective clothing. Before walking, they were exposed to hands and feet cooling for 30 min by either immersing into water at 20° C (WA), wearing frozen-gel mittens and slippers (FG), or wrapping phase-change materials melting at around 12° C (PC). In the pre-cooling period, decreases in skin temperature of hand and foot did not differ among the three techniques, but FG caused greater reduction in skin vascular conductance of hand and foot and augmentation in cold sensation and heat flux of hand and foot than that in WA and PC ($p<0.05$). Rectal temperature during walking was lower in the three techniques than in the control (without cooling, $p<0.05$). The attenuations in WA and PC were greater than that in FG ($p<0.05$). These results suggest that mild cooling by phase-change materials showed similar effectiveness to water immersion in reducing heat strain by hands and feet pre-cooling. COI:No

3P-140

Effects of The Short-Term of Growth Hormone Administration on Plasma Leptin in Male Diet-Induced Obesity Rats

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Long-term growth hormone (GH) treatment has been proposed to reduce fat mass and maintain lean mass. Plasma leptin also decreased in proportion to the adipose tissue mass (the major leptin secreted organs). However, the effect of short-term GH treatment (GHTxsh) on plasma leptin was unclear. The present experiments investigated the GHTxsh (1 mg/kg, twice daily, 5 days) on plasma leptin in 2 different types of adipose tissue in relation to insulin sensitivity. In the first experiment, basal plasma leptin from the DIO rats was decreased after GHTxsh, while it was unchanged in the lean control rats. In the second experiment, meal-induced plasma leptin from DIO rats was not changed by GH treatment. In addition, fasting plasma leptin was increased in control, but not in DIO, rats. Fasting plasma insulin was higher in the control rats after GH treatment. By contrast, the fasting plasma insulin effect could not be demonstrated in the DIO rats. Finally, glucose tolerance (GT) in control rats was unchanged; however, the GHTxsh could reverse GT in DIO rats. In conclusion, GHTxsh decreased basal plasma leptin only in DIO rats. GHTxsh could not change meal-induced plasma leptin. Finally, GHTxsh increased fasting plasma leptin only in control rats. The results revealed the different responses and perhaps the different mechanisms that GHTxsh influenced plasma leptin and the independent effect of GH on plasma leptin from insulin action. COI:No

3P-141

Effects of early insulin treatment on bone microstructure in Goto-Kakizaki type-2 diabetic rats

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Type 2 diabetes mellitus (T2DM), a common age-related metabolic disorder, has been known to impair the strength of bone microstructural. Insulin administration is the treatment of choice for T2DM patients with poor glycemic control or poor response to antidiabetic drugs, and it can induce anabolic effects on osteoblasts in vitro. However, whether early insulin replacement in the T2DM rats is effective in restoring bone structure has been elusive. In the present study, we therefore determined the effects of insulin injection on bone in adolescent female Goto-Kakizaki (GK) rats as compared with untreated GK rats and normal Wistar rats. The results showed that GK rats exhibited decreases in trabecular bone mineral density and cortical thickness with an increased medullary area, as determined by micro-computed tomography. However, 12-week insulin treatment did not restore cortical and trabecular structure in 7-month-old GK rats. Mean cortical area and periosteal perimeter were also not different among the 3 groups. In conclusion, despite being an effective diabetic therapy, insulin could not rescue bone microstructure in T2DM GK rats; therefore, early prevention of T2DM is the better way to protect against diabetic osteopathy. COI:No

3P-142

The anti-stress effect of the press tack needle - a comparative study on acupuncture points -

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Objectives: We have already reported that the press tack needle (PTN) treatment of the GV20 acupuncture point (Baihui) has an anti-stress effect in rats with social isolation stress. In this study, we investigated whether the effect would differ according to the acupuncture point.
Methods and results: Male Wistar rats were divided into a non-stress group (Control), a stress group (Stress) and a stress plus PTN treatment group (PTN). The rats in the PTN and Stress groups were housed alone for eight days. In the PTN group, a PTN (Pyonex; Seirin Co., Japan) was fixed on the GV20 or the BL21 acupuncture point (Weishu) on Day 7. We measured the stress behavior based on the period in which the rats showed aggressive behavior characterized by wrestling, boxing, or biting an intruder rat on Day 8. The duration of aggressive behavior in the Stress group was significantly increased in comparison to that in the Control group. The increase was inhibited in the PTN(GV20) group but not in the PTN(BL21) group. Both orexin and oxytocin are produced in the hypothalamus, and these secretions are reported to increase in these model animals. The levels of plasma orexin A and oxytocin in the Stress group were significantly increased in comparison to the Control group; however, these increases were inhibited in the PTN(GV20) group.
Conclusion: These results suggest that the effects of PTN may differ according to the acupuncture point that is used.
COI: No COI:No

3P-143

Circular acupuncture needles adjust cortisol secretion in mood disorder

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In mood disorders, excessive secretion of cortisol is observed. The aim of this report is to clarify whether acupuncture needles alter stress response. Subjects were recruited inpatients diagnosed unipolar or bipolar depression. Four days of circular acupuncture needles intervention was performed with the design of placebo control double blind trial.35 participants were allotted two groups (14 active needle group, 21 sham needle group). Each subject collected saliva four times a day (immediately after rising, 7AM, 4PM and 9PM), for two days (pre and post intervention). In active needle group, significant decrease of salivary cortisol secretion was observed at 4PM ($p=0.026$). In conclusion, acupuncture has a possibility of stabilizing stress response in mood disorders. COI:No

3P-144

Increased oxytocin-monomeric red fluorescent protein 1 fluorescent intensity with urocortin-like immunoreactivity in the hypothalamo-neurohypophysial system of aged transgenic rats

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Introduction
Oxytocin that is known to be involved in parturition and milk ejection reflex may be an important hormone in the aging process. Here, we examined the age-related changes of oxytocin-monomeric red fluorescent protein 1 (mRFP1) and urocortin of the hypothalamus, using oxytocin-mRFP1 transgenic rats.
Materials and Methods
Adult (3, 12, 18 and 24 months) male oxytocin-mRFP1 transgenic rats were used in the present study. Urocortin-like immunoreactivity (LI) was detected by immunohistochemistry.
Results and Discussion
The mRFP1 fluorescent intensity in the hypothalamo-neurohypophysial system was significantly increased in the aged transgenic rats. Age-related increased urocortin-LI cells almostly co-exist oxytocin-mRFP1-expressing cells in the hypothalamus of the transgenic rats. The physiological role of the co-existence of oxytocin and urocortin in the hypothamlamus of aged rats should be clarified by further study. COI:No

3P-145

The regulation of Arnt expression through thyroid hormone receptors

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Thyroid hormone (TH) has a critical role in human metabolism including liver. The changes of TH status affect the expression of several TH target genes in liver, and the effects induce chronic liver diseases such as non-alcoholic fatty liver disease (NAFLD). The expressions may be also changed by the exposure to several toxic compounds. Both Aryl hydrocarbon receptor nuclear translocator (Arnt) and Aryl hydrocarbon receptor (AhR) form a complex through the activation by ligands such as TCDD. Some reports showed that Arnt binds to the other nuclear receptors, but the interactions between Arnt and TH receptors (TRs) are still unclear. Previously, we reported that Arnt augmented TR-mediated transcription, and also Arnt mRNA expression were regulated by TRs. In this study, to clarify the mechanism, we investigated the interaction between Arnt and TRs further.To investigate TH response elements (TREs) on promoter region of Arnt, we performed ChIP assay with biotin-tagged TRs in HepG2 cells. The binding of the TR to an TREs on Arnt promoter region was observed as the control TREs of both Bcl3 and LDLR. Moreover, to confirm whether the TRE is specific to regulate Arnt expression, we utilized CRISPR-Cas9 system to knock down specific TREs.These results suggested that Arnt expression may be regulated by TR-mediated transcription through TREs on Arnt promoter region. Moreover, through the interaction between Arnt and TH system, metabolic systems in liver are maintained partly. COI:No

3P-146

Effects of food deprivation on the hypothalamic feeding-regulating neuropeptides gene expressions in the streptozotocin-induced diabetic rat

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We examined the hypothalamic feeding-regulating neuropeptides gene expressions in the arcuate nucleus (ARC) with/without fasting in the diabetic rats administered streptozotocin (STZ). STZ (80 mg/kg) was administered intraperitoneally (i.p.) in adult male Wistar rats. Rats were divided into 3 groups: Normal glucose (<300 mg/dl at light period) tolerance (N), Impaired glucose (>300 mg/dl at light period and <200 mg/dl after fasting for dark period) tolerance (I), and Diabetes (D) (>300 mg/dl at light period and >200 mg/dl after fasting for dark period). Two weeks after i.p. administration of STZ, they were decapitated after fasting for 12 hours. The gene expressions of *proopiomelanocortinPOMC, cocaine- and amphetamine-regulated transcriptCART, neuropeptide YNPY, agouti-related proteinAgRP* in the ARC were quantified by using in situ hybridization histochemistry. *POMC* and *CART* were significantly decreased in I and D compared to N. On the other hand, *NPY* and *AgRP* were significantly increased in D compared to N, whereas, that in I were not. The gene expression of the hypothalamic ARC anorexigenic peptide decreased in the rats with hyperglycemia after STZ administration but not hyperglycemia after fasting, and no significant change was observed in the orexigenic peptide. COI:No

3P-147

The role of D1 receptor in the nucleus accumbens in the expression of the odor preference of female rats

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Dopaminergic projections from the ventral tegmental area to the nucleus accumbens (NAcc) are considered essential in mediating the effects of natural rewards, and dopamine (DA) is believed to play a fundamental role in reward processes. It has been demonstrated that estrous female rats showed male-directed odor preference irrespective of presence or absence of prior sexual experience. Using in vivo microdialysis, we previously demonstrated that airborne odors from males induced a significant increase in extracellular DA in the NAcc in both sexually experienced and naive estrous female rats, suggesting that male odors are unconditioned rewards for female rats. The present study was conducted to investigate whether DA release in the NAcc is involved in the expression of the odor preference for male odors in sexually naive female rats. DA D1 (D1R) or D2 receptor (D2R) antagonist was injected into the NAcc of the female rats and the preference for male odors was examined. Administration of D1R, but not D2R, antagonist eliminated the odor preference for male odors. Neither of these antagonists impaired the locomotor activity and the ability to discriminate estrous odors from male odors. These results suggest that D1R in the NAcc plays a critical role in the expression of the odor preference for male odors in sexually naive female rats. COI:No

3P-148

allo-grooming behavior to distressed cagemate mice: activation of oxytocin receptor expressing cells

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Comforting someone in distress is commonly observed in human beings. Allo-grooming behavior is observed during consolation in various animals. Recently, monogamous prairie voles have been reported to show allo-grooming behavior to console their distressed partners.The purpose of this study is to establish a mouse model of consolation behavior toward a distressed conspecific cage mate so that neural mechanisms underlying the control of consolation behavior can be investigated in details.Pairs of C57BL/6J mice were housed together for 4 weeks. One mouse of each pair was exposed to an aggressive ICR mouse or kept isolated, and was returned to its home cage where its cage mate (a resident mouse) stayed. Mice exposed to aggressive ICR mice showed defeated postures during exposure to ICR mice. Defeated mice were returned to their home cages. Resident mice showed side-by-side contact and allo-grooming behavior toward socially defeated mice. The duration of allo-grooming behavior was significantly longer toward defeated mice compared to that toward non-defeated mice.Exposure to socially defeated mice increased expression of Fos protein in oxytocin receptor-expressing cells in the cingulate cortex and insular cortex. Grooming has been shown to have an anxiolytic action in rodents. Thus, the present data are consistent with a view that mice can show consolation-like behavior toward distressed cage mates. COI:No

3P-149

Relations between the orexin neural activity and the autonomic response by aversive stress stimulation

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Although orexin neurons are essential factor of inducing autonomic response by stress, the orexin neuronal activity in presence of stressor and its physiological role remain unclear. In this study, we investigated whether orexin neuron activity would be changed in association with stress-induced autonomic responses. To record two parameters of heart rate and the orexin neural activity from the free-moving-mouse at the same time in real time, we used the fiber photometry and telemeter systems. We used G-CaMP6 as a neural activity sensor, and mCherry for reference to exclude possible noise. Into the hypothalamus of orexin-tTA mice, AAV-TetO(3G) G-CaMP6 and AAV-TetO(3G) mCherry mixture were injected. Genetically specified neural activity in deep brain of those mice could be detected through the inserted optical fiber. Three acute aversive stresses were applied; intruder stress, aversive sound stress, aversive smell stress. The experiment showed that orexin neural activity immediately increased after three aversive stress stimulations. Additionally, they preceded the increases in heart rate. We conclude that an increase in orexin neural activity may be one of the causes but not a result from autonomic changes associated with fight-or-flight response. COI:No

3P-150

Prenatal treatment of bisphenol A causes some alterations on properties of odor-responsive neurons in rats

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Perinatal exposure of bisphenol A (BPA) cause several alterations in the brain and behavior. Our previous studies showed that prenatal treatment of low-level BPA impaired the gender differences of some behavioral parameters and enhanced depression-like behavior. In addition, in an experiment using a predator (fox) odor, BPA enhanced the behavioral response to the odor. It was suggested that BPA-treated rats were in increased anxiety and stress levels in the presence of the odor. This experiment focused on directly investigating the olfactory signaling system in the central nervous system. We made measurements in the area around the amygdala, which contains part of the olfactory pathway. Response patterns were examined by an extracellular recording system for three plant odorants and three predator odorants. Odor-responsive neurons of the BPA rats showed higher responses to fox odor than that did those of the control rats. This result was similar to those for a behavioral experiment in which BPA rats showed high responses to fox odor. In addition, although few neurons in the control rats responded to both plant and predator odors, a significant number responded in the BPA rats. There is a close relationship between the olfactory signaling system and the stress responsive system via the endocrine and autonomic nervous system. These results suggest that BPA modified some of the neuronal network involved in the olfactory pathway at the level of the amygdale, and also affected the corresponding stress response system. COI:No

3P-151

Changes in leg volume during an upright position

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It is well known that hydrostatic pressure pulls body fluid down into the lower extremities during standing and causes leg edema. Early studies show that the fluid accumulation increased and reached a plateau for about 40 minutes in upright position. However, it has been reported that the plateau was not reached. And they show that leg fluid increased linearly after more than 45 minutes in sitting position. This study aimed at investigating the time course of fluid accumulation for 90 minutes in upright (seated/standing) position.In order to clarify changes in leg volume, leg circumferences were measured at ankle, calf and thigh levels and leg fluid was estimated using a method of bioelectrical impedance. Subjects lay down in the supine position for 90 minutes, and then sat with their legs still for 90 minutes or they stood still for 90 minute. When they lay down, leg volume gradually decreased in the first 60 minutes, and the rate of decrease declined from 70-90 minutes. The reduction almost fit a first order exponential curve, suggesting that it is possible to decide foreseeable point in which decumulation of leg fluid stops in the supine position. Leg volume gradually increased during the upright position. The increase in fluid was quite variable and did not fit well with an exponential function and/or a linear function, suggesting that it is difficult to predict an end in the time course of fluid accumulation. Further study will be needed to clarify whether or not leg fluid accumulates lineally during prolonged upright position. COI:No

3P-152

A 3-month walking training does not improve thermal sensation in healthy elderly men

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Whole body and skin thermal sensations are blunted with normal aging. We recently reported that skin warmth perception was more sensitive in young adults with regular exercise (> 3 days/wk) than those without regular exercise. In this study, we assessed if blunted thermal sensations in the elderly were improved by regular exercise.

Eight elderly (72 ± 2.4 yrs) healthy men underwent a 3-month walking training (brisk walking (n = 5), ≥ 60 min/wk; or daily walking (n = 3), ≥ 10,000 steps/day). Before and after intervention, cold and warmth detection thresholds (± 0.1 ° C/s) at chest and forearm skin (thermode, 6.25 cm^2) and whole-body thermal sensation (VAS) under normothermia (NT; esophageal temperature, Tes, 36.4 ± 0.3 ° C) and mild-hyperthermia with lower legs immersion in 42 ° C water (HT; Tes, 37.2 ± 0.3 ° C) conditions were measured. Peak oxygen uptake (VO_{2peak}; step-up maximal walking test), total hemoglobin mass (THb), plasma volume (PV), and blood volume (BV) (CO-rebreathing method) were also measured.

After intervention, VO_{2peak}; increased but not significantly with increased THb, PV, and BV ($P < 0.05$). Skin cold and warmth detection thresholds at both sites, and whole-body thermal sensation remained unchanged both under NT and HT.

A 3-month walking training improved aerobic capacity with an increased blood volumes while did not improve whole body and skin thermal sensations in elderly men. COI:No

3P-153

Influence of artificial high concentration CO_2-water leg-bath on vascular elasticity and blood flow

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In this study, the influence of artificial high concentration CO_2-water (CO_2>1000 ppm) leg-bath on peripheral blood flow and vascular elasticity was investigated. The healthy female college students (n=11, age; 21-23yrs, height; 156.1±4.5cm, weight; 56.2±13.7kg) participated in this study. The subjects were randomly divided into the CO_2-water leg-bath group (n=6) and the tap-water group (n=5). A laser Doppler flowmetry probe for recording skin blood flow were attached to the skin over right medial gastrocnemius (MG) ($BF_{immersion}$) and right middle fingertip ($BF_{non\ immersion}$). The subjects immersed lower legs into tap-water or artificial CO_2-water at 35° C for 10 min following to 5-min rest at room temperature. The arterial vascular elasticity was evaluated using Ankle-brachial index (ABI), and brachial-ankle pulse wave velocity (baPWV) at pre and post leg-bath. $BF_{immersion}$ in the CO_2-water leg-bath was significantly larger than in the tap-water leg-bath (CO_2-water: 4.1±1.5 ml/min/100g, tap-water: 0.3±0.2 ml/min/100g, $p<0.01$). The baPWV tended to decrease in the CO_2-water leg-bath compared with the tap-water leg-bath. The present results suggested that high concentration artificial CO_2-water bathing may contribute to the improvement of peripheral and central blood flow and vasodilation. COI:No

3P-154

EFFECTS OF IRRADIATION ON MICE GENE EXPRESSION IN SKIN AND BONE

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Long duration spaceflight reduces bone density in astronauts. The potential for exposure to space radiation to contribute to lasting decrements in bone mass is not yet understood. Sustained changes to bone mass have a relatively long latency for development, however skin is a radiation sensitive organ and changes in skin gene expression may serve as an early radiation biomarker of exposures. Previous studies have shown that FGF18 gene expression levels of hair follicles collected from astronauts. In bone, FGF18 appears to regulate cell proliferation and differentiation positively during osteogenesis and negatively during chondrogenesis. Cellular defense responses to radiation are shared by a variety of organs, hence in this study, we examined whether radiation induced gene expression changes in skin may be predictive of the responses of skeletal tissue to radiation exposure. We have examined growth arrest pathways in mouse skin and long bones by measuring gene expression levels via qPCR after exposure to total body irradiation (TBI). In skin samples one day after IR, skin expression of FGF18 was significantly greater than sham-irradiated controls, but did not differ 11 days post TBI. In bone, TBI significantly increased expression of the pro-bone resorption cytokine, MCP-1, one day after TBI. FGF18 expression in skin and MCP-1 expression in bone were found to be positively correlated. These results suggest that early radiation induced changes in FGF18 gene expression in skin may have value for predicting subsequent loss of cancellous bone mass. COI:No

3P-155

Rasd1 is an estrogen-responsive immediate early gene and modulates expression of late genes in rat anterior pituitary cells

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Dexamethasone-induced Ras-related protein 1 (Rasd1) is a member of the Ras superfamily of monomeric G proteins that play central roles in multiple cellular functions, including cell proliferation, differentiation, transformation, secretion and apoptosis. Here we investigated the role of Rasd1 in regulating estrogen-induced gene expression in primary cultures of rat anterior pituitary cells. Rasd1 mRNA expression in anterior pituitary cells decreased after treatment with forskolin or serum and increased after treatment with 17β-estradiol (E2). Increases in Rasd1 mRNA expression occurred as early as 0.5 h after E2 treatment, peaked at 1 h and were sustained for as long as 96 h. This rapid and profound increase in Rasd1 mRNA expression induced by E2 was also seen in GH4C1 cells, an estrogen receptor-positive somatolactotroph cell line. Among pituitary estrogen-responsive late genes studied, basal mRNA expression of Pim3 and Igf1 genes was decreased by RNA interference-mediated knockdown of Rasd1 expression, whereas basal expression of the Giot1 gene was increased. Moreover, Rasd1 knockdown enhanced stimulation of Pim3 mRNA expression and attenuated inhibition of Fosl1 mRNA expression 24 h after E2 treatment. These changes in mRNA expression were accompanied by enhanced activity of promoters containing CRE, AP-1 and SRE binding sequences. These results suggest that Rasd1 is an estrogen-responsive immediate early gene and modulates E2 induction of at least several late genes in anterior pituitary cells. COI:No

3P-156

Biological effect of low-intensity pulsed ultrasound sonication on the LM8 osteosarcoma cell line

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Previously, we revealed that the combination of pulsed electromagnetic field stimulation and doxorubicin is an effective treatment for osteosarcoma, which is the most common malignant bone cancer. Currently, we are attempting to develop a novel therapeutic approach for osteosarcoma by using non-invasive mechanical instruments. In this study, we focused on the biological effects of low-intensity pulsed ultrasound sonication (LIPUS), which promotes bone formation and accelerates bone maturation. The purpose of this study was to investigate the antitumor effects of LIPUS on osteosarcoma cells. The effects of LIPUS on cell viability, induction of apoptosis, mitochondrial membrane potential, and intracellular signaling molecules in the LM8 osteosarcoma cell line were examined. LIPUS inhibited cell viability in a stimulation time-dependent manner in LM8 cells. In addition, it significantly reduced mitochondrial membrane potential and increased the number of annexin V-expressing cells, suggesting the induction of apoptosis. LIPUS treatment significantly increased phosphorylated Akt and IκBα levels and significantly reduced phosphorylated TAK1 and phosphorylated Chk1levels. These results suggest that LIPUS is a non-invasive adjuvant therapy that can be used to inhibit the proliferation of osteosarcoma cells. COI:No

3P-157

Therapeutic potential for spinocerebellar ataxia type 1 (SCA1): Exploiting functional G protein-coupled receptor (GPCR) crosstalk between GABAb receptor and mGluR1 in SCA1 model mice

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Spinocerebellar ataxia type 1 (SCA1) is an inherited progressive neurodegenerative disease and exhibits cerebellar ataxia and atrophy of Purkinje cells (PCs). Our previous study has shown that SCA1 model mice exhibit progressive impairment of the cerebellar signaling mediated by one of G-protein coupled receptors (GPCRs), the metabotropic glutamate receptor type 1 (mGluR1), which is indispensable for synaptic plasticity and motor coordination. However, the cerebellar mGluR1 signaling is not completely abolished in SCA1 mice and enhancement of the remaining cerebellar mGluR1 signals could be a potential therapeutic strategy for the treatment of SCA1. Interestingly, there is a functional crosstalk between mGluR1 and GABAb receptor (GABAbR), a different type of GPCR, in normal PCs. Previous reports showed that mGluR1 is tightly coupled to GABAbR, and that the nanomolar range of baclofen (Bac), a GABAbR agonist, potentiates mGluR1 responses in PCs with minimal effects on classical GABAbR signaling such as reduction in presynaptic transmitter release in the cerebellum. Exploiting the mGluR1-enhancing effect of Bac (i.e. the functional crosstalk between GABAbR and mGluR1), we show that low-dose Bac improves motor performance in SCA1 mice. Because Bac is a clinically approved drug as a muscle relaxant, our strategy could be a novel and advantageous therapy for SCA1 patients in the future. COI:No

3P-158

Macitentan treatment ameliorate impaired right coronary vasodilative function in SuHx rat model of severe pulmonary arterial hypertension

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Pulmonary hypertension (PH) causes cardiac hypertrophy in right ventricles (RV), and eventually leads to RV failure due to persistently elevated ventricular afterload. We hypothesized that the mechanical stress of RV associated with increased afterload impairs vasodilative function of the right coronary artery (RCA) in PH. PH was induced in male rats by a single subcutaneous injection of Sugen 5416 followed by 3 weeks of hypoxia (SuHx) while a control group exposed to room air. At week 5 post-injection, the rats were then randomized to treatment or no treatment with Macitentan for 5 to 8 week. RV function was evaluated by magnetic resonance imaging at week 5 and 8 post-injection. Coronary endothelial function was assessed using microangiography at week 8 post-injection. RV ejection fraction (EF) was significantly decreased and RV hypertrophy was significantly increased in the SuHx rats. Endothelium-dependent and -independent vasodilative responses were significantly attenuated in the middle and small arteries in SuHx rats. Macitentan treatment reversed the decreased EF and development of RV hypertrophy, and impaired vasodilative function of RCA. The observed impaired vasodilative function of RCA in PH rats suggests that impaired RCA function may contribute to RV failure in the patients with severe PH. COI:Properly Declared

3P-159

The involvement of ryanodine receptors in depression-like model mouse.

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For the development of a remedy, it is important to elucidate unexplained parts of mechanism of depression. Last year, we reported that protein expression of ryanodine receptors (RyRs) and IP_3 receptor (IP_3R) increased in hippocampus of the mouse stressed by water immersion with restriction. This increase was improved only RyRs by electroconvulsive shock (ECS). In the present study, depression-like model mouse was administrated dantrolene, the antagonist of RyRs, by intraperitoneal injection. After 2 weeks, we investigated the effect of dantrolene with behavioral (forced swimming test, locomotion activity, novelty suppressed feeding test) and the number of DCX/BrdU (+) cells in hippocampal dentate gyrus. We will report whether dantrolene could improve the depression-like behavior. COI:No

3P-160

Contribution of tyrosine kinase FYN to vascular remodeling in pulmonary arterial hypertension.

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Background and Purpose: Pulmonary arterial hypertension (PAH) comprises a multifactorial group of pulmonary vascular disorders that lead to pulmonary vascular remodeling and right heart failure. Src family non-receptor tyrosine kinase FYN is known to play important roles in vascular remodeling. In this study, we investigated the effects of FYN manipulation on pulmonary vascular remodeling and the therapeutic potential of eicosapentaenoic acid (EPA), which inhibit FYN activity, for PAH by *in vitro* and *in vivo* experiments.
Method: We evaluated the effects of FYN activity on stress fiber formation and STAT3 phosphorylation in HPAEC and HPASMC cells by immunohistochemistry and western blot analyses. Moreover, we evaluated hemodynamic parameters of PAH model rats with and without EPA treatment.
Results: Immunocytochemical analysis indicated that the dominant negative form of FYN prevented TGF-β2-induced stress fiber formation. Dominant negative FYN and EPA significantly suppressed IL-6 induced STAT3 phosphorylation. EPA treatment of PAH model rat significantly improved PAH pathology: pulmonary arterial thickening, right ventricle function and cardiovascular fibrosis.
Summary: Our results suggest that FYN contributes to remodeling processes of PAH. This further points to the benefits of targeting this molecule for treating PAH. COI:No

3P-161

Sex and age differences in sphingosylphosphorylcholine-induced contraction in mice basilar arteries

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Introduction: Cerebral vasospasm after subarachnoid hemorrhage can develop severe and sometimes lethal cerebral infarction and is mediated by the Ca^{2+}-sensitization of vascular smooth muscle (VSM) contraction. As molecular mechanism for such an abnormal VSM hypercontraction, we previously identified a sphingosylphosphorylcholine (SPC)/Fyn/Rho-kinase pathway. Although genetically modified mice would be required to study the importance of this pathway *in vivo*, the Ca^{2+}-sensitization of abnormal cerebral VSM contraction has been unpublished in mice.
Aims: The aims of this study were to clarify if a spasmogen, SPC could induce abnormal VSM contractions of the mice basilar arteries and, if it could do, to assess the possible sex and age differences in it.
Methods: High K^+- and SPC-induced contractions of basilar arteries of C57BL/6J mice were observed in the presence of L-NAME, a nitric oxide synthase inhibitor. The extent of SPC-induced contractions was expressed as a percentage of high K^+-induced contractions.
Results: In the 15-22-week-old mice, SPC caused contractions of basilar arteries in a dose-dependent manner (0.01-10 μM), without the sex difference. In contrast, in the 8-10-week-old mice, 10 μM single-dose SPC-induced contractions were larger in female (30% of high K^+-induced contraction) than in male (15%), both of which were much smaller than the 10 μM SPC-induced contractions (50%) in the 15-22-week-old mice. These data demonstrate the sex and age differences in the SPC-induced abnormal VSM contractions of the mice basilar arteries. COI:No

3P-162

Analysis of redox state of albumin and carbonyl stress in human leukocyte cell lines

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Human serum albumin (HSA) is the most abundant extracellular protein. The buffering function of HSA in redox state is due to a free cysteine residue at position 34 from the N-terminus (Cys-34). HSA is a mixture of human mercaptoalbumin (HMA) in which the Cys-34 is not oxidized, reversibly oxidized human non-mercaptoalbumin (HNA-1) and strongly oxidized human non-mercaptoalbumin (HNA-2). The percentage of oxidized albumin increases in several diseases, such as chronic renal failure, hepatic disease, and diabetes mellitus. We previously showed that human aortic endothelial cells showed conversion of HNA to HMA. We investigated the effect of human leukocyte cell lines on oxidative stress and protein carbonylation. We used the redox state of HSA as a marker of oxidative stress and carbonyl content as a marker of protein carbonylation. Although the strength of reducing ability was different among human leukocyte cell lines, these cells showed conversion of HNA to HMA. On the other hands, human leukocyte cell lines increased or decreased carbonyl content. Human leukocyte cell lines showed conversion of HMA to HNA, and these cells may participate in redox regulation in blood serum or bone marrow. However, further study is required for carbonyl stress. COI:No

3P-163

Effect of different exercises on the differentiation of neural stem cells and motor recovery in rats with motor cortex infarction

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The exercise therapy in rehabilitation is known to promote the recovery from impaired motor function after cerebral infarction. However, the neurologic mechanism remained largely unknown. We established an experimental model rat with infarction confined to motor cortex and the rats experienced following different exercises from 1 day to 4 weeks after surgery: 1) low-intensity treadmill running (Low-T), 2) high-intensity treadmill running, 3) voluntary wheel running and 4) combined exercise programs. Beam-walking tests reveals that motor recovery in gaiting was significantly enhanced by the exercises including Low-T compared to others. To examine the relationship between exercises and the differentiation of neural stem cells, we have performed lineage analysis with BrdU and immunohistochemistry with various markers in ipsilateral side. The number of BrdU(+) cells labeled in a week after surgery was significantly increased in the Low-T group compared to other groups in the peri-infarct region in 4 weeks post-infarction. Interestingly, the differentiation appeared to be affected by distinct exercises. For example, Low-T group had more BrdU(+) /NeuN(+) cells than other groups. It suggests that the different exercise programs could affect motor recovery and neuronal differentiation. COI:No

3P-164

Anti-inflammatory effects of TGF-β1 on microglia/macrophages are sustained in ischemic brains

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Damage-associated molecular patterns (DAMPs) can induce inducible nitric oxide synthase (iNOS) expression in microglia/macrophages (MG/MPs). Yet, in spite of the abundant presence of DAMPs in ischemic brain lesions that were prepared by transient middle cerebral artery occlusion, few MG/MPs express iNOS on 7 days after reperfusion (dpr), when transforming growth factor-beta1 (TGF) was robustly expressed. We found that many MG/MPs expressing iNOS on 3 dpr. Rat primary microglial cells were incubated with lipopolysaccharide (LPS) and released NO level was measured after transient incubation with TGF for 24 h. The NO release was persistently suppressed even 72 h after removal of TGF. Boiled supernatants prepared from ischemic brain tissues showed the similar sustained inhibitory effects on LPS-treated microglia that were prevented by the TGF receptor-selective blocker SB525334. After incubation with TGF for 24 h and its subsequent removal, LPS-induced phosphorylation of IkappaB (IkB) kinases (IKKs), IkB degradation, and NFkB nuclear translocation were inhibited in a sustained manner. In consistent with the results obtained in culture, phosphorylated IKK-immunoreactivity was abundant in MG/MPs in ischemic brain lesion at 3 dpr, whereas it was almost disappeared at 7 dpr. These results suggest that abundant TGF in ischemic brain lesions exerts sustained anti-inflammatory effects on MG/MPs by persistently inhibiting endogenous TLR ligand-induced IkB degradation. COI:No

3P-165

Direct observation of ascorbyl free radicals (vitamin C radicals) in blood of perioperative animals

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Background Ascorbate is located the most downstream of the radical scavenging reactions. It reacts with various free radicals and transforms itself to ascorbyl free radical (AFR, vitamin C radical). The authors observed AFR in whole blood samples obtained from perioperative animals. **Methods** The blood samples were obtained from goats (n = 18) under cardiac surgery using the cardiopulmonary bypass (CPB). The whole blood sample was inserted in an electron spin resonance (ESR) spectrometer (JES-RE1X, JEOL) with no spin trap added. The radical scavenging activity of the plasma was also measured for hydroxyl radicals by spin trapping method. **Results** From the whole blood samples, ESR signals representing AFR (g value = 2.0055, hyperfine splitting constant 0.195 mT) were constantly observed during the perioperative period. The concentration of AFR significantly increased during the surgery using CPB (133% - 158%, $p < 0.05$) except for right after declamping the aorta. On the other hand, radical scavenging activity of the plasma did not change significantly. **Conclusion** The increase in AFR reflected the radical scavenging activity of ascorbate under the oxidative stress due to the cardiac surgery. Unchanged radical scavenging activity of the plasma despite of the increase in AFR may indicate non-negligible antioxidative mechanisms other than ascorbate in perioperative period. COI:No

3P-166

Withdraw

Lunchtime Session

Lunchtime Session 1

[Supported by Lion Co., Ltd.]
Committee for Promotion of Gender Equality

Various actions of the Gender Equality. Let's learn the benefits of the actions for step-up in your career!

March 28 (Wed) 12:00～13:00 Hall 6

(No Abstact)

Lunchtime Session 2

[Supported by Matsutani Chemical Industry Co., Ltd.]

Rare sugar D-allulose induces GLP-1-vagal afferent signaling to correct arrhythmic feeding, obesity and diabetes

March 29 (Thu) 12:20～13:20 Hall 6

(No Abstact)

Lunchtime Session 3

Committee for Women in Physiology of Japan

Dr. Aya Irisawa and Aya Irisawa Memorial Award for Women Physiologists

March 30 (Fri) 12:00～13:00 Hall 6

(No Abstact)

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