Symposia

Symposium 01
Neuronal plasticity: from molecule to behavior
[Collaboration Symposium with The Japan Neuroscience Society]

(March 27, 9 : 00–11 : 00, Room A)

1S01A-1
Neonatal chronic stress alters actin dynamics and experience-driven synaptic plasticity via ADF/cofilin inactivation in the rat barrel cortex

Tada, Hirobumi; Suyama, Kumiko; Takahashi, Takuya (*Department of Physiology, Yokohama City University Graduate School of Medicine*)

Experience-dependent neural plasticity is crucial for the establishment of neural circuits and cognitive functions. Abnormal environment early in life such as neonatal chronic stress could cause various psychiatric disorders by the disruption of circuit formation. However, the mechanisms underlying how early long-lasting stress alters circuit organization remain poorly understood. Here, we found that neonatal chronic stress with social isolation phosphorylated and inactivated ADF/cofilin, the actin depolymerizing factor, via the stress glucocorticoid hormone signaling in the increase of immobilized fraction of actin. This led to the prevention of experience-driven synaptic AMPA receptor delivery in the developing rat barrel cortex. Thus, neonatal chronic stress inactivates ADF/cofilin, alters actin dynamics, and results in the blockade of experience-driven synaptic AMPA receptor delivery in the sensory cortex, leading to the malfunctioning in sensory processing which constitutes prominent symptoms in psychiatric disorders.

1S01A-2
A retrograde axonal transport elicited by Semaphorin3 A drives AMPA receptor subunit GluA2 to dendrites

Yamashita, Naoya; Goshima, Yoshio (*Yokohama City University, School of Medicine, Laboratory of molecular Pharmacology and Neurobiology*)

Neurons are compartmentalized into two molecularly and functionally distinct domains, axons and dendrites. The precise targeting and localization of proteins within these domains is critical for every aspect of neuronal function. However, how this process is regulated remains to be elucidated. We here demonstrate that Semaphorin3A (Sema3A), a secreted factor that navigates axons and dendrites, induces a retrograde axonal transport signaling, which regulates AMPA receptor subunit GluA2 localization in dendrites. In cultured hippocampal neurons at axon outgrowth stage, Sema3A enhances immunofluorescence levels of GluA2, but not GluA1 and GluN1 in dendrites. Using local Sema3A stimulation, we determine that the site of action of Sema3A is restricted at the axonal growth cone. The signaling elicited in the axonal growth cone is propagated toward the cell body by dynein-dependent retrograde axonal transport coupled with ion-related signal. PlexinA (PlexA), a receptor component for Sema3A, interacts with GluA2 at the immunoglobulin like, Plexins, transcription factors domain (PlexA-IPT). Application of PlexA-IPT suppresses dendritic localization of GluA2 but not GluA1 *in vitro* and *in vivo*. The PlexinA-GluA2 interaction is therefore essential for GluA2 delivery to dendrites. Our results identify a novel control mechanism of the glutamate receptors and provide evidence for a Sema3A-induced retrograde signaling from axonal growth cone to dendrites through the trafficking of PlexA.

1S01A-3
Light induced inactivation of AMPA receptors toward an artificial memory erasure

Takemoto, Kiwamu[1,2]; Nagai, Takeharu[3]; Takahashi, Takuya[1] ([1]*Department of Physiology, Yokohama City University;* [2]*JST, PRESTO;* [3]*ISIR, Osaka University*)

Hippocampus is an essential brain region for memory formation. While many analyses for hippocampus synaptic response in vitro have been reported, mechanism of memory formation in vivo is poorly understood. If we could inactivate synaptic function to induce "artificial memory erasure", it should be strong strategy for decoding of brain information in living animals. Among molecules in synaptic function, AMPA type glutamate receptors are especially known as important molecules for memory formation that are transported to synapse in response to many types of learning.

Towards development of "artificial memory erasure" and "synapse mapping" technology, we focused on chromophore assisted light inactivation (CALI) to induce loss of function by light. CALI is desirable for loss of function experiment because of its acute and spatially targetable properties in living cells and animals. CALI allows the functional analysis of a target protein inside or outside living cells with high spatiotemporal resolution. In previous study, we have reported the successful CALI method using eosin as photosensitizer (Takemoto et al. ACS Chem.Biol. 2011).

In this session, we first introduce basic features of our CALI method. We also report a new technique for AMPA receptor inactivation in living neurons with light irradiation under microscope. We will report detail properties, specificity, validity and future of this technology.

1S01A-4

Identification and analysis of target genes of the Rett syndrome causative gene, *Mecp2*, in the cerebral cortex

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Rett syndrome (RTT) is a neurodevelopmental autistic spectrum disorder presenting almost exclusively in girls ; it is the second most common cause of mental retardation after Down syndrome in girls. The identification of mutation of the *methyl-CpG binding protein 2* (*MECP 2*) gene on the X chromosome as the cause of RTT enabled a new era of cellular and molecular analysis and understanding of RTT pathophysiology.

Based on our previous work, we performed microarray analysis for the identification and molecular analysis of target genes of MeCP2 in the brain.

One of the candidate genes is *Irak1*, a component of the NF-kB signaling pathway. Quantitative RT-PCR confirms approximately 3-fold over-expression of *Irak1* in *Mecp2*-null CPN. Both ChIP analysis and bisulfite genomic sequencing identify that MeCP2 binds to one highly methylated CpG in the promoter region, indicating that MeCP2 directly regulates *Irak1* in the brain. We also performed multiple experiments that functionally tie *Irak1* to central aspects of the MeCP2 loss-of-function phenotype. Importantly, reducing NF-kB signaling in *Mecp 2*-null mice partially rescues the neurological phenotype and ameliorates their shortened lifespan.

Taken together, these results indicate that *Irak1* is a central target of MeCP2, and that its over-expression is directly involved in the pathogenesis of *Mecp2* mutant mice.

Symposium 02
Why dose skeletal muscle have fast and slow muscle fibers?

[Collaboration Symposium with Japanese Society of Physical Fitness and Sports Medicine]

(March 27, 9 : 00–11 : 00, Room B)

1S02B-1

Regulation of contractile properties in skeletal muscle fibers

Wada, Masanobu (*Graduate School of Integrated Arts and Sciences, Hiroshima University*)

A unique characteristic of skeletal muscle is its diversity created by the fiber composition and the heterogeneity of the individual fibers. A classically used method to identify fiber types is based on differences in the pH stability of myofibrillar ATPase (mATPase) activity. Because mATPase resides in the heavy chain portion of the myosin molecule, the differential sensitivity of mATPase to pH correlates with specific myosin heavy chain (MHC) isoform profiles. According to MHC isoforms found in adult human skeletal muscles, the following fiber types can be delineated : slow type I with MHCI and two fast types, namely, type IIA with MHCIIa and type IIX with MHCIIx.

A major determinant of contractile and relaxation speed in muscle fiber is its catalytic activity of mATPase and sarcoplasmic reticulum (SR) Ca^{2+}-ATPase, respectively. The three fiber types exhibit the increasing mATPase and SR Ca^{2+}-ATPase activities in the order of type I<type IIA<type IIX. In contrast, an efficiency of conversion from chemical energy to mechanical work is highest in type I, intermediate in type IIA and lowest in type IIX. These contractile properties found in each fiber type reveal that during dynamic exercise, type IIX and I fibers are better suited to produce power at high and low velocities, respectively and type IIA fibers occupy an intermediate position. Skeletal muscles are capable of responding to altered functional demands, because they consist of all of three fiber types and since fiber types are not fixed units but can be transformed if necessary.

1S02B-2

Skeletal muscle hypertrophy and myofiber-type transition

Kawada, Shigeo (*Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo*)

Skeletal muscles have several types of myofibers with varying contractile properties and fatigue susceptibilities. The myofibers are primarily classified into fast-twitch glycolytic (fast) and slow-twitch oxidative (slow) fibers. Skeletal muscles can adapt well to physical exercise. High-intensity mechanical stress to skeletal muscles, such as resistance exercise, increases muscle strength concomitant with hypertrophy of muscles. Low-intensity prolonged exercise, such as marathons, increases endurance exercise capability concomitant with increase in oxidative enzyme activity in muscles, resulting in increased energy production. Moreover, it has been demonstrated that these physical exercises induce fast-to-slow fiber-type transition regardless of the exercise type. Skeletal muscles subjected to mechanical unloading conditions show slow-to-fast fiber-type transition. These phenomena indicate that mechanical stress is a factor inducing myofiber-type transition and that exercise induces fast-to-slow myofiber-type transition. Mechanical stress causes various changes in skeletal muscle cells, such as Ca^{2+} concentration, energy demands, and proteins phosphorylation levels. Because recent studies have shown that these factors induce fast-to-slow myofiber-type transition, slow-to-fast myofiber transition in skeletal muscles is considered difficult under mechanical loading conditions. In this symposium, I will review the relationship between myofiber type transition and physical exercise, and the mechanisms of such transition.

1S02B-3

Rapid atrophy of fast fibers–mechanical ventilation induced diaphragmatic atrophy

Ichinoseki-Sekine, Noriko; Naito, Hisashi (*Graduate School of Health and Sports Science, Juntendo University*)

It has been shown that inactivity causes muscle atrophy. The soleus, an antigravity muscle, is often used to investigate disuse muscle atrophy because it mainly consists of slow (Type I) fibers that are particularly susceptible to this type of atrophy. Compared with the soleus, other limb muscles consisting of fast fibers (e.g., plantaris) do not show great response to disuse. Therefore, it is important to determine whether this difference is caused by fiber type-specific physiological and biochemical characteristics.

The diaphragm is a skeletal muscle that primarily consists of fast (Type II) fibers. Compared with fast muscles in the limbs, the diaphragm displays rapid-onset atrophy, in which 12 h of disuse causes 15% atrophy in rat diaphragm. Type I fibers also show diaphragmatic atrophy induced by disuse. However, the rate of atrophy is greater in Type II fibers. Although the mechanism is still unclear, it is hypothesized that a greater rate of diaphragmatic atrophy ensues because the diaphragm is active in most species 24 h per day. If this is true, the difference in muscle atrophy may be caused by the activity pattern.

To date, experimental studies on diaphragmatic atrophy are limited, compared with those in limb muscles. However, the mechanism responsible for this atrophy should be clarified since diaphragmatic atrophy leads to difficulties in removing patients from a ventilator. Recently, we studied diaphragmatic atrophy using an animal model with a mechanical ventilator. In this presentation, basic aspects and recent findings of mechanical ventilation-induced diaphragmatic atrophy will be discussed.

1S02B-4

Neuromuscular activation of quadriceps femoris during fatiguing contractions

Akima, Hiroshi (*Research Center of Health, Physical Fitness & Sports, Nagoya University*)

Skeletal muscle fiber type among individual quadriceps femoris (QF) muscles is relatively similar compared with the triceps surae in humans (Johnson et al. 1973, Saltin & Gollnick 1983). Edgerton et al. (1976) reported that metabolic properties of muscle fibers between the vastus lateralis (VL) and vastus intermedius (VI) is almost identical (FOG 20±3%, FG 34±3%, SO 46±4% for VL ; FOG 15±2%, FG 33±5%, SO 52±5% for VI), suggesting that neuromuscular response to fatiguing contraction would be similar. Neuromuscular activation by surface electromyography (EMG) would be an ideal technique to evaluate fatigability of the motor units in the QF. Regarding the QF, the pattern of neuromuscular activation to fatiguing contractions is non-constant : it is sometimes similar among the four individual muscles, but sometimes dissimilar that may not be account for muscle fiber types in the previous studies. During fatiguing isometric knee extension, median frequency (MF) of EMG signal, which is closely related to conduction velocity of the action potential on muscle fibers, in the four individual QF muscles significantly decreased from the initial ; however, MF in the VL muscle was significantly lower than that of the VI muscle at the end of the task even though metabolic properties of muscle fibers in the two muscles are very similar (Watanabe & Akima 2010). In my presentation, I'll show neuromuscular activation pattern of the four individual QF muscles during fatiguing contractions, and discuss structural (including fiber types), physiological and/or biomechanical aspects.

1S02B-5

Lactate metabolism in slow type and fast type muscle fibers

Hatta, Hideo (*Dept. of Sports Sciences, The University of Tokyo*)

Fast type muscle fibers show higher glycolytic activity, lower mitochondrial content and higher production of lactate during exercise comparing with slow type muscle fibers. The higher production of lactate is not necessarily due to lack of oxygen supply but mainly due to increased glycogenolysis. Production of lactate shows close relation with muscle glycogen concentrations. Fast type fibers have higher lactate transporter subtype 4 (MCT4), which is suitable for extrusion of lactate under high lactate concentrations (Km 25-31 mM for lactate transport). On the other hand, slow type fibers have higher mitochondrial content and MCT1, which is related to oxidation of lactate (Km 3.5-8.3 mM). Myocardium also has high mitochondrial content and MCT1. These characteristics suggest that the produced lactate in fast type fibers during exercise is exported via MCT4 and is incorporated into slow type fibers and heart via MCT1 and oxidized as fuel. Therefore, production of lactate is to distribute oxidative fuel reserved as glycogen in fast type fibers to slow type fiber, heart and other tissues. Glycogen in fast type fibers is a kind of reservoir for whole body energy. Decreased muscle glycogen content by exercise can be one of the main causes of fatigue not only because glycogen can be an immediate energy for exercise but also is required for muscle contraction triggered by calcium ion. Endurance athletes get tired with decreased muscle glycogen content and also with concomitant less production of lactate. The recruitment of slow fibers only not fast fibers at low intensity can be considered as an efficient way to keep glycogen content in fast type fibers.

Symposium 03
Immuno-Physiology on Serious Trauma in Disaster

(March 27, 9 : 00–11 : 00, Room C)

1S03C-1

Primary care using physiological and anatomical evaluation for severely injured patients

Yanagawa, Youichi (*Department of Emergency and Disaster Medicine, Juntendo University*)

Excluding the incurable most severely injured cases, surgical repair for anatomical abnormalities and resuscitation for physiological abnormalities, such as hypotension or hypoxemia induced by trauma, are required as lifesaving measures. In severely injured cases, some patients may demonstrate stable vital signs initially, but later develop unstable vital signs. Accordingly, both physiological and anatomical evaluations are important to evaluate injured patient correctly. The commonly measured physiological data, such as the blood pressure, have not changed for many years. Early detection of anatomical abnormalities induced by trauma using ultrasound and radiological instruments, before the deterioration of physiological data, is now being investigated. Ultrasound is useful to evaluate the real-time estimated intravascular volume indirectly. A retrospective study reported that whole body CT for severely injured patients led to a favorable outcome. Accordingly, a trial of establishing CT examinations in the resuscitation room, even for patients with unstable vital signs, is ongoing in Japan to detect early anatomical traumatic abnormalities as a part of the initial resuscitation to improve the outcomes of severely injured patients.

1S03C-2

Artificial Platelet for Hemostasis in Severe Injury

Hagisawa, Kohsuke[1]; Kinoshita, Manabu[2]; Nishikawa, Kahoko[3]; Yanagawa, Rempei[4]; Nishida, Yasuhiro[1]; Seki, Shuhji[2]; Saitoh, Daizoh[5] (*[1]Departments of Physiology, National Defense Medical College; [2]Departments of Immunology and Microbiology, National Defense Medical College; [3]Departments of Traumatology and Critical Care Medicine, National Defense Medical College; [4]Departments of Military Medicine, National Defense Medical College; [5]Division of Traumatology, National Defense Medical College Research Institute*)

Background : We developed a fibrinogen γ-chain (HHLGGAKQAGDV, H12)-coated, adenosine-diphosphate (ADP)-encapsulated liposomes [H12-(ADP)-liposomes] that accumulate at bleeding site via interaction with activated platelets via GPIIb/IIIa and augment platelet aggregation by releasing ADP.

Objective : To evaluate the efficacy of H12-(ADP)-liposomes for liver hemorrhage in acute thrombocytopenic rabbits.

Methods : Thrombocytopenia was induced in rabbits by repeated blood withdrawal and isovolemic transfusion of autologous washed red blood cells. H12-(ADP)-liposomes with platelet-poor plasma (PPP), platelet-rich plasma (PRP), PPP alone, or ADP liposomes with PPP was administered to the thrombocytopenic rabbits, and liver hemorrhage was induced by penetrating liver injury.

Results : In thrombocytopenic rabbits (platelets<50,000/μL), administration of H12-(ADP)-liposomes as well as PRP rescued all animals from liver hemorrhage as a result of potent hemostasis in the liver bleeding site, although rabbits receiving PPP or ADP liposomes showed 20% survival in the first 24 hours. Administration of H12-(ADP)-liposomes as well as PRP suppressed both bleeding volume and time from the site of liver injury.

Conclusions : H12-(ADP)-liposomes may be a safe and effective therapeutic tool for acute thrombocytopenic trauma patients with massive bleeding.

1S03C-3

Hemodynamic mechanisms for anaphylactic shock in rats

Shibamoto, Toshishige (*Dept. of Physiol. II, Kanazawa Med. Univ., Ishikawa, Japan*)

Patients suffered from anaphylactic shock sometimes becomes fatal and those treated with a nonselective β-adrenoceptor blocker propranolol have increased severity of anaphylaxis. However, hemodynamic mechanisms for anaphylaxis are not fully clarified. The heart, lung and liver are target organs in anaphylaxis animal models. We here determined how/which β_1- or β_2-adrenoceptor antagonist augments the severity of anaphylactic shock, with emphasis on the vascular beds of the heart, lung and liver. Ovalbumin-sensitized male Sprague-Dawley rats were used *in vivo* or *ex vivo*. The following pretreatment was adopted appropriately:(1) propranolol, (2) the selective β_1-adrenoceptor antagonist atenolol, and (3) the selective β_2-adrenoceptor antagonist ICI 118,551. All rats pretreated with β_2-adrenoceptor antagonists ICI 118,551 or propranolol died within 50 min after antigen ; 40% of those pretreated with β_1-adrenoceptor antagonist atenolol died within 60 min. In pulmonary circulation, pretreatment with ICI 118,551 or propranolol, but not that with atenolol, augmented anaphylactic vaso- and broncho-constriction. In isolated perfused hearts excised from the sensitized rats, pretreatment with ICI 118,551, rather than that with atenolol, enhanced anaphylactic coronary vasoconstriction, resulting in increased cardiac dysfunction. In contrast, either β_1- or β_2-adrenoceptor antagonist did not affect anaphylactic hepatic venoconstriction. In conclusion, blockade of β_2-adrenoceptor, rather than β_1-adrenoceptor, exerts the principal detrimental action on heart and lung, but not liver, resulting in fatal outcome of rat with systemic anaphylaxis.

1S03C-4

Severe immunodeficiency following multiple trauma and burn injury, and immunoenhancing therapy for such immunocompromised hosts

Kinoshita, Manabu; Seki, Shuhji (*Department of Immunology and Microbiology, National Defense Medical College*)

Severe trauma- or burn-injured patients are highly susceptible to bacterial infections/sepsis, and their outcomes become extremely poor due to infectious complications. Their host defense systems against infections, such as Th1-mediated cellular immunity, Th2-mediated humoral immunity and neutrophil-mediated immunity, are severely and multifactorially impaired. Although simultaneous enhancement of these immune responses may be ideal for such immunocompromised patients, its achievement appears to be difficult because of the cross-regulating effect of Th1 and Th2 responses. Interleukin-18 (IL-18) was originally identified as an IFN-γ-inducing factor, indicating a potent Th1 cytokine. IL-18 expectedly augments Th1 response to bacterial infections in synergy with IL-12, while it augments Th2 response to allergic disorders in the absence of IL-12. It is noteworthy that our recent murine studies have demonstrated that multiple alternate-day IL-18 injections (but not a single injection) could augment not only the Th1 but also the Th2 immune responses, including immunoglobulin M production against bacterial infection. Multiple IL-18 injections into the burn-injured mice can effectively restore severely impaired cellular, humoral and neutrophil-mediated immune responses, thus improving their survival after bacterial infections. IL-18 treatment may be an attractive and useful therapeutic tool against bacterial complications in immunocompromised hosts after severe surgical stress.

Symposium 04
Multidisciplinary approaches to physiological and pathological conditions at synapses

[Korea–Japan–China Joint Symposium]

(March 27, 9 : 00–11 : 00, Room D)

1S04D-1

Stargazin regulates AMPA receptor trafficking from plasma membrane to early endosome and lysosome during long term depression

Matsuda, Shinji; Yuzaki, Michisuke (*Department of Physiology, School of Medicine, Keio University, Tokyo Japan*)

Activity dependent trafficking of postsynaptic AMPA receptors plays a central role in experience dependent plasticity, e.g., long-term depression (LTD). Here, we report that stargazin, a prototypical transmembrane AMPA receptor regulatory protein (TARP), controls AMPA receptor trafficking depending on its phosphorylation state. Inhibiting the dephosphorylation of stargazin disrupts NMDA induced AMPA receptor endocytosis, and the late endosomal/lysosomal trafficking of AMPA receptors. Similarly, stargazin's dephosphorylation is necessary for low-frequency stimulus-evoked LTD in CA1 hippocampal neurons. Moreover, inhibition of late endosomal function, as well as early endosomal function, disrupts NMDA induced AMPA receptor endocytosis. Thus, the dephosphorylation of stargazin regulates AMPA receptor-trafficking from the cell surface to early endosomes, and from early endosome to lysosomes.

1S04D-2

Cholinergic activation of caspase-3 induces synaptic pruning during neuromuscular synapogenesis

Luo, Zhen Ge; Wang, Jin-Yuan; Chen, Fei; Fu, Xiu Qing (*Institute of Neuroscience, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China*)

During the development of vertebrate neuromuscular junction (NMJ), agrin stabilizes, whereas acetylcholine (ACh) destabilizes AChR clusters, leading to the pruning of postsynaptic structures. The intracellular mechanism underlying this counteractive interaction is not completely understood. Here we show that caspase-3, the effector protease involved in apoptosis, mediates elimination of AChR clusters. We found that caspase-3 was activated by cholinergic stimulation of cultured muscle cells without inducing cell apoptosis and this activation was prevented by agrin. Interestingly, inhibition of caspase-3 attenuated ACh agonist-induced dispersion of AChR clusters. Furthermore, we identified Dishevelled1 (Dvl1), a Wnt signaling protein involved in AChR clustering, as the substrate of caspase-3. Specific blockade of Dvl1 cleavage also prevented dispersion of AChR clusters. Finally, inhibition or genetic ablation of caspase-3 resulted in stabilization of aneural AChR clusters in agrin mutant mice. Thus, caspase-3 plays an important role in the pruning of postsynaptic structures during the development of NMJs.

1S04D-3

A Critical Role of Central TRPV1 in the Nociceptive Circuitry of Spinal Dorsal Horn

Oh, Seog Bae (*National Research Laboratory for Pain, Department of Neurobiology and Physiology, School of Dentistry, Seoul National University, Seoul, Republic of Korea*)

Neuropathic pain and mechanical allodynia may arise from sensitization of central circuits. In this symposium, I will present a novel mechanism of disinhibition-based central sensitization resulting from long-term depression (LTD) of GABAergic interneurons as a consequence of TRPV1 activation in the spinal cord. Intrathecal administration of TRPV1 agonists led to mechanical allodynia that was not dependent on peripheral TRPV1 neurons. TRPV1 was functionally expressed in GABAergic spinal interneurons and activation of spinal TRPV1 resulted in LTD of excitatory inputs and a reduction of inhibitory signaling to spinothalamic tract (STT) projection neurons. Mechanical hypersensitivity after peripheral nerve injury was attenuated in TRPV1-/- mice but not in mice lacking TRPV1-expressing peripheral neurons. Mechanical pain was reversed by a spinally applied TRPV1 antagonist while avoiding the hyperthermic side effect of systemic treatment. Our results demonstrate that spinal TRPV1 plays a critical role as a synaptic regulator and suggest the utility of CNS-specific TRPV1 antagonists for treating neuropathic pain.

1S04D-4

Synapse maturation and autism : The role of synapse adhesion molecules

Tabuchi, Katsuhiko[1,2,3]; Chang, Wen Hsin[3];
Nur Farehan, Asgar Mohamed[3]; Thomas, Sudhof C.[4];
Shigemoto, Ryuichi[3] (*[1]Department of Neurophysiology, Shinshu University School of Medicine, Matsumoto, Japan; [2]PRESTO JST, Okazaki, Japan; [3]National Institute for Physiological Sciences, Okazaki, Japan; [4]Stanford University School of Medicine, USA*)

Neuroligins and Neurexins are distinct families of cell adhesion molecules localized at post- and pre-synaptic terminals, respectively. They bind each other at synaptic cleft via their extra cellular domains and induce synapse maturation. R451C mutation in neuroligin-3 is the first identified neuroligin mutation that had been shown to affect the surface localization of Neurolign-3 protein by in vitro studies. We generated knock-in mice that recapitulate this mutation to examine its relevance to autism. These mice grew normally without exhibiting obvious physical phenotypes but showed behavioral abnormalities relevant to autism including impaired social interaction and enhancement of spatial learning and memory. We studied synaptic function of these mutant mice and found inhibitory synaptic transmission was selectively enhanced in the cerebral cortex. Administration of GABA receptor blocker ameliorated the impaired social interaction suggesting this mutation could be the cause of autistic behavior in these mice. We further found ratios of NMDA/AMPA and NR2B/NR2A, and synaptic plasticity were increased in hippocampus indicating synaptic maturation was impaired in these mice. We hypothesized that disturbance of synaptic maturation causes impairment in social behavior and extraordinary memory ability in certain type of autism patients.

Symposium 05
Physical therapy for pain treatment and its physiological mechanisms
[Collaboration Symposium with Japanese Physical Therapy Association]

(March 27, 9 : 00–11 : 00, Room E)

1S05E-1

Peripheral neural mechanisms of nociception/pain in myofascial structures

Taguchi, Toru (*Dept. Neurosci. II, Res. Inst. Environ. Med., Nagoya Univ., Nagoya, Japan.*)

Deep tissue pain is a major medical problem all over the world. It is a challenging issue to be conquered, especially in physical therapy because it directly restricts one's activities in daily living and quality of life, and because it seriously prevents the process in rehabilitation. Pain research per se, on the other hand, made remarkable advances in a couple of decades in parallel with the progress of experimental approaches. These advances, however, have been obtained from knowledge about cutaneous pain. Namely, understanding of deep tissue pain originating in the muscle and the fascia is far behind that about skin pain although deep tissue pain is of more clinical importance than skin pain due to higher prevalence, severity and chronification (i.e. transition from acute pain to chronic). We have been explored neural mechanisms of muscular nociception/pain by developing a novel animal model and a method to study the mechanisms. Recently, our special focus is on the "fascia" that is a forgotten tissue to be explored in medical sciences. We are unveiling that the muscle fascia is important not only as a supportive tissue, but as a nociceptive sensory organ that elicits pain sensation. In this symposium, fundamental mechanisms of nociception/pain arising from myofascial structures will be provided for better understanding and treatment of myofascial pain in physical therapy.

1S05E-2

Peripheral mechanisms of immobilization-induced hypersensitivity : the role of skin tissue

Okita, Minoru[1]; Sekino, Yuki[1]; Hamaue, Yohei[1]; Nakano, Jiro[2]
(*[1]Department of Locomotive Rehabilitation Science, Unit of Rehabilitation Sciences, Nagasaki University Graduate School of Biomedical Sciences. Nagasaki, Japan; [2]Department of Physical Therapy, Unit of Physical & Occupational Therapy, Nagasaki University Graduate School of Biomedical Sciences. Nagasaki, Japan*)

This study was designed to investigate histological changes in skin tissue accompanying immobilization-induced hypersensitivity. Changes in mechanical sensitivity, epidermal thickness, and peripheral nerve profiles in the upper dermis were examined in glabrous skin of rat hind paw after 1, 2, and 4 weeks of ankle joint immobilization by plaster casts. Induction of mechanical hypersensitivity was confirmed after 2 and 4 weeks of joint immobilization. Epidermal thinning, increase in peripheral nerve profiles in both myelinated A fibers and unmyelinated C fibers, and up-regulation of nerve growth factor (NGF) in the keratinocytes were observed in skin tissues in immobilized rats. The time course of epidermal thinning and increase in peripheral nerve profiles were similar closely to that of hypersensitivity, with significant differences between the immobilized and control rats after 2 weeks of immobilization, which became even more remarkable at 4 weeks of immobilization. These findings suggest that joint immobilization by cast induces epidermal thinning and increases peripheral nerve profiles in the upper dermis and that these changes might be partly responsible for immobilization-induced hypersensitivity.

1S05E-3

Neural mechanisms of skeletal muscle blood flow response induced by noxious stimulation

Uchida, Sae (*Department of Autonomic Neuroscience, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan*)

In physical therapy, several interventions such as exercise, heat or electrical stimulation, are used to improve blood flow of skeletal muscles. The mechanism of muscle vasodilation during exercise has been well studied by Matsukawa's group. In contrast, little is known about the physiological bases of increased muscle blood flow without muscle contraction. We clarified the following two mechanisms for increased muscle blood flow induced by noxious somatic afferent stimulation, in anesthetized rats.

(1) Axon reflex-like vasodilation mediated by calcitonin gene-related peptide (CGRP) : Repetitive antidromic electrical stimulation of unmyelinated C fibers in ipsilateral dorsal roots at the 3rd-5th lumbar segments causes an increase in biceps femoris muscle blood flow (MBF) without changes in blood pressure. The MBF response is abolished by topical application of a CGRP receptor antagonist.

(2) Passive vasodilation due to reflex pressor response : Electroacupuncture stimulation of a hindpaw at a stimulus strength sufficient to excite Aδ and C afferent fibers produces significant increases in biceps femoris MBF and arterial pressure. This increase in MBF is caused passively by a reflex pressor response.

In conclusion, we showed two mechanisms of muscle vasodilation without muscle contraction ; (1) axon reflex-like vasodilation mediated by CGRP, and (2) passive vasodilation due to reflex pressor response ; these are elicited by noxious somatic afferent stimulation.

Symposium 06
Heat acclimatization after exercise training: the role of the central and periphery

(March 27, 9 : 00–11 : 00, Room F)

1S05E-4

Pain inhibitory mechanisms of physical therapy

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It had been reported that physical therapies such as exercise and modality approach could improve not only subjective pain experience but also pain-associated dysfunction in chronic pain cases. However, underlying physiological mechanisms of these therapies have not clarified enough. We therefore conducted to investigate physiological role of physical therapy in chronic pain conditions. As for the therapeutic interventions, low-load or motor-learning exercise such as treadmill walking, ergometer cycling, grip control task and two-ball rotation task as well as modality approaches such as invasive and/or non-invasive heat, electrical, vibratory and acupressure stimulation were conducted for both chronic pain cases and healthy volunteers. All subjects had assessed pain threshold, oxygenation of the muscle and muscle hardness on local and distal points of stimulations as well as autonomic nervous activity, pain-related disability and pain-related anxiety. Since the therapeutic interventions improve the widespread pain conditions, we suggest that these physical therapies could affect pain associated neuronal modulations in both CNS and peripheral sensory-motor systems and resulted to achieve widespread and long lasting pain inhibitory effects.

1S06F-1

Effects of blood volume and humoral factors on the improved thermoregulatory capacity with exercise training

Okazaki, Kazunobu (*Research Center for Urban Health and Sports, Osaka City Univ and Dept of Environmental Physiology for Exercise, Osaka City Univ Grad Sch of Med, Osaka, Japan*)

Exercise training increases plasma volume (PV) which has been suggested to be oncotically mediated and so to rely on an increase in plasma albumin content (Alb_{cont}). It has been shown that the increase in Alb_{cont} and PV after a given period of training is enhanced by post-exercise protein and carbohydrate (CHO) intake during training period compared with placebo intake. There are several evidences that the increased PV is a predominant mechanism of the improved heat tolerance and thermoregulatory capacity after exercise training. We have shown that increase in esophageal temperature (T_{es}) during exercise in the heat is attenuated more after training with enhanced cutaneous vasodilatation and sweating to increased T_{es}in subjects with a higher increase in PV by post-exercise protein and CHO intake compared with subjects with placebo intake. These observations are accompanied with a greater increase in cardiac stroke volume therefore the increased PV after training would enhance cardiac filling pressure during exercise to enhance thermoregulatory response via cardiopulmonary baroreflexes. We have also shown that the enhanced cutaneous vasodilatation and sweating with PV expansion after training are eliminated when PV expansion is removed. Thus, exercise training-induced improvement of heat tolerance and thermoregulatory capacity is critically dependent on PV expansion and the resultant changes in central blood volume during exercise, which would be enhanced by post-exercise protein and CHO intake.

1S06F-2

Heat acclimation and baroreflex control of skin blood flow in humans

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Aerobic training has been suggested to enhance heat tolerance by improving thermoregulatory responses. As for the mechanism, Ikegawa et al. (2011) suggested in young men that increased plasma volume expansion after aerobic training contributes to enhanced cutaneous vasodilation after training. Recently, we found in passively warmed men that a component synchronized with cardiac cycle was involved in skin sympathetic nerve activity (SSNA), the component increased with an increase in body temperature but the increase was suppressed by hypovolemia when cutaneous vasodilation was suppressed (Kamijo et al., 2011). The results suggest that the SSNA component is an efferent signal for cutaneous vasodilation modulated by baroreflex ; however, the possibility that the component involves signals of muscle sympathetic nerve activity (MSNA) was not excluded completely. So, we examined effects of 30°head-up tilt on right atrial volume, carotid artery diameter, SSNA, and MSNA in passively warmed men and found that the SSNA component was reduced while MSNA was enhanced by head-up tilt where right atrial volume and carotid arterial distensions with cardiac cycle were reduced. Moreover, latency of the SSNA component from peak right atrial volume and that of MSNA from valley of carotid arterial diameter were almost constant (0.7s and 1.2s, respectively). Thus, the SSNA component triggered by atrial distension may not include MSNA spikes. Our results support the idea that the SSNA component synchronized with cardiac cycle significantly contributes to improved cutaneous vasodilation after training.

1S06F-3

The effect of spontaneous running-wheel exercise on behavioral thermoregulation in heat and thermal preference in mice : a possible role of the central

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Aim We tested chronic exercise in mice modulates behavioral thermoregulation. ***Methods*** Mice housed with or without a running-wheel for 8 w (WR and NWR groups, n=47 and 40, respectively) were used. Implanted a body temperature (T_b) measurement device, the mice received s.c. injection of isotonic- or hypertonic-saline (1 ml/100 g body wt ; 154 or 2,500 mM, IS or HS subgroup) and were placed in a box with 5 Peltier boards at the bottom. Three experiments were conducted for 90 min, using different controlling programs : 1) constant board temperatures of 28℃ or 39℃ ; 2) an operant-behavior setting : each board was set at 39℃ and the right-end board was changed to 20℃ within 60 s only when the mouse moved to the left-end board ; and 3) a thermal mosaic setting : each board was set at either 15℃, 22℃, 28℃, 35℃, or 39℃ with a 6-min interval. ***Results*** In Experiment 1, T_b in both subgroups of the WR group became higher than that in the NWR group. In Experiment 2, the NWR group showed smaller operant counts in the HS subgroup than the IS subgroup ; however, the WR group did not. In Experiment 3, the WR group preferred lower temperatures than the NWR group without any differences between the subgroups (e.g., 33.4±0.3℃ and 34.7±0.1℃ in IS groups). ***Conclusion*** Exercise may alter thermal preference and behavioral responses, thereby increases thermal tolerance, diminishing the effect of dehydration.

1S06F-4

Improvement of Heat Tolerance by Hypothalamic Neurogenesis in Long-term Heat-acclimated Rats

Matsuzaki, Kentaro; Katakura, Masanori; Hara, Toshiko; Hashimoto, Michio; Shido, Osamu (*Shimane Univ. Sch. Med. Shimane, Japan*)

In humans and rodents, repeated exposure to moderate heat has been well known to result in the development of heat acclimation that improves heat tolerance. The present study investigated a relationship between the improvement of heat-tolerance and the heat exposure-induced hypothalamic neurogenesis in rats. Male Wistar rats, initially maintained at an ambient temperature (Ta) of 24℃, were subjected to a constant high Ta of 32℃ (heat-exposed rats, HE) or were constantly kept at 24℃ (control rats, CN). Bromodeoxyuridine (BrdU) was intraperitoneally injected daily for 5 consecutive days after commencing heat exposure. On the 6th, 13th, 23rd, 33rd, 43rd and 53rd day of heat exposure, rats'brains were removed. Immunohistochemical analysis showed that the numbers of BrdU-positive cells in the hypothalamus of HE were significantly and consistently greater than those of CN. In HE, the number of BrdU-positive cells double-stained by a mature neuron marker increased abruptly after 43 days of heat exposure by about 7 times. This was not the case in CN. Moreover, administration of cytosine arabinoside, a mitosis inhibitor, into rats'intra-cerebral ventricle significantly reduced heat exposure-induced improvement of heat-tolerance. These results suggest that heat exposure facilitates proliferation of neuronal progenitor cells in the hypothalamus and promotes differentiation into neurons, which might have a certain role in improvement of heat tolerance of long-term heat-acclimated rats.

Symposium 07
Dynamics of inhibitory transmission and its molecular components

(March 27, 9 : 00–11 : 00, Room G)

1S07G-1

Amibient GABA regulates the multidirectional tangential migration of GABAergic interneurons in living neonatal mice

Inada, Hiroyuki[1]; Watanabe, Miho[2]; Uchida, Taku[3]; Fukuda, Atsuo[2]; Yanagawa, Yuchio[4]; Nabekura, Junichi[1] ([1]*NIPS, Okazaki, Japan;* [2]*Hamamatu Univ. Med., Hamamatsu, Japan;* [3]*Fukuoka Univ., Fukuoka, Japan;* [4]*Gunma Univ., Maebashi, Japan*)

Cortical GABAergic interneurons originate from ganglionic eminences and tangentially migrate into the cortical plate at early developmental stage. Some previous reports demonstrated that the disruption of intracortical migration in the marginal zone (MZ) caused changes in the location of interneurons in mature cortex, suggesting that it has critical role for the normal development of the cortex. Thus, the examination of regulatory mechanism in the MZ is required to understand the organization principle of the neocortex. To elucidate the characteristics of the migration in living animals, we established experimental design specialized for in vivo time-lapse imaging with two-photon laser-scanning microscopy. In the MZ of vesicular GABA/glycine transporter (VGAT)-Venus transgenic mice at the age of P0 to P3, we observed multidirectional tangential migration of GABAergic interneurons and quantified their properties of neuronal migration. Motility rate of GABAergic neurons and GABA content within the neonatal cortex of VGAT-Venus transgenic mice were significantly greater than those of GAD67-GFP knock-in mice, respectively, suggesting that extracellular GABA concentration could facilitate the motility of multidirectional tangential migration. Indeed, diazepam applied to GAD67-GFP mice increased motility rate substantially. Thus, activation of GABAAR by ambient GABA positively regulate the multidirectional migration of GABAergic interneurons in vivo.

1S07G-2

Morphological Changes and movements of functional proteins during inhibitory synapse formation

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We have aimed to visualize the morphology and dynamics of inhibitory synapses. For this purpose, we used two probes selective to presynaptic and postsynaptic structures : one is genetically Venus-labeled inhibitory neurons as a presynaptic marker and the other mCherry-tagged gephyrin, a postsynaptic scaffolding protein, as a postsynaptic marker. Using primary culture of mouse hippocampal neurons and dual wavelength fluorescence microscopy, we found close contacts of Venus-positive varicosities with mCherry-labeled gephyrin clusters in the dendritic shafts of dissociated pyramidal neurons. Time-lapse imaging revealed that : (1) the presynaptic varicosity underwent a marked morphological change, and (2) the postsynaptic scaffolding protein gephyrin clusters exhibited coordinated movements in a tight association with the presynaptic varicosities during the initial stage of inhibitory synapse formation. This inhibitory synapse mobility was characterized as changes in the shape including fusion, split and movement. The results suggest that the dynamic behavior of hippocampal inhibitory synapses critically underlies the alignment of synaptic connections within the inhibitory neural network.

1S07G-3

Dynamic regulation of glycine/GABA cotransmission at inhibitory synapses

Ishibashi, Hitoshi; Yamaguchi, Junya; Nakahata, Yoshihisa; Nabekura, Junichi (*National Institute for Physiological Sciences, Okazaki City, Japan*)

Fast inhibitory neurotransmission in the CNS is mediated by GABA and glycine. These transmitters can be accumulated in the same presynaptic nerve terminals, and are coreleased from the same synaptic vesicles at synapses in several CNS regions. So far, only a single transporter responsible for filling of synaptic vesicles at inhibitory synapses has been identified, and the shared transport of GABA and glycine by this vesicular inhibitory amino acid transporter (VIAAT) enables to corelease GABA and glycine from the same synaptic vesicles. However, the mechanisms that specify packaging of GABA+glycine into synaptic vesicles are not fully understood. We show here that, in spinal cord and hippocampal cultured neurons, intracellular loading of GABA or glycine markedly increased the respective contribution to inhibitory synaptic transmission. Furthermore, glycinergic transmission could be evoked from GlyT2-transfected hippocampal inhibitory neurons, while native hippocampal neurons show only GABAergic transmission. In addition, the uptake of glutamate increased the GABAergic component at inhibitory spinal synapses. Interestingly, at high-frequency stimulation, glycinergic IPSC shows greater decrease in amplitude than GABAergic ones, and failure of glycinergic IPSC was markedly increased. Our findings suggest that the phenotype of an inhibitory synapse is regulated by the nature of the presynaptically released transmitter. GABA/glycinergic inhibitory transmission is dynamic, and can easily change the component in response to changes in extracellular glutamate level.

1S07G-4

Role of $GABA_A$ receptor mediated tonic conductance in phsyiology and pathology

Yamada, Junko (*Det of Neurophysiol, Hirosaki Univ. Grad Sch of Med, Hirosaki Japan*)

γ-aminobutyric acid type A ($GABA_A$) receptors mediate fast synaptic inhibition in the mammalian central nervous system and regulate neuronal firing either by hyperpolarizing the membrane potential or by shunting excitatory inputs. Conventionally, transient activation of synaptic $GABA_A$ receptors mediates phasic inhibition, however recently it has become apparent that distinct $GABA_A$ receptors also participate in another type of inhibitory role. This role involves mediation of tonic inhibition by the continuous activation of extrasynaptic $GABA_A$ receptors. These receptors can be activated by a spillover of GABA from the synaptic cleft. Phospholipase C-related, but catalytically inactive protein (PRIP) was first identified as a novel inositol 1,4,5-triphosphate binding protein. The PRIP-1 subtype is expressed predominantly in the central nervous system and binds directly to the GABAA receptor-subunit and several other proteins involved in the trafficking of $GABA_A$ receptors to the plasma membrane. We found that the PRIP-1 knockout mouse showed an epileptic phenotype, confirmed by electroencephalogram. We studied the electrophysiological properties of GABAergic transmission in hippocampal CA1 pyramidal neurons, using a slice patchclamp technique. The amplitude of the tonic GABA current in PRIP-1 knockout neurons was markedly reduced compared with that in wild-type neurons. Consequently, the effect of DZP on PRIP-1 knockout mice was reduced. Dysfunction of extrasynaptic GABAergic transmission probably is involved in the epileptic phenotype of PRIP-1 knockout mice.

Symposium 08
Novel pharmacological strategies for cardiovascular diseases based on the recent breakthrough in physiological regulatory mechanisms
[Collaboration Symposium with The Japanese Pharmacological Society]

(March 27, 9 : 00–11 : 00, Room H)

1S07G-5

Molecular mechanism underlying the inhibitory synaptic plasticity revealed by single molecule imaging

Bannai, Hiroko[1]; Niwa, Fumihiro[1]; Arizono, Misa[1]; Triller, Antoine[2] ([1]*BSI, RIKEN, Wako, Japan;* [2]*Ecole Normale Superieure, Paris, France*)

Synaptic plasticity, the ability of neurons to modulate synaptic strength, is a key mechanism for learning and memory, and its dysfunction is the underlying cause of neuronal diseases. Synaptic plasticity is exhibited by both excitatory and inhibitory synapses, however, the cellular and molecular mechanisms are less well understood at inhibitory synapses than at excitatory synapses. In this study, we have analyzed the link between the inhibitory synaptic strength and the diffusion properties of type-A GABA receptors ($GABA_AR$), which mediate fast inhibitory neuronal transmission in central nervous system. Neuronal activity modified the strength of GABAergic synapses in cultured hippocampal neurons : enhanced excitatory synaptic activity decreased the cluster size of $GABA_AR$ and GABAergic mIPSC, without reducing the expression level of $GABA_AR$ on the cell surface. Single molecule imaging of the $GABA_AR$ labeled with quantum dots revealed that the diffusion coefficient and the synaptic confinement domain size of $GABA_AR$ increases in parallel with neuronal activity, depending on Ca^{2+} influx and calcineurin activity. These results indicate that $GABA_AR$ diffusion dynamics underlies rapid and plastic modifications of inhibitory synaptic transmission in response to neuronal excitation accompanied by Ca^{2+} influx. We will also present our recent data suggesting that GABAergic synaptic plasticity induced by the modification of $GABA_AR$ diffusion is independent of its scaffold protein gephyrin.

1S08H-1

Pannexin and atrial remodeling and atrial fibrillation

Furukawa, Tetsushi; Oishi, Sakiko; Sasano, Tetsuo (*MRI, Tokyo Medical and Dental Univ. Tokyo, Japan*)

Cardiac remodeling is characterized by inflammation and fibrosis of atrium and ventricle, and underlies various types of cardiac disease including congestive heart failure. It has been reported that stretch of ventricular myocytes induces ATP release through a gap junction channel family, pannexin 1, resulting in ventricular remodeling (Nishida et al. EMBO J. 2008). Atrial remodeling provides the basis for atrial fibrillation, a most frequent arrhythmias, accounting a third of persistent arrhythmias. Stretch of atrium is one of the major risk factors for development of atrial fibrillation. However, it is unknown if stretch of atrial myocytes triggers ATP release through pannexin channel. Here we found that stretch of atrial myocytes released ATP via a gap junctional channel, pannexin 2, but not pannexin 1. Released ATP induced mobilization and recruitment of macrophages toward stretched atrial myocytes. In vivo, transverse aortic constriction (TAC) induced macrophage infiltration, fibrosis, hypertrophy, and induction of tachyarrhythmias in atrium. Pre-treatment of mice with carbenoxolone, a non-specific pannexin blocker, inhibited macrophage infiltration, fibrosis, hypertrophy, and inducibility of tachyarrhythmias in the atrium. Thus, ATP release through stretched cardiac myocytes stimulate cardiac remodeling, and thus a pannexin family is the potential target of drug development for not only congestive heart failure but also atrial fibrillation.

1S08H-2

cAMP medication for cardiovascular diseases

Minamisawa, Susumu[1]; Yokoyama, Utako[2]; Ishikawa, Yoshihiro[2]
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Cyclic AMP (cAMP) is known to play a central role in regulating cardiovascular function. A variety of neurohormonal stimulations such as catecholamines and prostaglandins differentially regulates the cardiovascular system via cAMP activation. Although the cAMP signal is a common second messenger signal, there is a diversity of its regulation in a tissue-specific manner. Therefore, it is important to investigate how we can control the cAMP activation as medication for cardiovascular diseases. One promising key is the differences in adenylyl cyclase (AC) isoforms that display a tissue-specific distribution. However, the physiological significance of expressing multiple AC isoforms in a tissue and how each specific isoform regulates the cAMP signal remains poorly understood. Another potential key is the diverse downstream pathway through PKA or Epac. We will discuss about the versatile utilization of cAMP medication for heart failure and congenital heart defects.

1S08H-3

Regulation of cardiac redox homeostasis by hydrogen sulfide anion

Nishida, Motohiro (*Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan*)

While reactive oxygen species (ROS) are typically viewed as toxic mediators of oxidative stress in aerobic organisms, it is also now apparent that ROS mediate signal transduction events during both basal metabolism and inflammatory responses. An emerging aspect of ROS signaling is those reactions mediated by electrophilic byproducts of redox reactions, such as the electrophilic nucleotide 8-nitroguanosine 3',5'-cyclic monophosphate (8-nitro-cGMP) generated via reactions of ROS, NO and their secondary products. Here we report that hydrogen sulfide anion (HS^-), but not hydrogen sulfide (H_2S) itself, negatively regulates the metabolism and signaling actions of endogenous electrophiles. HS^- reacts with electrophiles via direct sulfhydration by HS^- and modulates ROS-derived electrophilic signaling mediators, best represented by 8-nitro-cGMP. The relevance of this reaction is reinforced by the significant amounts of 8-nitro-cGMP formed in mouse failing hearts after myocardial infarction (MI). Beneficial pharmacological effects stem from the sulfhydration of 8-nitro-cGMP by HS^-, which potently ameliorates indices of chronic heart failure after MI, accompanying the suppression of cellular senescence caused by H-Ras activation in cardiomyocytes. These data support HS^--induced electrophilic sulfhydration as a mechanism for terminating electrophile-mediated signaling and reveals a novel therapeutic strategy for treating oxidative stress-related cardiovascular diseases.

1S08H-4

Druggability of TRP channels in cardiovascular disease

Inoue, Ryuji (*Dpt. Physiol., Grad. Sch. Med. Sci., Fukuoka Univ., Fukuoka, Japan*)

TRP channels are characterized by their unique activation and modulation by a broad spectrum of physicochemical stimuli, including G-protein-coupled receptor agonists, pungent, cooling and gustatory agents, natural ligands and pheromones, thermal and mechanical stresses, and membrane potential. Recent studies have shown that more than 10 members of TRP superfamily are ubiquitously expressed in the cardiovascular (CV) system (CVS), and involved in a variety of CV functions and diseases. Owing to this potential clinical significance, TRP channels have been regarded as promising targets for the development of a new generation of CVS-acting drugs. However, there are several hurdles that hinder it, such as their elusive nature of activation/modulation, intimate and complex association with diverse cellular signaling pathways and membrane lipid microenvironments/dynamics, and existence of large disordered regions in the channels which prevent the precise evaluation of structure-activity relationships for drug-target interaction. All these appear to hamper rational drug design based on the precise knowledge of the molecular mechanisms and structures of TRP channels which are requisite for the identification of critical recognition sites for drug actions. As the apparent consequence of these limitations, TRP channel blockers/activators so far available show rather poor specificity and efficacy, and the mechanisms of their actions have been insufficiently understood. In this symposium talk, I attempt to discuss about the future druggability of CV TRP channels as the new therapeutic targets for CV diseases from the viewpoints raised above.

Symposium 09
Recent development of cell motility research with novel experimental methods

(March 27, 9 : 00–11 : 00, Room I)

1S09I-1

Visualization and Measurement of the power stroke in individual myosin heads coupled with ATP hydrolysis using the gas environmental chamber

Sugi, Haruo (*Department of Physiology, Teikyo University Medical School, Tokyo, Japan*)

We have already succeeded in recording ATP-induced myosin head recovery stroke in hydrated synthetic myosin filaments (myosin-myosin rod copolymer)(Sugi et al.,PNAS 105 : 17396-17401, 2008 ; Minoda et al.,BBRC 405 : 651-656, 2011) using the gas environmental chamber (EC) which enables us to study dynamic structural changes of hydrated biomolecules retaining their physiological function under electron microscope. To visualize the myosin head power stroke responsible for muscle contraction, we added actin filaments to synthetic myosin filaments, in which a small fraction of myosin heads were position-marked with gold particles of 20nm diameter. Initially, myosin filaments were surrounded by actin filaments running in parallel with each other due to rigor linkage formation.When ATP was applied iontophoretically to myosin filaments surrounded by actin filaments, individual myosin heads were found to move parallel to the filaent long axis by about 3nm, and returned towards their initial position after exhaustion of applied ATP. The concentration of applied ATP around the filaments was estimated to be 1-5nM, so that only a limited proportion of myosin heads were activated with ATP to perform their power stroke, while the majority of myosin heads form rigor likkages with actin filaments. When the ionic strength of expreimental solution was reduced, the amplitude of ATP-induced myosin head power stroke increased to 4-5nm, in accordance with the report that isometric force in skinned muscle fibers increases about twofold at low ionic strength.

1S09I-2

Micro-mechanics of bio-motile systems : Auto-oscillation(SPOC)of striated muscle and cell division

Ishiwata, Shin'ichi[1,2] ([1]*Dept. Phys., Fac. Sci. & Engn., Waseda Univ., Tokyo, Japan;* [2]*Waseda Biosci. Res. Inst. in Singapore(WABIOS), Singapore*)

Muscle usually takes either relaxation or contraction state, which is regulated by Ca2+. On the other hand, we found the third state exists at intermediate activation conditions for skinned skeletal and cardiac muscles, which we named "SPOC"[1]. The micro-mechanics of SPOC has been studied by manipulating a myofibril with glass micro-needles. We have constructed a unit model to explain the dynamic properties of SPOC [2]. The theory is based on the kinetics of cross-bridge formation depending on the spacing of myofilament lattice, the force balance not only parallel, but also perpendicular to the long axis of myofibrils. Further, we extended the unit model by connecting sarcomeres in series. This model can explain almost all properties of SPOC including the phase diagram composed of contraction, SPOC and relaxation regions, and the traveling waves along a myofibril (SPOC wave). We have been studying the effects of mechanical perturbation on cell division and chromosome segregation, by using a pair of flat cantilever. Here, I will report the effects of mechanical impulse (MI) on the timing of chromosome segregation in HeLa cells [3], showing that the MI, applied perpendicular to the pole-to-pole axis of a spindle, accelerates the chromosome segregation, whereas the MI applied along the pole-to-pole axis decelerates it. Ref : [1] Ishiwata, S., Shimamoto, Y., Fukuda, N. 2011. Prog. Biophys. Mol. Biol. 105, 187-98. [2] Sato, K., Ohtaki, M., Shimamoto, Y., Ishiwata, S. 2011. ibid. 105, 199-207. [3] Itabashi, T. et al. 2012. PNAS. 109, 7320-5.

1S09I-3

Energetics of Mechano-Chemical Coupling in the Rotary Molecular Motor F_1-ATPase

Kinosita, Jr., Kazuhiko (*Faculty of Science and Engineering, Waseda Univ. Tokyo, Japan*)

The F_1-ATPase is a part of the ATP synthase that supplies ATP in most living organisms. Its central subunit γ rotates when ATP is hydrolyzed in the three catalytic sites in the surrounding subunits. When γ is forced to rotate in reverse by an external torque, the hydrolysis reaction is also reversed, leading to ATP synthesis. How F_1 mediates the reversible interconversion between chemical and mechanical energies is the question. We like to answer by providing a complete energy diagram : for each set of bound nucleotides XYZ in the three catalytic sites (X, Y, Z=ATP, ADP+Pi, ADP, Pi, or none), we determine the potential energy for rotation $\psi^{XYZ}(\theta)$ of which the slope-$\partial \psi^{XYZ}(\theta)/\partial \theta$ gives the torque that drives (downhill) or opposes (uphill) γ rotation. The diagram will allow prediction of F_1 behaviors, except for kinetic constants, under all nucleotide conditions in the medium and under any external torque : whether spontaneous rotation occurs smoothly without pauses and how much torque is associated, whether reverse rotation by an external torque leads to ATP synthesis or futile, uncoupled rotation, etc. Now we are measuring the torque as a function of θ with magnetic tweezers while watching the bound nucleotides XYZ through angle-resolved single-fluorophore imaging. Integration of the torque yields $\psi^{XYZ}(\theta)$. Alternatively, $\psi^{XYZ}(\theta)$-$\psi^{noneYZ}(\theta)$ is equal to $k_BT \cdot \ln K_a^X(\theta)$ where k_BT is the thermal energy and K_a^X the association constant for nucleotide X in site 1. Once we determine the empty potential $\psi^{nnn}(\theta)$, we can construct $\psi^{XYZ}(\theta)$ because we have determined $K_a^X(\theta)$ [Nat. Commun. 3 : 1022, 2012].

1S09I-4

Analysis on the intranuclear moving particles visualized with apodised phase contrast microscopy

Katoh, Kaoru (*Biomed.Res.Inst., AIST, Tsukuba, Japan*)

Cell nucleus plays important roles in gene expression. The expression process should require dynamic change in architectural framework of nuclear structures, but it has been difficult to observe it as dynamic images. For example, in phase contrast microscopy, bright boundaries between object and media ("halo" artifact) surround objects and obscure detailed structures. Otaki, therefore, proposed **a**podised **p**hase **c**ontrast (APC) microscopy to reduce the halo artifacts. Moreover, we developed **p**upil **p**rojection **a**podized **p**hase **c**ontrast (PPAPC) microscopy, (external phase contrast with spacial frequency filtering). The PPAPC revealed many moving particles in living cell nucleus, which were unable to observe with conventional phase contrast microscopy. The intranuclear moving particles were found in the cultured cells.

To understand characteristics of the intranuclear particles movement, we observed the distribution, the number, and the moving pattern of the particles in each phase of cell cycle. The particles were localized at the specific region in G1 and S phase, and were distributed over the nucleus in G2 phase. The number increased in order of G1<S<G2. Moving speed is larger in G2 phase than in G1 and S phases. MSD-δT plots of the moving particles gave parabolic curves, suggesting that the moving particles showed directed motion.

To identify fine structures and molecules of the particles, we interactively observed identical nucleus with optical (PPAPC) and electron microscopy. The identified structures and molecules will be shown in the presentation. The mechanisms of the intranuclear movements will be also discussed.

Symposium 10
Synapses and Circuits: From Formation to Disorder
[Collaboration Symposium with Chinese Association for Physiological Sciences]

(March 27, 13 : 20–15 : 20, Room B)

1S10B-1
Distinct mechanisms of protein kinase D1 in the establishment of neuronal polarity and synapse formation

Wang, Yun (*Neuroscience Research Institute, Peking University, Beijing, China*)

Neurons are polarized cell with a single axon and several dendrites. Axons send information while dendrites receive and integrate information. The morphology of a neuron must be properly regulated to ensure the precise formation of neural circuit. Numerous studies have revealed the involvement of protein kinases in the regulation of neuronal morphology during neuronal development ; disabled functions or abnormal activities of protein kinases resulting in the abnormal changes of the neuronal morphology that lead to the development of neurological diseases. We are interested in how protein kinases regulate the neuronal morphology during neuronal development. We demonstrate that protein kinase D (PKD), family of serine/threonine-specific protein kinases, plays a key role in the establishment and maintenance of neuronal polarity in the early stage of neuronal development, and that this effect is dependent on its activity in the Golgi apparatus. PKD is distinct from the other proteins regulating the neuronal polarity that affects the stability of the cytoskeleton in neuronal processes. However, in the late stage of neuronal development, PKD controls the number of neuronal dendritic spines through its function at the presynaptic sites, and this effect is independent of its activity in the Golgi apparatus. N-cadherin is one of the downstream targets. Taken together, our studies indicate that the regulation of PKD in the establishment of neuronal polarity and synapse formation through distinct mechanisms during the early and the late stage of neuronal development, highlighting the diverse roles of PKD in the regulation of neuronal functions. The elucidation of the roles of protein kinases in neural development will be helpful for understanding of the mechanisms of neural developmental defects and neurological diseases.

Key word : PKD ; neuronal polarity ; synapse formation ; Golgi apparatus

1S10B-2
DEVELOPMENTAL PLASTICITY OF NEURAL CIRCUITRY FOR SPATIAL CODING.

Chan, Ying-Shing (*Departments of Physiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China*)

Synaptic plasticity is vital for normal behavior but the link between behavioral indicative of spatial recognition and cellular mechanisms of synaptic plasticity has been elusive. We hypothesize that postnatal tuning of synaptic efficacy in the vestibular system is required for the recognition of head orientation and the expression of vestibular behavior. Sensory cues of orientations are transmitted from the inner ear to neurons in the vestibular nucleus via glutamate synapses. Whole-cell patch-clamp data from the rat vestibular nucleus indicated that developmental acquisition of specific glutamate receptor subtypes at silent synapses was crucial for their conversion into functional ones and this was accompanied by persistent enhancement of synaptic excitability. Also, GABA synapses were found to show a developmental change in efficacy from depression to potentiation. Neonatal blockade of glutamatergic or GABAergic transmission in the vestibular nucleus delayed developmental emergence of a gravity-triggered orienting behavior. We further identified a neonatal period of susceptibility during which such perturbation deterred the establishment of an internal spatial map in the mature central vestibular system. These mature rats also exhibited deficits in both spatial navigation and motor learning abilities. Taken together, we provide evidence that postnatal tuning of the excitatory and inhibitory components of the neural network for spatial coding is significant for acquisition of spatial behavior. [*Supported by HKRGC 761409M, 761710M, 761812M*]

1S10B-3
A kinase makes a connection

MA, Lan (*Institutes of Brain Science, Fudan University, Shanghai, China*)

G protein-coupled receptor kinases are known as crucial feed-back negative regulators of G protein coupled receptors, and their physiological functions have long been ascribed to their role of phosphorylating and desensitizing GPCRs. We recently found that GRK5 also serves as a molecular scaffold, coordinating actin cytoskeleton dynamics and membrane remodeling to control neuronal morphogenesis. GRK5 knockout mice exhibit impaired social behaviors, learning and memory, associated with abnormal dendritic spine morphology. GRK5 colocalizes with F-actin in high dynamic actin structures in cell and promotes filopodial protrusion, neurite outgrowth, dendrite branching, and spine maturation. Surprisingly, these effect mediated by GRK5 are independent of its kinase activity. Furthermore, GRK5 could cross-links F-actin into bundles through interacting with F-actin via its C-terminal domain, and it targets F-actin bundles to PI (4,5) P2-containing liposomes through the binding of its N-terminal basic residues with PI (4,5) P2. Uncoupling GRK5-mediated actin and membrane dynamics by disruption of either its lipids-binding or actin-bundling capability impairs neuronal filopodial protrusion, neurite outgrowth, dendrite branching, and spine formation. These results reveal a novel function of GRK5 as an actin-bundling protein and a scaffold to link actin cytoskeleton to PI (4,5) P2-enriched membranes and demonstrate its physiological significance in neuronal morphogenesis.

1S10B-4

Early B cell factor 1 controls tangential migration of nigral dopaminergic neurons through regulation of EphrinB

Zhou, Jiawei (*Institute of Neuroscience, Chinese Academy of Sciences, Shanghai, China*)

Mesodiencephalic dopaminergic (mDA) neurons are essential for the control of multiple brain functions. Dysfunction of the mDA system is involved in the pathogenesis of several mental and neurological diseases such as Parkinson's disease (PD) and schizophrenia, of which some are considered to have a neurodevelopmental origin. Because of poor understanding of the molecular mechanisms underlying mDA neuron development, attempts at restorative treatment of PD have been hampered in the last two decades. Thus studies on mDA neuron development will promote our understanding of mechanisms of brain development and have an impact on developing novel approaches for the treatment of neurodegenerative diseases. Previously, we identified a set of genes that show spatially and temporally restricted expression in the mesnecephalon and are potentially important for mDA neuron development. Functional analysis on mice lacking the mesnecephalon-enriched gene Early B cell factor 1 (*Ebf1*) revealed that *Ebf1* is essential for the terminal migration of mDA neurons (Yin et al. *J Neurosci*. 2009). To understand how *Ebf1* controls the migration of nigral mDA neurons, we compared the gene expression profiles between wild-type and *Ebf1*-null brain. We found that EphrinB expression was upregulated in *Ebf1*-null mice. Functional assays showed that Ebf1 controlled EphrinB expression which mediated the terminal positioning of mDA neurons. Taken together, Ebf1 is a newly identified regulator required for the formation of substantia nigra during development.

Symposium 11
Novel studies on trafficking of AMPA receptors

(March 27, 13 : 20–15 : 20, Room C)

1S10B-5

Postnatal refinement of the cerebellar climbing fiber to Purkinje cell synapse

Hashimoto, Kouichi (*Department of Neurophysiology, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan*)

Neuronal circuits in neonatal animals are initially redundant, but rearranged during postnatal development in the activity dependent manner. The postnatal development of the cerebellar climbing fiber (CF) to Purkinje cell (PC) synapse is regarded as the nice model system to analyze the mechanisms of the postnatal circuit refinement in the central nervous system. In the adult cerebellum, most of PCs are innervated by single CFs. In contrast, PCs are innervated by multiple CFs at birth. Surplus CFs are gradually eliminated until the end of the third postnatal week in mice or rats. Recent analyses have demonstrated that the postnatal refinement of CFs is mediated by at least four developmental phases. During the first postnatal week, single CFs are strengthened relative to other CFs in individual PCs ("functional differentiation" stage). This competitive process occurs on the PC soma. Then, only the strengthened CFs (the "winner" CF) selectively translocate to dendrites after P9, while terminals of other weaker CFs (the "loser" CFs) are confined to the PC soma ("translocation" stage). Massive elimination of the weaker CFs occurs in two distinct steps that is independent of synapse formation of parallel fibers (the early phase CF elimination) and is critically dependent on it (the late phase CF elimination). We recently found that the P/Q type voltage gated Ca^{2+} channel plays important roles for the functional differentiation and the early phase CF elimination in the postsynaptic PCs. In this symposium, I would like to discuss the molecular mechanisms regulating these developmental stages.

1S11C-1

Visualization of AMPA receptors around postsynaptic membrane during synaptic plasticity

Hirano, Tomoo (*Department of Biophysics, Graduate School of Science, Kyoto University, Kyoto, Japan*)

An increase in the number of AMPA-type glutamate receptors (AMPARs) is critical for the expression of hippocampal long-term potentiation (LTP), a cellular mechanism of learning and memory. However, when and how each subtype of AMPAR reaches the postsynaptic membrane remains unclear. We have developed a novel experimental method to form postsynaptic-like membrane (PSLM) on a glass surface to precisely visualize the location and movement of AMPARs with total internal reflection microscopy. Fluorescence-labeled AMPAR subunit (GluA1, GluA2 or GluA3) was expressed in cultured hippocampal neurons, and their changes during LTP induced by electrical or chemical stimulation were recorded and analyzed. The increases of GluA1-3 in PSLM showed different time courses after the LTP induction. Exocytosis and lateral movement of AMPARs were also observed. Our results suggest that during LTP induction, (1) exocytosis of GluA1 homo-tetramer to the postsynaptic membrane occurs first, followed by (2) exocytosis of GluA1/GluA2 hetero-tetramer in the extra-synaptic membrane, and then (3) that of GluA2/GluA3 hetero-tetramer occurs. Changes of AMPARs during chemically induced LTD will also be presented.

1S11C-2

Photochemical approach for analysis of AMPA receptor dynamics

Kamiya, Haruyuki (*Department of Neurobiology, Hokkaido University School of Medicine, Sapporo, Japan*)

Postsynaptic AMPA type glutamate receptors (AMPAR) are not static, but have been shown to exchange with extrasynaptic receptors or those in intracellular reserved pools in constitutive as well as activity-dependent manner. Evidence for dynamic receptor trafficking was mostly shown by optical tracking of AMPAR subunits labeled with GFP or other fluorescent proteins, although it remains uncertain whether mode and rate of synaptic delivery of native AMPAR are similar with exogenously transfected ones. To reveal real-time dynamics of native AMPAR, alternative photochemical approach using ANQX, a photoreactive irreversible blocker of AMPA receptor, was adopted in mouse hippocampal slices. A brief UV illumination with fast application of ANQX resulted in persistent suppression of excitatory postsynaptic potentials (EPSPs) for prolonged observation period up to several hours, suggesting stable postsynaptic expression of AMPAR and minimal exchange with intracellular reserved receptors at resting condition. Analysis of timing of synaptic delivery during expression of long-term potentiation (LTP) revealed AMPAR traffic is transiently accelerated soon after LTP induction. Another advantage of this photochemical approach is to block glutamatergic transmission with spatially-restricted manner. So far layer specific photoinactivation was tested in hippocampal CA3 region, and successfully reduced mossy fiber transmission persistently. This approach may help to understand specific roles of certain inputs in complex brain circuitry.

1S11C-3

Environment regulates experience-driven synaptic delivery of AMPA receptors

Takahashi, Takuya (*Department of Physiology Research Institute, Yokohama City Univ. Yokohama, Japan*)

When one type of sensory system is disrupted, other intact remaining sensory function can be improved. Although this form of plasticity, cross-modal plasticity, is widely known, the molecular and cellular mechanisms underlying it are poorly understood. In a recent study, we demonstrated that visual deprivation increases extracellular serotonin in the juvenile rat barrel cortex and resulted in facilitation of synaptic delivery of AMPA-type glutamate receptors (AMPARs) at layer4-2/3 synapses in the barrel cortex via the activation of serotonin 5HT2A/2C receptors and ERK. This caused sharpening of functional whisker-barrel map at layer2/3 of the barrel cortex. Thus, sensory dysfunction of one modality leads to improvement of remaining modalities by the refinement of cortical organization through serotonin signaling-mediated facilitation of synaptic AMPARs delivery.

1S11C-4

How does the δ2 glutamate receptor regulate cerebellar LTD?

Kohda, Kazuhisa; Kakegawa, Wataru; Matsuda, Shinji; Yuzaki, Michisuke (*Department of Physiology, Faculty of Medicine, Keio University, Japan*)

Long-term depression (LTD), a form of synaptic plasticity underlying learning and memory process, has been discovered in various brain regions and is commonly caused by clathrin-dependent endocytosis of postsynaptic AMPA-type glutamate receptors. Cerebellar LTD is unique in that it requires the presence of another class of glutamate receptors, the δ2 glutamate receptor (GluD2), which is predominantly expressed at parallel fiber (PF)-Purkinje cell synapses. GluD2-null mice display impaired LTD and motor learning in addition to morphological abnormalities in PF-Purkinje cell synapses. It was recently clarified that, in its N-terminal domain, GluD2 interacted with Cbln1 secreted from PF terminals and played crucial roles in formation and maintenance of PF-Purkinje synapses. On the other hand, expression of a mutant GluD2 transgene lacking the C-terminal seven amino acids, with which several PDZ proteins are known to interact, restored morphological defects in PF-Purkinje cell synapses of GluD2-null mice, but LTD and motor learning remained impaired. The results imply that the downstream signaling of GluD2 in LTD induction should be mediated by its interacting proteins. Nevertheless, the question how GluD2 regulates cerebellar LTD, activity-dependent endocytosis of AMPA receptors, is still unresolved. In the symposium, we will show our recent studies on GluD2 function and discuss the roles of GluD2 as a gatekeeper of LTD induction.

Symposium 12
Gaseous molecules: their sensing and involvement in physiological functions

(March 27, 13 : 20–15 : 20, Room D)

1S12D-1

Molecular Basis of CO_2 Sensing in the Mouse Olfactory System

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Carbon dioxide (CO_2) is an important environmental cue for many organisms. In mammal, mouse, rat and guinea pig have a CO_2 sensor in the olfactory epithelium (OE). Mice can detect CO_2 at concentrations around the average atmospheric level by olfaction. In the ventrolateral region of the mouse OE, there is a unique subset of olfactory sensory neurons (OSNs), termed GC-D OSNs, which express *carbonic anhydrase 2 (Car2)* and *guanylate cyclase-D (GC-D)*, instead of odorant receptor. In GC-D neurons, Car2 and GC-D function as a sensor for CO_2 and urinary peptides, respectively. Further, it was reported that GC-D OSNs also detect carbon disulfide (CS_2) and mediates food-related social learning. Here, we report that at least two novel subsets of OSNs, which are not expressing *Car2*, respond to CO_2 as well. In contrast to GC-D OSNs, these CO_2-responding neurons did not react to both urinary peptides and CS_2. Interestingly, acidic pH solution activated only about half of Car2-CO_2 sensor cells. This means that Car2$^-$-CO_2 sensing OSNs can be divided into two types : the one is CO_2-sensing ; the other acidic pH-sensing. The treatment of a carbonic anhydrase inhibitor, acetazolamide, suppressed the response to CO_2 in CO_2-sensing OSNs. Among 16 genes encoding the carbonic anhydrase family, we have found that *carbonic anhydrase 7 (Car7)* is expressed in a subset of OSNs, instead of expressing *Car2*. Car7 is a good candidate in Car2$^-$-CO_2-sensing OSNs. These results suggest that mice sense CO_2 not only with GC-D OSNs, but also with the novel subsets of OSNs in the OE.

1S12D-2

Physiological and pathological roles of hydrogen sulfide : a focus on its implication in visceral pain and inflammation

Tsubota, Maho; Kawabata, Atsufumi (*Div. Pharmacol. Pathophysiol., Kinki Univ. Sch. Pharm., Higashi-Osaka, Japan*)

Hydrogen sulfide (H_2S) is formed by multiple enzymes including cystathionine-γ-lyase (CSE), playing various roles in health and disease. We have shown that H_2S activates/sensitizes $Ca_v3.2$ T-type Ca^{2+} channels expressed in sensory nerves, leading to facilitation of pain signals, modulation of inflammation and neuritogenesis. Here we focus on implication of H_2S in visceral pain and inflammation. Intracolonic (i.col.) administration of NaHS, an H_2S donor, causes colonic pain and referred hyperalgesia, accompanied by rapid phosphorylation of ERK in the spinal dorsal horn. The pro-nociceptive effect of i.col. NaHS is blocked by pharmacological inhibition or genetic silencing of $Ca_v3.2$, and by an inhibitor of TRPA1, known as another target for H_2S. Our studies have also revealed the pro-nociceptive and pro-inflammatory roles of endogenous H_2S formed by CSE in mice with cyclophosphamide-induced cystitis accompanied by bladder pain, which is mediated by $Ca_v3.2$, but not TRPA1. In contrast, repeated i.col. administration of NaHS produces neurally mediated colonic mucosal protection via activation of T-type Ca^{2+} channels. Together, endogenous H_2S formed by CSE targets $Ca_v3.2$ and/or TRPA1 channels, implicating in visceral pain signaling and progression or modulation of inflammation in internal organs including the colon and bladder.

1S12D-3

Sensing of O_2 by TRPA1 channels

Mori, Yasuo[1,2]; Kozai, Daisuke[1]; Takahashi, Nobuaki[1,3] ([1]*Dept. Synth. Chem. and Biol. Chem., Grad. Sch. Engineer., Kyoto Univ., Japan;* [2]*Dept. Technol. and Ecol. Hall of Global Environmental Studies, Kyoto Univ., Japan;* [3]*Adv. Biomed. Engineer. Res. Unit, Kyoto Univ., Japan*)

Molecular oxygen (O_2) is a prerequisite for cellular respiration in aerobic organisms but also elicits toxicity. To understand how animals cope with the ambivalent physiological nature of O_2, it is critical to elucidate the molecular mechanisms responsible for O_2 sensing. Here our systematic evaluation of transient receptor potential (TRP) cation channels using reactive disulfides with different redox potentials reveals the capability of TRPA1 to sense O_2. O_2 sensing is based upon disparate processes : whereas prolyl hydroxylases (PHDs) exert O_2-dependent inhibition on TRPA1 activity in normoxia, direct O_2 action overrides the inhibition via the prominent sensitivity of TRPA1 to cysteine-mediated oxidation in hyperoxia. Unexpectedly, TRPA1 is activated through relief from the same PHD-mediated inhibition in hypoxia. In mice, disruption of the *Trpa1* gene abolishes hyperoxia- and hypoxia-induced cationic currents in vagal and sensory neurons and thereby impedes enhancement of *in vivo* vagal discharges induced by hyperoxia and hypoxia. The results suggest a new O_2-sensing mechanism mediated by TRPA1.

1S12D-4

Hydrogen peroxide(H_2O_2)-mediated functional regulation and physiological role of Transient Receptor Potential Melastatin 2(TRPM2)

Kashio, Makiko[1]; Sokabe, Takaaki[1]; Mori, Yasuo[2]; Tominaga, Makoto[1] ([1]*Cell Signaling, OIIB(NIPS), Okazaki, Aichi, Japan;* [2]*Kyoto Univ., Kyoto, Japan*)

For many years, Reactive Oxygen Species (ROS) were viewed as the undesirable hazardous molecules. However, ROS are now considered significant signaling molecules and H_2O_2 has the best qualified property among them. These cellular "redox" signals are considered to modulate various proteins through modification such as cysteine/methionine oxidation, and play a role in physiological functions. ROS-producing enzymes such as NADPH oxidase (Nox) and Dual oxidase (Duox) become activated by diverse signals including cytokines, growth factors and elevation of intracellular Ca^{2+} concentrations. Therefore, ROS can be generated in a lot of physiological conditions and exert redox-mediated regulation.

TRPM2 is a non-selective cation channel expressed in various tissues such as brain, spleen and immune cells in which TRPM2 is surrounded by body temperature. We have found a novel mechanism for TRPM2 activation whereby H_2O_2 lowers temperature threshold for TRPM2 activation causing its activation even under body temperature. At the site of infection, where Nox enzyme is activated, TRPM2 is considered to be activated and involved in macrophage function such as cytokine release and fever-enhanced phagocytosis. Therfore, TRPM2 can function as a sensor for both temperature and redox signals, and integrate the information in macrophages.

As mentioned above, TRPM2 is expressed in various tissues and can be regulated by environmental redox state. Possible roles in other tissues will be discussed in this session.

Symposium 13
Development and application of cell type–specific transgene expression in the cerebellum

(March 27, 13 : 20–15 : 20, Room E)

1S13E-1

Virus-mediated highly efficient and cell-type specific gene expression in the cerebellum — Modulation of cathepsin K activity and development of unique promoters—

Hirai, Hirokazu (*Dept. Neurophysiol. Gunma Univ. Grad. Sch. Med. Maebashi, Japan*)

Expression of a foreign gene into the brain tissue in vivo is a powerful method for gene therapy as well as basic research. Recent marked advances in lentiviral vectors and adeno-associated viral (AAV) vectors allowed us highly efficient gene expression in neuronal and glial cells in vivo. We have been using lentiviral vectors for the past decade and recently AAV vectors to transfer a foreign gene into the brain, especially, into the cerebellar cortical cells. Cell types transduced by viral vectors are determined initially by viral tropism and, after the entry into host cells, by promoters accommodated. We found that cathepsin K released from HEK 293T cells during viral production drastically shifted lentiviral tropism from Purkinje cells to Bergmann glia, and that blockade of the enzyme activity or removal of cathepsin K from viral solution restored the viral tropism for Purkinje cells. On the other hand, we recently succeeded to develop unique promoters that permit strong gene expression specifically in Purkinje cells, interneurons or Bergmann glia. In this presentation, I introduce our recent works about our new methods using viral vectors, by which cell-type-specific gene expression is attained.

1S13E-2

Cell-type specific gene transfer in olivo-cerebellar coculture preparation for the study of developmental synapse elimination

Uesaka, Naofumi[1]; Mikuni, Takayasu[1]; Hirai, Hirokazu[2]; Kano, Masanobu[1] ([1]*Dept. of Neurophysiol., Grad. Sch. of Med., Univ. Tokyo, Tokyo, Japan;* [2]*Dept. of Neurophysiol., Grad. Sch. of Med., Gunma Univ., Maebashi, Japan*)

To study the cellular and molecular mechanisms of synapse formation and refinement during brain development, it is important to develop methods for effective gene transfer. *In vitro* culture preparations have large advantages in terms of the accessibility and the manipulation flexibility, which enables efficient and reproducible gene transfer. We developed an organotypic coculture preparation allowing the elucidation of mechanisms for developmental synapse elimination in mammalian brain. This coculture consists of a cerebellar slice obtained from rat or mouse at postnatal day 9 (P9) or P10 and a medullary explant containing the inferior olive dissected from rat at embryonic day 15. We verified that climbing fibers (CFs), the axons of inferior olivary neurons, formed functional synapses onto Purkinje cells (PCs) in the cerebellum of cocultures. PCs were initially reinnervated by multiple CFs with similar synaptic strengths. Surplus CFs were eliminated subsequently, and the remaining CFs became stronger. These changes are similar to those occurring in developing cerebellum *in vivo*. Using this coculture preparation, we demonstrate that gain- and loss-of-function analyses can be efficiently performed in specific cell types by lentivirus-mediated gene transfer. Thus, our coculture preparation and gene transfer by lentiviral vector will greatly facilitate the elucidation of mechanisms of synapse elimination.

1S13E-3

A transgenic approach to target inhibitory neurons in the cerebellar cortex

Yanagawa, Yuchio (*Gunma University Graduate School of Medicine, Maebashi, Japan*)

GABAergic cells are major inhibitory neurons in the cerebellar cortex, and these cells play an important role in motor function. The cerebellar cortex is a simple three-layer structures consisting of mainly five types of neurons : the GABAergic stellate, basket, Purkinje, and Golgi neurons ; and the glutamatergic granule cells. Two isoforms of glutamate decarboxylase, GAD65 and GAD67, and vesicular GABA transporter (VGAT) are specifically expressed in GABAergic neurons. GABAergic neurons are primarily scattered in the cerebellar cortex, and thus it is difficult to selectively label or manipulate them. We suggest that a transgenic approach may overcome these barriers. The GAD67-GFP knock-in mouse has been widely used for the identification of GABAergic neurons. However, the overall GABA content in the GAD67-GFP knock-in mouse brain was reduced because of the destruction of the endogenous GAD67 gene, and heterozygous GAD67-GFP knock-in mice caused the impairment of synaptic innervation from climbing fiber to Purkinje cell during development. To overcome such a problem and to highlight the function and morphology of GABAergic neurons, we generated the VGAT-Venus transgenic mouse. Double immunostaining analysis in the transgenic mouse showed that Venus-expressing cells were primarily immunoreactive for GABA in the cererbellar cortex. These results demonstrate that the VGAT-Venus transgenic mouse should be useful for studies on GABAergic neurons in the cerebellar cortex.

1S13E-4

Diversity of inhibitory interneurons in the cerebellar granular layer

Hirono, Moritoshi (*Organization for Advanced Research and Education, Doshisha University, Kyoto, Japan*)

In the cerebellar granular layer, Golgi and Lugaro cells have been identified as large inhibitory interneurons playing specific roles in the cerebellar function. Other subtypes, small Golgi cells and small fusiform Lugaro cells, have recently been distinguished, which was followed by addition of globular cells identified as unique smaller-sized inhibitory interneurons based on their characteristic morphology. We electrophysiologically investigated synaptic activities of these small granular layer interneurons, particularly globular cells. We used a strain of gene-manipulated mice expressing GFP specifically in GABAergic neurons which allowed us to clearly target small and dispersed inhibitory interneurons under the microscope. Globular cells exhibited marked inhibitory synaptic activity together with monosynaptic inputs from the axon collaterals of Purkinje cells (PCs). IPSCs evoked at PC-globular cell synapses showed paired-pulse facilitation. In contrast, small Golgi cells or small fusiform Lugaro cells displayed fewer and smaller spontaneous IPSCs. Globular cells were silent at rest but became active resulting in spike discharges in response to application of monoamines, either serotonin or noradrenaline. The two amines also excited small Golgi cells, but small fusiform Lugaro cells was activated only by serotonin. Furthermore, globular cells appeared to be activated by excitatory mossy fiber inputs. Our findings suggest that globular cells play a unique role for neural information flow in the cerebellum.

Symposium 14
Cutting-edge Technologies for Exploring Life Sciences
[Collaboration Symposium with The Biophysical Society of Japan]

(March 27, 13 : 20–15 : 20, Room F)

1S13E-5

Studies on the longitudinal compartmentalization of the cerebellum using aldolase C－Venus knock－in mice

Sugihara, Izumi[1]; Fujita, Hirofumi[1]; Aoki, Hanako[1]; Yamazaki, Maya[2]; Sakimura, Kenji[2] ([1]*Dept Systems Neurophysiol, Tokyo Med & Dental Univ, Tokyo, Japan;* [2]*Dept Cellular Neurobiol, Brain Research Inst, Niigata Univ. Niigata, Japan*)

The adult cerebellar cortex is subdivided longitudinally by about 40 compartments of Purkinje cell subsets that are defined by different expression levels of certain molecules, such as aldolase C (zebrin II). Individual longitudinal compartments have specific projection patterns of efferent and afferent axons, and are involved in different aspects of movement control and other cerebellar functions. To visualize the longitudinal compartments with fluorescence for physiological and anatomical studies related to cerebellar compartmentalization, we developed knock-in mice in which a Venus gene sequence was inserted into exon 2 of the aldolase C gene (aldc-vns mice). In heterozygous aldc-vns mice, the longitudinal striped pattern was clearly visible in living and in fixed cerebella. Histological examination showed that the Venus expression followed the aldolase C expression pattern exactly. We carefully reexamined the organization of the aldolase C compartments by serial section alignment analysis of the entire cerebellar cortex of aldc-vns mice. We could then identify a few stripes that have not been previously recognized in the flocculus and in the central cerebellum. Aldc-vns mice were particularly useful in recording neuronal activities and labeling axonal projections in identified aldolase C stripes. We clarified some detailed topography in the corticonuclear and olivocortical projections in labeling studies with these mice.

1S14F-1

Observation of Single-Molecule Dynamics with High-Speed AFM

Uchihashi, Takayuki[1,2]; Ando, Toshio[1,2] ([1]*Physics Department, Kanazawa University, Japan;* [2]*Bio-AFM Frontier Research Center, Kanazawa University, Japan*)

Life process is the integration of extraordinarily diverse networks comprised of various biomolecules such as proteins, nucleic acids and related signaling chemicals. When we focus on a biological phenomenon and hypothesize a simplified model, a schematic cartoon might be drawn showing structure-function relation and interaction kinetics of multiple biomolecules as if we directly look at them through our eyes.

Currently-prospering single molecule analysis by fluorescence microscopy can detect dynamic behavior of protein at work but the spatial resolution is not high enough to visualize protein structure. Atomic force microscopy (AFM) possess very unique in its ability to visualize individual protein molecules in solution at (sub) nanometer resolution. However, its imaging rate is too low to capture dynamic events of molecules because of the slow mechanical responses of the cantilever and scanner.

In order to afford AFM to trace moving protein molecules, we have been developing various devices over the past decade. High-speed AFM is now routinely used to study dynamic processes of purified single proteins under physiological conditions, such as conformational change of motor and membrane proteins at work, protein-protein interaction, protein crystal dynamics. Further, very recently we apply HS-AFM to observe dynamics process on a living cell. In this talk, we s introduce recent success capturing dynamic biomolecular processes with high-speed AFM.

1S14F-2

Probing the micromechanics of the vertebrate metaphase spindle

Shimamoto, Yuta[1,2]; Maeda, Yusuke[2,4]; Libchaber, Albert[1]; Ishiwata, Shin'ichi[3]; Kapoor, Tarun[1] ([1]*The Rockefeller Univ., New York, USA;* [2]*JST, PRESTO, Tokyo, Japan;* [3]*Waseda Univ., Tokyo, Japan;* [4]*Kyoto Univ., Kyoto, Japan*)

The metaphase spindle is a micrometer-sized, microtubule-based structure that is assembled to segregate chromosomes during cell division. This structure is subjected to a variety of mechanical forces that act in diverse orientations and over a wide-range of timescales. Despite our extensive knowledge about the molecular and genetic aspects of spindle assembly and function, it still remains unclear how this essential cytoskeletal structure generates and responds to forces while maintaining overall stability, as we have a poor understanding of its micromechanical properties. Here, we have developed an assay system, in which timescale- and orientation-dependent mechanical properties of the metaphase spindle can be quantitatively analyzed by using force-calibrated microneedles and high-resolution microscopy. We find that the spindle structure is mechanically anisotropic, and alters its property from solid-like to fluid-like and vice versa depending on the timescale of force application. We also find that spindle's solid-like property can be linked to the bending elasticity of spindle microtubules, and spindle's fluid-like property depends on the dynamics of microtubule crosslinking. These data suggest a quantitative model for the micromechanics of this cytoskeletal architecture and provide insight into how structural and functional stability is maintained in the face of different forces, such as those that control spindle size and position, and can result from deformations associated with chromosome movement.

1S14F-3

The spatial pattern of cochlear amplification

Nin, Fumiaki[1,2]; Fisher, Jonathan[2]; Reichenbach, Tobias[2]; Uthaiah, Revathy[2]; Hudspeth, James[2] ([1]*Physiology II, Graduate School of Medicine, Niigata University, Japan;* [2]*Rockefeller University, New York, US*)

Sensorineural hearing loss, which stems primarily from the failure of mechanosensory hair cells, is associated with changes in the traveling waves that transmit acoustic signals along the cochlea. However, the connection between cochlear mechanics and the amplificatory function of hair cells remains unclear. Using a novel optical technique that permits the targeted inactivation of prestin, a protein of outer hair cells that generates forces on the basilar membrane, we demonstrate that these forces locally interact with cochlear traveling waves to achieve enormous mechanical amplification. By perturbing amplification in narrow segments of the basilar membrane, we further show that a cochlear traveling wave accumulates gain as it approaches its peak. Analysis of these results indicates that cochlear amplification produces negative damping that counters the viscous drag impeding traveling waves ; targeted photoinactivation locally interrupts this compensation. These results reveal the locus of amplification in cochlear traveling waves and connect the characteristics of normal hearing to molecular forces.

1S14F-4

Measuring Temperature in a Living Cell

Okabe, Kohki[1]; Uchiyama, Seiichi[1]; Inada, Noriko[2]; Harada, Yoshie[3]; Funatsu, Takashi[1] ([1]*Grad. Schl. of Pharm Sci., Univ of Tokyo, Tokyo, Japan;* [2]*Grad. Schl. of Biol. Sci., NAIST, Ikoma, Japan;* [3]*iCeMS, Kyoto Univ., Japan*)

Temperature is a fundamental physical quantity that governs every biological reaction within living cells, and temperature distribution reflects cellular thermodynamics and function. In medical studies, the cellular pathogenesis of diseases (e.g., cancer) is characterized by extraordinary heat production. Therefore, intracellular temperature imaging of living cells should promote better understanding of cellular events and the establishment of novel diagnoses and therapies. However, imaging of temperature distributions in living cells has never been achieved. Here we demonstrate the first intracellular temperature imaging based on a fluorescent polymeric thermometer and fluorescence lifetime imaging microscopy (FLIM). The spatial and temperature resolutions of our thermometry were at the diffraction limited level (200 nm) and 0.2℃, respectively. The intracellular temperature distribution we observed indicated that the nucleus and centrosome of a COS7 cell both showed a significantly higher temperature than the cytoplasm and that the temperature gap between the nucleus and the cytoplasm differed depending on the cell cycle. The heat production from mitochondria was also observed as a proximal local temperature increase. These findings demonstrate an intrinsic connection between temperature and organelle function. Thus, our intracellular temperature imaging has a significant impact on the comprehension of cell function and will provide insights into the regulatory mechanisms of intracellular signaling.

1S14F-5

Real-time imaging of single sarcomeres in the mouse heart *in vivo*

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Active force in cardiac muscle is highly dependent on sarcomere length (SL), known as the Frank-Starling mechanism of the heart. Indeed, a change of ~0.1 μm in SL causes a dramatic change in its contractile performance, especially under partial activation states. However, because of technical difficulties, no stuides have been conducted hitherto to quantitatively analyze sarcomere dynamics in the living heart *in vivo*. In the present study, we conducted an experimental system allowing for the real-time imaging of sarcomeric motions in ventricular myocytes in the anesthetized mouse. We expressed GFP at sarcomeric Z-disks (α-actinin-GFP) by using the adenovirus vector system in the left ventricle of the adult mouse, and measured the length of a single sarcomere at ~100 fps in various regions of cardiomyocytes in the anesthetized open-chest mouse under a fluorescence microscope (combined with a confocal unit and a piezo scanner). Likewise, we successfully recorded electrocardiogram and left ventricular pressure simultaneously with the sarcomeric motions. At the meeting, we will discuss how the cardiac excitation-contraction coupling is organized *in vivo*.

1S14F-6

Make biomagnetic fields realistic : Application of pulse-driven magnetoimpedance sensor to physiology

Nakayama, Shinsuke[1]; Atsuta, Satoshi[1,3]; Kondo, Masao[1]; Uchiyama, Tsuyoshi[2] (*[1]Nagoya Univ. Grad. Sch. Med., Nagoya, Japan; [2]Nagoya Univ. Grad. Sch. Eng., Nagoya, Japan; [3]Div. Technol. Develop., Fujidenolo, Komaki, Japan*)

Biomagnetic fields have, so far, been measured with SQUID (superconducting quantum interference device)-based sensors, but their applications are nearly limited in living bodies. As the name indicates, this technology requires conditions of extremely low temperature, at least -200℃, far apart from our body temperature. The total system is thus so large and expensive that only a limited number of central hospitals and research institutes enjoy the benefit of SUQID magnetic sensors. To make a breakthrough in the biomagnetism field, we have employed a magnetic sensor referred to as a magnetoimpedance (MI) sensor. Since the probe of this magnetic sensor is constructed solely from ordinary electromagnetic materials, such as detector coils and magnetic amorphous wires, it is operated at body temperature, and accessible close to living systems. Also, excitation pulse is applied at 1 μs intervals, thereby quasi-real time recordings are carried out in measurements of biological activity. In this presentation, we carefully explain the theoretical backgrounds of MI technology, and possible procedures to improve this sensor. Actually, compared to ordinary one, the sensitivity is increased to approx. 100 μV/nT, and the detection limit is less than 0.1 nT. Also, we show recent measurements of magnetic activity in small isolated samples and in human chest, using an improved detector circuit. In some measurements of biomagnetic activity, electric activity is simultaneously measured.

1S15G-1

Analysis of molecular mechanism of platelet dense-core granule secretion by a semi-intact system

Horiuchi, Hisanori; Shirakawa, Ryutaro (*Institute of Development, Aging and Cancer, Tohoku Univ. Sendai, Japan*)

Platelets store self-agonists such as ADP in the dense granules and secrete the granules to contribute to explosive activation of platelets by a positive feedback mechanism at the site of vascular injury. We established a dense granule secretion assay with platelets permeabilized by streptolysin-O. The secretion in the assay was temperature- and ATP-dependent with similar calcium sensitivity to intact platelets. It was also cytosol-dependent and we identified an essential factor in cytosol that was PKCalpha. We found that small GTPase Rab27 regulated the secretion, which was present predominantly in the GTP-bound form in unstimulated platelets due to constitutive GDP/GTP exchange activity. Therefore, we considered that the function of Rab27 is to maintain the granules in a primed status for the secretion. We then identified Munc13-4, a homologue of Munc13-1 known as an essential priming factor for neurotransmitter release, as an effector of Rab27. Addition of Munc13-4 in the assay enhanced the Ca2+-induced secretion while addition of anti-Munc13-4 antibody strongly inhibited it. Further, platelets from patients lacking Munc13-4 exhibited impaired the thrombin-induced secretion. Munc13-4 contains two Ca2+-binding C2 domains and mutation in either domain abolished the secretion-enhancing activity and the SNARE-containing liposome fusion enhancing-activity, induced by Ca2+. These results indicated that Munc13-4 mediates the Ca2+ signal in the secretion. Thus, Ca2+ would mediate the secretion through PKCalpha and Munc13-4, found using the semi-intact assay.

Symposium 15
Novel approaches to platelet functions *in vivo*

(March 27, 13 : 20–15 : 20, Room G)

1S15G-2

Structural basis of integrin activation and integrin-targeted therapeutics

Shimaoka, Motomu (*Mie University Medical School, Tsu-city, Japan*)

Integrins represent a foremost family of cell adhesion molecules that mediate cell-to-cell and cell-to-matrix interactions in a wide range of biology. Integrin-mediated adhesive interactions play a critical role in platelet aggregation and thrombus formation as well as in immune cell trafficking to sites of inflammation and tissue injury. What makes integrins very unique in many cell adhesion molecules is their ability to transmit signals across the plasma membrane bi-directionally. Allosteric machinery has been revealed that controls such integrin bi-directional signaling, thereby supporting the dynamic and reversible transformation of integrins between non-adhesive and adhesive states. This makes integrins as an ideal platform to understand the mechanistic basis of how small-molecules modulate functionalities of large membrane proteins with complex domain organization.

We have found and characterized novel classes of integrin allosteric antagonists that bind to the extracellular "hot spot" and, thereby, perturb the activation-dependent conversion to the high-affinity conformation. The small-molecule allosteric antagonists to integrins have been classified into three types based on their targeting profile : the conformational changes of the alpha I domain ; the conformational signal transmission between the alpha and beta I domain ; or the conformational changes of the beta I domain. These antagonists have demonstrated the potential to block platelet and leukocyte adhesion in response to stimulation.

The presentation will cover the molecular and structural basis of allosteric perturbation of integrin activation by targeting the extracellular parts as well as a novel cytoplasmic target that regulate integrin activation.

1S15G-3

A role of platelet activation receptor CLEC−2 in tumor metastasis, lymphangiogenesis, and thrombus formation

Suzuki-Inoue, Katsue (*Department of Clinical and Laboratory Medicine, Faculty of Medicine, University of Yamanashi, Yamanashi, Japan*)

We have identified the novel class of platelet activation receptor C-type lectin-like receptor 2 (CLEC-2) as a receptor for rhodocytin, a platelet activating snake venom. CLEC-2 activation leads to tyrosine phosphorylation of YITL motif in its cytoplasmic tail, binding of Syk, initiation of downstream tyrosine phosphorylation events, and activation of phodpholipas Cγ2, which result in platelet aggregation. We also identified podoplanin as an internal ligand for CLEC-2. Podoplanin is expressed on the surface of tumor cells and facilitates tumor metastasis by inducing platelet aggregation. We proved that an antibody that blocks the binding between CLEC-2 and podoplanin inhibited tumor metastasis using an experimental lung metastasis model in mice. Podoplanin is also expressed in lymphatic endothelial cells. We generated CLEC-2-deficient mice and found that these mice die at the embryonic/neonatal stages associated with disorganized and blood-filled lymphatic vessels and severe edema. Moreover, by transplantation of fetal liver cells from CLEC-2 + / + or CLEC-2-/- embryos, we were able to demonstrate that CLEC-2 is involved in thrombus stabilization in vitro and in vivo without apparent increase in bleeding tendency. These findings revealed that CLEC-2 plays a crucial role not only in tumor metastasis, but also in lymphangiogenesis and thrombus stabilization. We propose that CLEC-2 could be a novel target protein for an antiplatelet drug without increasing bleeding tendency and that for antimetastatic drug.

1S15G-4

Novel approaches to platelet functions by in vivo molecular imaging

Nishimura, Satoshi (*Department of Cardiovascular Medicine, Translational Systems Biology and Medicine Initiative, The University of Tokyo, Japan*)

The mechanism by which thrombotic vessel occlusion occurs independently of plaque development or endothelial cell (EC) disruption remains unclear, largely because of an inability to visualize thrombus formation, especially at the single-platelet level in real time. Therefore, we developed in vivo imaging technique based on single- and multiphoton microscopy, and we assessed dynamic cellular interplay in thrombosis models. We visualized that rapidly developing thrombi composed of discoid platelets without EC disruption was triggered by ROS photochemically induced by moderate power laser irradiation. Using this technique, we elucidated that Lnk (adapter protein) regulates integrin signaling leading to stabilization of developing thrombus in vivo (2010 JCI). We analyzed the in vivo function of artiricial, iPS derived platelets (2010 JEM). In addition, we elucidated the contribution of inflammatory cytokines, ROS, and integrin signaling to our thrombosis models (2011 Blood).

We also visualized the platelet biogenesis in bone marrows. We utilized the actin-GFP mice, which enabled us to visualize and analyze effectively the megakaryocytes (MKs) dynamics in scalp bone marrows of living animals.

In sum, using our imaging system can be a powerful tool to analyze thrombus formation. We clarified the mechanism of discoid platelet aggregations on undisputed endothelium. The initial platelet aggregation subsequently leads to irreversible integrin- and actin-dependent thrombus development. Inflammatory cytokine signaling in ECs also played pivotal role.

Symposium 16
Ion channels leading to functional alteration in pancreatic beta cell

(March 27, 13 : 20−15 : 20, Room H)

1S16H-1

Genetic and functional analyses of K_{ATP} channel gene mutations in patients with neonatal diabetes in Japan

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K_{ATP} channels are critical metabolic sensors in acute metabolic changes, including hyperglycemia, hypoglycemia, ischemia, and hypoxia. Pancreatic β-cell type K_{ATP} channel, composed of Kir6.2 (*KCNJ 11*) and SUR1 (*ABCC8*) subunits, plays key roles in glucose-stimulated insulin secretion. The common etiology of permanent neonatal diabetes mellitus (NDM) is a mutation in one of three genes, *KCNJ11*, *ABCC 8*, or *INS*. We conducted a nationwide survey of patients with NDM and revealed the estimated incidence of NDM is approximately 1 in 90,000 live births in Japan. By candidate gene sequencing of *KCNJ11*, *ABCC8*, and *INS* using the genomic DNA isolated from peripheral blood leukocytes of patients and the evaluation of the mutations as a cause of NDM, we identified numerous susceptibility mutations including novel ones in *KCNJ11* and *ABCC8*. Furthermore, we detected the mutations in juvenile- and adult-onset diabetes. Functional analyses were carried out and the variations in spontaneous Po, nucleotide- and SU-sensitivity, and functional cell surface expression among the mutant channels were detected. The phenotype or severity of diabetes caused by mutations in K_{ATP} channel genes could reflect the overall effect of each variable factor in each mutation. These results broaden the spectrum of diabetes mellitus caused by K_{ATP} channel gene mutations, and also suggest a possibility of these mutations should be taken into consideration in the cause of neonatal, juvenile-onset, and adult-onset diabetes.

1S16H-2

K_{ATP} channel and insulin secretion. Studies of mutated channels

Shimomura, Kenju; Yada, Toshihiko (*Division of Integrative Physiology, Department of Physiology, Jichi Medical University, Tochigi, Japan*)

Mutations in pancreatic β-cell K_{ATP} channel subunits Kir6.2 and SUR1 can affect insulin secretion. Gain/loss-of-function mutations of Kir6.2 and SUR1 are known to cause hyperglycaemia/hypoglycaemia. In case of gain-of-function mutations, we have found that sulfonylureas (SU), selective blocker of K_{ATP} channels, remain effective at closing mutated channels. This has enabled many patients to switch from insulin injection therapy to oral sulfonylurea therapy. On the other hand, many hypoglycaemia caused by loss-of-function mutations can also be treated by using oral diazoxide, selective opener of K_{ATP} channels. However, in some cases of loss-of-function hypoglycaemia, patients gradually show hyperglycaemia tendency in the later life. To understand the functional effects of these mutations, we have generated transgenic mice expressing Kir6.2 mutations. Induction of gain of function mutation Kir6.2-V59M in adult mice led to diabetes. This elevation of blood glucose was treatable by implanting glibenclamide pellet under the skin of mice. In perifused islets from transgenic mice, insulin secretion was completely lost in response to high glucose. However, glibenclamide was able to stimulate insulin secretion. Importantly, in the presence of 2μM glibenclamide, both elevation of basal insulin secretion and restoration of glucose-stimulated insulin secretion (GSIS) was observed. These results may produce insights into mechanism and treatment of patient with diabetes.

1S16H-3

Ghrelin attenuates insulin release via Kv channel activation in islet β-cells

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Voltage-dependent potassium channels are involved in repolarization of excitable cells. In pancreatic β-cells, activation of delayed rectifier K^+ (Kv) channels possibly repolarize cells and attenuate glucose-stimulated action potentials to suppress insulin secretion. Inhibition of the β-cell Kv current would be expected to prolong action potentials and enhance glucose-induced insulin secretion. Ghrelin, an acylated 28-amino acid peptide, reportedly restricts insulin release in islet β-cells via pertussis toxin-sensitive G-proteins and thereby regulates glucose homeostasis. Ghrelin suppressed glucose (8.3 mM)-induced insulin release in rat perfused pancreas and isolated islets, and these effects of ghrelin were blunted in the presence of cAMP analogues or adenylate cyclase inhibitor. Glucose-induced cAMP production in isolated islets was attenuated by ghrelin. Ghrelin also attenuated glucagon-like peptide-1 action to increase cAMP production and insulin release in isolated islets. Furthermore, ghrelin potentiated Kv channel currents without altering Ca^{2+} channel currents and attenuated glucose-induced $[Ca^{2+}]_i$ increases in rat β-cells in a cAMP signal-dependent manner. These results suggest that ghrelin directly interacts with islet β-cells to attenuate glucose-induced cAMP production, which lead to activation of Kv channels and suppression of glucose-induced $[Ca^{2+}]_i$ increase and insulin release. Ghrelin and its receptor signaling in β-cells may be potential therapeutic target to counteract the progression of type 2 diabetes.

1S16H-4

5-HT receptor 3a signaling regulates insulin release from pancreatic beta cells during pregnancy

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In response to the increased insulin demands due to insulin resistance during pregnancy, pancreatic islets undergo not only the increase in β cell proliferation but also the increase in glucose-stimulated insulin secretion (GSIS), though the mechanism of the greater insulin secretion during pregnancy is still not well known. Here we explored that 5-HT synthesized in β cells during pregnancy acts to regulate the increase in GSIS from β cells with enhanced glucose sensitivity through 5-HT receptor 3a (Htr3a), a ligand-gated ion channel. GSIS from isolated wild-type (WT) pregnant mouse islets showed a marked increase, but not from the Htr3a gene knockout (KO) mouse. However, Htr3a KO did not affect β cell proliferation and 5-HT production. Electrophysiological studies showed that 5-HT produces a depolarizing shift of resting membrane potential in β cells through Htr3a, which stimulated the glucose-induced Ca^{2+} influx with enhanced glucose sensitivity. This causes enhanced glucose sensitivity of glucose low responsive β cells, as a result, the population of the high secretory responsive β cells and eventual fusion events were increased in pregnant mouse islet. Thus, our data indicate that 5-HT-Htr3a signaling in a paracrine-autocrine fashion plays an essential role in the dramatic increase of GSIS during pregnancy.

Symposium 17
Optogenetics marks a new era of *in vivo* physiology
[Collaboration Symposium with The Japan Neuroscience Society]

(March 27, 13 : 20–15 : 20, Room I)

1S17I-1
Repertoire of optogenetically targeting projection neurons which should modulate psychiatric conditions

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Optogenetics has proven to be a powerful tool capable of manipulating the activity of a specific population of cells in a complex multicellular organism. This approach is enthusiastically pursued in recent neuroscience field and the causal relationship between neural activity and behavior is finally starting to become unveiled. However, most studies utilize virus mediated gene transfer for the induction of light-sensitive proteins, such as channelrhodopsin-2 (ChR2), and such method inevitably introduces variability of expression between trials. Therefore, transgenic approach has long been sought, however, satisfying the demands of the specificity as well as the abundance of expression were difficult.Here, we established Knockin-mediated ENhanced Gene Expression by improved tetracycline-controlled gene induction system (KENGE-tet). We found that high levels of tTA-mediated transcription can be achieved by knocking in tetO-ChR2 cassette into a locus at a housekeeping gene, beta-actin. We respectively crossed this tetO-ChR 2 knockin mouse with serotonergic, dopaminergic, and noradrenergic-specific tTA lines, and achieved ChR2 expression in specific cell-types. In all cases, the level of ChR2 expression was high enough to allow manipulation of cell activity.

1S17I-2
Identification of thermogenesis-driving central mechanism by optogenetic activation of projection neurons that connect specific brain regions

Nakamura, Kazuhiro; Kataoka, Naoya (*Career-Path Promotion Unit for Young Life Scientists, Kyoto Univ., Kyoto, Japan*)

Central regulation of metabolic heat production (thermogenesis) in brown adipose tissue (BAT) is important for the control of body temperature and energy expenditure. Central output driving BAT thermogenesis is controlled by sympathetic premotor neurons in the rostral medullary raphe (rMR). However, how sympathetic premotor neurons are controlled from upper brain sites is unknown. Here we examined whether an axonal projection from the dorsomedial hypothalamus (DMH) to the rMR provides an excitatory input to drive activation of sympathetic premotor neurons. Virus-mediated delivery of channelrhodopsin-2 (ChR2) gene into rat DMH resulted in localization of ChR2 proteins in cell bodies in the DMH and in their axon terminals in the rMR. *In vivo* photostimulation of ChR2-containing axons in the rMR consistently increased BAT thermogenesis, blood pressure and heart rate. Photostimulation of ChR 2-containing cell bodies in the DMH also elicited similar responses, which were eliminated by nanoinjection of glutamate receptor antagonists into the rMR. The responses to photostimulation of ChR 2-containing axon terminals in the rMR were inhibited by local warming of the thermoregulatory center, preoptic area (POA). These results indicate that the DMH-rMR projection provides a glutamatergic input to sympathetic premotor neurons to drive thermogenic and cardiovascular outflows and that this glutamatergic activation of sympathetic premotor neurons is influenced by thermal information from the POA.

1S17I-3
Optogenetic control of serotonergic neurons and anxiety-related behavior

Ohmura, Yu (*Department of Neuropharmacology, Hokkaido University Graduate School of Medicine, Sapporo, Japan*)

It has generally been thought that serotonin release in the forebrain attenuates anxiety. However, there is so far no direct evidence proving this hypothesis. Although there is extensive indirect evidence, it is mixed. For example, while selective serotonin reuptake inhibitors (SSRIs) are first-line agents for anxiety disorders, increased anxiety is often observed during the acute phase of treatment. Therefore, in the present study, we aimed to obtaining direct evidence about the causal relationship between serotonin and anxiety using recently developed optogenetic tools. We obtained transgenic mice expressing channelrhodopsin-2 (ChR2) mutant (C128S) only in central serotonergic neurons by crossing tetO-ChR2 (C128S)-EYFP knock-in mice with Tph 2-tTA BAC transgenic mice. The activation/deactivation rates of C128 S mutant with blue light are slow (τon=20 ms, τoff=108 s).We inserted an optical fiber to the median raphe nucleus (MRN). We applied blue light to open ChR2, and measured extracellular serotonin levels in the ventral hippocampus and recorded behavioral changes in the elevated plus maze. Yellow light was used as a negative control because it will not open ChR2.We demonstrated that blue light illumination to the MRN significantly increased extracellular levels of serotonin in the ventral hippocampus while yellow light did not. Moreover blue light illumination affected anxiety-like behavior in the elevated plus maze while yellow light did not. Thus we obtained direct evidence of the causal relationship between serotonergic activity in the MRN and anxiety.

1S17l-4

Use of RNA interference, DREADD and optogenetics to address the role of adenosine A_{2A} receptors in the nucleus accumbens for sleep–wake regulation

Lazarus, Michael; Urade, Yoshihiro; Huang, Zhi-Li (*Osaka Bioscience Institute, Japan*)

Adenosine promotes sleep through the activation of A_{2A} receptors. A_{2A} receptors are densely expressed on striatopallidal neurons of the basal ganglia, where dopamine D_2 receptors are co-expressed with A_{2A} receptors and involved in motor function, habit formation, and reward/addictive behaviors. The extent to which A_{2A} receptors in the basal ganglia contribute to the regulation of sleep and wakefulness is not known. We investigated the role of A_{2A} receptors in the basal ganglia for wakeful consciousness by using powerful tools for site-specific gene manipulations, including A_{2A} receptor knockout mice based on the Cre/lox technology ; focal A_{2A} receptor knockdown in rats through the local infection with adeno-associated virus carrying short-hairpin RNA of A_{2A} receptors ; and modulation of neuronal activity through in-vivo stimulation with optogenetic technologies and receptor-channel systems. Our studies have revealed that the arousal effect of caffeine is mediated by A_{2A} receptors on neurons in the shell of the nucleus accumbens (NAc) and that transient activation of NAc neurons promotes sleep. These observations strongly suggest that A_{2A} receptors in the NAc are key structural elements for the control of sleep and wakefulness. These findings further suggest the intriguing possibility that the ventral striatum may be a key site through which sleep and wakefulness are regulated by behavioral processes and, by extension, that motivational state may be an important fundamental regulator of sleep and wake (Trends Neurosci, doi : 10.1016/j.tins.2012.07.001).

1S18J-1

Disrupted cortical function underlies behavior dysfunction due to social isolation

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Stressful events during early childhood can have a profound lifelong influence on emotional and cognitive behaviors. However, the mechanisms by which stress affects neonatal brain circuit formation are poorly understood. Here, we find that neonatal social isolation disrupts molecular, cellular and circuit developmental processes leading to behavioral dysfunction. Neonatal isolation prevents long-term potentiation and experience-dependent synaptic trafficking of AMPA receptors normally occurring during circuit formation in the rodent barrel cortex. This is mediated by an increase of the stress glucocorticoid hormone, associated with reduced CaMKII signaling, and results in the attenuation of the whisker-sensitivity at the cortex. These effects lead to defects in whisker-dependent behavior in juvenile animals. These results indicate that neonatal social isolation alters neuronal plasticity mechanisms and perturbs the initial establishment of a normal cortical circuit, potentially explaining the long lasting behavioral effects of neonatal stress.

Symposium 18
Mother–child interaction influences the brain functions of mother and child

(March 27, 13 : 20–15 : 20, Room J)

1S18J-2

Effects of early life stress on brain activity : implications from maternal separation model in rodents

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Adverse experiences in early life can affect the formation of neuronal circuits during postnatal development and exert long-lasting influences on neural function. Many studies have shown that daily repeated maternal separation (RMS), an animal model of early life stress, can modulate the hypothalamic-pituitary-adrenal axis (HPA-axis) and can affect subsequent brain function and emotional behavior during adulthood. However, the molecular basis of the long-lasting effects of early life stress on brain function has not been completely elucidated. In this mini-review, we introduce various cases of maternal separation in rodents and illustrate the alterations in HPA-axis activity by focusing on corticosterone (CORT), an end-product of the HPA-axis in rodents. We then present the characterization of the brain regions affected by various patterns of MS, including RMS and single time maternal separation (SMS) at various stages before weaning, by investigating c-Fos expression, a biological marker of neuronal activity. These CORT and c-Fos studies suggest that repeated early life stress may affect neuronal function in region- and temporal-specific manners, indicating a critical period for habituation to early life stress. Furthermore, we introduce changes in behavioral aspects and gene expression in adult mice exposed to RMS.

1S18J-3

Maternal experience alters the hippocampal function related to learning and memory

Furuta, Miyako; Fukushima, Atsushi; Funabashi, Toshiya; Akema, Tatsuo (*Department of Physiology, St. Marianna University School of Medicine, Japan*)

Reproductive experiences such as pregnancy, lactation and maternal behavior, results in significant alterations in subsequent hormone levels in females. Several studies have demonstrated that circulating hormones can significantly affect hippocampal neural structure and functions such as learning and memory. These results led us to hypothesize that reproductive experiences alters the spatial function related to the hippocampus. We first examined the hippocampus-dependent behavioral tests. We found that primiparous rats showed better performance than nulliparous rats in Y maze test, but not Morris water maze. Morphological studies, such as the density, length, or shape of spines in the hippocampus, failed to detect the changes between both. Next, we examined functional alteration of the hippocampus by whole-cell patch-clamp method. In acute hippocampal slice preparation, we recorded CA1 neurons stimulating schaffer collateral by voltage-clamp. LTP was induced by paired protocol. We found that the LTP was successfully induced in primiparous rats but not in nulliparous rats, suggesting that the induction of LTP was occluded in nulliparous rats but not in primiparous rats. The data suggests that reproductive experience like maternal behavior alters special learning and these effects are caused by the synaptic enhancement and/or ability in the hippocampus. To confirm our hypothesis, we have currently studied PSD 95 and phosphorylation of glutamine receptors by western blotting.

1S18J-4

Oxytocin works as a key factor for emotion and memory control, and it is a therapeutic target for mental disorders

Matsui, Hideki (*Dept. Physiol., Okayama Univ. Grad. Sch., Okayama, Japan*)

Oxytocin (OT) is an essential hormone for mammalian labor and lactation. It is synthesized in magnocellular and parvocellular neurons of the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus. The hormone in magnocellular neurons is secreted in the posterior lobe of the pituitary, whereas the one in parvocellular neurons is transported to various areas of the brain including hippocampus and amygdala. OT acts as a neurotransmitter/neuromodulator to regulate a range of CNS functions in males and females, including emotional, parental, affiliative, and sexual behaviors.

We have shown OT causes long-lasting, long-term potentiation (L-LTP) through CREB phoshorylation in hippocampal synapses and induce memory potentiation during motherhood in mice.

We further show OT is released into blood and within distinct brain regions in response to stressful and social stimuli, and the hormone has an antidepressant-like effect in animal studies. Physiological activities such as sexual activity and mating induce the release of OT in the central nervous system. A drug for the treatment of sexual dysfunction, sildenafil, enhances the release of OT from the posterior pituitary. This drug has antidepressant-like effects through activation of an OT signaling pathway.

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1S18J-5

Neural correlates of maternal love, paternal love and children's love for their parents

Shinohara, Kazuyuki; Nishitani, Shota; Takamura, Tsunehiko (*Dept. Neurobiol. Behav., Grad. Sch. Biomedical Sci., Nagasaki Univ. Nagasaki, Japan*)

Neural substrates for parent-child bonding/attachment in humans have not been clarified. We examined patterns of prefrontal cortex (PFC) activity in mothers and fathers or in children while they are watching their own child or parent video clip. We performed near-infrared spectroscopy (NIRS) measurements while mothers and fathers (or children) viewed silent video clips of their own child (or parent) facial expressions and other age-matched child's (or adult's) facial expressions. Children participated in the present study are boys before, during and after puberty. We found that the right ventromedial PFC (vmPFC) was activated in mothers whereas any region of the PFC was not activated in fathers during viewing the smiling facial expression of their own child. However, when fathers were divided into two groups according to AVPR1A polymorphisms, the left vmPFC activation was observed in fathers without the 334 allele of the RS3 but not in fathers with the allele. On the other hand, boys before puberty showed an increase in the right vmPFC activity whereas boys during puberty showed increases in the left vmPFC and dorsolateral PFC activity during watching their own parent smiling. However, boys after puberty did not show any increase in PFC activity. These results suggest that an important role of vmPFC in both maternal and paternal bonding and sexual laterality of neural substrates for parental bonding although all fathers do not always respond to child'smile. Furthermore, neural substrates for attachment may vary through the pubertal development.

Symposium 19
Rethinking how pain is generated from nociception: morphofunctional approaches
[Collaboration Symposium with The Japanese Association of Anatomists]

(March 27, 15 : 20–17 : 20, Room B)

1S19B-1

Structure and function of interneurons in the substantia gelatinosa of the spinal cord and their role in the circuitry processing nociceptive information

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The substantia gelatinosa (SG) of the spinal dorsal horn is known to play a role in modulating and transmitting incoming sensory (including nociceptive) information. However, its structural and functional organization, and its role in the neuronal circuitry for processing pain information, remain poorly understood due to the difficulty in identifying populations of interneurons. Virtually all SG neurons are excitatory or inhibitory interneurons because none of the axons arising from these cells reach supraspinal areas. Thus it is very important to dissect the local neuronal circuitry, which involves different types of SG neurons, in order to understand the output signal from spinal cord. Recently, we investigated these interneurons by using a combined electrophysiological and anatomical approach. This included tests for discharge patterns, responses to neuromodulators, and excitatory and inhibitory inputs evoked by dorsal root stimuli, as well as examination of morphological features and neurochemical phenotypes. We found substantial correlations among these properties. We also revealed possible mechanisms involving abnormal pain states, and interestingly it seems that particular types of SG neurons have specific roles in modulating local circuitry, so that the input-output relation could be changed through interactions among these interneurons.

1S19B-2

Site-specific and phase-specific activation of macrophage/microglia in the primary afferent system in neuropathic pain model mice

Senba, Emiko; Kami, Katsuya(*Department of Anatomy & Neurobiology, Wakayama Medical University, Wakayama, Japan*)

Proliferation and activation of macrophages (mϕs)/microglia in the primary afferent system may be critical for the development and maintenance of neuropathic pain. Activated mϕs have been classified into M1 (pro-inflammatory) and M2 (anti-inflammatory) subtypes. In the present study we focused on the polarity and origin, i.e. resident or bone marrow (BM)-derived, of these activated mϕs/microglia in mice subjected to partial sciatic nerve ligation (PSL) according to the method of Seltzer. At day 3 to 28 post-operation, C57BL/6J and EGFP-chimeric mice were perfused transcardially with 4% PFA-0.1M PBS. At day 3 to 21 post-PSL, Iba-1 (+) mϕs/microglia were significantly increased in the ipsilateral dorsal horn, and they were CD 68 (+)/CD 86 (+)/Arginase-1 (-)/CD163 (-)-M1 mϕs/microglia. In analysis of EGFP-chimeric mice, we first found EGFP (+) BM-derived cells infiltrated in spinal dorsal horns at day 21 post-PSL, and these cells were not polarized yet or polarized into CD206 (+)-M2 subtype. On the other hand, EGFP (+)-mϕs were detected at day 3 in injured sciatic nerves. Our previous study using PSL model has shown that iNOS (+)/Arginase-1 (-)-M1 mϕs were markedly increased in injured sciatic nerves, and mϕs activated in the DRGs were all iNOS (-)/Arginase-1 (+)-M2 phenotype. These findings may indicate that resident or BM-derived activated mϕs/microglia in the primary afferent system of PSL model mice may enhance or modulate neuropathic pain by changing their polarity in a site-specific and phase-specific manner.

1S19B-3

The role of C fiber afferents in the establishment of synaptic potentiation in the nociceptive amygdala

Takahashi, Yukari; Kato, Fusao(*Lab. Neurophysiol., Dept. Neurosci., Jikei Univ. Sch. Med. Tokyo, Japan*)

Of several brain regions proposed to underlie the link between nociception and emotion, the capsular part (CeC) of the central amygdala, aka, "the nociceptive amygdala", is strategically situated to be a direct target of peripheral nociceptive system, because it receives information arising from the lamina I neurons of the dorsal horn that receive C fiber afferents through the spino-parabrachio-amygdaloid pathway (Gauriau & Bernard, 2001 ; Todd, 2010). Indeed, in animal models with arthritis, colitis or formalin-induced inflammatory pain, robust synaptic potentiation at the synapses between fibers from the lateral parabrachial nucleus (LPB) and CeC neurons has been described, in agreement with the increased C fiber-mediated inputs in these models. However, we have demonstrated that such LPB-CeC potentiation also occurs in a neuropathic pain model with the spinal nerve ligation, in which Aβ fiber-triggered tactile allodynia, not the C fiber-mediated nociception, is the principal nocifensive behavior (Ikeda et al, 2007). Using this model together with the neonatal capsaicin treatment that selectively ablates C fibers expressing TRPV1 channels, we examined the role of C fiber afferents in establishment of LPB-CeC potentiation. Despite clear manifestation of the allodynia in the capsaicin-treated rats losing responses to ocular capsaicin application, the LPB-CeC transmission in these rats was not potentiated. We concluded that C fiber-mediated information is necessary for enhancing the link between nociception and emotion during the course of chronic pain.

1S19B-4

Regulation of TRPV1/A1 channel expression in sensory neurons after inflammation by artemin

Noguchi, Koichi; Miyagawa, Yasuko(*Department of Anatomy and Neuroscience, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan*)

NGF is known to be one of the main regulators of the sensitivity of sensory neurons by up-regulating TRPV1 and TRPA1. However, its receptor, TrkA, is only co-localized with TRPV1/A1 in less than 50% of cases. In contrast TRPV1/A1 show a high degree of co-localization with GFR alpha 3, which is a receptor of artemin, a member of GDNF family. We thus focused on investigating the relationship between artemin and TRPV1/TRPA1 in inflammatory hypersensitivity. Male SD rats were used for all procedures. CFA was injected into the plantar of left hindpaw, and the plantar skin was collected at various time points for 7 days after CFA injection. Temporal expression pattern of artemin mRNA was different from that of NGF and GDNF. Up-regulation of NGF mRNA was transient and peaked at 3h after CFA injection, whereas artemin mRNA showed long lasting increase at least up to 7day after injection. To know the effect of artemin on the nociception, a recombinant mice artemin was injected into the plantar for 5 consecutive days and mechanical and thermal behavioral analyses were performed. Hypersensitivity was found in both mechanical and thermal testing compared to PBS control. Moreover, we could detect significant increase of TRPV1/A1 mRNA after 5-day injection of artemin. These changes were exclusively observed in the phosphorylated p38 MAP kinase positive neurons that showed the upregulation after artemin injection. These results suggested that peripheral artemin in inflamed tissue might be involved in the generation of long-term hypersensitivity via up-regulation of TRPV1/A1.

1S19B-5

In vivo analysis of spinal GABAergic inhibition of nociceptive transmission

Furue, Hidemasa (*Information Physiol, NIPS, Okazaki, Japan*)

Recent studies have shown that plastic changes in the chloride gradient of superficial dorsal horn neurons lead to a reduction in GABA-mediated inhibition in neuropathic pain models. However, the physiological significance of spinal GABAergic transmission in nociceptive modulation remains to be determined. We examined how spinal GABAergic neurons are excited by naturalistic sensory stimulation or optogenetic stimulation of the descending noxious inhibitory system. In vivo whole-cell patch-clamp recordings were made from superficial dorsal horn neurons. Under voltage clamp conditions, superficial dorsal horn neurons exhibited spontaneous inhibitory postsynaptic currents (IPSCs). Cutaneous innocuous touch stimulation elicited a barrage of IPSCs and inhibited action potentials elicited by noxious stimulation. The receptive field for touch-evoked IPSCs was larger than that for noxious stimulation-evoked excitatory responses. Immunohistochemical analysis demonstrated that small-sized afferent fibers made a direct synaptic contact with spinal GABAergic neurons. A selective activation of locus coeruleus neurons in the brain stem with optogenetic approaches also activated spinal GABAergic neurons. The descending GABA activation was mediated through $\alpha 1$ receptors. These results suggest that tactile cutaneous stimulation and pontospinal noradrenergic activation increase inhibitory GABAergic synaptic responses in the superficial spinal dorsal horn to reduce noxious transmission.

Symposium 20
Compartmentalization in Smooth Muscle Organs

(March 27, 15 : 20–17 : 20, Room D)

1S20D-1

Role of distinct layers in the bladder wall in regulating bladder function

Hashitani, Hikaru (*Dept. of Cell Physiology, Nagoya City Univ. Grad. School of Med. Sci., Nagoya, Japan*)

The bladder spends most of its time storing urine at low intravesical pressure, while only transiently contracting during voiding. The two opposed functions are achieved by the fine-gained integration of various cell populations within the bladder wall. Thus, signal transmissions within and amongst different layers are becoming relevant in terms of physiology and disease of the bladder. The urothelium which previously had been thought of as a passive barrier, is now recognized as paracrine cells releasing several substances, including ATP that plays a critical role in sensing bladder fullness. The suburothelial layer consists of heterogeneous cell populations including interstitial cells that may act as an intermediary of complex signal transmissions amongst urothelium, nerves and detrusor smooth muscle. Besides these anatomical characteristics, there is an extensive network of microvasculature with spontaneous venular constrictions that appear to be driven by pericytes activity as a functional feature in the microcirculation. The arrangement of detrusor smooth muscle layer is not very definitive, but the inner layer muscle appears to be more spontaneously active than the outer layer, and may have a close interaction with suburothelial layer. On the other hand, outer detrusor muscle contraction is largely reliant on efferent nerve activity. Fibroblasts that have been considered to be slender cells extending thin bipolar processes may have a sheet-like morphology, and thus could act as partitions to compartmentalize the distinct layers in the bladder wall.

1S20D-2

Cellular Interactions Controlling Tonic and Peristaltic Contractions of Gastric Smooth Muscle

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Gastric function involves both tonic nerve-mediated contraction of the gastric fundus and nerve-modulated slow waves that underlie gastric peristalsis of the gastric corpus and antrum. Cells termed interstitial cells of Cajal (ICCs) are fundamental to this process. There are two dominant types of gastric ICCs, these being ICCs present intramuscularly (ICC-IM) and ICCs present in the myenteric plexus (ICC-MY). ICC-IM strongly influence muscle contraction through being directly innervated and also contribute to generation of slow waves. ICC-MY which are dominantly present in the gastric antrum have long been considered to be the pacemaker cells, however this has been challenged given the finding that slow waves which first generate in the corpus may be initiated by ICC-IM, these being the dominant ICC cell type in the corpus. However, given that there are some ICC-MY in the gastric corpus, it remains possible that these are focally present at the pacemaker initiation site and help initiate slow waves. Slow wave propagation is also fundamental for gastric function in generating the peristaltic rings of constriction down the stomach. This requires considerable fine-tuning given the stomach is highly asymmetric. Interestingly, the asymmetric distribution ICC-MY could underpin this, subserving to proportionally enhance slow wave propagation rates down the greater curvature of the stomach.

1S20D-3

Spread of electrical and calcium signals within vascular endothelium

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For the effective control of peripheral blood circulation against the blood pressure, not a limited portion of but a certain length of blood vessel should be synchronously regulated. A signal conducting mechanism along the blood vessel is thus required. The vascular endothelium seems to be a good conduction pathway of electrical signals because the endothelial cells are connected to each other with a lot of gap junctions. In the present study, a sheet of endothelium acutely prepared from the guinea-pig mesenteric artery was employed. The membrane potentials of two different cells were measured using two patch electrodes and the intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$), was monitored using a Ca^{2+}-sensitive dye. Upon application of acetylcholine (ACh), the $[Ca^{2+}]_i$ increased and the membrane hyperpolarized. At the beginning of ACh-application the input resistance of the patched cell decreased reflecting the activation of K^+ channels. Then it increased above the control level while ACh was still present and slowly returned after the washout of ACh. This increment of the input resistance might be due to Ca^{2+}-inactivated channels. The junctional resistance between cells seemed to be slightly increased during ACh-application. In the Ca^{2+}-imaging, the $[Ca^{2+}]_i$ in individual cell was not constant but fluctuated and transient increase occasionally occurred. As such an increase in one cell was often accompanied by a simultaneous increase in the neighboring cells, Ca^{2+} seems to diffuse through the gap junctions rather quickly.

1S20D-4

Stromal cells play important roles in tissue compartmentalization : A FIB/SEM study

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By using a novel scanning electron microscope, FIB/SEM (Quanta 3D FEG, FEI), we investigated guinea-pig seminal vesicle wall, in which recently spontaneous intrinsic peristaltic movement was found, for cells related to the regulation of the movement. FIB/SEM is a scanning electron microscope (SEM) developed with a totally different concepts from conventional SEM, which enables high resolution observations like TEM of broad areas, as well as, to obtain stacks of serial images by repeating gallium-ion beam ablation and viewing material contrast images of flat surfaces. In the vertical sections, fragments of stromal cells were identified in submucosa and muscular layer. Tracing those fragments and cell bodies revealed in a single slice that the stromal cells located in submucosa separating epithelial layer and smooth muscular layer as two physically isolated compartments. In smooth muscular area, traced cellular processes tended to surround each muscular bundle, forming loose circle around each bundle. When those cellular fragments were traced in each serial image and reconstructed, the shape of those cells appeared to be cells with undulated thin sheet-like broad processes, but not stellate with finger-like long processes. Taken together, the sheet-like stromal cells form septa that compartmentalize tissue elements. Some other instances of other organs are to be demonstrated and the functional significance of the newly revealed structures will be discussed.

1S20D-5

Oxygenation-induced remodeling of postnatal rat ductus arteriosus with basic fibroblast growth factor signaling

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The ductus arteriosus (DA), a fetal arterial connection between the pulmonary artery and the aorta, normally closes several days after birth. In addition to contraction of DA smooth muscle, intimal thickening (IT) leads to eventual DA closure. Our previous studies have demonstrated that placental prostaglandin E induce IT during fetal period. However, it remains unknown how IT of the DA is enhanced after birth. We hypothesized that raising oxygen tension promotes IT and anatomical DA closure. We found that basic fibroblast growth factor (bFGF) was highly expressed in the part of IT in human and rat neonatal DA. Oxygenation increased production of bFGF {1.6-fold (1h), n=4, P<0.001}, H2O2 {1.8-fold (10 min), n=6, P<0.01} and phosphorylation of ERK1/2 {2.2-fold (15 min), n=5, P<0.001} in rat DA SMCs, but not in aortic SMCs. Oxygenation and recombinant bFGF promoted DA SMC migration (1.8-fold, n=6, P<0.001). In vivo study, intraperitoneal administration of anti-bFGF antibody, ROS inhibitor or ERK1/2 inhibitor attenuated postnatal IT in full-term rat DA (0.8-fold, 0.8-fold, or 0.8-fold, respectively, n=4-10, P<0.01). Furthermore, administration of bFGF promoted IT in preterm rat DA (1.4-fold n=4, P<0.01). These results suggest that raising oxygen tension and subsequent induction of ROS-mediated bFGF production via ERK1/2, lead to postnatal anatomical closure of the DA.

Symposium 21
Rehabilitation and motor functional recovery

(March 27, 15 : 20–17 : 20, Room E)

1S21E-1

Neural plasticity underlying the training-induced recovery of gripping after primary motor cortex lesion in macaque monkeys

Murata, Yumi (*Hum. Tech. Res. Inst., AIST, Tsukuba, Japan*)

We previously reported that motor training after primary motor cortex (M1) lesion promotes recovery of precision grip in macaque monkeys. We also showed that the regional cerebral blood flow during the precision grip task, as observed by $H_2{}^{15}O$-positron emission tomography (PET), was increased in the ipsilesional ventral premotor area (PMv) during the functional recovery. In the present study, we evaluated the contribution of the ipsilesional PMv to functional compensation using a pharmacological inactivation experiment by microinjections of muscimol, a $GABA_A$ receptor agonist. After ibotenic acid lesion of the M1 digit area, monkeys underwent a post-lesion training using the precision grip task. Muscimol injection into the ipsilesional PMv after recovery of precision grip impaired the recovered precision grip in affected hand, while the muscimol-induced inactivation of the same region had a small effect before lesion. This result suggests that the recovery of precision grip depends on increased activity of the ipsilesional PMv. Moreover, we investigated the plastic changes of neurons during the functional recovery using histochemical analysis of a plasticity-related protein (GAP-43), which may mediate axonal growth and presynaptic plasticity. *In situ* hybridization histochemistry revealed the increased gene expression of GAP-43 in the ipsilesional PMv during the recovery phase after M1 lesion. The results of the present study may indicate that plastic changes in the ipsilesional PMv are involved in functional compensation of gripping after M1 lesion.

1S21E-2

Neurophysiological effects of robot-assisted stepping on excitabilities of spinal and supraspinal neural circuits

Nakazawa, Kimitaka (*Graduate School of Arts and Sciences, The University of Tokyo, Japan*)

It has been well established that reorganization of locomotor related neural circuitries can be induced with properly designed locomotor training. However, neural mechanisms underlying the reorganization are still not fully understood. To address this issue we have conducted a series of experiments, which aimed to reveal effects of both sensory inputs and descending commands on excitability modulation of neural circuits involving human bipedal locomotion. In this symposium a part of results we have so far obtained in the experiments focusing on the effect of sensory inputs will be shown. By using a robotic gait trainer, Lokomat we tested effects of sensory inputs evoked during robot-assisted stepping (RAS) on corticospinal (CS), stretch (H-) reflex and cutaneous reflex excitabilities. The results showed that CS excitability of tibialis anterior (TA) was facilitated phase-dependently during the RAS in partially loaded condition, while in 100% unloaded condition no facilitation was observed. The H-reflexes of soleus, TA and wrist flexor were all inhibited during the RAS in both unloaded and partially loaded conditions. The cutaneous reflex of TA was modulated phase-dependently only during partially loaded RAS. These results demonstrated that sensory inputs, especially body weight related somatosensory input have a facilitatory effect on both CS and cutaneous reflex pathways of TA, whereas those sensory inputs regardless of body weight related or not have an inhibitory effect on spinal stretch reflex circuits of upper and lower limb muscles during the RAS.

1S21E-3

Changes in human motor cortex excitability during motor imagery and its implications for rehabilitation

Liang, Nan (*Department of Integrative Physiology, Graduate School of Biomedical and Health Sciences, Hiroshima Univ. Hiroshima, Japan*)

Motor imagery (MI) is a dynamic cognitive process without overt movements. To date, MI has been utilized not only in the athletes but also in the patients with central nervous system disorders. Functional imaging techniques have revealed that specific motor-related areas in the brain may be engaged in MI and therefore contribute to the improvements of motor performance. Transcranial magnetic stimulation (TMS) has further demonstrated that the human primary motor cortex, which functions nearby the final common pathway, plays a crucial role in the generation of motor outflows involving MI. We have examined using TMS techniques the facilitatory and inhibitory effects of MI on the motor cortex as well as the modulation of the intracortical inhibition and facilitation. We also conducted experiments to explore the inter-hemisphere interaction in association with MI. Our results indicate that the facilitatory effects of MI on the motor cortex excitability are modulated owing to different motor strategies in the contributions of agonist and synergist muscles, and that a phenomenon of surround inhibition could also be observed during MI. Furthermore, it is suggested that the enhancement of the motor cortex excitability driven by MI of the contralateral limb is interfered with by isodirection and forceful movement of the ipsilateral limb, which may be due to an increase in the transcallosal inhibitory effects. The novel findings regarding the effects of MI on the motor cortex excitability and the clinical implications will be discussed.

1S21E-4

Brain plasticity and therapeutic exercises

Domen, Kazuhisa (*Department of Rehabilitation Medicine, Hyogo College of Medicine, Japan*)

Neuroscience research has revealed the phenomenon of use-dependent plasticity (UDP) of the brain. Constraint-induced movement therapy (CIMT) is one of the most successful examples of the clinical applications of UDP. CIMT is an evidence-based neurorehabilitative approach designed to improve upper limb (UL) function in hemiplegic patients. CIMT involves restraining the unaffected UL with intensive training of the affected UL for 6 h a day for 10 days with tasks of increasing difficulty called shaping. The chief principles of CIMT are restraint of the unaffected UL, intensive training of the affected UL, and shaping. CIMT facilitates use of the affected limb, contributing to a cycle of increased motivation to use the affected UL. These processes lead to the reversal of learned nonuse phenomenon through UDP. Recently, a behavioral technique called transfer package has been designed to transfer gains obtained in a laboratory into the real-life setting. The mechanism of CIMT can be explained using the computational neuroscience-based motor learning principles, supervised learning, reinforcement learning, and unsupervised learning. We also speculate that the transfer package may have a role in meta-learning. We are now investigating whether the outcome of CIMT predicted by corticospinal tract integrity can be assessed by diffusion tensor imaging. A preliminary study demonstrates that fractional anisotropy ratio of the posterior limb of internal capsule can be used to predict the outcome of CIMT. The mechanism of CIMT can be applied to other therapeutic exercises in rehabilitation. The application of UDP will be discussed in this symposium.

Symposium 22
Long-term and multi-functional imaging reveals novel functions of the biological clock

(March 27, 15 : 20–17 : 20, Room F)

1S22F-1

Revolutionary bioimaging with super-duper luminescent proteins

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Fluorescent protein technology revolutionized our understanding of biological processes. However the requirement for external illumination definitely precludes its universal application to all biological processes in all tissues. Although chemiluminescence does not have this problem, light emissions from conventional probes are too weak to realize the potential of this imaging modality to work where fluorescence cannot. In the symposium, we will introduce development of an extremely bright luminescent protein, which is a chimeric protein of enhanced *Renilla* luciferase and fluorescent protein with a high BRET efficiency. It enables not only real-time imaging of intracellular structures in living cells with spatial resolution equivalent to fluorescence but also sensitive tumor detection in freely moving unshaved mouse. Functional indicators based on this chimeric protein can image Ca^{2+}, cAMP, or ATP dynamics in environments where fluorescent indicators have failed. These super-duper luminescent proteins allow visualization of biological phenomena not seen before at the single-cell, organ, and whole-body level, in animals and plants.

1S22F-2

Large-Scale Circadian Calcium Imaging in Neuronal Network of the Suprachiasmatic Nucleus

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The circadian pacemaker in the hypothalamic suprachiasmatic nucleus (SCN) is a hierarchical multi-oscillator system in which neuronal networks play crucial roles in expressing coherent circadian rhythms. Using a new large-scale calcium imaging method with genetically-encoded calcium sensors and Nipkow spinning disk confocal imaging system, we visualized intracellular calcium from the entire surface of SCN slice in culture including the regions where autonomous clock gene expression was undetectable. We found circadian calcium rhythms at a single-cell level in the SCN, which were topologically specific with a larger amplitude and more delayed phase in the ventral region than the dorsal. The robustness of the rhythm was reduced but persisted even after blocking the neuronal firing with tetrodotoxin (TTX). Notably, TTX dissociated the circadian calcium rhythms between the dorsal and ventral SCN. These results reveal the topological specificity of the circadian calcium rhythm in the SCN and the presence of coupled regional pacemakers in the dorsal and ventral regions. Neuronal firings are not necessary for the persistence of the calcium rhythms but indispensable for the hierarchical organization of rhythmicity in the SCN.

1S22F-3

ES cell-based in vitro evaluation system of circadian clock phenotypes in mammals

Yagita, Kazuhiro (*Neuroscience and Cell Biology, Kyoto Pref. Univ. Med., Kyoto, Japan*)

The molecular oscillations underlying the generation of circadian rhythmicity in mammals develop gradually during ontogenesis. Recently, using mouse embryonic stem (ES) cells and their in vitro differentiation method, we have demonstrated that cell-autonomous system developed circadian molecular clocks in mammals. Here, we established the genetic screening system evaluating circadian phenotype using the bi-allelic mutant ES cell bank. First, we analyzed the rhythms of differentiated cells from Casein Kinase I delta (CKIδ) mutant ES cell line which did not express CKIδ gene. Strikingly, their differentiated cells showed ～3 hours longer period-length than wild type cells, which was compatible with recently published data using CKIδ deficient mice tissues. Moreover, revertant allele re-gaining CKIδ expression recovered their circadian period-length to similar level of wild type allele. These results supported an idea that ES cell-based circadian clock formation assay should be available for the genetic screening to evaluate the circadian phenotypes before generating knock-out mice. Thus, we have started the screening by in vitro circadian clock formation assay using the mutant ES cell bank. Moreover, we could detect the 2～3-hour lengthening of the circadian clock in cells differentiated from Casein Kinase 2 alpha subunit (CK2α) deficient ES cell. Getting together, we propose the ES cell-based in vitro evaluation system of mammalian circadian phenotypes.

Symposium 23
New functions and regulatory mechanisms of a voltage sensor domain

(March 27, 15 : 20–17 : 20, Room G)

1S22F-4

Internal representation of external environment : light–response program in the mammalian circadian clocks

Ueda, Hiroki R. (*RIKEN, Kobe, Japan*)

Mammalian clock systems can be sensitively and stably entrained by environmental cycles on the rotating Earth, in spite of the diverse light conditions that vary according to climate, latitude of location and season of the year. To explain this entrainment capacity, parametric and non-parametric mechanisms have been hypothesized to sense the intensity and transition of light signals, respectively. However, it has not been fully explored what kind of information in light signals is actually extracted and represented in mammalian central clocks. To address this issue, we also performed a whole-genome transcriptional profiling of circadian and light-response programs in mammalian central clocks and found that light-induced and light-repressed genes tend to be rhythmically expressed during the day and during the night, respectively. Principle component analysis of light-induced genes further revealed both early-type and late-type light-induced genes, which can encode the transition and intensity of light signals, respectively. These results, thus, provide us with a comprehensive database of molecular probes for various light responses including parametric and non-parametric response mechanisms in mammalian circadian clocks.

Reference *Nature* 418 : 534-9 (2002), *PNAS* 101 : 11227-32 (2004), *Nature Genetics* 37 : 187-92 (2005), *Nature Genetics* 38 : 312-9 (2006), *Nat Cell Biol.* 9 : 1327-34 (2007) , *Nature* 452 : 317-22 (2008), *PNAS* 105 : 14946-51 (2008), *Nat Cell Biol.* 10 : 1154-63 (2008), *PNAS* 106 : 9890-5 (2009), *PNAS* 106 : 15744-9 (2009), *Curr Biol.* 20 : 2199-206 (2010), *Cell* 144 : 268-81 (2011), *PNAS* 109 : 15036-41 (2012), *Cell Reports* (2012).

1S23G-1

Gating modulation of KCNQ channels via the voltage–sensing domains

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KCNQ1 is a *Shaker*-type voltage-gated potassium channel α subunit which is widely expressed in various human tissues. Four KCNQ1 subunits can form a single K^+ channel, containing a single pore (S5-S6 segments) and four peripheral voltage sensor domains (VSD ; S1-S4 segments). Tetrameric KCNQ1 channel can have up to four KCNE proteins, which are the single-transmembrane auxiliary subunits for KCNQ1 channel. Each KCNE protein drastically changes the gating properties of KCNQ1 channel in a quite different way. KCNE1, for example, makes the activation kinetics of KCNQ1 current two-orders of magnitude slower compared to KCNQ1 current without KCNE1. KCNE3, on the other hand, makes KCNQ1 channels constitutively open. We previously identified that the movement of VSDs of KCNQ1 channel was largely affected by the presence of KCNE proteins : KCNE1 stabilizes the VSDs in the down state while KCNE3 stabilizes them in the up state. There have been growing evidences showing that KCNE1 can interact with the VSD of KCNQ1 channel and affects the VSD movement. However, how KCNE3 controls KCNQ1 gating remains largely unknown. By using a KCNQ1 ortholog from *Ciona intestinalis* Ci-KCNQ1, which is not modulated by any KCNE proteins, we successfully identified that a pair of phenylalanine resides (Phe127 and Phe130) on the S1 segment were required for the KCNE3 modulation. Interestingly, they were not important for the KCNE1 modulation. Different interaction manners may be the reason why KCNE1 and KCNE 3 stabilize the VSD in the opposite states.

1S23G-2

Exploring the gating mechanism of the Hv1 proton channel with intracellular blockers

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The voltage-gated proton channel Hv1 is known to play important roles in proton extrusion, pH homeostasis, and production of reactive oxygen species in a variety of cell types. It has been recently implicated in cancer development and neuronal death during ischemic stroke. The channel lacks the pore domain typical of Nav, Kv, and Cav channels, and it is made of two voltage sensing domains (VSDs) each containing a gated proton permeation pathway. We have identified guanidine derivatives that inhibit the Hv1 VSDs with a mechanism similar to pore block in other voltage-gated channels. We find that each VSD in the Hv1 channel has its own binding site facing the inner side of the membrane and accessible only in the open state. As long as the inhibitor is bound, the gate in the VSD cannot close. We also find that inhibitor unbinding from one VSD is controlled by the neighboring VSD via tight allosteric coupling between gates. Understanding how compounds like guanidine derivatives interact with Hv1 and block proton conduction is an important step toward the development of pharmacological treatments for diseases caused by Hv1 hyperactivity. To this end, we are exploring the molecular determinants of the channel-blocker interaction to produce Hv1 inhibitors with improved affinity and specificity. This work is supported by NIH (grant GM 098973) and by the American Heart Association (grant 09BGIA 2160044).

1S23G-3

Regulatory roles of the dimeric structure in the voltage −gated H^+ channel

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The voltage-gated H^+ channel (Hv) is a voltage-sensor like four transmembrane (S1-S4) protein, and functions in a dimer form. Each channel protomer has its own permeation pathway. The functional role of dimer assembly, hence, is distinct from formation of the permeation pathway. One characteristic derived from the dimer assembly is the cooperative channel gating, i.e.- the gating movement of one channel subunit affects the gating of the other subunit within the dimeric unit. Here we report that the C-terminus ends downstream of the S4 voltage sensor helix form a dimer coiled-coil architecture which underpins the dimeric assembly. Thermodynamic analysis showed that the stability of the coiled-coil domain protein regulated the channel gating. Systematic mutation of the linker region between S4 and the coiled-coil uncovered that the two regions were linked helix-wise. Trimeric and tetrameric Hv channels were able to be engineered by mutating the coiled-coil domain, and showed broken cooperativities in the gating ; suggesting that the orientation of the transmembrane domains also carries weight. Cross-linking analysis toward the S1-S4 region revealed that two S4 helices were situated closely in the dimeric channel. Thus, the rigid structure of uninterrupted helices, which projects from the transmembrane and are twisted in the cytoplasmic region, regulates the gating properties of the Hv channel dimer.

1S23G-4

Exploring the proton permeation pathway by using homology modeling and molecular dynamics simulation of the Hv1 proton channel

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The voltage-gated proton channel (Hv1) has a proton-conducting transmembrane domain, which is homologous to the voltage-sensing domains (VSDs) of various ion channels. Since its crystal structure is not solved, the atomistic details of proton permeation through Hv1 have to be explored based on homology modeling of Hv1 structure and on molecular dynamics simulation with the model. Here we modeled the structure of Hv1 using the VSD of sodium channel NavAb (PDB 4 kew) as a template. NavAb was preferred because it has a higher sequence identity to Hv1 than the VSDs of potassium channels, such as Kv1.2, which were employed in preceding analyses. The model structure was then embedded in POPE membrane and fully solvated, and molecular dynamics simulation was performed for more than 400 ns. As a result, Hv1 included two clefts filled with solvent, one on the extracellular and the other on the intracellular sides. The water molecules in the clefts met at a constriction point, which lay between the side chains of Asp112 and Arg211 and a few water molecules intervening them were connecting the two aqueous clefts on both sides. We observed hydrogen bond networks in the Hv1 channel, which was compatible with the Grotthus mechanism proton permeation. The stable interactions of water with the polar side chains of Hv1 were also described.

Symposium 24
Mechanism of respiratory rhythm generation: from the forefront of research

(March 27, 15 : 20–17 : 20, Room H)

1S24H-1

Respiratory rhythm is driven by astrocytes in the pre-Botzinger complex

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In the beginning of my talk, I introduce the anatomical localization of the circuitries involved in respiratory rhythm generation and the previously proposed theories of the rhythmogenic mechanism. In the whole animal, the rhythm is generated in a large network that consists of mutually connected local circuitries, i.e., the pontine and parafacial respiratory groups and preBotzinger complex (preBotC) as well as the high cervical spinal cord respiratory group. Among those, the preBotC is considered the most important site for rhythmogenesis. Several theories have been proposed as the rhythmogenic mechanism, including the network, pacemaker neuron, and group pacemaker theories. However, none of these theories could perfectly explain all the experimental findings. Although all of these theories have assumed that the rhythmogenic mechanism consists of only neurons, glial physiology has been revealing that glial cells actively control the neuron network function in the brain. Therefore, we tested whether respiratory rhythm is generated by glial cells in the rhythmically active slice. Calcium imaging of preBotC cells revealed that a subset of astrocytes exhibit rhythmic calcium elevations preceding the inspiratory neuronal activity by 0.5-2 sec. These preinspiratory astrocytes maintained their rhythmic activities during blockade of neuronal activity. Optogenetic stimulation of preBotC astrocytes induced a burst firing of inspiratory neurons. These findings, together with the previous observation that blockade of astrocytic metabolism abolishes inspiratory neural output, indicate that astrocytes trigger inspiratory activity.

1S24H-2

Multiple modes of respiratory rhythm and pattern generation in the brainstem

Koizumi, Hidehiko (*NINDS, National Institutes of Health(NIH), Bethesda, USA*)

Rhythmic respiratory activity originates within excitatory and inhibitory circuits in the bilateral ventral respiratory column of the brainstem. The pre-Botzinger complex (pBC) is a distinct subregion with circuits functionally specialized for inspiratory rhythm generation. The pBC can operate in multiple modes of rhythmic pattern generation under different physiological and pathophysiological conditions. To unravel various mode of network operation, we defined the functional synaptic interactions of different types of respiratory neurons. We reconstructed temporal patterns of excitatory and inhibitory synaptic conductances of inspiratory and expiratory neurons in in situ perfused rat brainstem-spinal cord preparations, where the pBC interacts with numerous other respiratory neuron populations, and in in vitro slices from neonatal rats that isolate the pBC circuits. The different profiles of excitatory and inhibitory synaptic inputs found under in situ and in vitro conditions reflect the functionally interacting neuron populations in the different modes of rhythmic pattern generation. We also comparatively analyzed electrophysiological and morphological properties of excitatory and inhibitory respiratory neurons, and revealed structural-functional features that distinguish excitatory and inhibitory subpopulations and explain the different modes of respiratory rhythmogenic function. Our results collectively constitute the concepts of a compartmental organization, a bilaterally coupled excitatory rhythmogenic kernel, and an inspiratory-expiratory pattern generation function of pBC microcircuits.

1S24H-3

New mechanisms of modulation of respiratory rhythm : Effects of TRP channel related substances

Onimaru, Hiroshi (*Dept of Physiol, Showa Univ. School of Med., Tokyo, Japan*)

It is not well understood whether chemicals that are known as transient receptor potential (TRP) channel agonists or antagonists exert any effects on the medullary respiratory center. Recently, we have examined effects of transient receptor potential (TRP) channel-related substances on respiratory rhythm generation in the brainstem-spinal cord preparation from newborn rats. These substances are divided into roughly two groups ; inducing excitatory effects or inhibitory effects, whereas some of them induced transiently biphasic effects. Capsaicin (TRPV1 agonist) and cinnamaldehyde (TRPA1 agonist) induced biphasic effects ; initial inhibition and subsequent excitation. Menthol (TRPM8 agonist), carvacrol and eugenol (TRPV3?) induced strong inhibitory effects. One of characteristics of capsaicin effects is an induction of desensitization. Notably, cinnamaldehyde (as well as allyl isothiocyanate) induced long-lasting facilitation of respiratory rhythm for more than 2 hrs after washed out. In contrast, carvacrol and eugenol induced strong inhibition of respiratory rhythm followed by extremely shortening of inspiratory burst duration (i.e. inspiratory phase composed of a single spike activity in inspiratory neurons) and this continued for more than 1 hr after washed out. These results suggest that eugenol or carvacrol inhibited cellular (and/or network) mechanisms that are essential for maintenance of burst duration of respiratory neurons. Effects of these compounds may illuminate new aspects of cellular mechanisms of respiratory rhythm generation, although the detailed mechanisms are unknown yet.

1S24H-4

In silico reconstruction of the respiratory neuronal network

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The preBötzinger complex (preBötC) is essential for respiratory rhythm generation, and in vitro transverse slice preparations containing preBötC generate rhythmic activity. The preBötC is composed of both excitatory and inhibitory neuronal populations, and within each population, a subpopulation with bursting property exists. Conventional models of rhythm generation within preBötC require only excitatory neurons : Functional roles of inhibitory neurons have not yet been understood. The frequency of rhythmic activity increases and seizure-like activity is produced when $GABA_A$ or glycine receptor antagonist is bath-applied to rhythmic slice preparations. Randomly connected network models consisting of excitatory and inhibitory neurons cannot reproduce this experimental result. Here we show that specific topology of network connectivity is required to reproduce the phenomena, where inhibitory bursters inhibit ectopic neuronal bursts. Most of previous studies assumed a fixed fraction of all-to-all connectivity, randomly connecting the constituent neurons of the neuronal network. This assumption leads to homogeneity in excitatory and inhibitory postsynaptic currents among constituent neurons, which is unlikely for real networks. In the present study, we reconfigured the synaptic connectivity, which was initially allocated randomly, using mutational algorithms so as to result in improved synchronicity among the neurons. Eventual network topology reproduced some of the features obtained experimentally. Supported by JST strategic Japanese-German cooperative program in computational neuroscience.

1S25I-1

Neuroendocrine Control of Feeding Behavior and Psychomotor Activity by Orexin in Fish

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Orexin is a neuropeptide distributed widely among vertebrates. In mammals, orexin and its receptor system are involved in the regulation of food intake, locomotion and psychomotor activities including the sleep/wakefulness cycle. With regard to non-mammalian vertebrates, there has also been intensive study aimed at the identification and functional characterization of orexin and its receptor, and recent investigations of the role of orexin have revealed that it exerts behavioral effects in teleost fish. Goldfish and zebrafish are excellent teleost fish models, and in these species it has been demonstrated that orexin increases food consumption as an orexigenic factor and enhances locomotor activity, as well as being involved in the regulation of active and rest status (circadian rhythmicity and the sleep/wakefulness cycle), as is the case in mammals. This presentation shows current knowledge of orexin derived from studies of teleost fish, as representative non-mammals, focusing particularly on the role of the orexin system, and examines its significance from a comparative viewpoint.

1S25I-2

Orexin System is Involved in Ultradian Episodic Increase of Heart rate and Locomotor Activity

Miyata, Kohei; Ootsuka, Youichirou; Kuwaki, Tomoyuki (*Dept. Physiol. Grad. Sch. Med. Dent. Sci. Kagoshima Univ. Kagoshima, Kagoshima, Japan*)

We reported that body temperature, heart rate and brown adipose tissue temperature in unrestrained conscious rats episodically increase approximately every 90 min. This oscillation is called *Ultradian Rhythm*. Physiological parameter changes occur synchronously and are preceded by increasing of hippocampal theta wave. These two points suggest that ultradian rhythm is coordinated by central nervous system. Hippocampus theta wave correlates with arousal level. We assume that central arousal system contribute to generate ultradian rhythm. Orexin system is known as sleep-arousal control system. Therefore we hypothesized that orexin system is involved in generating ultradian rhythm. To investigate this hypothesis, we examined ultradian rhythms in two different orexin-deficient mice (orexin knockout (KO) mice (n=8), orexin/ataxin-3 transgenic (Tg) mice (n=14)) and control wild-type (WT) mice (C57BL/6, n=8). Orexin neurons are ablated in Tg mice. We measured core body temperature, heart rate and locomotor activity, in conscious freely moving mice for 24h (dark-light 12-h alternation). Ambient temperature was kept constant during recording. We compared WT and orexin deficient mice ultradian rhythm. Similar ultradian rhythm of heart rate was observed in all three genetic types of mice, while the amplitude of ultradian rhythm in body temperature and locomotor activity was attenuated in orexin deficient mice ($p<0.05$).

Symposium 25
Control of autonomic function and instinctive behavior: a role of orexin

(March 27, 15 : 20–17 : 20, Room I)

1S25I-3

Cerebellar microcomplex in the folium p controls orexin-modulated cardiovascular defense reactions

Nisimaru, Naoko[1,2]; Ito, Masao[1] ([1]*RIKEN, BSI, Wako, Japan;* [2]*Dept. of Physiol., Faculty of Med., Oita Univ., Oita, Japan*)

We investigated a small discrete area of the cerebellum, that is, folium-p of the flocculus (fp). In fp, Purkinje cells were excited by stimulation of the classic defense areas in the hypothalamus and mesencephalic periaqueductal grey. This stimulation elicited a transient increase of the arterial blood pressure, as a sign of cardiovascular defence reactions. We showed immunocytochemically and pharmacologically that orexinergic axons mediate the excitation of fp Purkinje cells. fp Purkinje cells project their axons to the lateral edge of the ipsilateral parabrachial nucleus, and appear to control cardiovascular defense reactions. We found also that climbing fiber signals to folium-p Purkinje cells were elicited by high arterial blood pressure or a high potassium concentration in muscles, implying errors in control of blood circulation. Finally, we evoked actual defense reactions by applying electric foot shock stimuli to a rabbit freely moving in a cage. Measurements were conducted for femoral and celiac arterial blood flow and blood pressure. Foot shock stimuli induced an increase in arterial blood flow in contracting muscles and a reciprocal decrease in visceral organs. Both the increase and decrease were attenuated by systemic administration of orexin antagonists. Their mutual balance was impaired by folium-p in flocculus lesioning (after kainite treatment). We conclude that folium-p is a unique microcomplex that adaptively controls cardiovascular defense reactions under orexin-mediated neuromodulation.

1S25I-4

Hypothalamic Orexin Stimulates Feeding-Associated Glucose Utilization in Skeletal Muscle via Sympathetic Nervous System

Minokoshi, Yasuhiko[1,2]; Shiuchi, Tetsuya[3]; Okamoto, Shiki[1,2] ([1]*Div Endocrinol Metab, NIPS, Okazaki, Japan;* [2]*Dep Physiol Sci, Graduate Univ Adv Studies(Sokendai), Okazaki, Japan;* [3]*Dep of Integ Physiol, Inst of Health Biosci, Univ of Tokushima, Tokushima, Japan*)

Hypothalamic neurons containing orexin (hypocretin) are activated during motivated behaviors and active waking. We show that injection of orexin-A into the ventromedial hypothalamus (VMH) of mice or rats increased glucose uptake in skeletal muscle and promoted insulin sensitivity in the tissue, but not in white adipose tissue (WAT), by activating the sympathetic nervous system. These effects of orexin were blunted in mice lacking β adrenergic receptors but were restored by forced expression of the β_2-adrenergic receptor in both myocytes and nonmyocyte cells of skeletal muscle. Orexin neurons are activated by conditioned sweet tasting and directly excite VMH neurons, thereby enhancing insulin-induced glucose uptake and glycogen synthesis in muscle, but not WAT, via sympathetic nerve and β_2-adrenergic pathway. Suppression of orexin signaling in the VMH impaired glucose metabolism during oral glucose ingestion. Orexin and its receptor in VMH thus play a key role in the regulation of muscle glucose metabolism associated with highly motivated behavior by activating muscle sympathetic nerves and β_2-adrenergic signaling.

1S25I-5

Hypothalamic Sirt1 overexpression leads to a negative energy balance

Sasaki, Tsutomu; Shimpuku, Mayumi; Kikuchi, Osamu; Susanti, Vina Yanti; Yokota-Hashimoto, Hiromi; Kobayashi, Masaki; Kitamura, Tadahiro (*Institute for Molecular and Cellular Regulation, Gunma Univ., Maebashi, Japan*)

Anorexigenic proopiomelanocortin (POMC) -positive neurons and orexigenic agouti-related peptide (AgRP) -positive neurons, located in the arcuate nucleus of the hypothalamus (ARC), play key roles in the hypothalamic control of the whole body energy balance. The NAD^+-dependent deacetylase Sirt1 is implicated in energy metabolism regulation, and its expression decreases with age in ARC. The results of murine Sirt1 loss-of-function studies in POMC and in AgRP neurons have been inconsistent, and the roles of hypothalamic Sirt1 in regulating the whole body energy balance remain controversial. Here we show that conditional overexpression of Sirt1 in POMC or AgRP neurons in mice leads to a negative energy balance. Sirt1 overexpression in POMC neurons stimulated energy expenditure and lipolysis via improved leptin sensitivity and increased sympathetic activity to adipose tissues whereas overexpression in AgRP neurons suppressed food intake ; these effects resulted in a lean phenotype. Notably, the suppression of age-dependent weight gain by Sirt1 overexpression in these neurons was countered by a high-fat, high-sucrose diet. Our results indicate that in the central melanocortin neurons, Sirt1 helps achieve a negative energy balance by modulating leptin sensitivity and regulating both food intake and energy expenditure. These functions can be suppressed by diet-induced obesity.

1S25I-6

Orexin receptor 1 in the Locus coeruleus plays an important role in establishing fear memory

Soya, Shingo; Hasegawa, Emi; Sakurai, Takeshi (*Department of Molecular Neuroscience and Integrative Physiology, Kanazawa Univ. Ishikawa, Japan*)

The noradrenergic projection arising from the locus coeruleus (LC) to central nucleus of amygdala and bed nucleus of the stria terminalis has been implicated in the formation of emotional memory. Since noradrenergic neurons in the LC abundantly express one of orexin receptors, OX1R, and orexin neurons densely project to them, we hypothesized that OX1R-mediated pathway is involved in physiological fear learning. To address this, we used $Ox1r^{-/-}$ mice to examine a classical cued and contextual fear-conditioning test. We found that $Ox1r^{-/-}$ mice showed impaired freezing responses in both cued and contextual fear conditioning paradigms. Double immunolabeling of c-fos and tyrosin hydroxyrase (TH) showed that activation of the NA neurons in the LC was lower in $Ox1r^{-/-}$ mice after test session against both cue and contextual stimuli. When OX1R expression was restored in the NA neurons in the LC, the freezing behavior to the auditory cued was resqued to the levels comparable to wild type mice. These observations support the hypothesis that orexin system modulates the retrieval of cue-dependent fear memory via NA neurons in the LC. This study shows that lateral hypothalamus plays a crucial role in the output of auditory fear memory through orexinergic system. This is the first report of abnormality found in the $Ox1r^{-/-}$ mice.

Symposium 26
Molecular and neural mechanisms of chemoreception–induced behavior responses

(March 27, 15 : 20–17 : 20, Room J)

1S26J-1
Endogenous humoral modulators of behavioral preference for sweet and salty tastes

Ninomiya, Yuzo; Niki, Mayu; Yoshida, Ryusuke; Shigemura, Noriatsu
(*Sect. Oral Neurosci., Gard. Sch. Dent. Sci., Kyushu Univ. Fukuoka, Japan*)

Gustatory system plays the important role in maintaining homeostasis in animals. If animals lack essential nutrients for their survival such as sugars, minerals and essential amino acids, they may be able to find out these insufficient nutrients by using taste cues. Such hunger preference behavior for particular taste cues has been reported to link with the action of humoral factors via activation of their cognate receptors in the central nervous system, especially in the hypothalamus. For example, preference for sweet or salt taste is associated with orexegenic and anorexegenic mediators, such as leptin and endocannabinoids, or a major mediator of body fluid and sodium homeostasis, angiotensin II, respectively. Recently, we found that receptors for these humoral factors are also expressed in the taste bud, and these factors influence the preference behavior not only via the central nervous system, but the peripheral taste system as well. In this talk, I will summarize recent advances in our knowledge on modulation of humoral factors on ingestive behavior induced by taste cues.

1S26J-2
Neural circuit basis of olfactory behavior in zebrafish

Yoshihara, Yoshihiro(*RIKEN Brain Science Institute, Japan*)

Zebrafish has become one of the most useful model organisms in neurobiology. In addition to its general advantageous properties (external fertilization, rapid development, transparency of embryos, etc.), zebrafish is amenable to various genetic engineering technologies such as transgenesis, mutagenesis, gene knockdown / knockout, and transposon-mediated gene transfer. Our transgenic approach unraveled two segregated neural pathways originating from ciliated and microvillous sensory neurons in the olfactory epithelium to distinct regions of the olfactory bulb, which likely convey different types of olfactory information (e.g. pheromones and odorants). Furthermore, the two basic principles (one neuron-one receptor rule and axon convergence to target glomeruli) are essentially preserved also in zebrafish, rendering this organism a suitable model vertebrate for the olfactory research. In this talk, I will summarize recent advances in our knowledge on functional architecture of the zebrafish olfactory circuits mediating specific odor-induced behaviors. In particular, I will focus on molecular genetic dissection of the neural elements involved in the attraction to food odorants, the aversion from alarm pheromones, and the social response to sex pheromones.

1S26J-3
Molecular mechanisms for sexual behaviors elicited by chemosensory signals

Touhara, Kazushige(*Department of Applied Biological Chemistry, The University of Tokyo, Japan*)

In terrestrial animals, a variety of social and sexual behaviors are regulated by chemosignals called pheromones that act via the olfactory or vomeronasal system. Pheromones could be volatile or non-volatile as long as they convey biological information to different individuals within the same species. We recently demonstrated that a male-specific peptide, exocrine gland-secreting peptide 1 (ESP1), which was released into tear fluids, was a non-volatile sex pheromone that enhanced female sexual receptive behavior. We then revealed the molecular mechanisms and the neural pathway involved in decoding the ESP1 signal in the vomeronasal system. We recently identified a novel volatile compound, Z5-14 : OH, that was excreted in a sex-specific manner into male urine and acted as a physiological odorant receptor ligand in the main olfactory system. Z5-14 : OH appears to be one of fatty acid metabolites, and turned out to enhance the attractiveness of male urine to female mice, suggesting that it is a volatile pheromone that conveys a male signal. Mice utilize both volatile and non-volatile cues to recognize the opposite sex, and these signals regulate various sexual behaviors.

1S26J-4

Modulation of feeding behavior by nutritional value evaluation in Drosophila

Tanimura, Teiichi; Toshima, Naoko (*Division of Biological Sciences, Graduate School of Sciences, Kyushu University, Fukuoka, Japan*)

Gustation is an essential chemical sense for organisms to discriminate edible foods. Nevertheless gustatory information alone is not always enough for proper decision-making of feeding behavior, as animals need to modulate their feeding behavior depending on the nutritional requirment in the body. Recent studies revealed that *Drosophila* do not simply respond to taste stimulus, but have an ability to regulate the feeding behavior through a decision-making process. Intake of amino acids is necessary for egg production and longevity. We studied the feeding preference for amino acids and performed two-choice preference tests. Results indicated that flies prefer amino acids to a low concentration of glucose and significantly increase their preference for some amino acids when flies were placed in amino acid-deprived condition for several days. Amino acid-deprived flies ingest amino acids even when they were replete with glucose. Some of those amino acids induced proboscis extension reflex only in amino acid-deprived flies. We found that mutant *poxn* mutant flies with no external taste organs preferred amino acids over sugar in two-choice preference tests, suggesting that the external taste sensilla are not always necessary to detect amino acids. These data suggest that *Drosophila* have amino acid receptors in the gustatory receptor neurons on the proboscis and might have an internal amino acid sensor to regulate their feeding behavior depending on the internal amino acid level.

Symposium 27
Current issues on circulation; collaboration between basic and clinical scientists
[Joint Symposium between Physiological Society of Japan and Japanese Circulation Society]

(March 28, 9 : 00–11 : 00, Room A)

1S26J-5

CALHM1 ion channel mediates purinergic neurotransmission in the taste bud during sweet, bitter and umami perception

Taruno, Akiyuki[1,2]; Vingtdeux, Valerie[3]; Li, Ang[1]; Ma, Zhongming[1]; Ohmoto, Makoto[4]; Matsumoto, Ichiro[4]; Leung, Sze[5]; Abernethy, Maria[5]; Dvoryanchikov, Gennady[6]; Civan, Mortimer M.[1]; Chaudhari, Nirupa[6]; Hellekant, Goran[5]; Tordoff, Michael G.[4]; Marambaud, Philippe[3]; Foskett, J. Kevin[1] ([1]*Dept Physiol, Univ Pennsylvania, Philadelphia, USA;* [2]*Dept Mol Cell Physiol, Kyoto Pref Univ Med, Kyoto, Japan;* [3]*Feinstein Inst for Med Res, Manhasset, NY, USA;* [4]*Monell Chem Senses Ctr, Philadelphia, PA, USA;* [5]*Dept Physiol Pharmacol, Univ Minnesota Duluth, Duluth, MN, USA;* [6]*Dept Physiol Biophys, Miller Sch Med, Univ Miami, FL, USA*)

Recognition of sweet, bitter, and umami tastes requires the nonvesicular release from taste bud cells of ATP, which acts as a neurotransmitter to activate afferent neural pathways. However, how ATP is released is uncertain. Here we show that a recently identified ion channel, calcium homeostasis modulator 1 (CALHM1), is indispensable for taste-evoked ATP release from sweet/bitter/umami-sensing type II taste bud cells. *Calhm1* knockout mice have severely impaired perceptions of sweet, bitter and umami compounds, whereas sour and salty taste recognition remains mostly normal. *Calhm1* expression is confined to type II taste bud cells. Its heterologous expression induces a novel ATP permeability that releases ATP from cells in response to maneuvers that activate the CALHM1 ion channel. Knockout of *Calhm1* strongly reduces voltage-gated currents in type II cells and taste-evoked ATP release from taste buds without affecting the excitability of taste cells to taste stimuli. Thus, CALHM1 is an ATP release channel required for sweet, bitter, and umami taste perception.

2S27A-1

The role of the second messenger system in regulating cardiac function

Ishikawa, Yoshihiro (*Cardiovascular Research Institute Yokohama City University Graduate School of Medicine Yokohama 236–0022, Japan*)

Sympathetic nerve activity is a major mechanism of regulating cardiac function. Norepinephrine released from the synaptic terminal binds to the beta adrenergic receptor on cardiac membrane, leading to the activation of the stimulatory G protein and thus adenylyl cyclase. Adenylyl cyclase, a membrane-bound enzyme, catalyzes the conversion of ATP to cAMP, which then activates protein kinase A and Epac, the latter of which was identified more recently and is known to regulate the Rap pathway independent of protein kinase A. Although the major effector role of cAMP signal has been attributed to protein kinase A, an increasing body of evidence has suggested important contribution of Epac to regulating cardiac function. Classically, by the use of Gs overexpression mouse model, we have demonstrated the activation of cAMP signal enhances cardiac function in a short time scale, but deteriorates it, in a long time scale, by increasing cardiac apoptosis and thus fibrosis. There is no doubt that protein kinase A plays an important role in this process, however, through the study of various transgenic mouse models, such as Epac-overexpression or Epac-knockout model, we have identified the new role of this target molecule of cAMP. Epac not only plays a role in regulating cardiac hypertrophy, it also regulates the response of cardiac myocytes against various stresses. Epac also plays a role in the cross talk between cytokine and catecholamine signal. Accordingly, Epac may serve as an attractive target for drug development in the treatment of heart failure.

2S27A-2

The role of autophagy in heart disease

Sadoshima, Junichi (*University of Medicine and Dentistry of New Jersey, New Jersey Medical School Newark, New Jersey, USA*)

Autophagy is a bulk degradation process in which proteins/organelles are sequestered into double membrane vesicles termed autophagosomes and degraded at lysosomes. Autophagy is activated during nutrient starvation in order to restore the cellular level of ATP. Autophagy is also activated during oxidative stress in order to eliminate protein aggregates and damaged intracellular organelles. Although autophagy is generally protective in the heart, excessive autophagy may induce myocardial damage. We have shown previously that autophagy is protective during myocardial ischemia, whereas it rather promotes myocardial injury during reperfusion. Thus, in order for autophagy to protect the heart, it should be maintained in appropriate levels. We have shown recently that autophagy is suppressed below physiological levels in the heart in response to high fat diet feeding through inadvertent activation of mTOR, which in turn increases the susceptibility of the heart during myocardial ischemia. Autophagy is also suppressed below physiological levels through activation of Mst 1, a pro-apoptotic protein kinase, in the heart after myocardial infarction, which in turn enhances accumulation of aggresomes and damaged mitochondria and the development of heart failure. Molecular interventions to restore the level of autophagy normalize the susceptibility against ischemia and prevent cardiac remodeling. In this lecture, I will discuss the signaling mechanisms controlling the level of autophagy in the heart and its patho-physiological significance and therapeutic implications.

2S27A-3

Future treatment of the heart failure and pathophysiological analysis of various heart diseases using human iPS cell−derived cardiomyocytes

Fukuda, Keiichi (*Department of Cardiology, Keio University School of Medicine*)

Although heart transplantation can drastically improve the survival, shortage of the donor heart is a serious problem. The regenerative medicine of the failing heart had been long awaited. To address this question, we had developed novel methods to induce human iPS cells from circulating human T lymphocytes using Sendai virus containing Yamanaka 4 factors. We had screened the factor that were expressed in future heart forming area of the early mouse embryo, found several growth factors and cytokines that can induce cardiomyocytes differentiation and proliferation, and applied them to human iPS cells. We performed transcriptome of the metabolic enzymes and fluxome analysis using ^{13}glucose and ^{13}lactic acid on ES/iPS cells and cardiomyocytes, and found that their metabolic pathways were completely different. Based on these findings, we purified cardiomyocytes using glucose-free lactate-supplemented medium. Purity of the cardiomyocytes was>99%, and they did not make teratoma formation. The transplanted cardiomyocytes using our technique can survive in the heart with more than 90%, and can show physiological growth after transplantation. We expect the combination of these techniques can achieve future heart regeneration. We also developed human disease model cardiomyocytes using human iPS cells from the patients with long QT syndrome and other hereditary heart disease. These disease model cardiomyocytes represented the phenotype of the disease, and might be helpful for drug screening and pathophysiological analysis.

2S27A-4

Role of inflammation in cardiovascular remodeling : from bench to bed side

Isobe, Mitsuaki (*Department of Cardiovascular Medicine, Tokyo Medical and Dental University*)

Inflammation is critically involved in the pathophysiology of cardiovascular remodeling. Recent investigations have revealed crucial roles of T cell-mediated immunity and inflammation in the development of atherosclerosis, cardiac allograft vasculopathy, and restenosis after stent implantation. Intracellular signals through T cell receptor cause activation of NFκB. The focus of our investigation is to clarify the pathophysiological role of NFκB in the development of occlusive arterial lesions. We used mice models including cardiac allograft vasculopathy after heart transplantation and wire-injured femoral arteries. Coculture of smooth muscle cells (SMC) and activated T cells from mice with cardiac allograft rejection resulted in proliferation of SMCs. Treatment of cardiac allografts or femoral artery with NFκB decoy gene transfected by either HVJ-liposome method or ultrasound-microbubble method attenuated development of intimal hyperplasia after heart transplantation or wire injury. These data indicate that NFκB are critically involved in the development of a variety of vascular remodeling through activation of SMCs. Based on these in vivo and in vitro data we developed translational research. Patients with coronary artery disease were treated with locally-delivered NFκB decoy after stent implantation. Results of 18 patients showed safety of this gene therapy and favorable results on prevention of restenosis after stenting. Treatment targeting inflammation through this molecule is promising in the prevention of cardiac allograft vasculopathy and other vascular diseases.

Symposium 28
Structural biology: Physiology in the next era

(March 28, 9 : 00–11 : 00, Room D)

2S28D-1

Cryo-electron microscopic studies of eukaryotic flagella motors

Kikkawa, Masahide (*Department of Cell Biology and Anatomy, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan*)

Eukaryotic flagella are microtubule-based cellular organelles that play important roles in eukaryotic cells. The core of flagella is typically made of nine outer doublet microtubules and two central pair microtubules. In flagella, two biological motors play critical roles : kinesins are used for intraflagellar transport and dyneins generate beating motion of flagella. Here, we will present two of our recent results about controlling these two motors.

First, we elucidated the structural basis of the kinesin processivity. To this aim, we engineered a monomeric kinesin head that mimics the front head state. Single molecule fluorescent imaging showed that the mutated kinesin motor domain stably bound to the microtubule and its neck linker is in the undocked state. Cryo-electron microscopy was used to obtain the 3D structure of the mutant kinesin-microtubule complex at 7 Å resolution. It revealed that the structure of kinesin motor domain is similar to that in the nucleotide-free state, even the presence of ATP analog. These results showed that the counter-clockwise rotation of kinesin head requires both ATP and docking of the neck linker, suggesting that the rotation works as an "AND gate" to enable kinesin's processive movement.

Second, we demonstrated by cryo-electron microscopy and chemical crosslinking that intermediate chain 2 (IC2) of ODA interacts with the dynein regulatory complex in the axoneme and constitutes part of the outer-inner dynein (OID) linker. Furthermore, we identified IC2 as a functional hub between ODA and IDA based on the phenotypes of *Chlamydomonas* mutants expressing biotinylation-tagged IC2. The flagella of the IC2 mutant showed activated microtubule sliding and enhanced ATPase activities of ODA, as well as an altered waveform, indicating attenuated IDA activity. We concluded that the OID linker controls both ODA and IDA and regulates flagellar beating.

2S28D-2

Structures and Mechanisms of G-protein couple receptors

Iwata, So (*Graduate School of Medicine, Kyoto University*)

I will present following two topics in my talk. Histamine H_1 receptor : Histamine is an important pharmacological mediator involved in pathophysiological processes such as allergies and inflammations. Histamine H_1 receptor (H_1R) antagonists are very effective drugs alleviating the symptoms of allergic reactions. We have solved the crystal structure of the H_1R complex with doxepin, a first-generation H_1R antagonist. The doxepin pocket is associated with an anion-binding region occupied by a phosphate ion. Docking of various second-generation H_1R antagonists reveals that the unique carboxyl group present in this class of compounds interacts with Lys 191 and/or Lys 179, both of which form part of the anion-binding region. This region is not conserved in other aminergic receptors, demonstrating how minor differences in receptors lead to pronounced selectivity differences with small molecules. Adenosine A_{2A} receptor : The adenosine A_{2A} receptor ($A_{2A}AR$) is responsible for regulating blood flow to the cardiac muscle. We have successfully raised a mouse monoclonal antibody against human $A_{2A}AR$ that prevents agonist but not antagonist binding to the extracellular ligand-binding pocket, and solved the structure of $A_{2A}AR$ in complex with the antibody Fab fragment (Fab2838). This structure reveals that Fab2838 recognizes the intracellular surface of $A_{2A}AR$ and that its complementarity-determining region, CDR-H3, penetrates into the receptor. CDR-H3 is located in a similar position to the G-protein carboxy-terminal fragment in the active opsin structure and to CDR-3 of the nanobody in the active β_2-adrenergic receptor structure, but locks $A_{2A}AR$ in an inactive conformation.

Symposium 29
Physiology of acupuncture: translational research
[Collaboration Symposium with The Japan Society of Acupuncture and Moxibustion]

(March 28, 9 : 00–11 : 00, Room E)

2S29E-1

Efficacy and Safety of Acupuncture : Clinical Evidence and Bidirectional Translational Research

Yamashita, Hitoshi (*Graduate School of Health Sciences, Morinomiya Univ. of Medical Sciences, Osaka, Japan*)

Systematic reviews and some rigorously conducted RCTs suggest that acupuncture is effective for some conditions such as chronic low back pain and chemotherapy induced nausea beyond placebo effect. However, many RCTs fail to show the superiority of real needling over sham. Main reason for this would be that sham needling is not physiologically inert and therefore it is different from placebo pill used in drug RCTs. If the sham needling has substantial clinical effect, it is difficult to detect specific effect of real acupuncture needling in sham-controlled RCTs. For this important issue, basic research on physiological activity of sham interventions has been performed by physiologists in Japan (Kawakita and colleagues).

Regarding the safety of acupuncture, serious adverse events are rare in standard practice according to some large-scale prospective surveys. However, little is known about biological responses to acupuncture stimulation : for example, what kind of physiological, chemical, and immunological reactions occur during electroacupuncture using stainless steel needles.

Thus, many basic researches have to be done in the future to support the clinical evidence of acupuncture in both aspects of efficacy and safety. This is rather different meaning of what we call "translational research" in the field of drug development. However, in the field of traditional medicine in which clinical practice has been already performed for a long time, we need "bidirectional translational acupuncture research" approach, as pointed out by Langevin and colleagues (eCAM, 2011).

2S29E-2

Neural mechanisms of inhibition of vesical micturition contractions by gentle cutaneous stimulation

Hotta, Harumi (*Dept. Auton. Neurosci., Tokyo Metropol. Instit. Gerontol., Tokyo, Japan*)

Recently we found a gentle mechanical skin stimulation technique for inhibition of micturition contractions of the urinary bladder using anesthetized rats. That stimulation excites low threshold cutaneous mechanoreceptive myelinated and unmyelinated afferent fibers ; the frequency of discharge is greater in unmyelinated afferents than in myelinated afferents. To clarify central nervous system (CNS) mechanisms of this inhibitory effect by gentle skin stimulation, we recorded activity from and stimulated to both the Barrington's nucleus (micturition center in pons) and the spinal cord in CNS intact and spinal cord transected rats, respectively. The skin stimulation depressed bladder contraction induced by electrical stimulation of the Barrington's nucleus or its descending tract, whereas it depressed bladder distention-induced neuronal activation in both pontine and spinal cord micturition centers. These results suggest that the skin stimulation inhibits both ascending and descending transmission of micturition reflex pathway at the spinal cord. This would lead to shutting down a positive feedback between bladder and brain. Endogenous opioids in the spinal cord appear to have an essential role, since intrathecal naloxone abolished the inhibitory effect by the skin stimulation. These results may help to understand mechanisms of clinical outcome of physical therapy, including acupuncture, for overactive bladder.

2S29E-3

Acupuncture affects gut motility and secretion via autonomic nerves

Noguchi, Eitaro (*Graduate School of Technology and Science, Tsukuba University of Technology, Japan*)

In Japan it has long been considered that the autonomic nervous system is affected by acupuncture and moxibustion therapies. In literature from the middle of the Meiji Era, when the effects of acupuncture and moxibustion were just beginning to be understood in the light of modern medicine, we can already find statements in "Shinji-shinsho" (published in 1892) that acupuncture acts on visceral organs via the autonomic nervous system. In 1973, Sato and Schmidt published a review entitled "Somato-sympathetic reflexes" in the journal. That research had an extremely important influence on the subsequent development of other basic research on acupuncture and moxibustion therapy. We present here some recent experimental work on the mechanism of acupuncture for regulating somato-gastric or duodenal reflexes in anesthetized rats. And in anesthetized rats, it has been proven that acupuncture to the abdomen causes inhibition of motility by excitation of sympathetic nerves via spinal reflexes, while acupuncture of the limbs causes an increase in motility by excitation of vagus nerves via supraspinal reflexes. Also, studies on the effect of acupuncture on gastric acid secretion have confirmed that somato-autonomic reflexes are involved (1996), and it has also been shown that endogenous opioids play a role (1996). In spite of the knowledge gained from recent studies, the actual mechanisms at work remain unclear. Therefore, our understanding of the mechanisms involved with the effects of acupuncture on autonomic functions is still uncertain and open to investigation.

2S29E-4

Effect of acupuncture on the immune system

Hisamitsu, Tadashi (*Dept. of Physiol., Sch of Med., Showa Univ. Tokyo, Japan*)

We study the effect of acupuncture and Moxibustion on immune activity using arthritis model animal. And we also examine the effect of acupuncture on the blood fluidity. I present some of our finding In this symposium. (1) The influence of electro-acupuncture (EA) and Moxibustion (Mox) on collagen-induced arthritis (CIA) animal was examined. DBA/1J mice were immunized with bovine type II collagen (CII). EA stimulation or Mox, begun on day 21 simultaneously with the second immunization, was applied three times a week for 3 weeks at the acu-point equivalent to GV4 (Meimon). The results showed that EA and Mox delayed the onset, attenuated the severity of arthritis, and reduced the anti-collagen antibody level. Furthermore, these stimulation significantly increased serum IL-6 concentration and regulatory T cell ($CD4^+$ $CD25^+$ $Foxp3^+$ T cell) number, and decreased splenic endogenous IL-1 β and serum prostaglandin E2 (PGE2) concentration. These data suggest that EA has an inhibitory effect on murine CIA, and the partial mechanism of its therapeutic result may be attributed to inhibiting the productions of IL-1β and PGE2 and activation of regulatory T cell. (2) In the Oriental Medicine, reduction of the blood fluidity is one of the important pathological symptom. In the pain and stress model animal, the blood fluidity is markedly lowered like a stagnant blood. Increase of platelet adhesion and/or reduction of erythrocyte deformability results from sympathetic activation, increase of blood ATP level and increase of oxidative stress may have important role on this changes. EA applied several acu-points significantly improved these changes.

Symposium 30
Present status and perspective of space physiology

(March 28, 9 : 00–11 : 00, Room F)

2S30F-1

Influence of gravity on the characteristics of motor learning and memory : prism adaptation in reaching as an example

Hirata, Yutaka[1]; Wada, Yoshiro[2] (*[1]College of Engineering, Chubu Univ. Kasugai, Japan; [2]Nara Medical Univ. Kashihara, Japan*)

Micro-gravity imposes various effects on human body such as malfunctioning of cardiovascular system, weakening of muscle strength, reducing calcium concentration in bones, and inducing space motion sickness [1]. Further, under micro-gravity, physical movements are slowed down and become somewhat awkward as seen in astronauts and cosmonauts. Motor control systems of our body are continuously calibrated by interacting with gravity on the earth, thus require re-adjustments in the brain motor areas when gravitational environment is changed. This is not only due to the direct effects of gravity on the mass of our body, but to the effects on sensory systems such as vestibular and proprioceptive systems as well. Although some astronauts have made subjective reports informally, scientific evidence on motor learning and memory retention under different gravitational environments is missing. In the present study, we address this issue by evaluating learning and memory retention curves of prism adaptation in a hand-reaching task under different gravitational conditions. We compare upright versus supine positions, and 1G versus 2G hyper-gravity conditions. The upright vs. supine, and the 1G vs. 2G experiments were conducted respectively at the Neural Cybernetics Lab of Chubu Univ., and the Aeromedical Laboratory, Japan Air Self-Defense Force (JASDF). We demonstrate that quicker learning, less forgetting and greater memory retention rates are obtained in a spine position and under 2G in comparison with their counter part in most of the subjected we tested.

2S30F-2

RESPONSES OF THE CHARACTERISTICS OF BONES TO GRAVITATIONAL UNLOADING

Ohira, Yoshinobu; Kawano, Fuminori (*Graduate School of Medicine, Osaka University, Osaka, Japan*)

It has been a serious concern that detrimental effects on the characteristics of weight-bearing bones, caused by chronic exposure to microgravity environment, may not be fully normalized after return to the Earth. However, the number of human subjects or animals, exposed to microgravity environment in each flight, is limited generally. Further, the flight duration is not constant, either. Therefore, ground-based control studies, such as hindlimb suspension of rodents, have been utilized often to investigate the responses of bones to inhibition of antigravity activity. Here we report the effects of gravitational unloading by hindlimb suspension or loading at 2-G using animal centrifuge from postnatal day 4 to month 3 on the growth and development of hindlimb bones in rats. Growth-related increases of bone weight and mineral density were inhibited by unloading. But they were gradually recovered toward the control levels. None of the parameters were influenced by 2-G exposure. However, irreversible external bend of the shaft and rotation of the distal end of tibia, which limit the dorsi-flexion of ankle joints, were induced following chronic unloading. It was suggested that such phenomena were caused by the abnormal mechanical forces imposed by the ankle dorsi-flexors and plantar-flexors.

2S30F-3

Some aspects of slow- and fast-twitch skeletal muscles in response to long-term spaceflight

Goto, Katsumasa[1]; Yoshioka, Toshitada[2]; Ohira, Yoshinobu[3] (*[1]Department of Physiology, Graduate School of Health Sciences, Toyohashi SOZO Univ., Japan; [2]Hirosaki Gakuin Univ., Japan; [3]Graduate School of Medicine, Osaka Univ., Japan*)

Exposure to microgravity environment causes atrophy of skeletal muscle, especially antigravitational slow-twitch muscle. Drastic changes in the expressions of mRNAs and proteins in atrophied skeletal muscle have been reported. Recently, it has been reported that the effects of 91-day-exposure to microgravity on skeletal muscles in mice by using the mouse drawer system (MDS), sponsored by Italian Space Agency and housed in the International Space Station. Drastic muscle atrophy, as well as slow-to-fast transition of myosin heavy chain phenotypes, was observed in soleus muscle, but not in fast-twitch extensor digitorum longus (EDL) muscle. Gene expressions of the atrophy-related ubiquitin-ligases were up-regulated in both soleus and EDL muscles. Insulin-like growth factor was down-regulated in soleus, but up-regulated in EDL. In addition, stress-related genes, such as heat shock proteins (HSPs), were up-regulated in EDL, not in soleus. Results from this study strongly suggested that up-regulation of HSPs could be a countermeasure for long-term spaceflight. This study was supported, in part, by Grant-in-Aid for Challenging Exploratory Research (24650411, KG ; 24650407, YO), and Grants-in-Aid for Scientific Research (B, 20300218, KG ; A, 22240071, TY ; S, 19100009, YO) from the Japan Society for the Promotion of Science, and the Science Research Promotion Fund from the Promotion and Mutual Aid Corporation for Private Schools of Japan (KG).

2S30F-4

Recent Advances in Cardiovascular Research for Space Physiology

Kawai, Yasuaki; Matsuo, Satoshi (*Adaptation Physilogy, Faculty of Med., Tottori Univ., Yonago, Japan*)

Animals on the earth have been evolved under the environment of Earth gravity (1 G). Advances in technology have provided human with opportunities to meet a different environment, low- or micro-gravity, when astronauts go up to the space, 0.16 G on the moon and almost 0 G in the space station. The effect of micro-gravity on cardiovascular functions is greater in human than in other animals because human being usually keeps upright standing or sitting position in which the direction of gravity is parallel to the long axis of the circulatory system. Previous reports demonstrated that many of astronauts suffered from facial edema and nasal congestion due to headword fluid shift during space flight, and from orthostatic intolerance after returning to the earth. In this talk, we will present recent advances of space physiology in cardiovascular research field. The mechanism of orthostatic intolerance has been extensively examined in the past 50 years. Many factors are implicated in the mechanism, including hypovolemia, decreased function of sinoaortic baroreceptor reflex, reduction of heart mass, reduced reactivity of peripheral arteries and veins, increased permeability in leg capillaries, alteration of cerebrovascular autoregulation, and so on. A role of vestibulosympathetic reflex has been also pointed out as one of the mechanism. Furthermore, recent studies are going to clarify the molecular mechanisms such as gene expression in vascular endothelium and smooth muscles which may result in changes in nitric oxide synthase and ryanodine receptor subtype.

2S30F-5

Does spaceflight attenuate sensitivity of vestibulo−cardiovascular?

Morita, Hironobu[1]; Abe, Chikara[1]; Tanaka, Kunihiko[2] ([1]*Gifu University Graduate School of Medicine;* [2]*Gifu University of Medical Science*)

The vestibular system is known to have an important role in controlling arterial pressure upon posture transition (vestibulo-cardiovascular reflex). However, this system is known to be highly plastic, i.e., if subjects are exposed to different gravitational environment, the sensitivity of the system is altered. Thus, it is possible that the sensitivity of vestibulo-cardiovascular reflex is diminished after spaceflight, and then orthostatic hypotension is induced. To test this hypothesis, we applied for "Utilization of International Space Station in the Fields of Life Science" and the proposal was adopted. Experiments began in 2012 and will be over in 2014 ; during this period six astronauts will be examined. The experiments just began, and enough data have not been obtained, but the post-flight data about one astronaut are going to be obtained in January, 2013. This data will be presented in this symposium.

2S30F-6

The effectiveness of artificial gravity with ergometric exercise on spaceflight deconditioning

Iwase, Satoshi[1]; Nishimura, Naoki[1]; Sugenoya, Junichi[1]; Paloski, William[1]; Young, Laurence[1]; van Loon, Jack J.W.A.[1]; Wuyts, Floris[1]; Clément, Gilles[1]; Rittweger, Jörn[1]; Gerzer, Rupert[1]; Lackner, James[1]; Akima, Hiroshi[2]; Katayama, Keisho[2]; Qi, Fu[1]
([1]*Department of Physiology, School of Medicine, Aichi Medical University, Nagakute, Japan;* [2]*Research Center of Health, Physical Fitness & Sports, Nagoya University, Japan*)

Artificial gravity project proposes the first in-flight testing of the effectiveness and acceptability of short radius centrifuge as a countermeasure to human deconditioning on orbit. The concept is a very old one, although the implementation using a short radius centrifuge is relatively new. The ground based research supporting the in-flight AG validation we propose has been extensive, and includes research at ground centrifuges under the direction of the members of the investigator team. We propose to use the unique opportunity of testing astronauts on the International Space Station for this purpose. For human space voyages of several years duration, such as those envisioned for exploration of Mars, crews would be at risk of catastrophic consequences should any of the systems that provide adequate air, water, food, or thermal protection fail. Beyond that, crews will face serious health and/or safety risks resulting from severe physiologic deconditioning associated with prolonged weightlessness. The principal physiologic deconditioning risks are related to physical and functional deterioration of the loss of regulation of the blood circulation, decreased aerobic capacity, impaired musculo-skeletal systems, and altered sensory-motor system performance. These physiologic effects of weightlessness are generally adaptive to spaceflight and present a hazard only following G-transitions upon return to Earth or landing on another planet. However, they may present hazards in flight in the event of a traumatic bone fracture, alterations in the heart's rhythm, development of renal stones, or sensory-motor performance failure during piloting, extra vehicle activities, or remote guidance tasks. Our previous rod-like centrifuge system have proved the effectiveness of artificial gravity in the ground-based study, however, it was too large to install in the International Space Station. Therefore, we remodeled the centrifuge to the size to be fixed in it. In the present session, we will describe how our new system of centrifuge-induced artificial gravity device functions to prevent spaceflight deconditioning due to weightlessness.

Symposium 31
Recent progress and future prospects of physiological research using ionized radiation

(March 28, 9 : 00–11 : 00, Room G)

2S31G-1
Current status and future of heavy ion cancer therapy

Nakano, Takashi (*Heavy ion Medical Center, Department of Radiation Oncology, Gunma University Graduate School of Med. Gunma, Japan*)

Currently, carbon ions are used for heavy ion cancer therapy. Carbon ions have superior dose distribution which increases tumor control with sparing side effect of surrounding normal organs. Additionally carbons have 2-3 times stronger cell killing effects which effectively control tumors irrespective of various radiation resistant nature originated by hypoxic condition, p53 mutant status and cancer stem like cells etc. Hence, carbon ion therapy allows the tumors controlled effectively and successfully without using invasive procedures such as surgery. The short treatment time (some by one fraction in one day, by 3 weeks on average) in compared to conventional X-ray radiotherapy which requires 6-7 weeks is another significant clinical advantage in carbon therapy. Carbon ion cancer therapy started first at NIRS in 1994 in Japan and more than 5000 patients with various cancers have been treated. Among them, especially, superior clinical results were obtained in cancers of lung, liver and prostate, bone and soft tissue sarcomas, and recurrent rectal cancer. At Gunma University, carbon ion cancer therapy started in March 2010 and more than 470 patients with prostate cancers, lung cancers, liver cancers and osteosarcomas etc. have been treated safely without unexpectedly strong side effects.

2S31G-2
Status and Future Plan of Heavy-Ion Cancer Radiotherapy Facility HIMAC

Noda, Koji (*Department of Accelerator and Medical Physics, National Institute of Radiological Sciences, Japan*)

The first clinical trial with a carbon-ion beam generated from HIMAC was conducted in June 1994. The total number of patients treated was about 6,500 as of August 2012. The impressive advance of carbon-ion therapy using HIMAC has been supported by high-reliability operation and by the developments of accelerator and beam-delivery technologies. Based on more than ten years of experience with HIMAC, we carried out design studies and R&D works toward a standard carbon-ion radiotherapy facility downsized from the HIMAC. As a result, collaborating NIRS with Gunma University, a pilot facility was constructed at Gunma University, and the treatments have carried out successfully since March 2010. On the other hand, NIRS proposed a new treatment research facility for the further development of radiotherapy with HIMAC, based on the pencil-beam 3D scanning. On the basis of the design study and the related R&D work, the new treatment research facility was constructed, as an extension of the existing one. The treatments have been successfully carried out with a pencil-beam 3D scanning since May 2011. As a future plan, we have developed a superconducting rotating gantry, and we are going to just start a study of a superconducting accelerator for the ion radiotherapy. The status and future plan of the heavy-ion cancer radiotherapy HIMAC is reported.

2S31G-3
Defective DNA Repair System and Neurological Disorders

Enokido, Yasushi (*Department of Pathology, Institute for Developmental Research, Aichi Human Service Center, Aichi, Japan*)

Defective DNA repair machinery may be a more common pathology underlying various neurological disorders than we have ever thought. Emerging evidence suggests that DNA damage affects transcriptional expression of some genes involved in learning, memory and neuronal survival to regulate a program of brain development, ageing and pathogenesis throughout the life. Classical studies have suggested the link between the defect of DNA repair/damage response and neuropathology, directly and indirectly. In fact, a few inherited human neurodevelopmental and neurodegenerative diseases have direct link with defects in the defense system against DNA damage caused by various environmental stresses including radiation. Here, I will focus on the recent advances in defining the molecular basis of DNA repair and damage responses associated with some human neurological diseases, that may provide us a new insight into the unique characteristics of DNA repair machinery in the nervous system. I hope these may also provide us effective strategies for developing new therapeutics and preventive medicine against neurological disorders associated with radiation damage.

2S31G-4

Effect of X-irradiation on Mouse Brain

Shirao, Tomoaki (*Dept of Neurobiology and Behavior, Gunma Univ. Grad. Sch. of Med., Maebashi, Japan*)

Brain irradiation is an effective therapeutic tool for cancer, but it is possible that X-irradiation generates unfavorable influences on the higher brain function. Therefore it is necessary to explore the effects of X-irradiation on the higher brain function and elucidate its mechanism. We showed by in vitro experiments that immature neurons had higher radiosensitivity (Shirai et al, 2006) and indicated that irradiation inhibits neural structural development in both pre- and post-synaptic terminals (Okamoto et al., 2009). Further, we examined the effects of X-irradiation in vivo. Behavior analysis showed that 10 Gy X-irradiation suppress the formation of fear conditioning memory. In addition, immunohistochemical study showed the neuronal cell death of newly-generated neurons and the decrease of drebrin immunostaining in the neruopil region. In this talk, I will show the time course of induced apoptosis and the decrease of drebrin in the neuropil region, and discuss the X-irradiation effect on the neurons by putting the in vivo and in vitro data together.

Symposium 32
Plasticity in the brain: From physiological functions to disease

(March 28, 9 : 00–11 : 00, Room H)

2S32H-1

The active zone protein CAST regulates synaptic vesicle recycling and quantal size

Kobayashi, Shizuka[1]; Hida, Yamato[2]; Ishizaki, Hiroyoshi[3]; Inoue, Eiji[3]; Tanaka-Okamoto, Miki[4]; Yamasaki, Miwako[5]; Miyazaki, Taisuke[5]; Fukaya, Masahiro[5]; Kitajima, Isao[6]; Takai, Yoshimi[7]; Watanabe, Masahiko[5]; Ohtsuka, Toshihisa[2]; Manabe, Toshiya[1] (*[1]Div. of Neuronal Network, Inst. of Med. Sci., Univ. of Tokyo, Tokyo, Japan.; [2]Dept. of Biochem., Univ. of Yamanashi, Chuo, Japan.; [3]KAN Res. Inst., Kobe, Japan.; [4]Mol. Biol. Res. Group, Res. Inst., Osaka Med. Center for Cancer and Cardiovascular Diseases, Osaka, Japan.; [5]Dept. of Anat. and Embryol., Hokkaido Univ. Grad. Sch. of Med., Sapporo, Japan.; [6]Dept. of Clinical Lab. and Mol. Pathol., Grad. Sch. of Med. and Pharmaceutical Sci. for Res., Univ. of Toyama, Toyama, Japan.; [7]Div. of Mol. and Cell. Biol., Kobe Univ. Grad. Sch. of Med., Kobe, Japan.*)

It is essential for stable synaptic transmission that the quantal size is kept within a constant range. It is also important that synaptic efficacy during and after repetitive synaptic activation is sufficiently maintained by replenishing the release site with synaptic vesicles. However, the mechanisms for these fundamental properties of synaptic transmission have still been undetermined. Here, we found that the active zone protein CAST played pivotal roles in both presynaptic regulation of quantal size and recycling of endocytosed synaptic vesicles. In CAST KO mice, miniature synaptic responses were increased in size, and synaptic depression after prolonged synaptic activation was larger than that in wild-type mice, which was attributable to selective impairment of vesicle trafficking via the recycling endosome. Therefore, CAST serves as a key molecule that regulates dynamics and neurotransmitter contents of synaptic vesicles.

2S32H-2

Dynamic distribution of synaptic strengths in simple networks

Goda, Yukiko (*RIKEN Brain Science Institute, Wako, Saitama, Japan*)

We address how synaptic strengths are dynamically distributed across a dendritic tree of a hippocampal pyramidal neuron. Long-term synaptic plasticity, such as LTP and LTD, is thought to modify synaptic strengths in an input-specific manner where plasticity is confined to synapses belonging to active inputs while sparing synapses associated with inactive inputs. Nonetheless, input specificity can break down as heterosynaptic plasticity could be observed following LTP induction. How input-specificity is controlled, particularly at the level of individual synapses, is not well understood. We have been examining the extent of input specificity of long-term synaptic plasticity in postsynaptic neurons and its regulation using dissociated hippocampal neuronal cultures as a model system, in which the exact synaptic connectivity of the pre and the postsynaptic neurons and the strengths of individual synapses belonging to each connection could be readily mapped. Our recent progress will be discussed.

2S32H-3

Neocortical adult neurogenesis and its neuroprotective effects against ischemia

Ohira, Koji[1]; Takeuchi, Rika[1,2]; Miyakawa, Tsuyoshi[1,2,3] (*[1]Divi Sys Med Sci, ICMS, Fujita Hlth Univ, Toyoake, Japan.; [2]CREST, JST, Kawaguchi, Japan; [3]Ctr for Gen Anal Behav, NIPS, Okazaki, Japan.*)

Adult neurogenesis in the hippocampal subgranular zone (SGZ) and the subventricular zone (SVZ) is regulated by various factors. Chronic treatment with selective serotonin reuptake inhibitors (SSRIs) modulates adult neurogenesis in the SGZ, which is hypothesized to mediate the antidepressant effect of these substances. Layer 1 inhibitory neuron progenitor cells (L1-INP cells) were recently identified in the adult cortex, but it remains unclear what factors other than ischemia affect the neurogenesis of L1-INP cells. Here, we show that chronic treatment with an SSRI, fluoxetine (FLX), stimulates production of GABAergic interneurons from L1-INP cells in the cortex of adult mice. Immunofluorescence analysis revealed that FLX treatments increased the number of L1-INP cells in all examined cortical regions in a dose-dependent manner. A retroviral vector containing an enhanced synapsin I promoter-driven Venus reporter expression cassette was constructed and revealed that Venus-expressing GABAergic interneurons were generated from retrovirus vector-labeled L1-INP cells. The neuroprotective effects of new GABAergic interneurons on ischemic excitotoxicity were examined. The number of apoptotic cells in the ischemic cortices of FLX-treated mice was significantly lower than that in cortices in which adult cortical neurogenesis was inhibited by local infusion of arabinosylcytosine. This study indicates that FLX can increase the number of cortical GABAergic interneurons, which have neuroprotective functions against ischemia.

2S32H-4

Dematuration of hippocampal neurons as a cellular basis for antidepressant action

Kobayashi, Katsunori[1,2]; Imoto, Yuki[3]; Suzuki, Hidenori[1,2]; Segi-Nishida, Eri[4] (*[1]Dept. Pharmacol., Nippon Med. Sch., Tokyo, Japan; [2]JST, CREST, Saitama, Japan; [3]Dept. Physiol Chem., Kyoto Univ. Pharm. Sci., Kyoto, Japan; [4]Dept. Syst. Biosci. for Drug Discov., Kyoto Univ. Pharm. Sci., Kyoto, Japan*)

Antidepressant medication and electroconvulsive therapy have been used to treat major depression. However, it is not known whether these treatments share a common cellular mechanism of action. We have recently shown a distinct form of neuronal plasticity induced by chronic antidepressant treatment, that is, a reversal of maturation of granule cells (GCs) in the adult hippocampal dentate gyrus. In the present study, we examined whether electroconvulsive stimulation (ECS), an animal model of electroconvulsive therapy, can also cause this dematuration of GCs in adult mice. Repeated ECS increased the excitability of GCs and decreased prominent frequency facilitation that characterizes functional maturation of GC output synapses. The frequency facilitation recovered to the baseline level in 2 weeks after 3 times of ECS, but stayed suppressed after 11 times of ECS. ECS also reduced the expression of the mature GC marker calbindin and the activity-dependent expression of c-fos, an indicator of the mature in vivo responsiveness of GCs. Single ECS was sufficient for the downregulation of calbindin gene expression. These results suggest that ECS can rapidly induce dematuration of GCs, and that repetitive treatments convert it into the stable form. Our findings suggest that the granule cell dematuration is involved in the mechanism of action of both pharmacological antidepressant treatment and electroconvulsive therapy.

Symposium 33
Cutting edges of neuroscientific studies on face perception and recognition

(March 28, 9 : 00–11 : 00, Room I)

2S33I-1

Face Mosaics : Neuronal Organization Linking Visual Features and Face Category

Sato, Takayuki[1]; Uchida, Go[1]; Lescroart, Mark[2]; Kitazono, Jun[3]; Okada, Masato[3]; Tanifuji, Manabu[1] (*[1]Lab. for Integrative Neural Systems, RIKEN BSI, Wako, Japan; [2]Helen Wills Neuroscience Institute, UC Berkeley, California, USA; [3]Grad. Sch. of Frontier Sciences, The Univ. of Tokyo, Kashiwa, Japan*)

Higher mammals use different hierarchical levels of visual information to guide goal-oriented behavior. In the brain, visual features, objects and categories are encoded in inferotemporal (IT) cortex, but the neuronal organization linking these three levels of information is unknown. Using dense untargeted electrophysiological recordings and intrinsic signal imaging, we found that the exposed cortex is subdivided into functionally distinct contiguous domains, each spanning several millimeters ; one of these domains represented the face category. Remarkably, we also identified domains with low responsiveness to the face category, revealing that anti-visual category preference coding participates in cortical processing. These face sensitive domains were observed to contain heterogeneous local activity for the face category corresponding to feature columns ; thus we term these domains mosaics. These findings demonstrate that hierarchical representation of facial features, faces, and face category are tightly coordinated in face mosaics.

2S33I-2

Face－inversion affects time course of hierarchical categorization in monkey inferior temporal cortex

Matsumoto, Narihisa[1]; Sugase-Miyamoto, Yasuko[1]; Ohyama, Kaoru[2]; Kawano, Kenji[3] (*[1]AIST, Tsukuba, Japan; [2]Univ. of Tsukuba, Tsukuba, Japan; [3]Kyoto Univ, Kyoto, Japan*)

We previously reported that face-responsive neurons in the inferior temporal cortex initially represent information about global category, i.e. human vs. monkey vs. shapes, and they later represent information about fine categories about the faces, e.g. facial expression. To investigate the effect of face inversion upon the neuronal activity, we recorded activities of 128 single neurons in the inferior temporal cortex of two rhesus monkeys (Macaca mulatta). The monkey performed a fixation task. Test stimuli were colored pictures of monkey faces (with 4 different facial expressions), human faces (with 4 different expressions), inverted images of the human and monkey faces, and geometric shapes. Population activity vectors were calculated from the mean firing rates of the 128 neurons for each stimulus using a time window of 50 ms that slid across the trial in 1-ms steps. Cluster analysis was applied to the vectors in each time window. Three clusters, i.e. human, monkey, and shapes, appeared initially, i.e. global categorization. Later the human and monkey clusters separated, representing human individuals and monkey expressions, i.e. fine categorization. After face inversion, human, monkey, and shape clusters appeared initially. Later, the human, monkey, and shape clusters were still mainly observed. These results suggest that the representation by the neuronal population is varied in accordance with the characteristic effect observed in the research on face perception : the "face inversion effect".

2S33I-3

What facial information is important for rapid detection of the face？—Visual Search of the Face in Humans and Monkeys—

Nakata, Ryuzaburo; Tamura, Ryoi; Eifuku, Satoshi (*Department of Integrative Neuroscience, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan*)

PURPOSE : Based on previous work suggesting that participants efficiently detected the faces of their own species in visual search tasks, and that inner features (e.g., eyes) of the face were not important for efficiency, this study further explores what contributes to efficient face detection. METHOD : Subjects were two Japanese macaques and human participants. Stimuli consisted of several types of faces and non-face distracter objects. Subjects were asked to detect an odd element (the face) in an array of distracters (non-face objects) that were of different sizes (4-20). RESULTS AND DISCUSSION : Both humans and monkeys efficiently detected the face with low spatial frequency components, and the face with which they had fewer visual experiences (the other race faces for humans, and rhesus monkey faces for Japanese macaques) ; however, they did not efficiently detect the face with high spatial frequency components, the silhouettes of faces, and the back of the heads. These results suggest that the information of low spatial frequency components contained within outer features of their own species face was possibly affected as antecedent information for detecting the face in the face-processing mechanism.

2S33I-4

Spatial tuning of voice location is altered with spatially deviated facial movie stimulus in the lateral belt region of marmoset auditory cortex

Miyakawa, Naohisa; Banno, Taku; Suzuki, Wataru; Ichinohe, Noritaka (*Dept Ultrastructural Research, National Institute of Neuroscience, National Center for Neurology and Psychiatry, Kodaira, Japan*)

Our perception of sound (e.g. voice) position is shifted toward an accompanying visual stimulus (e.g. face). For example, a ventriloquist can make us believe that his voice comes from his puppet, not from him, by handling the puppet while minimizing his own lip movement. However, the neural mechanisms of this shift of spatial perception are still an open question. The caudal lateral belt area (CL) is a subregion in the auditory association cortex that contains neurons tuned to spatial location of sound, and is suggested to be the "where pathway of sound". CL is also known to receive visual inputs, and audiovisual integration of sounds were reported previously. In order to evaluate whether spatial tuning of sound in CL neurons can shift by deviated visual input, we recorded neural activities in anesthetized marmoset CL while presenting spatially parameterized auditory and visual stimuli. Recorded marmoset voice was delivered to the animal through one 7 speakers in front the animal aligned in an arc with 20 degrees interval, and spatial tuning curve for voice location was computed. Movie stimulus was displayed on a monitor in front of the animal at one of 2 positions with 15 degrees lateral shift from the paralyzed gaze center. We found that spatial tuning of some CL neurons to the voice stimuli were deviated toward the location of the movie stimuli, suggesting contribution of CL to the perceptual shift of sound location by visual input.

Symposium 34
Neural regulation of arterial pressure: Basic and frontline topics

(March 28, 9 : 00–11 : 00, Room J)

2S34J-1
Exercise pressor reflex : a sympathoexcitatory mechanism originating in contacting skeletal muscle

Koba, Satoshi (*Division of Integrative Physiology, Tottori University Faculty of Medicine, Yonago, Japan*)

Sympathetic nerve activity, blood pressure, and heart rate as well as respiration increase in response to exercise. These cardiorespiratory adjustments during exercise are partially mediated by a reflex originating from contracting skeletal muscle. This sympathoexcitatory reflex, termed the exercise pressor reflex, is evoked as thin fiber muscle afferents are stimulated by mechanical deformation of the afferents' receptive fields as well as by metabolic by-products due to contraction. Signals from the nerve endings project to the dorsal horn of the spinal cord via group III and IV muscle afferent fibers and then to the brain stem. For the last decade, much research attention has been paid to roles the exercise pressor reflex plays in abnormal regulation of circulation seen in cardiovascular disease such as heart failure and hypertension. In these diseases, the exercise pressor reflex is exaggerated. Of note, in heart failure, the mechanical component of this reflex becomes exaggerated while the chemical component is attenuated. In hypertension, both mechanical and chemical components of the reflex are exaggerated. Recent studies from our laboratory have suggested that oxidative stress in these diseases contributes to the exaggerations of the exercise pressor reflex and its mechanical component. Updates on our understandings of the exercise pressor reflex in health and cardiovascular disease are presented.

2S34J-2
Central mechanisms of arterial pressure regulation during exercise : integrative functions of the nucleus of the solitary tract

Waki, Hidefumi (*Department of Physiology, Wakayama Medical Univ, Wakayama, Japan*)

A single bout of exercise induces a moderate increase in arterial pressure (AP) with marked tachycardia as a result of sympathoexcitation. In this symposium, the potential brain mechanisms underlying cardiovascular regulation during exercise will be introduced, with a focus on the functions of the nucleus of the solitary tract (NTS). The NTS is known as a pivotal region which integrates the baroreceptor sensory information with other inputs such as muscle afferents and descending signals from the hypothalamic defense area, making it an ideal site for generating cardiovascular controls during exercise. Indeed, the GABAergic inter-neurons within the NTS are likely involved in baroreceptor reflex resetting by limiting the degree of excitation of barosensitive NTS neurons, and thus are capable of continuous increases in sympathetic nerve activity with a high level of AP during exercise. We recently found that the tuberomammillary nucleus (TMN) of the posterior hypothalamus, which is known as the histaminergic center of the brain, may also be involved in exercise-induced cardiovascular responses. Because activation of histamine receptor H1 expressed in the NTS of rats induced pressor and tachycardiac responses, and these responses exhibit functional plasticity after long-term daily exercise, we postulates that the TMN-NTS pathways is involved in the central command and has an important role in regulating the cardiovascular system during exercise. This study was supported by the JSPS (21300253) and the Takeda Science Foundation.

2S34J-3
Blood pressure adaptation and the defense areas in the brain

Horiuchi, Jouji (*Department of Biomedical Engineering, Toyo University Kawagoe, Japan*)

Stress evokes powerful autonomic response. The autonomic response to stressors is mediated by the sympathetic nervous system. The sympathetic response to the stress is mediated by 2 hypothalamic areas. Inhibition of the hypothalamic areas reduces the pressor response evoked by the stressor. In addition, the midbrain area also plays a crucial role in mediating cardiovascular response to the stress. On the other hand, these areas in the hypothalamus and the midbrain are also essential brain centers of the defense reaction that accompanies sympathoexcitatory response. Activation of neurons in the areas evokes increases in arterial pressure and sympathetic activity that are similar to the response to the stress. Therefore, the cardiovascular response to the stress has been evolved as a survival strategy to adopt an environmental change. Animal has 2 homeostatic mechanisms, which are maintenance and adaptation. During the resting period, the maintenance system is dominant to the adaptation. Once a circumstance is changed, the adaptation system overcomes the maintenance. It has been believed that the maintenance system, such as baro- and chemo-reflexes, is inhibited during the adaptation to the environment. However, recent studies have shown that the baro-and chemo-reflexes still work even under the condition of the stress or the exercise. Therefore, the blood pressure adaptation during the stress and the exercise receive sympathetic control from the defense areas and also get an influence of blood pressure maintenance such as baro- and chemo-reflexes.

2S34J-4

AT1 Receptors and Oxidative Stress in the RVLM Are the Possible Therapeutic Target for Hypertension

Hirooka, Yoshitaka (*Department of Advanced Cardiovascular Regulation and Therapeutics, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan*)

Recent studies indicate that activation of the sympathetic nervous system plays an important role as previously thought. We found that oxidative stress is increased in the brain in hypertensive rats, such as spontaneously hypertensive rats (SHR) and stroke-prone SHR (SHRSP). Mn-SOD overexpression in the rostral ventrolateral medulla (RVLM) of the brainstem reduced blood pressure through sympathoinhibition in SHR as well as SHRSP. In contrast, Mn-SOD overexpression in the paraventricular nucleus (PVN) of the hypothalamus decreased heart rate, but did not decrease blood pressure. AT1 receptor stimulation in the RVLM induced activation of the NAD (P) H oxidase/Rac1 pathway thereby causing reactive oxygen species generation. We also found that caspase-3 activity in the RVLM was significantly higher in SHRSP than in Wistar-Kyoto (WKY) rats. ICV infusion of an AT1 receptor blocker in SHRSP inhibited the caspase-3 pathway in the RVLM. ICV angiotensin II stimulation elicited TLR4 activation. Furthermore, we suggest that the decreased numbers of astrocytes in the RVLM are involved in the enhanced sympathetic outflow in SHRSP. Oral treatment with telmisartan, an AT1 receptor blocker, decreased blood pressure with sympatho-inhibition probably acting on the RVLM, because this sympatho-inhibitory response was associated with reduction of oxidative stress in the RVLM. We conclude that AT 1 receptors and oxidative stress in the RVLM may be an important therapeutic target for hypertension.

Symposium 35
Physiology Research and Teaching in Rehabilitation Medicine

(March 28, 13 : 20–15 : 20, Room B)

2S34J-5

Differential control of sympathetic nerve activity during sleep, exercise and, mental stress

Miki, Kenju (*Nara Women's University, Nara, Japan*)

Sympathetic nerve activity plays a critical role in regulating systemic arterial pressure in our daily activity, including sleep, wakefulness, exercise and stress.. There is a growing body of evidence showing that sympathetic nerve activity is regulated differently and in an organ-specific manner, suggesting that differential changes in sympathetic outflows may be involved in behavioral state dependent changes in arterial pressure responses. We have demonstrated that each behavior state generates a state-specific patter of sympathetic nerve activity in rats. Sympathetic nerve activity apparently changes in a global fashion in the states NREM sleep, quiet awake, moving, voluntary movement and exercise. However, it changes in non-uniform fashion during REM sleep and mental stress. REM sleep resulted in diverse changes in sympathetic outflows ; renal sympathetic nerve activity decreased while lumbar sympathetic nerve activity and systemic arterial pressure increased. Freezing behavior evoked an immediate and a sustained increase in RSNA while LSNA and systemic arterial pressure remained unchanged. We have observed that acute shifts in baroreflex control of sympathetic outflow occurred in a state-dependent and region-specific manner. It was suggested that there might be discrete subgroups of neuronal networks with baroreflex pathway. It is therefore likely that the arterial baroreflex pathways may be modulated in a regionally directed manner, resulting differential changes sympathetic nerve activity, which acts in concert to orchestrate the adjustments of systemic arterial pressure for the whole body in daily activity.

2S35B-1

Restoration of Hemiparetic Upper Limb after Stroke with Brain–Machine Interface Technology

Liu, Meigen (*Department of Rehabilitation Medicine, Keio University School of Medicine*)

Because recovery of upper extremity (UE) functions to a practical level has been considered difficult after stroke, compensatory approaches have been emphasized. Based on researches indicating greater potential for neural plasticity, approaches targeted to functional restoration are popularized. Recent meta-analysis indicates effectiveness of several available interventions for arm functions, but not for hand functions. We therefore devised two new interventions to improve paretic hand. One is Hybrid Assistive Neuromuscular Dynamic Stimulation therapy designed to facilitate daily use of the hemiparetic UE by combining EMG triggered electrical stimulation with a wrist splint. We demonstrated improvement of motor function, spasticity, functional scores and electrophysiological parameters in chronic as well as subacute stroke. To be its candidates, however, EMG must be recorded from finger extensors. For patients with no detectable EMG, we devised EEG-based BMI neurofeedback training that provides real time feedback based on analysis of volitionally decreased amplitudes of sensory motor rhythm (SMR) during motor imagery of affected finger extension. In a pilot study, we found appearance of voluntary EMG in the affected finger extensors, improvement of finger function, greater suppression of SMR over both hemispheres during motor imagery and increased cortical excitability as assessed with transcranial magnetic stimulation. These interventions offer promising neurorehabilitative tools for hemiparetic UE.

This work was supported by the MEXT Strategic Research Program for Brain Sciences.

2S35B-2

Higher brain functions and measurement of brain activities by fNIRS

Morioka, Shu (*Kio University, Koryo, Japan*)

Traditionally, the effects of rehabilitation have been determined by evaluating only the activities of daily living and motor function. However, recently, the measurement results of brain imaging studies have been used to determine outcome. This is based on the fact that neural plasticity mechanisms are involved in the recovery of motor and perceptual functions of subjects who receive rehabilitation. In therapeutic exercise in rehabilitation, the therapist requires the subjects to perform dynamic movements. In most cases of therapeutic exercise in medical rehabilitation, the therapist may request the subjects to perform dynamic movements. fNIRS has allowed the measurement of brain activity during dynamic movements, such as gait, by using brain function imaging devices. We have measured changes in cerebral blood flow by using fNIRS for developing clinical interventions, determining the effects of therapeutic exercise on recovery of motor function after stroke, and evaluating the improvement in higher brain dysfunctions and pain. We found some evidence of the effects of improvement of cognitive and motor imagery ability on motor learning and recovery of motor function after stroke. In rehabilitation research, examining the activation of motor-related brain areas during motor imagery and perceptual learning is important to validate the usefulness and effectiveness of interventions. In addition, fNIRS allows real-time observation of brain activities of the subject and is of clinical value from a neurofeedback perspective. We will highlight research on higher brain functions, such as imagery, and the effect of interventions in patients with disabilities.

2S35B-3

Mechanisms of immobilization-induced muscle contracture : investigation of the molecules associated with muscle fibrosis

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A recent review proposed that the mechanism of immobilization-induced muscle contracture is related to the onset of fibrosis, based on the observed overexpression of intramuscular collagen. However, the molecular mechanism involved in the progress of muscle contracture is still unclear. Our study investigated the role of molecules associated with fibrosis—type I and type III collagen, COL1 and COL3 ; transforming growth factor-β1, TGF-β1 ; hypoxia-inducible factor-1α, HIF-1α ; and α-smooth muscle actin, α-SMA—in immobilized rat soleus muscle at 1, 2, 4, 8, and 12 weeks following muscle immobilization. As opposed to the control rats, the immobilized rats showed that the expression of HIF-1α mRNA was significantly higher during 4, 8, and 12 weeks following muscle immobilization, whereas TGF-β1, α-SMA, and COL1 and COL3 mRNAs were significantly higher during the entire immobilization period. Furthermore, COL1 mRNA and a number of α-SMA positive cells were significantly higher at 4, 8, and 12 weeks than during the first 2 weeks of immobilization. These findings suggest that the up-regulation of TGF-β1 may have activated the fibroblasts and promoted their differentiation into myofibroblasts ; these changes also correspond to an increase in the levels of COL1 and COL3. In addition, the muscle tissue was seen to become hypoxic after 4 weeks of immobilization—a change that accelerated the production of COL1. In conclusion, we speculate that all these alterations may influence the progress of muscle contracture.

Key words ;
immobilization, muscle contracture, fibrosis, molecular mechanism

2S35B-4

Development of Assistive Technology for Person with Physical Disabilities

Hatakeyama, Takuro (*Dr. of Engineering Faculty of Human Sciences, Waseda University*)

There are four considerations which I think important in developing and providing assistive devices for person with disabilities.

The first point is a precise identification of the user's needs. It is common that users can not specify their exact needs. In this case, some of the staff should reinforce them to describe what they desire. In case the user can not fully identify any, the clues are often found in the users' occupation, roles in their family, hobbies and future dreams. Next, the staff integrate all information obtained, and clarify the user's needs and available services.

The second point is the respect for a user's self determination.

In the process of development and provision of assistive devices, any final decision should be made by the user. To ease this self determining, we have to provide options as many as possible. Encouraging self determination usually improve the user's independence.

The third point is an appropriate selection of the level of technology utilized for the device. The highly advanced technology is not necessarily the best choice. The selection has to be done through the careful assessment of the user's physical, perceptual and cognitive functions. We also need to know too much support by assistive devices may cause the user to lose his or her feeling of being alive (motivation of living). To minimize this disadvantage, any interface should be designed by utilizing the residual function appropriately.

Finally, to improve the quality of assistive devices, the most advanced technology that are developed in various fields need to be applied.

Symposium 36

Cutting-edge of *in vivo* science

[Symposium Supported by Science Council of Japan]

(March 28, 13 : 20–15 : 20, Room D)

2S36D-1

Synapse Remodeling in Pathological Condition in vivo

Nabekura, Junichi[1,2] ([1]*NIPS, Okazaki,Japan;* [2]*SOKENDAI, Hayama, Japan*)

Recent advance of two photon excitation of fluorescent molecules enables us to observe the fine structures and neuronal activity in vivo with a high resolution. Here, I would show two examples of a real-time and a long-term time lapse imaging of synaptic structures of mouse cortex in pathological condition. In ischemic brain, there was massive remodeling of synaptic structures, generation and elimination. Resting microglial processes, which directly contact onto synaptic structures, change in contact duration from 5 minutes in healthy brain to over one hour in damaged brain. Prolonged microglial contact was frequently followed by the disappearance of synaptic structures. Such microglial-synapse contacts determine the subsequent fate of damaged synapses-to remain, or to be eliminated. Peripheral nerve injury altered nociceptive signal processing, represented by tactile allodynia. Time lapse imaging at an interval of 3 days revealed that the rate of spine turnover in the primary somatosensory cortex increased limited in a developmental phase of neuropathic pain. Preexisting stable spines survived less following injury, and new spine preferentially survived. Thus, injury-induced hyperactivity of sensory afferents induces rapid and selective remodeling of cortical synapses. Astrocyte was enhanced in the developmental phase. Photo-activation of astrocyte accelerated synapse remodeling, suggesting an involvement of astrocyte in synapse remodeling in chronic pain. An advance in imaging of fine structures in the living brain contributes to better understand brain function in terms of synaptic and neuronal dynamics.

2S36D-2

Activity manipulation of neurons and control of instinctive behaviors using optogenetics

Yamanaka, Akihiro[1,2,3] ([1]*Research Institute of Environmental Medicine, Nagoya Univ., Nagoya, Japan;* [2]*JST, PRESTO, Saitama, Japan;* [3]*NIPS, Okazaki, Japan*)

Instinctive behaviors, such as sleep/wakefulness, feeding and sexual behaviors are regulated by the hypothalamic neurons. Recent research revealed that the hypothalamic neurons containing neuropeptides are implicated in the regulation of these instinctive behaviors. It is essential to study neural regulatory mechanisms of these instinctive behaviors using a whole animal since these instinctive behaviors are exhibited only therein. Optogenetics enable control of the activity of specific type of neurons in the whole body animal using light. We apply optogenetics to Orexin-producing neurons (orexin neurons). Orexin neurons are located in the hypothalamus but project their efferents throughout the brain. Intriguingly, mice lacking the prepro-orexin gene showed behavioral characteristics similar to human sleep disorder Narcolepsy, that is a fragmentation of sleep/wakefulness and sudden muscle weakness. Human clinical studies also showed that orexin neurons are specifically ablated in the narcoleptic patient's brain. These results suggest that the orexin neurons play a critical role in the regulation of sleep/wakefulness. Previous studies using electrophysiological in vitro techniques have identified potential neuronal pathways or networks connecting orexin neurons with other neurons which are known to be involved in sleep/wakefulness regulation. Our current research involves applying optogenetics in the hypothalamic peptide-containing neurons to reveal regulatory mechanisms of these instinctive behaviors.

2S36D-3

Undesranding of molecular mechanism underlying cardiovascular development by imaging of zebrafish embryogenesis

Mochizuki, Naoki; Fukuhara, Shigetomo (*Natl. Cerebr. & Cardiovasc. Ctr. Res. Inst. Suita, Osaka, Japan*)

Heart and vessels are the first organs to be developed during embryogenesis and originate from lateral plate mesoderm. To establish the circulation, both organs are concertedly formed. However, it is still unclear how cardiogenesis and vascular development are regulated. We have tried to investigate how these organ development is molecularly regulated by looking at the signaling and morphology simultaneously. Morphological changes required for forming tissues and organs must be precisely controlled by the specific signaling. Therefore, we assume that visualizing the signal controlling the morphology and assembly of the cells is fundamental to exploring the organogenesis including cardiovascular development.

We have used transgenic zebrafish expressing cardiac-specific fluorescent probes or endothelial cell-specific fluorescent probes to monitor the cell movement and signaling. These probes include the proliferation monitoring probes, Rho family GTPase activity monitoring probes, and β-catenin-dependent transcription monitoring probes. In this symposium, I would like to introduce how we use the these transgenic zebrafish to understand the cardiovascular development.

2S36D-4

Brain Imaging Analyses of Brain–Gut Interactions

Fukudo, Shin (*Department of Behavioral Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan*)

Irritable bowel syndrome (IBS) provides excellent model for research not only on many functional gastrointestinal disorders but also on pain and/or emotion. Patients with IBS are characterized by chronic and recurrent abdominal pain and/or abdominal discomfort linked with diarrhea and/or constipation without any structural or chemical abnormalities by routine medical examination. IBS patients often show stress-induced colonic hypermotility as well as anxiety disorders or depressive disorders. The mutual interactions between immune system and nervous system are present behind the post-infectious IBS. Although brain-to-gut efferent signal was initially focused in IBS research, much attention was paid to gut-to-brain afferent signal later on. That is because visceral hypersensitivity was detected in the majority of IBS patients. Brain imaging techniques including positron emission tomography, functional magnetic resonance imaging, and viscerosensory evoked potential enable us to depict visceral pain pathway as well as relating emotional circuit. There are some candidate substances which have salient roles in pathophysiology of IBS. Corticotropin-releasing hormone (CRH) is a major mediator of stress response in the brain-gut axis. We showed that administration of CRH aggravated visceral sensorimotor response in IBS patients. Conversely, administration of CRH antagonists likely alleviate IBS pathophysiology. Serotonin (5-HT) is another candidate in association to brain-gut function in IBS. Further studies on visceral neuropathways using brain imaging are warranted.

Symposium 37
Functional diversity of Store-operated Ca^{2+} entry (SOCE)

(March 28, 13 : 20–15 : 20, Room E)

2S37E-1

The physiological function of SOCE in B cells

Baba, Yoshihiro; Kurosaki, Tomohiro (*Laboratory for Lymphocyte Differentiation, WPI Immunology Frontier Research Center(IFReC), Osaka University*)

Alterations in cytosolic concentration of Ca^{2+} are essential signals for a variety of physiological events. The engagement of B cell receptor (BCR) results in the transient release of Ca^{2+} into the cytosol from endoplasmic reticulum (ER) stores. In turn, the decrease of ER luminal Ca^{2+} concentration triggers the opening of Ca^{2+} channels in the plasma membrane, which induces a sustained influx of extracellular Ca^{2+}. These processes are referred to as store-operated Ca^{2+} entry (SOCE), which is an essential pathway for continuous Ca^{2+} signaling. While the ER calcium sensor STIM1 and STIM2 are crucial components for SOCE activation, its physiological role in B cells is unknown. Here we uncover a physiological function for SOCE in B cells by analyzing mice with B cell-specific deletions of STIM1 and STIM2. Our findings indicate that STIM1 and STIM2 are critical for BCR-induced SOCE, NFAT activation and subsequent IL-10 production. Although STIM proteins are not essential for B cell development and antibody responses, these molecules are required to suppress experimental autoimmune encephalomyelitis via an IL-10-dependent mechanism. Thus, STIM-dependent SOCE is a key signal for B cell regulatory function required to limit autoimmune inflammation.

2S37E-2

Inhibition of TRPC channels underlies biological activities of a pyrazole compounds

Mori, Yasuo[1]; Kiyonaka, Shigeki[1]; Nishida, Motohiro[2] ([1]*Dept. Synth. Chemi. and Biol. Chem., Grad. Sch. Engineer., Kyoto Univ., Japan;* [2]*Dept. Pharmacol. Toxicol., Grad. Sch. Pharmaceu. Sci., Kyushu Univ., Japan*)

Canonical transient receptor potential (TRPC) channels control influxes of Ca^{2+} and other cations that induce diverse cellular processes upon stimulation of plasma membrane receptors coupled to phospholipase C (PLC) activation and downstream IP_3-induced Ca^{2+} release from internal Ca^{2+} store ER and its depletion. Invention of subtype-specific inhibitors for TRPCs is crucial for distinction of respective TRPC channels that play particular physiological roles in native systems, and as well for development of novel therapeutic strategies of diverse types of diseases. We have characterized series of pyrazole compound which show different selectivity in inhibiting TRPC channels. Structure-function relationship studies of pyrazole compounds showed that the trichloroacrylic amide group is important for the TRPC3 selectivity of Pyr3. In DT40 B lymphocytes, Pyr3 potently eliminated the Ca^{2+} influx-dependent PLC translocation to the plasma membrane and late oscillatory phase of B cell receptor-induced Ca^{2+} response. Moreover, Pyr3 attenuated activation of nuclear factor of activated T cells, a Ca^{2+}-dependent transcription factor, and hypertrophic growth in rat neonatal cardiomyocytes, and *in vivo* pressure overload-induced cardiac hypertrophy and fibrosis in mice. Thus, TRPC-selective inhibitors are powerful tools to study *in vivo* function of TRPCs, which can be activated in a store-dependent and store-independent fashion, suggesting a pharmaceutical potential of pyrazole componds in treatments of TRPC-related diseases.

2S37E-3

The function of Store-operated Ca^{2+} entry (SOCE) in melanoma

Umemura, Masanari[1]; Fujita, Takayuki[1]; Yokoyama, Utako[1]; Ishikawa, Yoshihiro[1]; Iwatsubo, Kousaku[2] ([1]*Cardiovascular Research Institute Yokohama City University School of Medicine, Graduate School of Medicine;* [2]*Department of Cell Biology and Molecular Medicine, New Jersey Medical School-University of Medicine and Dentistry of New Jersey*)

Although Ca^{2+} is the one of major second messenger, the role of Ca^{2+} remains unknown in the cancer research field. Store-operated calcium entry (SOCE) is the major mechanism to induce extracellular Ca^{2+} into cytosolic space especially in non-excitable cells. SOCE is initiated by depletion of Ca^{2+} in the endoplasmic reticulum (ER) followed by Ca^{2+} influx from the extracellular space. This phenomenon is regulated by interaction of two molecules, STIM1 (stromal interaction molecule 1) in the ER and Orai (ORAI calcium release-activated calcium modulator) in the plasma membrane. Previous reports demonstrated the roles of Orai1 and STIM1 in migration of various cell types. However, a few reports have suggested their roles in cancer cells.

Since melanoma is one of the most aggressive cancers, we have focused on the role of SOCE in melanoma cell migration. Our major findings are ; 1) SOCE exists in melanoma. 2) STIM1 and Orai1 regulate SOCE in melanoma 3) Inhibition of SOCE suppresses melanoma proliferation and migration. Our results suggested that Ca^{2+}, especially induced by SOCE, is the target of the next generation of cancer therapy.

2S37E-4

Role of SOCE in vascular endothelial cells

Hirano, Katsuya (*Division of Molecular Cardiology, Graduate School of Medical Sciences, Kyushu University*)

The store-operated Ca^{2+} entry (SOCE) plays a crucial role as a major Ca^{2+} entry pathway in vascular endothelial cells and contributes to the regulation of various endothelial functions, including production of vaso-relaxing/contracting factors, vascular permeability, cell proliferation and angiogenesis. Understanding of the molecular mechanisms underlying SOCE has greatly advanced following the identification of the STIM proteins as a primary sensor of the amount of the stored Ca^{2+} content and the Orai proteins as channels proteins mediating SOCE. As a result, the basic mechanism of activation of SOCE by the store depletion has been substantially elucidated. However, the mechanism regulating the SOCE activity has not been fully understood. In this symposium, I would like to discuss the role of SOCE in the production of nitric oxide and endothelium-dependent vasorelaxation with some emphasis on the involvement of STIM1. Furthermore, I would like to discuss the role of phosphorylation of STIM1 in the regulation of SOCE. We found STIM1 to be phosphorylated depending on the degree of Ca^{2+} depletion of the stores, by using a Phos-tag SDS-PAGE analysis, which allows the quantitative analysis of protein phosphorylation. The phosphorylation of STIM1 appears be related to the sustained phase of the SOCE. Although the precise mechanism for the phosphorylation-mediated regulation of SOCE and its functional significance still remains to be investigated, the STIM1 phosphorylation may play a critical role in regulating SOCE.

Symposium 38
Oral health care contributes to the general health status

(March 28, 13 : 20–15 : 20, Room F)

2S38F-1

Multidisciplinary Approach to Sleep Apnea Syndrome

Nejima, Jun[1]; Yamanaka, Hiroyuki[1]; Ishikawa, Chieko[2]; Ohkubo, Chikahiro[2]; Hirai, Shinya[3]; Ogawa, Takumi[3]; Takamatsu, Tomoya[4]; Hamada, Yoshiki[4] (*[1]Department of Internal Medicine, Tsurumi University School of Dental Medicine, Yokohama, Japan; [2]Department of Removal Prosthodontics, Tsurumi University School of Dental Medicine, Yokohama, Japan; [3]Department of Fixed Prosthodontics, Tsurumi University School of Dental Medicine, Yokohama, Japan; [4]Department of Oral and Maxillofacial Surgery, Tsurumi University School of Dental Medicine, Yokohama, Japan*)

Obstructive sleep apnea syndrome (OSAS) is a common disorder which causes recurrent hypoxic episodes and decreases in intra-thoracic pressure, and consequently sympathetic activation, increased levels of plasma cytokines, and increase in venous return, resulting in hypertension, arrhythmias, aortic aneurysm, insulin resistance, and cardiovascular disorders. Therapeutic option includes, in addition to the correction of the daily lifestyle, nasal continuous positive airway pressure, oral appliance (OA), otolaryngeal surgery, and oral and maxillofacial surgery, which are applied to the patient depending on the severity in terms of apnea hypopnea index (AHI), and underlying pathophysiologic mechanisms. From 2006 to 2008, 201 patients with snore visited Tsurumi University Dental Hospital. Of these, 141 patients visited prosthodontic division for OA treatment. 34.3% of the patients had hypertension, 10.0% had cardiac disorder, and 4.5% had diabetes mellitus. AHI ranged from 0 to 116.8 with an average of 27.5±24.7 (events/hour). In 75.5% of the patients, AHI reduced to less than 5, and/or less than 50% of the baseline value, after the advancement with OA during sleep. Thus, multidisciplinary approach, oral and maxillofacial approach in particular, for OSAS would be potentially beneficial for systemic disorder including obesity, diabetes mellitus, hypertension, and cardiac diseases.

2S38F-2

Animal model studies to investigate the relationship between periodontal disease and atherosclerosis

Ochiai, Tomoko (*Dept. of Microbiology and Immunology, Nihon Univ. Sch. of Dent. at Matsudo, Japan*)

Periodontitis was recently shown to increase the risk of atherosclerosis, and accumulating evidence suggests that chronic infection with periodontal pathogens, such as *Porphyromonas gingivalis*, is associated with increased risk of atherosclerosis. We have assessed the relationship between periodontopathic bacterial infection and atherosclerosis in apo E-deficient spontaneously hyperlipidemic and C57BL/6 mice. Progress of atherosclerosis was seen in bacterial infection and also needed the condition of hyperlipidemia. Although periodontal infection enhanced inflammatory cytokines, such as IL-6 and MCP-1 levels, in normolipidemic mice, there was slight atherosclerotic plaque formation. The inflammatory response may therefore be unrelated to lipid metabolism. These findings suggest that inflammation caused by periodontopathic bacteria may play a synergistic role with other preexisting factors, such as hyperlipidemia, resulting in the development of atherosclerosis. We also assessed the potential of a nasal vaccine against the 40-kDa outer membrane protein (40k-OMP) of *P. gingivalis* for the prevention of atherosclerosis accelerated by *P. gingivalis*. In mice, nasal immunization against the 40k-OMP significantly reduced atherosclerotic plaque accumulation in the aortic sinus and lowered the serum cytokine levels compared with nonimmunized mice. These findings suggest that nasal immunization with 40k-OMP could be an effective vaccine for prevention of atherosclerosis accelerated by *P. gingivalis* under hyperlipidemic conditions.

2S38F-3

Chewing ameliorates autonomic imbalance and prevents poststress arrhythmias in rats

Ono, Yumie (*Health Science and Medical Engineering Lab, School of Science and Technology, Meiji University, Kanagawa, Japan*)

Reducing stress is important in preventing sudden death in patients with cardiovascular disease, as stressful events may cause autonomic imbalance and trigger fatal arrhythmias. In order to investigate whether chewing could ameliorate stress and prevent arrhythmias, we analyzed changes in radiotelemetered electrocardiograms in rats that were allowed to chew a wooden stick during a 1-h period of immobilization stress. Chewing significantly reduced the occurrence of ventricular premature beats (VPBs) and complex ventricular ectopy after immobilization and prevented stress-induced prolongation of the QT interval of VPBs throughout the 10-h experimental period. It also prevented prolongation of the QRS complex and fluctuations in the QT interval in normal sinus rhythm beats preceding VPBs during both immobilization and in the poststress period. Spectral analysis of heart-rate variability further showed that chewing significantly inhibited the stress-induced increase in the power ratio of low-to-high frequency activity (LF/HF : a marker of sympathetic activity) during immobilization and in addition was associated with blunting of the stress-induced increase in plasma noradrenaline observed at the termination of immobilization. These results indicate that chewing can ameliorate sympathetic hyperactivity during stress and prevent poststress arrhythmias and suggest that chewing may provide a nonpharmacological and costeffective treatment option for patients with a high risk of stress induced fatal arrhythmia.

2S38F-4

TCTP/Fortilin regulates survival of carcinoma cells and cardiomyocytes

Fujita, Takayuki; Cai, Wenqian; Hidaka, Yuko; Jin, Huiling; Jin, Meihua; Suita, Kenji; Ishikawa, Yoshihiro (*Cardiovascular Research Institute, Yokohama City Univ. Yokohama, Japan*)

TCTP (translationally controlled tumor protein), also known as Fortilin is an anti-apoptotic protein. TCTP is highly expressed in tumor tissues including oral squamous cell carcinoma. Recently, we demonstrated that TCTP is a p53 inhibitor. TCTP binds directly to the DNA binding domain of p53, thereby preventing it transcriptionally activating Bax. TCTP overexpression inhibited p53 induced cell death of U2OS osteosarcoma cells. Moreover, downregulation of TCTP by SiRNA transfection enhanced the susceptibility of U2OS cells to UV-induced DNA fragmentation. Subsequently, we examined whether TCTP can regulate survival of cardiomyocytes. In contrast to antitumor therapy, inhibition of apoptosis can be an effective treatment strategy for heart failure. The analysis of transgenic mice with cardiomyocyte-specific overexpression of TCTP revealed that TCTP upregulation protected against the doxorubicin induced heart failure. In cultured cardiomyocytes, downregulation of TCTP enhanced the susceptibility to Doxorubicin induced cell death. These findings indicate that TCTP may be a potent therapeutic target for both cancer and heart failure.

2S38F-5

Teeth and Health : interpretation of nutritional epidemiologic study results of adults and elderly people

Hanada, Nobuhiro (*Translational Research, School of Dental Medicine, Tsurumi Univ. Yokohama, Japan*)

Teeth and Health : interpretation of nutritional epidemiologic study results of adults and elderly people Nobuhiro Hanada Tsurumi University School of Dental Medicine The objectives of my talk will be to : 1) Discuss the relationship between food selection and teeth 2) Discuss the relationship between the metabolic syndrome and oral health. The nutritional epidemiologic studies of the adults and elderly people were performed in various fields supported by the health labour science research grant. Average bite force of denture wearers is, less than half of the natural teeth. Eating with dentures is quite different from eating with natural teeth. Mastication efficiency is drastically decreased for denture wearers. Dentures are unstable. So, it will tend to upset in the oral cavity during eating time. Certain foods are often avoided by denture wearers. Tiny, hard particles can be painful if they get under the dentures. Sticky foods can stick to the dentures and should be avoided. Difficulty in speaking is another problem of denture wearers. There are many elderly people whose teeth problems are associated with limitation of food choice and decreased nutrient intake. Tooth loss results in individuals selecting a diet that they can masticate in comfort. The technology of prosthetic dentistry has advanced, and new materials have improved ability to replace missing teeth. However, it must be remembered that the dentures cannot be replaced the natural teeth sufficiently. Clinical research evidence show that we need to keep at least 20 teeth at the end of our lives.

Symposium 39
Cardiac impulse propagation and arrhythmias

(March 28, 13 : 20–15 : 20, Room G)

2S39G-1

Gap Junction Remodeling and Arrhythmogenesis during Development of Heart Disease

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The intercalated disc (ID) contains different junctional complexes, adhesion junctions (AJs) and connexin (Cx) gap junctions (GJs). GJs provide the pathway for intercellular current flow, and the AJs mediate intercellular coupling. We investigated ID remodeling (ID-R) and its potential role in arrhythmogenesis. 1) Cultured rat ventricular myocytes were subjected to rapid electrical stimulation. A short-term RES caused upregulation of Cx43 and an increase of conduction velocity (CV) through an autocrine action of Angiotensin II to activate MAPKs. 2) We investigated changes in ID-R in UM-X7.1 cardiomyopathic hamster, and associated alterations in the electrophysiological properties. UM-X7.1 at heart failure stage showed significant reduction of cardiac space constant, a decrease in CV, and an increase in action potential duration dispersion. The expression of Cx43 was reduced and Ser255-phosphorylated Cx43 was increased. A decrease of β-catenin at nucleus, which functions as TCF/LEF binding factor transcriptional activator of Cx43, preceded Cx43 alteration and modified Cx43 transcription. These alterations were prevented by RAAS blockade. In conclusions, ID-R might contribute to arrhythmogenesis during development of heart failure, and RAAS blockade might be an upstream therapy for ventricular arrhythmias.

2S39G-2

Dynamics of cardiac excitation wave propagation and electrophysiological mechanisms of sustained tacharrhythmias

Honjo, Haruo; Kodama, Itsuo; Kamiya, Kaichiro (*Dept. Cardiovasc.Res., Res. Inst. Environ. Med., Nagoya Univ., Nagoya, Japan*)

Coordinated propagation of action potentials in the heart depends on intercellular current flow through gap junction (GJ) channels. Deranged expression and/or organization of GJ proteins (connexines) in cardiac muscle have been demonstrated in a variety of pathological conditions, such as myocardial ischemia, inflammation and hypertrophy, and alterations of GJ function are known to provide electrophysiological substrates for sustained cardiac arrhythmias. In addition, propagation of cardiac excitation waves is affected by source-to-sink balance of intercellular current through GJ channels. For example, when the excitation wave front is convex, conduction velocity waves is decreased compared to that of a flat wave front, because the local excitatory current supplied by upstream excited cells distributes a large unexcited area downstream. Such wave front curvature-dependent source-to-sink mismatch is supposed to play essential roles in the genesis of spiral-type functional reentry of myocardial excitation waves that maintains cardiac fibrillation and tachycardia. Recent advances in high-resolution optical action potential mapping techniques with the aid of voltage-sensitive dyes enable quantitative assessment of cardiac excitation wave dynamics in isolated heart preparations, and provide useful information to reveal electrophysiological mechanisms underlying cardiac sustained tachyarrhythmias.

2S39G-3

Simulation study of excitation conduction in human atrioventricular node using action potential models constructed from messenger RNA data

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Because of difficulty to obtain human heart for electrophysiological study, there are few models of the human cardiac action potential. Recently, we have developed action potential models for single human cardiac conduction system cell based on the human right atrial cell action potential using the expression of ion channel messenger RNAs (mRNAs). In this study, we focused on action potential conduction between atria and ventricles, especially in the atrioventricular (AV) node. We constructed simplified a one-dimensional anatomical model from the right atrium to the bundle of His via the AV node with fast and slow conduction pathways. Using this model, we simulated action potential conduction. During sinus rhythm, the fast pathway acted as a primary conduction pathway. When stimuli with short coupling interval corresponding to atrial fibrillation (AF) seen frequently in clinically were applied to the atrium, conduction in the slow pathway was also observed. In addition, we could also simulate a Wenckebach periodicity and effects of ion channel blockers and β blocker to control ventricular rate during AF. Our model is useful to investigate the characteristics of AV node and the effects of antiarrhythmic drugs.

2S39G-4

Theoretical Studies on the Mechanisms of Cardiac Excitation Propagation under Atrial Structural Remodeling

Ashihara, Takashi (*Dept. of Cardiovascular Medicine, Shiga Univ. of Medical Science, Otsu, Japan*)

Background : It is widely believed that collagen accumulation in atria is the fundamental mechanism of structural remodeling under chronic atrial fibrillation (AF). However, little is known about the precise role of the atrial structural remodeling in the chronicity of AF. **Methods :** To elucidate this issue, we repeated simulations of excitation propagation in the model of human atrium with or without structural remodeling (gap junction remodeling, fibroblast proliferation, and collagen accumulation), and we analyzed details of the electrophysiological changes of atrial tissue. **Results :** (1) The gap junction remodeling markedly decreased the longitudinal-to-transverse ratio of conduction velocity (CV) from 3.5 to 2.2 ; however, this did not cause conduction disturbance, resulting in AF. (2) Due to electrotonic effects of fibroblasts, of which resting membrane potential was around -50 mV, on myocytes, the fibroblast proliferation shortened the action potential duration (APD) and depolarized the resting membrane potential of the myocytes. (3) As the result of the fibroblast electrotonic influence, 4 or more 6.5-pF fibroblasts coupled to a 100-pF myocyte decreased CV, and more than 10 fibroblasts caused conduction block. (4) Both the APD shortening and the CV decrease by the fibroblast proliferation were more pronounced at shorter diastolic intervals during AF. (5) Such electrophysiological changes were not attributed directly to collagen accumulation alone. **Conclusion :** This study provides mechanistic insight into the role of structural remodeling in the AF chronicity.

Symposium 40
Molecular mechanisms of oxidative stress–resistance induced by a new gas mediator

(March 28, 13 : 20–15 : 20, Room H)

2S40H-1

Molecular hydrogen influences gene expression profiles of rodent organs in healthy and diseased states

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Molecular hydrogen is a hopeful agent for oxidative stress-related and/or inflammatory disorders. However, the molecular mechanism for these therapeutic effects of hydrogen still remains poorly understood. To elucidate possible mechanism of in vivo effect of hydrogen, we examined whether molecular hydrogen alters gene expression levels in normal mouse livers by DNA microarray analysis. We identified 140 mouse genes that were upregulated (31 genes) or downregulated (109 genes) by administration of hydrogen in the form of hydrogen-containing air (HCA) and hydrogen-rich water (HRW). Ingenuity Pathway Analysis revealed that hydrogen influenced expression of NF-κB- and NFAT-regulated genes. We next examined whether the gene expression levels were influenced by the route of hydrogen administration, and found that HRW had potent effects on gene expression in systemic organs, even though only rapid and transient increase of hydrogen concentration was observed in arterial blood after oral HRW administration, suggesting that hydrogen may be a systemic gene-expression modulator that acts in a concentration-independent manner. In addition, we investigated gene expression profiles after hydrogen administration using NASH and ARDS model animals and observed that hydrogen were effectively suppressed disease-related gene expressions in these model animals.

2S40H-2

Molecular mechanism of the inhibitory effect of hydrogen on inflammation

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Molecular hydrogen exhibits beneficial effects in a number of disorders. The effect of hydrogen has been ascribed to the reduction of oxidative stress. Based on our studies on type I allergy, we previously proposed modulation of signal transduction as another mechanism for the hydrogen effect. In an attempt to determine if hydrogen inhibits signal transduction also in other disease models, we examined the hydrogen effect on lipopolysaccharide / interferon-γ (LPS / IFN-γ)-stimulated inflammatory responses in murine macrophage RAW264 cells. Hydrogen treatment reduced LPS/IFN-γ-stimulated induction of inducible isoform of nitric oxide synthase (iNOS) and production of nitric oxide (NO). Hydrogen inhibited LPS/IFN-γ-stimulated phosphorylation of apoptosis signal-regulating kinase 1 (ASK1) and its downstream signaling molecules including p38 MAP kinase, JNK and IκBα. However, hydrogen did not affect LPS/IFN-γ-stimulated activation of NADPH oxidase and production of reactive oxygen species (ROS). Finally, oral intake of hydrogen-rich water ameliorated anti-type II collagen antibody-induced arthritis in mice, a model for human rheumatoid arthritis. These results suggested that hydrogen inhibits inflammation in part through modulation of signal transduction. Taken together, our results supported our hypothesis that molecular hydrogen modulates signal transduction and acts as a signal modulator.

2S40H-3

Molecular hydrogen as a radioprotector : Possible mechanisms of hydrogen antioxidant activity

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Because prompt elimination of radiation-induced reactive oxygen species should protect lung tissue from damaging effects of irradiation, we investigated the possibility that H_2 could serve as a radioprotector. Indeed, *in vitro* experiments showed that a high concentration of molecular hydrogen (H_2) reduced irradiation-induced hydroxyl radicals in media and in cultured cells, and protected lung epithelial cells from damage caused by oxidative stress. We also found that H_2-treatment reduced the severity of irradiation-induced acute oxidative injury and apoptotic response in mouse lungs. Five months after irradiation, chest micro-CT and pathological findings revealed that consumption of hydrogen-rich water suppressed lung fibrosis. However, molecular mechanisms underlying the remarkable effect with a small amount of H_2 remain to be elucidated. Using strict regulation of H_2 and O_2 concentrations, we found that pretreatment of cells with H_2 suppressed the H_2O_2-induced cell death, whereas posttreatment did not. H_2-treatment enhanced mitochondrial membrane potential and cellular ATP accompanying a decrease in reduced glutathione and an increase in superoxide. Nuclear translocation of Nrf2 and increase in antioxidative enzymes of the treated cells indicate the possibility that a mild stress with H_2 induce an increased resistance to exacerbated oxidative stress. We propose here that H_2 functions both as a radical scavenger and "a hormetic effector" against oxidative stress.

2S40H-4

The effect of hydrogen-enriched dialysate on redox state of albumin

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Background : Oxidative stress characterized by decrease of reduced human serum albumin (HSA) is closely related to high incidence of cardiovascular disease and mortality among chronic kidney disease (CKD) patients treated with dialysis therapy. Since effective method to suppress oxidative stress in CKD patients is limited in the clinical setting, novel and safe approach is needed.
Methods : We applied hydrogen-enriched dialysate to CKD patients treated with hemodialysis and peritoneal dialysis as a novel method to reduce oxidative stress. The effect of hydrogen-enriched dialysate on HSA-redox was studied.
Results : Single administration of hydrogen-enriched peritoneal dialysate significantly increased reduced HSA fraction. Such effect was not observed after administration of standard peritoneal dialysate. Hemodialysis using hydrogen-enriched dialysate reduced oxidized HSA more effectively than standard hemodialysis. Such reductive effect of dissolved hydrogen was not observed in *in-vitro* experiment, suggesting hydrogen enhances reductive property of living cells such as endothelial and blood cells.
Conclusion : Hydrogen-enriched dialysate offers CKD patients safe and effective anti-oxidative treatment.

2S40H-5

Oxidative stress-resistance induced by molecular hydrogen

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The central nervous system (CNS) white matter (WM) ischemia is an important clinical problem and may produce injury, in part, by ROS-induced mitochondrial dysfunction. Using the mouse optic nerve (MON) WM model, we tested whether hydrogen (H_2) in drinking water reduced functional WM ischemic injury. Functional integrity of MON was determined by quantitatively monitoring the area of MON compound action potential (CAP) in vitro. A 60 min period of oxygen and glucose deprivation (OGD) caused prompt loss of the CAP followed by an average 20% recovery. After 10-14 days of H_2-water, the CAP area did not disappear during ischemia and recovered to a significantly great extent during reperfusion. Immunostaining of axonal neurofilament also showed significant protection by previous drinking of H_2-water. Accumulation of nuclear 8-oxoguanine (8-oxoG), a marker of oxidative DNA damage, was observed mainly in oligodendrocytes after OGD. The level of 8-oxoG and lipid peroxidation after OGD were significantly reduced in optic nerves from H_2-water drinking mice. The importance of these observations is that ischemic protection of myelinated CNS WM by drinking H_2-water provided partial protection in a novel manner, suggesting oxidative stress-resistance and intriguing therapeutic options.

Symposium 41
From translation to molecular chaperone— Physiological function and pathology

(March 28, 13 : 20–15 : 20, Room I)

2S41I-1

The biological regulation by the endoplasmic reticulum stress response

Imaizumi, Kazunori (*Depart. Biochemistry, Graduate School of Biomedical & Health Sciences, University of Hiroshima, Hiroshima, Japan*)

Eukaryotic cells can adapt to endoplasmic reticulum (ER) dysfunction by producing diverse signals from the ER to the cytosol or nucleus. These signaling pathways are collectively known as the unfolded protein response (UPR). The canonical branches of the UPR are mediated by three ER membrane-bound proteins : PERK, IRE1 and ATF6. These ER stress transducers basically play important roles in cell survival after ER stress. Recently, novel types of ER stress transducers that share a region of high sequence similarity with ATF6 have been identified, Luman, OASIS, BBF2H7, CREBH, and CREB4. Despite their structural similarities with ATF6, differences in activating stimuli, tissue distribution and response element binding indicate specialized functions of each member on regulating the UPR in specific organs and tissues. In this symposium, I would like to present that both these new members of ER stress transducers and canonical UPR signaling are involved in functional regulation such as osteogenesis, chondrogenesis, and development of goblet cells in intestine. Furthermore, regulatory mechanisms for the activation of new types of ER stress transducers including OASIS and BBF2H7 will be referred.

2S41I-2

Gamma-oryzanol, a major component of brown rice, improves feeding behavior by decreasing hypothalamic endoplasmic reticulum stress in mice

Kozuka, Chisayo[1]; Yabiku, Kouichi[1]; Takayama, Chitoshi[1]; Matsushita, Masayuki[1]; Oyadomari, Seiichi[2]; Shimabukuro, Michio[3]; Masuzaki, Hiroaki[1] (*[1]Graduate School of Medicine, University of the Ryukyus, Japan; [2]Institute for Genome Research, University of Tokushima, Japan; [3]University of Tokushima Graduate School of Health Biosciences, Japan*)

Compared to refined white rice (WR), brown rice (BR) is known to prevent obesity and type 2 diabetes in humans. However, the underlying mechanisms still remain unclear. We thus investigated the effects of BR and its component, γ-oryzanol (Orz), on feeding behavior in mice. To assess the preferences for dietary fat, mice were simultaneously allowed free access to chow diet and high fat diet (HFD). BR significantly attenuated the preference for dietary fat, thereby leading to suppression of body weight gain. Under HFD, expression levels of endoplasmic reticulum (ER) stress-related gene in hypothalamus were significantly decreased in BR-fed group compared with WR-fed group. Compared with vehicle-treated mice, the preference for dietary fat was decreased in mice treated with 4-phenyl butyrate (a chemical chaperone), raising the possibility that hypothalamic ER stress influences the preference for dietary fat. In vitro studies showed that Orz significantly reduced ER stress. To examine the effect of Orz on feeding behavior, mice were treated with Orz. Orz did attenuate the preference for dietary fat. (Kozuka C et al. *Diabetes* in press, **2012**)
These data suggest that Orz may open a fresh avenue to treat obesity-diabetes syndrome thorough modifying feeding behavior.

2S41I-3

Transformation of protein elongation factor into heat shock response transcription factor during the stress responses

Matsushita, Masayuki (*Graduate School of Medicine, University of the Ryukyus, Okinawa, Japan*)

In the process of clarifying stress response mechanisms via protein translational regulation, we discovered a change brought about by splicing modified elongation factor 1Bδ (eEF1Bδ), a known translation factor, to a molecule with a domain that directly binds to heat shock element (HSE) allows the molecule to function as a transcription factor. While eEF1Bδ is specifically localized in the cytoplasm, the long isoform of eEF1Bδ (eEF1BδL is localized in the nucleus and induces heat shock element (HSE)-containing genes in cooperation with heat-shock transcription factor 1 (HSF1). In addition, eEF1BδL directly binds to HSE oligo DNA in vitro and associates with HSE containing the HSPA 6 promoter region in vivo. Splicing has been known to play an important role in finite genetic diversification. In flies, sex determination is known to be based on a change brought about by splicing cascade, but in mammals, prior to the present discovery, there have been no reports of splicing resulting in clear changes in protein function (translation and transcription factors). The importance of splicing in organisms to achieve finite genetic diversification can clearly be seen by the fact that splicing modifies a translation factor into a transcription factor that induces chaperon proteins, which regulates necessary processes such as protein folding after stress responses.

2S41I-4

Fine-tuning of protein translation by tRNA modification and its pathological relevance

Wei, Fanyan (*Dept. Mol. Physiol. Faculty of Life Sci. Kumamoto. Univ. Kumamoto, Japan*)

All transfer RNAs contain chemically modified nucleotides. Particularly, methylthiolation in adenine of postion 37 is found through bacteria to human. However, the modification enzyme and the physiological role of methylthiolation have remained largely unknown. We have identified two enzymes, Cdkal1 and Cdk5rap1, which catalyzes methylthiolation of cytosolic and mitochondrial tRNAs in mammalian cells respectively. Cdkal1 has been identified as a risk gene for type 2 diabetes. The enzyme exclusively methythiolates cytosolic $tRNA^{Lys}$ (UUU) and regulates the decoding fidelity of Lys codon. Genetic deletion of Cdkal1 in mouse pancreatic b-cells compromised incorporation of lysine residue in proinsulin, resulting in decrease of mature insulin secretion and development of diabetes. On the other hand, Cdk5rap1 is a mitochondria-localizing enzyme which specifically methylthiolates mitochondrial tRNAs. The methylthiolation is critical to prevent frameshifting during translation of the corresponding codons in mitochondria. Genetic deletion of Cdk5rap1 in mice attenuated mitochondrial protein translation, resulting in decrease of mitochondrial oxidative phosphorylation activity. Because of the mitochondrial dysfunction, Cdk5rap1 knockout mice showed abnormal metabolic profiles that were resemble to symptoms observed in human mitochondria diseases. Taken together, our results demonstrate that methylthiolation of tRNAs is critical for fine-tuning of protein translation, which is indispensable to maintain cellular homeostasis.

2S41I-5

Regulation of local protein synthesis in axons by trans-acting factors for translation : implication in neurological disorder

Sasaki, Yukio (*Dept Mol Pharmacol Neurobiol, Yokohama City Univ Grad Sch Med, Japan*)

Local protein synthesis in distal portion of elongated axons has been recognized as a fundamental mechanism to supply immediately required proteins for axon extension and pathfinding in response to extracellular stimuli such as neurotrophic and axon guidance factors. The molecular mechanism of local protein synthesis involves the recognition of cis-acting elements in the 5'- and/or 3'-untranslated region (UTR) by trans-acting factors such as specific binding proteins and microRNAs (miRNAs). However, it is unclear how trans-acting factors regulate local translation in axons and growth cones for axonal functions. We found that phosphorylation of zipcode binding protein 1 (ZBP 1), a β-actin mRNA binding protein, in growth cones play a critical role in translational regulation of β-actin mRNA for growth cone turning. Furthermore, Fragile X Mental Retardation Protein (FMRP), a protein binding specific mRNAs and miRNAs, regulate growth cone morphology. Aberrant regulation of local translation in axons is one of possible mechanism of neurological disorders such as Fragile X syndrome. These findings provide new insight into mechanism of fine-tuning for axonal function by local protein synthesis.

Symposium 42
The mechanisms of the optimization and breakdown of the stratified circulating system

(March 28, 16 : 00–18 : 00, Room D)

2S42D-1

Multicellular networks in coronary artery formation and their pathophysiological implication

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Recent progress in cell lineage analysis has revealed that the heart is composed of various cell types of different origins. The coronary artery, although previously thought to develop by outgrowth from the aortic root, now proved to be formed by ingrowth of angiogenic precursors. Three different embryonic tissues, the proepicardium, the sinus venosus and the endocardium, have been reported as the origins of coronary endothelial cells in mice and birds, whereas their relative contributions remain controversial. The proepicardium has also been reported to give rise to coronary smooth muscle cells. On the other hand, experimental and clinical evidences have suggested heterogeneity of smooth muscle cell populations in the coronary artery. In our recent study, we found that neural crest cells from the preotic region migrate into the heart and differentiate into coronary artery smooth muscle cells in the proximal region. Ablation of the preotic neural crest causes abnormalities in coronary septal branch and orifice formation. Appropriate migration and deployment of neural crest cells and subsequent smooth muscle differentiation require multicellular interactions involving endothelin signaling possibly through G 12 / 13-dependent mechanisms. These findings on cellular origins and heterogeneity will provide a fundamental basis for understanding the pathophysiology of coronary artery disease.

key-word :
Heart Development, Coronary Artery, Smooth Muscle, Neural Crest, Endothelin

2S42D-2

A novel concept on transcription mechanism derived from epigenomics on vascular cells

Wada, Youichiro (*LSBM, RCAST, the Univ. of Tokyo, Japan*)

Since chronic inflammation of endothelial cell is the first stage of atherogenesis, we stimulated endothelial cells using a representative inflammatory stimulant, tumor necrosis factor-alpha (TNFa), and observed it regulates the induction and reduction of more than 500 genes in a orchestrated time course manner. To obtain a comprehensive view of a single transcription cycle caused by TNFa, we switched on transcription of five long human genes (longer than 100 kbp) with TNFa and monitored (using microarrays, RNA fluorescence in situ hybridization, and chromatin immunoprecipitation) the appearance of nascent RNA, changes in binding of Pol II and two insulators (the cohesin subunit RAD21 and the CCCTC-binding factor CTCF), and modifications of histone H3. Activation triggers a wave of transcription that sweeps along the genes at approx. 3.1 kbp/min ; splicing occurs cotranscriptionally, a major checkpoint acts several kilobases downstream of the transcription start site to regulate polymerase transit, and Pol II tends to stall at cohesin/CTCF binding sites. 3C data revealed transcription of one of the five big genes is accompanied with smaller TNFa responsive genes on the same chromosome. These results suggested that transcription of TNFa responsive genes is performed by a single transcription complex, which provides a platform for both transcription and splicing. By identifying special proximity of TNFa responsive genes by 3C-based technique and by proteomic approach combined with chromatin immunoprecipitation, we are trying to elucidate the identity of transcription complex in TNFa stimulated endothelial cells.

2S42D-3

Molecular Mechanisms of Dynamic Cardiovascular Adaptation from Fetal to Neonatal Life

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Increase in oxygen tension and decline in placental hormones, such as prostaglandin E2 (PGE2), are major factors that make a rapid transition from fetal to adult circulatory system, such as the closure of ductus arteriosus (DA), a fetal bypass vessel. We found that, during fetal period, PGE2-EP4 signaling decreased elastic fiber formation through degradation of the cross-linking enzyme lysyl oxidase, and increased hyaluronan-mediated intimal thickening in the DA. After birth, decline in serum concentration of PGE2 together with raising oxygen tension leads to constriction of the DA. We also found that, once PGE2 concentration was decreased, lysyl oxidase was no longer degraded, resulting in increasing cross-linking of collagen fibers to make the DA fibrous tissue. Oxygenation further enhanced intimal thickening via secretion of basic fibroblast growth factor in the neonatal DA. The transient receptor potential melastatin 3 (TRPM3) which was inhibited by progesterone in utero promoted constriction of the postnatal DA by decrease in plasma osmolarity. These data suggest that fetal environment promotes vascular remodeling as a preparation for transition to neonatal life, and that drastic environmental change at birth further promotes the remodeling to complete the adaptation.

2S42D-4

Pathophysiological role of blood and lymphatic vessels in the skin

Hirakawa, Satoshi (*Dept. of Dermatology, Hamamatsu Univ. School of Medicine, Hamamatsu, Japan*)

Vascular system plays a crucial role in promoting physiological maintenance and pathological alteration of the skin. Vascular endothelial growth factor (VEGF)-A induces vascular permeability for cutaneous tissue homeostasis. Meanwhile, the targeted overexpression of VEGF-A in mouse skin promotes enhanced leakage from blood vessels, leading to the development of chronic skin inflammation. Cutaneous lymphatic vessels play an essential role in absorbing and transporting interstitial tissue fluid in physiological condition. However, during cancer progression, tumor cells induce new lymphatic vessel growth within the primary site, leading to an accelerated formation of tumor metastasis. This presentation provides recent topics to better understand the molecular mechanism of skin disease.

Symposium 43
Why do microglia exist in the brain? As a target for treatment of neurological disorders

(March 28, 16 : 00–18 : 00, Room E)

2S42D-5

Use of nanoparticle to analyze vasculature in diseases

Kano, Mitsunobu R (*Department of Pharmaceutical Biomedicine, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan*)

Effective treatment of "intractable" solid tumors is one of important goals of chemotherapy using nano drug delivery system (nanoDDS). To realize this, we need to investigate why intractable tumors, such as pancreatic cancer, are "intractable". Our study suggests that the structure of vasculature in those tumors is different from what we observe in popular tumor animal models.

We used xenografts of BxPC3 cell line derived from pancreatic cancer as a model of intractable cancer, and those of C26 cell line derived colon cancer as an ordinary cancer model, and compared them. The BxPC3 model has more fibrotic stromal components, whereas the C26 model has less. Moreover, the former has more pericyte-covered vasculature than the latter. As a result, nanoparticle accumulated autonomously in the latter, whereas it accumulated hardly in the former. To overcome this difficulty in accumulation in the pancreatic cancer model, which is a factor of intractability, we used TGF-beta inhibitor. The inhibitor reduces pericytes, and increased accumulation of nanoparticle in the model and led to significant growth-inhibitory effect. Yet, use of VEGF inhibitor, reported to normalize vasculature, did not increase accumulation in the stroma rich models. VEGF inhibitor did, however, increase distribution of nanoparticle in the colon cancer model, although TGF-beta inhibitor did not in the model.

We further analyzed vascular structure in human tissues of pancreatic or colon cancers. Vasculature in pancreatic cancer was firmly covered by pericytes, whereas that in colon cancer was not covered by pericytes, as in the animal models.

According to these observations, we may need to know the structure of tumor vasculature in various cancers, and to optimize it to maximize the effect of nanoDDS.

2S43E-1

Microglial Circadian Clock Controls Microglia–Synapse Interactions in the Healthy Brain

Nakanishi, Hiroshi (*Department of Aging Science and Pharmacology, Faculty of Dental Sciences, Kyushu University, Fukuoka, Japan*)

Resting microglia in the healthy brain are very dynamic, constantly extending and shrinking their processes. Why dose the brain invest so much energy to microglia? There is increasing evidence that resting microglia play many physiological functions including regulation of neurogenesis through the phagocytosis of adult new born cells, maintenance of synaptic homeostasis and reorganization of synapses by elimination of synapses with low activities. More recently, we have found that a circadian clock in microglia tightly regulates the total length of microglial processes, which in turn regulate synaptic activities through interaction with synapses. During the light period, microglia retract their processes resulting in decreased synaptic activities, which may be necessary for intensive synaptic activities during the subsequent dark period in mice. Our results suggest that microglial processes operate synapses to generate circadian synaptic activities in the healthy brain. Microglia utilize two microglia-specific molecules, P2 Y_{12} receptors and cathepsin S, which are tightly regulated by the circadian clock. Our findings may aid in understanding not only microglial physiology but also synapse homeostasis in the normal adult brain. Therefore, microglia are worth investing so much energy.

2S43E-2

Optogenetical Control of Microglial Activation

Sawada, Makoto (*Dept. Brain Function, Research Institute of Environmental Medicine, Nagoya Univ., Nagoya, Japan*)

Microglia, macrophage-like cells in the CNS, are multi-functional cells ; they play an important role in removal of dead cells or their remnants by phagocytosis in the CNS degeneration as well as are one of important cells in the CNS cytokine network. They are thought to be originated from mesoderm, and to be similar cells to other tissue-resident macrophages. As macrophages, activated microglia have been shown to remove potentially deleterious debris and promote tissue repair by secreting neurotrophic factors at the neuronal injury sites, however, they can release potentially cytotoxic substances in vitro, and at least so-called fully activated form of microglia which are observed at the injury site in AIDS dementia is neurotoxic. These suggest that some factor (s) may contribute to change microglial phenotype from protective to toxic, but the detail is not clear. Recently we generated channelrhodopsin-mutant protein expressing microglia, Ra2_GR and 6-3_GR. Channelrhodopsin is an ion channel activated by light irradiation. Intracellular sodium ion increased by light irradiation in both Ra2 _GR and 6-3_GR accompanied by increase of mRNA expression such as pro-inflammatory cytokines, chemokines and iNOS. This technique can control microglial activation, therefore, it may provide a new strategy for repair/regeneration of neural and oligodendrocytic damages.

2S43E-3

Agents modulating neuroprotective and neurotoxic functions of microglia and their application to the pathologic brains

Tanaka, Junya (*Graduate School of Medicine, Ehime University, Ehime, Japan*)

Microglia have long been known have both neurotoxic and neuroprotective effects. Lipopolysaccharide (LPS), a Toll-like receptor (TLR) 4 ligand, is the most common agent used to induce the neurotoxic phenotype of microglia in vitro. LPS induces microglial expression of proinflammatory mediators and abolishes neuroprotective phenotypes. Thus, LPS induces the neurodestructive nature of microglia. When a co-culture of microglia and neurons was incubated with LPS, neurons underwent degeneration, mainly through NO-dependent damage. To prevent this neurodegeneration, many agents were added to the co-culture, and their anti-inflammatory effects were examined. The glucocorticoid dexamethasone, a cytokine mixture (GM-CSF+IL-3), beta-adrenergic agonists, and a sedative/hypnotic, bromvalerylurea, were found to markedly suppress LPS-induced iNOS expression and neuronal degeneration. Furthermore, these agents were applied to a 6-OHDA-induced rat Parkinsonism model, and their effects on microglia in the substantia nigra were investigated. The agents prevented nigral dopaminergic neuronal loss in the rat model and modulated microglial reactions in terms of morphology and function. The responses of other glial cells, astrocytes and NG2 cells, in the affected substantia nigra were also investigated. In this symposium, the potential of these anti-inflammatory agents as novel treatments for neurological disorders accompanied by neuronal degeneration will be discussed.

2S43E-4

Therapeutic strategies against neurodegenerative disorders targeting microglia

Suzumura, Akio (*Department of Neuroimmunology, RIEM, Nagoya University, Aichi, Japan*)

Microglia are monocyte-macrophage lineage cells, while other glial cells are neuroectodermal origin. Accumulation of microglia is commonly observed around degenerating neurons. There, microglia produce a variety of factors and function both neurotoxic and neuroprotective. Thus, accumulation of glia in various neurological disorders is not a static scar, gliosis, but more actively involved in degeneration and regeneration as neuroinflammation. We have shown previously that the most neurotoxic factor from activated microglia is glutamate, and that the suppression of glutamate release from microglia results in amelioration of disease progression in animal models of neurodegenerative disorders. On the other hands, when exposed to harmful stimuli, neurons also produce various factors as help me signals. Recently, we found that a CX3C chemokine, fractalkine (FKN), interleukin-34 (IL-34) and fibroblast growth factor 2 (FGF2) were secreted from damaged neurons. FKN, IL-34 and FGF2 differently activated microglia to rescue neurons by upregulating phagocytosis of toxicants or damaged debris, and production of anti-oxidant enzyme. The bi-directional interaction between neurons and microglia is important for understanding of chronic neuroinflammation, and gives us clues for future therapeutic strategy against neurodegenerative disorders.

Symposium 44
New development of H^+ dynamics and its role in cell function

(March 28, 16 : 00–18 : 00, Room F)

2S44F-1

Roles of hydrogen and chloride ions in proliferation of MKN28 Human Gastric Cancer Cells

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Cancer cells generate acidic microenvironments by producing a lot of acidic metabolites due to high metabolic condition, but keep cytosolic pH (pH_c) normal or higher than normal cells, suggesting that activity/expression of H^+ transporting systems in cancer cells is higher than normal cells. In the present study, we tried to identify roles of Na^+/H^+ exchanger (NHE), one of the most important H^+ transporters, in proliferation of human gastric cancer MKN28 cells expressing NHE. Ethyl-isopropyl amiloride (EIPA, an NHE inhibitor) caused G_0/G_1 arrest suppressing proliferation of MKN28 cells with no effects on pH_c, but reduction of $[Cl^-]_c$. Co-application of EIPA with DIDS (an inhibitor of Cl^-/HCO_3^- exchangers such as anion exchanger (AE) and Na^+-driven Cl^-/HCO_3^- exchanger (NDCBE)) decreased pHc, suggesting that DIDS-sensitive AE and/or NDCBE keep pH_c normal via stimulation of HCO_3^- uptake coupled with Cl^- release under NHE-inhibited conditions. EIPA-induced lowered $[Cl^-]_c$ phosphorylated MAKPs, leading to up-regulation of p21 expression, resulting in G_0/G_1 arrest. Based on these observations and ionic environment-based electro-chemical potentials, we conclude that EIPA suppresses proliferation of MKN 28 cells through up-regulation of p21 expression via reduction of $[Cl^-]_c$ caused by NDCBE- but not AE-mediated compensation for keeping pHc normal under NHE-inhibited conditions. This is the first report that NHE inhibition suppresses proliferation of cancer cells via reduction of $[Cl^-]_c$ but not pH_c.

2S44F-2

Anti-tumor cell effects of vacuolar H^+-ATPase inhibition

Hiruma, Hiromi (*Dept. of Physiol., Kitasato Univ. Sch. of Med., Sagamihara, Japan*)

Vacuolar H^+-ATPase (V-ATPase) is expressed in acidic organelle and maintains low pH inside the organelles. This enzyme is recently suggested to be a target for cancer therapy, since V-ATPase inhibitors inhibit tumor cell proliferation and tumor growth. This study is conducted to investigate the effects of V-ATPase inhibitor bafilomycin A1 (Baf) on intracellular pH distribution, cell death, and cell division in human osteosarcoma cell line Saos-2. The pH indicator Lisotracker yellow/blue showed strongly acidic organelles and almost neutral cytosol in Saos-2 cells. Treatment with Baf caused extrusion of H^+ from the organelles, transient formation of vacuoles around the individual organelles, and acidification of cytosol. Time-lapse microscopy revealed that continuous treatment of Saos-2 cells with Baf caused both acute and delayed cell death. This treatment also resulted in a marked decrease in cell division associated with a decrease in phospho-histone H3 (an M-phase marker)-positive cells and with a slight decrease in 5-bromo-2'-deoxy-uridine (BrdU, an S-phase marker)-incorporated cells. Time-lapse cell cycle protein expression showed that most Baf-treated cells remained expressing Cdt1 protein (a G1 marker) but did not progress to express Geminin (an S/G2/M-phase marker). These results indicate that inhibition of V-ATPase by Baf induces cell death and inhibition of cell division, which may be attributed to cell cycle arrest before M-phase in addition to the tumor cytotoxicity of Baf. All of these effects may be involved in the acidification of cytosol. Blockade of V-ATPase can be a therapeutic approach for cancer.

2S44F-3

pH regulation by the Na^+/H^+ exchanger 1 : upstream and downstream signaling pathways leading to cardiac hypertrophy

Wakabayashi, Shigeo (*National Cerebral and Cardiovascular Center, Osaka, Suita, Japan*)

Since the first molecular cloning of the ubiquitous pH-regulating transporter Na^+/H^+ exchanger 1 (NHE1) by Dr. Pouyssegur's group in 1989, particular attention was focused on its transport mechanism, regulation and pathological significance. However, there are two fundamental, but yet fully unresolved questions on NHE1 molecule, i.e., i) how NHE1 is activated in response to external stimuli such as hormones, and ii) how activation of NHE1 regulates the downstream target molecules via its cytosolic ionic changes. For the first question, we provided evidence that hormonal activation of NHE1 occurs via direct interaction of diacylglycerol (or its potent analogue, phorbor esters) with the lipid-interacting domain (aa 542-598) of NHE1, but not via protein kinase C. For the second question, we indentified a novel NHE1-binding partner, Ca^{2+}-dependent phosphatase calcineurin (CaN), which interacts with the 6-residues motif ^{715}PVITID^{720} of NHE1, and found that their interaction mediates the amplification of the downstream signaling pathway via CaN-dependent transcription factor NFAT. We show that such hormone-induced NHE1 activation and subsequent downstream signal amplification can be a mechanism of NHE1-dependent cardiac hypertrophy, which is an adaptive response of hearts to mechanical stress. We propose that the cytoplasmic domain of NHE1 serves as a platform to transmit the ionic signals produced by NHE1 to the downstream targets, as well as the regulatory machinery for ion transport.

2S44F-4

Hypoxia Signalling, pHi Regulation & Tumour Metabolism. Novel Therapeutic Approaches

Pouyssegur, Jacques; Marchiq, Ibtissam; Le Floch, Renaud; Chiche, Johanna; Roux, Daniele (*Institute of Research on Cancer and Aging, Nice(IRCAN), Univ. of Nice, Centre A. Lacassagne, Nice, France*)

Early on in evolution, oxygen sensing emerged, as a central control mechanism of energy metabolism and vasculogenesis. At the heart of this regulatory system is the Hypoxia-Inducible Factor, HIF-1, which controls the expression of, among other gene products, VEGF-A, Angiopoietin-2 and Notch-ligand, three key angiogenic factors in vertebrates. This finding has placed the hypoxia-signaling pathway at the forefront of nutritional control. HIF-1 can induce a vast array of gene products controlling glycolysis, intracellular pH (pHi), angiogenesis, cell migration and invasion, and so has become recognized as a strong promoter of tumor growth. It is therefore not surprising that HIF-1 also promotes access to another source of nutrients by inducing macroautophagy. In this presentation, we will highlight some of the HIF1-induced gene products, carbonic anhydrases IX and XII (CAs) and monocarboxylate transporters (MCTs), which regulate intracellular pH (pHi) by controlling export of metabolically-generated acids (carbonic and lactic acids). We report that targeting pHi-regulated processes severely restricts tumour growth, a process that compromises glycolysis-generated ATP levels. We propose that membrane-bound carbonic anhydrases (CAIX, CAXII), monocarboxylate transporters (MCT1 and MCT4) as well as their chaperon Basigin/EMMPRIN/CD 147), which are associated with exacerbated tumor metabolism represent new potential targets for anticancer therapy.

3S45A-1

iPS technology-based cell therapy for damaged CNS and investigation of neural disorders

Okano, Hideyuki (*Department of Physiology, Keio University School of Medicine, Japan*)

The 2012 Nobel Prize of Physiology or Medicine was awarded for Shinya Yamanaka and Sir John B Gurdon for their discovery that "*for the discovery that mature cells can be reprogrammed to become pluripotent*". Stimulated by their achievements, there is an increasing interest in iPS technology for their application in medical science. We have been investigating the development of cell therapy for injured spinal cord using iPS cells-derived neural stem/progenitor cells (Okano et al., Circulation Res, 2013 ; Nakamura and Okano, Cell Res, 2013). So far, we have induced the differentiation of mouse and human iPS cells toward neural stem cells (Miura et al., Nat Biotech, 2009) and transplanted them into mouse and non-human primate spinal cord injury models (Tsuji et al., PNAS, 2010 ; Nori et al., PNAS, 2011 ; Kobayashi et al., PLoS ONE, 2012). Consequently, the transplantation of these cells resulted in functional recovery without tumor formation upon selection of appropriate cell lines. The transplanted cells differentiated into neurons, astrocytes and oligodendrocytes. Both cell replacement and non cell autonomous trophic actions are likely to be responsible for the graft-induced functional recovery. In this symposium, I will also introduce our recent results on the characterization of patients-derived iPS cells as diseases models of Parkinson disease and Alzheimer disease (Yagi et al. Hum Mol Genet, 2011 ; Imaizumi et al., Mol Brain, 2012 ; Ito et al., Annals Neurol, 2012).

3S45A-2

Towards regenerative medicine strategy and new drug development for kidney diseases using iPSC technology

Osafune, Kenji (*Center for iPS Cell Research and Application(CiRA), Kyoto University*)

Regenerative medicine strategies using induced pluripotent stem cells (iPSCs) have been vigorously studied in multiple cell types and disorders. However, the differentiation method from iPSCs or embryonic stem cells (ESCs) into kidney lineage has not been fully developed. We have recently established efficient induction methods from human iPSCs into intermediate mesoderm (IM), an embryonic germ layer that gives rise to kidneys. The human iPSC-derived IM cells show the developmental potential to differentiate into multiple renal cell types included in adult kidney, such as glomerular podocytes and renal tubular epithelia, and to form three-dimensional renal tubular structures. We are now establishing induction methods from the human IM cells into renal progenitors to develop replacement therapies for chronic kidney diseases. On the other hand, it has been demonstrated that disease models using patient-derived iPSCs can be used to understand pathological mechanisms and discover new drug compounds in some intractable disorders. We have derived disease-specific iPSC lines from patients with hereditary renal diseases, such as autosomal dominant polycystic kidney disease (ADPKD), autosomal recessive polycystic kidney disease (ARPKD) and Alport syndrome, in parallel with developing efficient differentiation methods from human iPSCs into renal lineage cells, to create novel *in vitro* models for the intractable kidney diseases. I would like to talk about recent advances and future perspectives of regenerative nephrology and disease modeling research for kidney disorders.

Symposium 45
Current state and future of Regenerative Medicine

(March 29, 9 : 00–11 : 00, Room A)

3S45A-3

In vitro Reconstruction of Functional Mouse Seminiferous Tubules Supporting Germ Cell Differentiation

Ogawa, Takehiko (*Department of Urology, Yokohama City University Graduate School of Medicine*)

It is known that cells of testis tissues in fetal or neonatal periods have the ability to reconstruct the testicular architecture even after dissociation into single cells. This ability, however, has not been demonstrated effectively *in vitro*. In this study, we tried to reconstruct seminiferous tubules *in vitro* which could support spermatogenesis. Testis cells of neonatal mice were dissociated enzymatically into single cells. The cells formed aggregates in suspension culture and were transferred to on agarose gel to continue the culture with a gas-liquid interphase method, where a tubular architecture gradually developed during the following 2 weeks. Immunohistological examination confirmed Sertoli cells forming tubules and germ cells inside. With testis tissues of *Acr*-GFP transgenic mice, whose germ cells express GFP during meiosis, 38 out of 40 cell aggregates formed a tubular structure and 19 showed GFP expressions in their reconstructed tissues. Meiotic figures were observed in many tissues and round spermatids were occasionally confirmed histologically. In addition, we mixed cell lines of spermagonial stem cells (Germline stem cells, GS cells) into the testis cell suspension, and found the incorporation of GS cells in the tubules in 20 out of 32 reconstructed tissues. Those GS cells differentiated up to meiotic phase. This *in vitro* reconstruction technique will be a useful method for the study of testis organogenesis and spermatogenesis.

Symposium 46
Invitation to Translational research of Neurocardiology—Hyaperactivity of central sympatheticnerve

(March 29, 9 : 00–11 : 00, Room B)

3S45A-4

Generation of human liver tissue from an induced pluripotent stem cell–derived organ bud transplant

Takebe, Takanori (*Department of Regenerative Medicine, Yokohama City University Graduate School of Medicine*)

Since the discovery of embryonic stem cells in1981, decades of laboratory studies have failed to generate a complex vascularized organ such as liver from pluripotent stem cells, giving rise to the prevailing belief that in vitro recapitulation of the complex interactions among cells and tissues during organogenesis is considered to be essentially impractical. One possible approach to create a complex and vascularized organ is to recapitulate the cellular interactions during early organogenesis. Here, we show the generation of vascularised and functional human liver tissue from hiPSCs by transplantation of liver buds created in vitro (hiPSC-LBs). Specified hepatic cells self-organised into three-dimensional hiPSC-LBs by recapitulating organogenetic interactions between endothelial and mesenchymal cells. Immunostaining and gene expression analyses revealed resemblance between in vitro grown hiPSC-LBs and in vivo liver buds. Human vasculatures in hiPSC-LB transplants became functional by connecting to the host vessels within 48 hours. The formation of functional vasculatures stimulated the maturation of hiPSC-LBs into tissue resembling the adult liver. Highly metabolic hiPSC-derived tissue performed liver-specific functions such as protein production and human-specific drug metabolism without recipient liver replacement. Furthermore, transplantation of hiPSC-LBs onto mesentery rescued the drug-induced lethal liver failure model. To our knowledge, this is the first report demonstrating the generation of functional human organ from pluripotent stem cells. Although efforts must ensue to translate these techniques, our proof-of-concept, i.e. organ bud transplantation, provides a promising new approach towards regenerative medicine.

3S46B-1

Clinical implication of blood pressure variability in hypertension

Kario, Kazuomi (*Division of Cardiovascular Medicine, Jichi Medical University School of Medicine, Shimotsuke, Japan*)

There is growing evidence that excess variability in blood pressure (BP) is an independent risk of cardiovascular events. There are various BP variabilities such as visit-to-visit variability in clinic BP, day-by-day variability in home BP, and morning surge in ambulatory BP.

We first defined morning BP surge (MBPS) by ambulatory BP monitoring (ABPM), and demonstrated that excess MBPS is the risk of stroke independent of 24-br BP level and the nocturnal BP dipping status in hypertensives (Kario et al Circulation 2003 ; 107 : 1401-6). Excess MBPS is associated with the activation of sympathetic nervous system and renin angiotensin system, and makes vicious cycle with both large and small artery disease.

Both type of disrupted circadian BP rhythm, such as extreme-dipper pattern with excess nocturnal BP falls and non-dippers/riser pattern with nocturnal hypertension are more closely associated with stroke events independently of the 24-hr BP level, when compared with dipper pattern. The non-dipper/riser BP pattern is also associated with subclinical cerebrovascular disease such as silent cerebral infarcts and deep white matter disease, and brain atrophy, which are risk for cognitive and physical dysfunction in the elderly.

In the J-HOP (Japan Morning Surge Home Blood Pressure) study, we measured sleep BP using home BP monitoring (HBPM), and found that the HBPM-measured sleep BP was comparable to ABPM-measured sleep BP. The 24-hr perfect BP control including BPs during sleep and morning periods would achieve more effective prevention of cardiovascular disease.

3S46B-2

Abnormal Sympathoexcitaion Associated with 'Brain −Heart Interaction' Causes Cardiovascular Diseases

Kishi, Takuya (*Department of Advanced Therapeutics for Cardiovascular Diseases, Kyushu University Graduate School of Medical Sciences, Japan*)

In the cardiovascular diseases, abnormal prolonged sympathoexcitation is important. Sympathetic nerve activity is regulated by brain, and we have focused on rostral ventrolateral medulla (RVLM) in the brainstem, which is known as a vasomotor center. We have demonstrated that angiotensin II type 1 receptor (AT1R)-induced oxidative stress in the RVLM causes prominent sympathoexcitation in hypertensive model rats, and that apoptosis and inflammation iin the RVLM cause sympathoexcitation. Furthermore, we also recently have determined that "neuron-astrocyte uncoupling" associated with oxidative stress, chronic inflammation, and apoptosis in the RVLM causes abnormal sympathoexcitation in hypertensive rats. These findings could indicate that abnormal sympathoexcitation associated with "neuron-astrocyte uncoupling" in the RVLM causes cardiovascular diseases (Brain-Heart axis). Interestingly, the "neuron-astrocyte uncoupling" in the RVLM could be also occurred in ischemia-induced heart failure or dietary-induced metabolic model rats. These results suggest that abnormal sympathoexcitation mediated by "neuron-astrocyte uncoupling" in the RVLM is induced by cardiac ischemia or systemic adipocytokines (Heart-Brain axis). In conclusion, we consider that abnormal sympathoexcitation associated with "Brain-Heart interaction" would cause cardiovascular diseases.

3S46B-3

Assessment of Sympathetic Over Activity by the Analysis of Nonlinear Heart Rate Dynamics

Hayano, Junichiro (*Medical Education, Nagoya City Univ., Nagoya, Japan*)

Decreased heart rate variability (HRV) after acute myocardial infarction (AMI) is associated with an increased risk of mortality. HRV indices reported as post-AMI risk predictors are 24-hr standard deviation of normal-to-normal R-R intervals (SDNN), very low frequency power (VLF), deceleration capacity (DC), and heart rate turbulence (HRT). These indices, however, mainly reflect cardiac vagal activity or reflex function. To date, there is little evidence for the possibility that cardiac sympathetic over activity can be detected by HRV. Recently, we have developed a new HRV index called non-Gaussianity index (λ) to detect increased probability of the occurrence of large intermittent tachycardia during daily life. Among 670 post-AMI patients, we performed 24-hr Holter monitoring to assess λ and other HRV predictors, including SDNN, VLF, DC, and HRT. The λ showed no substantial correlation with other HRV indices ($|r|<0.4$) and was decreased in patients taking β-blockers ($P=0.04$). During a median follow up for 25 months, there were 45 (32 cardiac and 13 non-cardiac) deaths (6.7%). Increased λ predicted cardiac death (RR [95% CI], 1.6 [1.3-2.0] per 1 SD increment, $P<0.001$) and the predictive power was independent not only of established clinical risk factors but also of other HRV predictors. The combination of increased λ and abnormal HRT, a measure of vagal reflex dysfunction, provided the best predictive model for cardiac death. Our observations support the hypothesis that increased λ reflects cardiac sympathetic over activity that precipitates fatal cardiac events after AMI in combination with vagal dysfunction.

3S46B-4

Evaluation of sympathetic autonomic function and its implications in neurological disorders

Asahina, Masato (*Department of Neurology, Chiba University, Japan*)

Autonomic investigation is of value in diagnosing neurological disorders and predicting the prognosis, as well as assessing the severity of autonomic failure. From an aspect of diagnostic biomarkers, it is noteworthy that autonomic involvements precede onset of motor symptom in Parkinson's disease (PD) ; a common neurodegenerative disorder characterised by motor dysfunction (parkinsonism) and several non-motor features. There is a possibility that detection of autonomic dysfunction is helpful for diagnosis of PD in the early or premotor stage. Moreover, PD is a clinical phenotype of Lewy body disease, as well as dementia with Lewy bodies (DLB), therefore, evaluation of autonomic function may be useful to differentiate DLB from other dementia diseases, such as Alzheimer's disease. In terms of clinical management, assessment of autonomic dysfunction may be useful to predict prognosis in patients with neurological disorders, because autonomic dysfunction is considered to be associated with critical events, such as sudden death. For instance, cardiac mortality after stroke is common, particularly right insular lesion, which is considered to mediate sympathetic activities, may be a predictor of cardiac events. Autonomic function tests may be able to detect abnormal sympathetic activity which could be related to cardiac events. For early diagnosis and more adequate management of neurological disorders, it is necessary to provide further details of disease-specific autonomic abnormalities and autonomic involvements related with disease prognosis, and that requires a collaboration of basic physiology and clinical neurology.

Symposium 47
Recent issues on research ethics

(March 29, 9 : 00–11 : 00, Room D)

3S47D-1

A Decade of Neuroethics : Impact on Neuroscience Community in Japan and Asia

Fukushi, Tamami (*Center for Research Development and Strategy, Japan Science and Technology Agency, Japan*)

An academic discipline of neuroethics was originated in 2002 in United States. In 2004, neuroethics research group was launched in Research Institute of Science and Technology for Society (RISTEX) at Japan Science and Technology Agency (JST). Since then, Japanese researchers from various fields such as philosophy of science, bioethics, science communication, and neuroscience, have collaborated together to assess the feasibility of Japanese neuroethics. In 2006, the Japan Neuroscience Society and Japan Bioethics Society started the neuroethics session at their annual meeting. In 2008, the Ministry of Education, Culture, Sports, Science and Technology established Strategic Research Program for Brain Sciences (SRPBS). In SRPBS neuroethics research unit were established together with neuromodulation and brain-machine Interface research & developing group. The first topic of this presentation is to summarize the impact of neuroethics on Japanese neuroscience communities including SRPCS and surrounding researchers working neuromodulation and psychiatric fields. In this presentation the author also introduce recent progress in neuroethics in Asia. Since the year of 2008, in order to attract potential talent in Asia to neuroethics research, Japanese researchers had collaborative activity with Asian researchers those who are suffering the practical problem in neuroscience research or interested in the philosophical/sociological approach to the relationship between neuroscience and society.

3S47D-2

Importance of information disclosure in animal experiment ethics and conflict of interest

Kurata, Kiyoshi (*Dept Physiology Hirosaki Univ Sch Med, Japan*)

Since the current animal welfare law regulating experimental animals in Japan was effective in 2006, the law has been revised this year, 2012. The five years between 2006 and 2011 were regarded as a period to improve our regulation of each Japanese institution based on 3R principles (replacement, reduction, and refinement), such as providing better circumstances for animals and compulsory training programs for every people who take care of experimental animals. It is important to notice that, in the revision, exemption from registration of the institution have not been included in the revision. However, scientists using experimental animals should not take the exemption for granted, and should remind that experiments using animals are always under information disclosure upon official requests. Furthermore, mutual inspection programs between universities and/or institutions have started to evaluate how animal experiments are being conducted appropriately, and the inspections are also carefully reviewed by our government. Another important issue is conflict of interest (COI). COI is primarily concerned in clinical research, but it is becoming one of major issues in our physiological field. This is because many basic scientists conduct translational researches collaborating with clinicians. The Physiological Society of Japan, as well as many medical societies, require its members to declare their COI and disclose it if necessary, when they submit papers to its annual meetings and to the society journal, the Journal of Physiological Sciences. Again, we should be aware that our experiments are always under public eyes.

3S47D-3

Responsible Conduct of Research and Ethics of Scientific Publishing

Iriki, Atsushi (*RIKEN BSI, Wako, Saitama, Japan*)

"Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication". (Declaration of Helsinki ; Clause 30)

Researchers are members of a community where their achievements are recognized through publications, that have an immediate impact on society. Thus, responsible professional conduct and ethical manner throughout the collection, analysis, and publication of data are mandatory. Irresponsible practices induce negative impacts-undermine reliability of the study, critical inquiry to the field, undermine public and funding support, pose a risk to research-based health care decisions, and many more. In this symposium, the editor's viewpoints on the pitfalls that authors may encounter upon publication will be discussed. Those include classes of irresponsible scientific practices, such as fabrication, falsification, and plagiarism. To prevent such pitfalls, professional standards-'morality as a person' and 'quality as a scientist'-need to be established, over regulations, mandates and guidelines for institutional assessments.

3S47D-4

New Trends in Clinical Research Ethics : Eight Ethical Principles and Research Ethics Consultation

Tashiro, Shimon (*Office for Promoting Medical Research, Showa University, Tokyo, Japan*)

Systematic inquiry in clinical research ethics was developed as a branch of bioethics in the 1970s in the US. The Belmont Report published in 1979 introduced three ethical principles for clinical research ethics and clarified their applications. This framework, together with the concept of independent review, has been widely accepted all over the world. In the 1990s, researchers mainly from the department of bioethics at NIH started to organize previous studies in research ethics into readings and textbooks and published them in the 2000s.
In this presentation, a new ethical framework called eight ethical principles for ethical clinical research will be discussed. The new ethical framework reflected the debate on ethics of multinational research in the 1990s. As a result, it includes two characteristic principles, namely, collaborative partnership and respect for human research participants. I maintain that these principles have already influenced Japanese guidelines for clinical research. In addition, recent developments of research ethics consultation service in the US and Japan will be examined. Clinical researchers today are facing complex ethical and regulatory problems and need individual advice even in early phases of research. Therefore, some universities and institutions have created a department for this service apart from the conventional system of independent review. However, there are still many obstacles for developing this service especially in Japan. I review the present status of research ethics consultation service in Japan and point out issues that must be resolved.

Symposium 48
Consideration of diversity through sex difference in physiological sciences

[Symposium by the Committee of equal opportunity for women physiologist]

(March 29, 9 : 00–11 : 00, Room E)

3S48E-1

Introduction : Understanding of diversity from the view of physiology

Sekino, Yuko[1]; Shirao, Tomoaki[2] ([1]*Div. Pharmacol. NIHS,Tokyo,Japan;* [2]*Dept Neurobiol and Behav, Univ of Gunma, Grad Sch Med, Gunma, Japan*)

The committee of Equal Opportunity for Women Physiologist has missions to analyze current situations of women physiologists in their working places and to find out a solution to the discrimination between men and women for the opportunity to get professional positions and grant funds. In this symposium, we will discuss the physiological differences between women and men, and keep our awareness of important physiological differences. As approaching to the goal, the understanding of the differences will help us to understand the diversity needed in our society. The understanding will lead us to achieve an equal employment opportunity for career development in scientific working places.

3S48E-2

Behavioral gender difference, a view from the gene and neurons

Yamamoto, Daisuke (*Tohoku Univ. Grad. Sch. Life Sci., Sendai, Japan*)

In 1990, we isolated a Drosophila muatnt, satori, the males of which display homosexual courtship and do not copulate with females. Subsequently, satori was found to be an allele of fruitless (fru), a mutant known by bisexual courtship in males. The fru gene encoding BTB-Zn-finger transcription factors organizes male sexual behavior by controlling the development of sexually dimorphic neuronal circuitry. However, the molecular mechanism by which fru controls the sexual fate of neurons has been unknown. Our recent study represents the first step to clarify this mechanism. We have shown that : i) Fru forms a complex with the transcriptional cofactor Bonus (Bon) which recruits either of two chromatin regulators, Histone deacetylase 1 (HDAC1) or Heterochromatin protein 1a (HP1a) to Fru-target sites ; ii) the Fru-Bon complex has a masculinizing effect on single sexually-dimorphic neurons when it recruits HDAC1, whereas it has a demasculinizing effect when it recruits HP1a ; iii) HDAC1 or HP1a thus recruited to Fru-target sites determines the sexual fate of single neurons in an all-or-none manner, as manipulations of HDAC1 or HP1a expression levels affect the proportion of male-typical neurons and female-typical neurons without producing neurons of intersexual characteristics. Here, we further discuss the possible molecular mechanisms whereby HDAC1 and HP1a accomplish the sex-switching function in the brain.

3S48E-3

Neuroendocrine Basis of Sex Differences in Social and Emotional Behavior

Ogawa, Sonoko (*Lab. Behavioral Neuroendocrinology, University of Tsukuba, Tsukuba, Japan*)

We have been studying brain mechanisms of social behavior, particularly regulation of sex-specific sexual and aggressive behavior by gonad steroids. Recently, we also have focused on social interactive behaviors including social preference, social recognition and social memory as well as emotional and anxiety-related behavior in social context. This wide range of social behavior may also be regulated by steroid hormones through both organizational action on perinatal and peripubertal sexual differentiation of neural circuitries and direct activational action on behavioral expression later in life. Moreover, our recent studies have revealed that environmental factors such as neonatal adverse experience by maternal separation might influence, in a sex-specific manner, later responses measured in behavioral paradigms focused on various aspects of social and emotional behavior. In this talk, we will overview what we have known about hormonal and environmental regulation of sex-typical expression of social behavior and discuss possible brain mechanisms. (Supported by Grant-in-Aid for Scientific Research 23240057).

3S48E-4

Sex differences in rodent in vivo toxicity studies

Ogawa, Kumiko (*Pathology, National Inst. Health Sci., Japan*)

We have noted that the susceptibility to development of certain diseases may differ between males and females. The sensitivity to drugs and chemicals can also vary with the gender. In this presentation, I would like to introduce some examples of sex differences in rodent toxicity studies and the impact of its outcome and discuss the possible cause of difference. In the OECD guidelines for toxicity studies used for the safety evaluation of various chemicals, it is a requirement that male and female rats (or mice) are equally examined in repeated dose 28-, 90-day toxicity, chronic toxicity and carcinogenicity studies. Examination of general clinical parameters, body/organ weights and food/water consumption is mandated, along with performance of hematology and clinical biochemistry, gross necropsy, and a full histopathological assessment. While the majority of normal ranges of related parameters differ with the gender, the acceptable daily intake (ADI) is determined from the lowest value for the no observed adverse effect level (NOAEL) among the data in both sexes. It has been well known that the susceptibility of Typ-P1 to mice hepatocarcinogenicity is higher in females than males. Degawa et al revealed that androgen suppressed the expression of CYP1A2, a critical N-hydroxylation enzyme for activation of heterocyclic amines causing liver carcinogenesis. However, interestingly, when another heterocyclic amine, PhIP was fed to rats, the incidence of the colon carcinomas was higher in males while mammary adenocarcinomas were observed only in females. These facts indicate that it is not possible to choose one gender for toxicology studies conducted for safety evaluation.

3S48E-5

Sex/gender differences in musculoskeletal pain and issues to be considered in their research

Mizumura, Kazue (*Dept.Phys.Ther, Coll. Life Health Sci, Chubu Univ., Japan*)

Musculoskeletal pain such as low back pain, tender shoulder, articular pain is one of the top health complains of Japanese, especially high in women. There are several painful pathological conditions in musculoskeletal system incidence of which is much higher in women. Those are osteoarthritis, rheumatoid arthritis, fibromyalgia, temporomandibular disorders etc. Several factors inducing such difference can be considered : 1) genetic factors, 2) physiological and structural differences, 3) different sensitivity to pain, 4) sex hormones, 5) different neural functions, 6) difference in life cycle/style, and 7) difference in sociocultural role. In addition, sensitivity to or effectiveness of drugs for pain relief is also different between males and females. Despite existence of clear sex/gender differences in musculoskeletal pain, pain research had been and is still now being done mainly on male animals. Main reason for this might be existence of menstrual cycle in female animals. In my talk I will briefly review above mentioned factors that influence the incidence of painful conditions and pain sensitivity, thereafter I will touch my experimental results on muscular pain. One is related with a method evaluating deep pain threshold through the skin that may cause a problem in comparing the pain sensitivity between females and males with different body structure (e.g. fat thickness). The other is hyperalgesia induced by repeated cold stress (model for fibromyalgia). It is severe and long-lasting in female rats than in males. This difference might be induced by hormone or size of the body.

Symposium 49
Advanced glial strategy on neuronal circuits

(March 29, 9 : 00–11 : 00, Room F)

3S49F-1

Control of local synthesis and initial events in myelination by action potentials

Wake, Hiroaki[1]; Fields, R Douglas[2] (*[1]National Institute for Basic Biology, NINS, Okazaki, Japan; [2]National Institute of Health, Bethesda, Japan*)

Neural activity may stimulate myelin formation, the electrical insulation on nerve fibers, in association with learning and postnatal experience. Oligodendrocytes, the myelinating glia of the CNS, are morphologically complex cells that are capable of myelinating multiple axons independently from many different cellular extensions and induce rapid conduction of electrical impulses in the vertebrate brain. Myelin formation is essential for information processing because myelin increases conduction velocity at least 50 times. We have shown that oligodendrocytes, the myelinating glia of the CNS, exhibit elevated Ca2+ responses in their fine processes and cell soma, in response to action-potential (AP) firing in axons. Inhibition of the Ca2+ responses in oligodendrocyte processes significantly inhibits myelin formation without affecting oligodendrocyte differentiation. We have demonstrated that elevated Ca2+ responses in oligodendrocyte processes promotes the turnover of cholesterol rich domains as visualized by a pH sensitive GFP fused with transferrin receptor. We have visualized myelin basic protein (MBP) local translation using a photo-convertible GFP fused to the 3'UTR of MBP. Using this system we have shown that de novo translation of mRNA for MBP, the major constituent of myelin sheaths occurs in direct response to electrically active axons. These findings provide new insight into how myelination, and thus conduction velocity and function of neural circuits, can be regulated by nervous system activity.

3S49F-2

A local calcium influx pathway in astrocytes and its role in synaptic plasticity

Shigetomi, Eiji[1,2]; Jackson-Weaver, Olan[2]; Thomas, O'Dell J[2]; Baljit, Khakh S[2,3] (*[1]Dept. Pharmacol., Univ. Yamanashi, Japan; [2]Dept. Physiol., UCLA, USA; [3]Dept. Neurobiol., UCLA, USA*)

Astrocytes may actively regulate synaptic function during intracellular Ca^{2+} elevations that occur spontaneously or during activation of receptors on astrocytes. However, understanding of Ca^{2+} signals has been hindered by lack of methods to measure Ca^{2+} in small compartments such as near the plasma membrane and within astrocyte processes. Recently, we refined a genetically encoded Ca^{2+} indicator to monitor Ca^{2+} signals near the plasma membrane[1-3], leading to the discovery of a microdomain-like Ca^{2+} influx pathway mediated by TRPA1 channels and underlying spontaneous and localized Ca^{2+} signals in astrocytes. Genetic and pharmacological approaches suggest that TRPA 1 channels mediated Ca^{2+} fluxes in astrocytes[3]. TRPA1 channel-mediated Ca^{2+} influx regulates basal Ca^{2+} levels in astrocytes without affecting store-mediated Ca^{2+} release, suggesting that astrocyte Ca^{2+} is regulated by at least two independent pathways. It has been proposed that astrocyte Ca^{2+} regulates synaptic plasticity, although the source of Ca^{2+} is unclear. We investigated if the TRPA1 channel-mediated Ca^{2+} influx pathway regulates synaptic plasticity. Pharmacological blockade and genetic deletion of TRPA1 channels significantly reduced LTP in a D-serine dependent manner. Our data suggest that basal Ca^{2+} levels regulated by TRPA1 may control D-serine release from astrocytes, which in turn regulates LTP.

References
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3S49F-3

Exploring the causal relationship between glial activity and mind

Matsui, Ko (*Division of Cerebral Structure, National Institute for Physiological Sciences, Okazaki, Japan*)

There is a general assumption that information processing in the brain is mediated predominantly by neuronal activity. Recent evidence shows that there are direct and rapid mechanisms for neurons to communicate with glia cells ; however, without the evidence for a signaling pathway leading back from glial activity to neuronal activity, we remain uncertain of the glial participation in rapid information processing. Extracellular electrical stimulation used to study synaptic transmission between neurons inevitably stimulates glial cells as well, thus gliotransmitter release could have been unintentionally evoked in these studies but its effect overlooked. Here, we introduced a transgenic mouse line in which channelrhodopsin-2 was selectively expressed in astrocytes including cerebellar Bergmann glial cells. Selective photostimulation of these astrocytes lead to release of glutamate which was sufficient to activate AMPA receptors on Purkinje cells (PCs) and to induce long-term depression of parallel fiber to PC synapses through activation of mGluRs on PCs. We also show that neuronal activation by glial stimulation also works in vivo and can lead to perturbation of cerebellar modulated motor behavior. In contrast to the point-to-point communication provided by neuronal release of synaptic vesicles, glial activation likely causes preferential activation of perisynaptic and extrasynaptic receptors expressed on neurons as these receptors directly apposes glial membrane. These results provide evidence that glial activation can serve as a modulatory mechanism for setting the tone of neuronal activity and behavior.

3S49F-4

Functional impact of glial glutamate receptors in vivo

Kirchhoff, Frank (*Department of Molecular Physiology, University of Saarland, Homburg, Germany*)

Neurotransmitter receptors expressed by glial cells are seen as essential components of bidirectional neuron-glia communication. In the cerebellum Bergmann glial cells express α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors (AMPARs) of (BG) that are composed solely of GluA1 and/or GluA4 subunits. To study their function in vivo, genetically modified mice were generated to selectively and inducibly ablate both GluA1 and GluA4 subunits in astrocytes. During the late phase of cerebellar development the deletion of AMPARs in BG resulted in retraction of glial appendages from Purkinje cell (PC) synapses, increased amplitude and elongation of evoked PC currents as well as delayed formation of glutamatergic synapses. In adult mice the inactivation of AMPARs revealed also a retraction of processes though at a later onset. These physiological and structural changes were accompanied at the behavioral level by impairment in fine motor coordination. Our data suggest an active contribution of glial transmitter receptors in the control of cerebellar output function.

Symposium 50
Physiology of GLP-1, a molecular target of diabetes treatment —What we know and what we don't—

(March 29, 9 : 00–11 : 00, Room G)

3S50G-1

Mechanism of sugar-induced glucagon-like peptide-1 secretion from enteroendocrine L-cells

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An intestinal hormone glucagon-like peptide-1 (GLP-1), secreted from enteroendocrine L cells, potentiates insulin secretion from pancreatic β-cells in a glucose-dependent manner, thereby inhibiting the postprandial rise in blood glucose levels. Secretion of GLP-1 is triggered by oral ingestion of nutrients including fat, protein, and sugars. Since the inhibition of GLP-1 inactivation by dipeptidyl peptidase 4 (DPP4) inhibitors are now widely used in treatment of type 2 diabetes mellitus, the clarification of the mechanism of GLP-1 secretion by sugars is essential. However, lack of suitable cell lines to study GLP-1 secretion and the difficulty in measuring plasma GLP-1 levels in vivo have hampered clarification of the mechanism. Nevertheless, recent progress in measuring active GLP-1 levels in plasma enabled us to assess the GLP-1 secretion in human in vivo. Pancreatic β-cell and eneteroendocrine L-cells share several common features ; both elicit regulated exocytosis to secrete hormones in response to glucose. The molecular mechanism for glucose-induced insulin secretion from pancreatic β-cells has been studied extensively and the process involves many molecules/systems, such as ATP-sensitive K^+ channels, sweet receptors, and neuronal input. By analogy with the insulin secretion by glucose, the mechanism of sensing luminal sugars in L were evaluated in healthy Japanese male volunteers. In this symposium, the mechanism of glucose sensing mechanisms in L-cells and their similarity with and differences from pancreatic β-cell will be discussed.

3S50G-2

The role for GLP-1 in feeding regulation

Date, Yukari (*Frontier Science Research Center, University of Miyazaki, Japan*)

Glucagon-like peptide-1 (GLP-1) and leptin are anorectic hormones produced in the small intestine and white adipose tissue, respectively. Investigating how these hormones act together as an integrated anorectic signal is important to elucidate a mechanism to maintain energy balance. We here demonstrate that coadministration of subthreshold GLP-1 and leptin dramatically reduces feeding in rats. Although coadministration of GLP-1 with leptin did not enhance leptin signal transduction in the hypothalamus, it significantly decreased phosphorylation of AMP-activated protein kinase (AMPK). In addition, coadministration of GLP-1 with leptin significantly increased proopiomelanocortin (POMC) mRNA levels. Considering that alpha-melanocortin stimulating hormone (alpha-MSH) is derived from POMC and functions through the melanocortin-4-receptor (MC4-R) as a key molecule involved in feeding reduction, the interaction of GLP-1 and leptin on feeding reduction may be mediated through the alpha-MSH/MC4-R system. As expected, the interaction of GLP-1 and leptin was abolished by intracerebroventricular preadministration of the MC4-R antagonists agouti-related peptide and SHU9119. Taken together, GLP-1 and leptin cooperatively reduce feeding at least in part via inhibition of AMPK following binding of alpha-MSH to MC4-R. Furthermore, we present that this interaction of GLP-1 and leptin was canceled in rats with midbrain transaction. This finding indicates that the brain stem would be important to integrate the information of interaction of GLP-1 and leptin.

3S50G-3

Molecular mechanism by which DPP4 inhibitor suppresses glucagon secretion

Kitamura, Tadahiro (*Institute for Molecular and Cellular Regulation, Gunma Univ. Maebashi, Japan*)

Prevention of the inactivation of GLP-1 by inhibiting enzyme DPP4 is a strategy that is currently used for the treatment of diabetes. In addition to increase in glucose-induced insulin secretion, DPP4 inhibitor is also known to suppress glucagon secretion. However, the latter mechanism is still unclear. Therefore, we tried to elucidate the mechanism by which DPP4 inhibitor vildagliptin suppresses glucagon secretion in mice. We administered vildagliptin (60mg/kg/day) daily using oral gavage to mice for 12 weeks. Controls were given plain water. We confirmed the increase in plasma GLP-1 levels in the vildagliptin treated mice. Oral glucose tolerance test showed better glucose tolerance without change of plasma insulin levels in vildagliptin treated mice. Importantly, plasma glucagon levels were significantly decreased in vildagliptin treated mice compared to the control mice. Histological analysis revealed that both pancreatic alpha and beta cell mass were unchanged in vildagliptin treated mice. We then isolated islets from the mice and assessed the expression level of genes related to glucagon secretion using real-time RT-PCR. Islets from the vildagliptin treated mice showed significantly lower expression level of proglucagon than control mice, which was associated with decreased MafB, FoxA2 and NeuroD, regulators for proglucagon transcription. PC2, a convertase for glucagon, was unaltered in the vildagliptin treated mice. We therefore conclude that administration of vildagliptin inhibits proglucagon gene transcription, which leads to suppressed glucagon secretion and better glucose tolerance.

3S50G-4

Ghrelin attenuates GLP−1 action to stimulate cAMP signaling and insulin secretion in islet β−cells

Yada, Toshihiko[1]; Boldbaatar, Damdindorj[1]; Kurashina, Tomoyuki[1]; Sone, Hideyuki[1]; Rita, Rauza Sukma[1]; Kakei, Masafumi[2]; Dezaki, Katsuya[1] (*[1]Div. Integrative Physiol., Dept. Physiol., Jichi Med. Univ., Sch. Med., Shimotsuke, Japan; [2]First Dep. Med., Saitama Med. Center, Jichi Med. Univ. Sch. Med., Shimotsuke, Japan*)

Glucagon-like peptide-1 (GLP-1) and ghrelin are, respectively, physiological potentiater and inhibitor of glucose-induced insulin secretion from pancreatic islet β-cells. This study aimed to clarify whether exogenous ghrelin administration counteracts and endogenous ghrelin blockade enhances insulinotropic action of GLP-1, and to elucidate the underlying signalling mechanism for interaction of the two hormones in rat islet β-cells. GLP-1 enhanced glucose-induced increases in insulin release and cAMP synthesis in isolated islets and $[Ca^{2+}]_i$ increases in single β-cells. The GLP-1-enhanced activities were all attenuated by administration of ghrelin. Ghrelin also suppressed $[Ca^{2+}]_i$ responses to an adenylate cyclase activator forskolin. Moreover, GLP-1-induced insulin release and cAMP production were markedly enhanced by [D-lys^3]-GHRP-6, a ghrelin receptor antagonist, in isolated islets. These results indicate that both islet-produced and exogenously applied ghrelin counteracts glucose-dependent GLP-1 action to increase cAMP production, $[Ca^{2+}]_i$ and insulin release in islet β-cells. This finding positions ghrelin as a potent modulator of insulinotropic GLP-1.

Symposium 51
Front line of investigation of pathophysiology of heart failure in animal models

(March 29, 9 : 00−11 : 00, Room H)

3S51H-1

Inherited dilated cardiomyopathy(DCM)model mouse with no symptom, high risk of sudden death and congestive heart failure, and factors affecting the progression of the disease

Kurebayashi, Nagomi (*Dept Pharmacol, Juntendo Univ Sch Med, Japan*)

Inherited DCM is reported to result primarily from mutations that cause weakness in force production. However, carriers of inherited DCM mutation do not always develop symptoms of HF at birth. Instead, many are aware of symptoms of HF at some point in their life, which varies from young to old age, and the symptoms thereafter worsen. Alternatively, some die suddenly before HF becomes evident. These reports raise questions about how and when these symptoms appear in inherited DCM carriers. Because data in humans are confounded by various factors, investigations with animal models are required. To address the above questions, we investigated a knock-in mouse model with ΔK210 in cardiac troponin T (TNNT2), which is identical to one of the human DCM mutations. Young DCM mice at 1 month or before had already enlarged hearts, but showed no signs of HF and a much lower mortality than at 2 months or later. At around 2 months, some would die suddenly with no clear signs of HF, whereas at 3 months, many of the survivors developed congestive HF. Expression analyses of HF markers and current measurements revealed multi-step structural and functional remodeling proceeds in this mouse model. Interestingly, some of the changes were considerably suppressed by drug administration or exercise. Our results suggest that early initiation of therapy may be important in inherited DCM.

3S51H-2

Mechanism underlying GTP−binding protein αq−induced heart failure and cardiac tachyarrhythmia

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The $G\alpha_q$ protein-coupled receptor (GPCR) signaling pathway plays a critical role in the development of cardiac hypertrophy and heart failure (HF). To elucidate the mechanism underlying $G\alpha_q$-induced HF and cardiac tachyarrhythmias, we investigated the electrical and structural remodeling and arrhythmia induction in mice with transient transgenic cardiac expression of activated G protein αq ($G\alpha_q$-TG). $G\alpha_q$-TG mice induced HF, and atrial and ventricular remodeling and tachyarrhythmias. As the structural remodeling, ventricular myocyte hypertrophy and the extensive interstitial fibrosis were observed in $G\alpha_q$-TG hearts. As the electrical remodeling, all of the electrocardiogram parameters measured were prolonged and atrial action potential duration prolongation and impulse conduction slowing were observed in $G\alpha_q$-TG mice. Moreover, early afterdepolarization-induced triggered activity was frequently observed in single $G\alpha_q$-TG ventricular myocytes. Protein expressions of canonical transient receptor potential (TRPC) channels 3 and 6 increased in $G\alpha_q$-TG hearts. Interestingly, cardiac-specific overexpression of diacylglycerol kinase ζ restored all of the electrical and structural remodeling and inhibited cardiac tachyarrhythmias in $G\alpha_q$-TG mice. Moreover, SK&F96365, a TRPC channel blocker, prevented EAD and VT in $G\alpha_q$-TG mouse hearts. These results suggest that diacylglycerol and TRPC play important roles in $G\alpha_q$-induced cardiac remodeling and arrhythmias.

3S51H-3

Identification of novel drug targets based on the elucidation of molecular mechanisms underlying chronic heart failure using genetically－engineered mouse model

Kuwahara, Koichiro; Nakao, Kazuwa (*Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine*)

Despite recent progress in pharmacological and non-pharmacological interventions, prognosis in patients with chronic heart failure still remains poor. Identification of novel therapeutic targets based on knowledge of the molecular basis underlying the development of heart failure is anticipated. In the study of molecular mechanisms regulating the production of cardiac hormones, atrial and brain natriuretic peptides, we identified a transcriptional repressor NRSF to be an important transcriptional regulator of these hormones. To evaluate the role played by NRSF in the heart, we generated transgenic mice expressing a dominant-negative mutant of NRSF in a heart-specific manner (dnNRSF-Tg). The dnNRSF-Tg showed progressive cardiomyopathy and sudden arrhythmic death. Using this model, we studied molecular mechanisms underlying the development of arrhythmias associated with heart failure. We have demonstrated the contribution of fetal type cardiac ion channels, T-type Ca^{2+} channels and HCN channels to sudden death of dnNRSF-Tg. In addition, we further found that activation of sympathetic nervous system and renin-angiotensin-aldosterone system also affect the increased arrhythmicity in dnNRSF-Tg. Collectively, our studies using dnNRSF-Tg as a mouse model of chronic heart failure and sudden arrhythmic death reveal molecular mechanisms underlying the development of lethal arrhythmias associated with heart failure and potentially novel therapeutic targets for heart failure.

Symposium 52
New approach for comprehensive understanding of sleep/wakefulness by young scientists

(March 29, 9 : 00–11 : 00, Room I)

3S51H-4

The molecular mechanism by which chronic and excessive β_1–adrenergic stimulation remodels ventricular excitation–contraction coupling

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In heart failure, chronic and excessive stimulation of β_1-adrenergic receptors (β_1AR) remodels ventricular excitation-contraction coupling. To delineate the molecular mechanism underlying this remodeling, we investigated the cardiac function and L-type Ca^{2+} channel (LTCC) activity in ventricular myocytes (VM) of mice chronically treated with isoproterenol (ISO mice). ISO mice exhibited cardiac hypertrophy and failure. As assessed in the whole-cell configuration of the patch clamp method, t-tubular LTCC activity was halved by activation of protein phosphatase (PP) 2A whereas surface sarcolemmal LTCC activity was doubled by inhibition of PP1 in isolated ISO VM. These abnormalities were completely prevented by metoprolol administrated with ISO, indicating that β_1AR mediated the deleterious effects of ISO. However, these abnormalities were also prevented by pertussis toxin (PTX) applied with ISO, indicating that chronic receptor-mediated activation of $G_{i/o}$ proteins also participates in the remodeling. Indeed, chronic treatment of ISO mice with inverse agonists for β_2AR and M_2-muscarinic receptors (M_2R) but not A_1-adenosine receptors normalized the basal LTCC activity almost completely and cardiac function partially. Thus, chronic and excessive β_1AR stimulation results in chronic β_2AR- and M_2R-mediated activation of $G_{i/o}$ proteins, which in turn causes abnormal basal LTCC activity and cardiac contractility in heart failure.

3S52I-1

Long term bioluminescence measurement from the suprachiasmatic nucleus with an optical fiber in freely moving mice

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In mammal, the circadian rhythms are generated by the central clock located in the hypothalamic suprachiasmatic nucleus (SCN). The mechanism of circadian oscillation is an autoregulatory transcription and translation feedback loop involving several clock genes and their protein products. A bioluminescence reporter, such as firefly luciferase, provides a powerful tool for long-term recording because of its low toxicity and high quantitativeness. Since sleep and wakefulness can only be assessed in conscious animals, we developed a method to monitor bioluminescence reporter activity for long term with an optical fiber in the discrete brain areas of freely moving mice. We successfully recorded clock gene expression rhythms from the SCN *in vivo*.
We used *Per1-luc* and *Bmal1-Eluc* transgenic mice expressing a *Per1* and *Bmal1* promoter driven luciferase reporter, respectably, and PER 2 : : LUC knock-in mice carrying a PER2 fusion luciferase reporter. We inserted an optical fiber into the brain just above the SCN through a guide cannula fixed on the skull. Bioluminescence and spontaneous behavioral activity were simultaneously measured by a Photomultiplier tube and an infrared thermal sensor, respectively, for more than 4 weeks. The phase relation among *Per1-luc*, *Bmal1-Eluc*, and PER2 : : LUC rhythms were similar as those of *ex vivo* measurement. The system is useful to understand the relationship between molecular functions and behaviors in living animals.

3S52I-2

Cortical neuronal activities during acute optogenetic induction of sleep

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Sleep is a state that can be measured using electroencephalography (EEG), which is greatly affected by the cortical state. Orexin neurons, which are important for the maintenance of wakefulness, have direct projections to the cerebral cortex. To reveal cortical neuronal activities during acute optogenetic inhibition of orexin neurons, we used transgenic mice in which orexin neurons expressed archaerhodopsin-3 (orexin/Arch mice), a green light-activated neuronal silencer. We recorded local field potentials (LFPs) from somatosensory and motor cortex and EEG in orexin/Arch mice. During acute (60 s) optogenetic inhibition of orexin neurons, delta band power of EEG rapidly increased within ten seconds, while that of LFP tended to increase gradually. Cross correlations between LFPs of ipsilateral somatosensory and motor cortex increased rapidly, indicating rapid increase in synchronization of cortical oscillations. EEG reflects neuronal activities from larger brain regions than LFP does. During acute optogenetic inhibition of orexin neurons, the rapid enhancement in LFP synchronization between cortical regions should support the rapid increase in EEG delta band power.

3S52I-3

New model mice for Narcolepsy : timing controlled ablation of orexin neurons

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Orexin is a neuropeptide which is produced in small number of neurons in the hypothalamus, which is orexin neuron. It is reported that specific loss of these neurons causes sleep disorder "narcolepsy". Narcolepsy is typically onset in adolescence or early adulthood. However, it takes about a decade from onset to correct diagnosis. This delay makes it difficult to follow the progress of the symptoms which appeared in the early stage of narcolepsy. There is no perfect mice model for narcolepsy so far. Here we generated new narcolepsy model mice using tetracycline gene expression control system. In these mice, orexin neurons were specifically ablated at any timing by expressing diphtheria toxin A fragment (DTA) when the chow was replaced from including doxycycline (DOX (+), 100 mg/kg) to without DOX (DOX (-)). Immunohistochemical study revealed that 95% of orexin-immunoreactive neurons were ablated at 2 weeks after DOX (-). During DOX (-), sleep/wakefulness pattern were analyzed by continuous recording of EEG and EMG. We revealed the relationship between number of orexin neurons and progress of symptoms, fragmentation of sleep/wakefulness and cataplexy-like behavioral arrest.

3S52I-4

The function of rapid eye movements and brain activities during REM sleep

Ogawa, Keiko (*Graduate School of Integrated arts and sciences, Hiroshima Univ. Hiroshima, Japan*)

Rapid eye movements (REMs) are the most prominent physiological features of REM sleep. However the function of REMs during REM sleep is still unclear. It is known that REMs during REM sleep are analogous in shape to REMs during wakefulness (saccades). In our studies, we investigated event-related brain potentials time-locked to the onset and offset of eye movements during wakefulness and REM sleep. During wakefulness, the presaccadic negativity (PSN) occurs before saccade with maximal amplitude over centroparietal region. It is similar to readiness potential and reflects the voluntary readiness activity of eye movements. Following saccades, positive cerebral potentials (lambda response) appear at occipital sites. The lambda response is assumed to correspond to visual potential after fixation. During REM sleep, although no PSN was found, lambda-like responses were observed in the cortical visual area, as in wakefulness. In addition, we have also recorded the another phasic brain potentials accompanying REMs, that is, before REMs, pre REM-negativity (PRN) appeared in the limbic area. Then, positive potentials (P200r) occurred time locked to the onset of REMs in the premotor and parietal cortecies. These potentials were not observed during wakefulness. Our findings suggested that REMs are initiated without preparation, but elicit some brain activity. These phasic brain activities might play a key role in explaining the function of REMs during REM sleep, and also in approaching to the function of REM sleep (dreaming, memory consolidation).

3S52I-5

Genetic analysis of the REM sleep center and its developmental origin

Hayashi, Yu[1]; Sakai, Kazuya[2]; Yasuda, Kosuke[1]; Nguyen, Julia[1]; Ando, Reiko[1]; Itohara, Shigeyoshi[1] (*[1]Lab for Behavioral Genetics, Riken BSI, Wako, Japan; [2]Neuroscience Research Center, INSERM, Lyon, France*)

Sleep in mammals has evolved into a complex state composed of REM (rapid eye movement) sleep (or paradoxical sleep) and non-REM sleep (or non-paradoxical sleep). REM sleep has received much attention as it is the major source of dreams. Little is known, however, about the evolutionary origin or physiologic significance of REM sleep. Furthermore, the neurons responsible for the transition between the two sleep states are controversial due to the heterogeneity and complexity of the brainstem neurons. Here, we established a genetic method which enables postnatal manipulation of neurons that derive from a specific cell lineage. This method adopts the Cre-loxP system and tetracycline inducible system to "tag" neurons that originate from a specific cell lineage, and the DREADD pharmacogenetic tool to manipulate the activity of the tagged neurons. Using this method, we genetically identified neurons in the brainstem pontine area that robustly regulate transitions between REM and non-REM sleep. Furthermore, we show that these neurons share a common developmental origin with neurons that promote arousal. Finally, we identified the vertebrate-specific molecule Netrin-G1 as a factor required for normal REM sleep. These results are expected to provide critical evidence about the neurons that regulate sleep states and provide implications about the evolutionary origin of REM and non-REM sleep.

3S52I-6

Roles of noradrenergic and histaminergic neurons in arousal mechanisms

Takahashi, Kazumi (*Fukushima Med. Univ. Fukushima, Japan*)

Based on our findings from single-unit recording during the transition from wakefulness to sleep in unanesthetized mice, we have proposed that sleep-process does not start with the activation of forebrain sleep-promoting neurons, but starts with deactivation (disfacilitation) of waking-promoting neurons including noradrenergic (NA) neurons in the locus coeruleus and histaminergic (HA) neurons in the tuberomammillary nucleus. We have also demonstrated that, at the onset of wakefulness, NA and HA neurons started firing, respectively, before and after both the onset of EEG desynchronization and the onset of firing in other wake-promoting neurons, suggesting that NA neurons may play some roles in both initiation and maintenance of wakefulness, while HA neurons may function only in maintenance. To further explore the functions of NA neurons in sleep-wake mechanisms, we selectively ablated NA neurons by immunotoxin-mediated cell targeting and found that the bilateral ablation caused reduction in the duration and increase in the number of wakefulness bouts, while the amount of wakefulness did not change. These results suggest that NA neurons may play an important role in maintenance of wakefulness and that NA function initiating wakefulness could be modulatory.

Symposium 53
Recent progress in pathophysiology on oxygen and ROS in the brain
[Collaboration Symposium with Japanese Society of Pathophysiology]

(March 29, 13 : 20–15 : 20, Room F)

3S53F-1

Oxidative damage in brain genomes and neuroprotective mechanisms

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8-Oxoguanine (8-OxoG), a major oxidized base lesion produced by reactive oxygen species, is associated with various pathological conditions including carcinogenesis and neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease. Although the mechanism by which 8-oxoG causes carcinogenesis is well understood, the mechanism by which it causes neurodegeneration is unknown. We recently demonstrated that excision repair of adenine inserted opposite 8-oxoG by adenine DNA glycosylase encoded by *Mutyh* triggers neurodegeneration under oxidative stress. Mutant mice lacking 8-oxo-dGTPase encoded by *Mth1* and/or 8-oxoG DNA glycosylase encoded by *Ogg1* exhibited severe neurodegeneration, whereas mutant mice lacking *Mutyh* or *Ogg1/Mutyh* were resistant to neurodegeneration when mitochondrial neurotoxin, 3-nitropropionic acid was administered. These results indicate that OGG1 and MTH1 protect brain while MUTYH promotes neurodegeneration under oxidative stress. 8-OxoG accumulated in mitochondrial DNA of neurons and caused calpain-dependent neuronal loss, while delayed nuclear accumulation of 8-oxoG in microglia resulted in PARP-dependent activation of apoptosis-inducing factor and exacerbated microgliosis. These results reveal that neurodegeneration under oxidative stress is a complex process caused by 8-oxoG accumulation in the genomes of neurons and microglia in the brain. Different signaling pathways were triggered by the accumulation of single-strand breaks in each type of DNA generated during base excision repair initiated by MUTYH.

3S53F-2

Dietary habits affect the functional development of brain

Wada, Keiji[1,2] ([1]*National Institute of Neuroscience, NCNP, Tokyo, Japan;* [2]*CREST, JST, Kawaguchi, Saitama, Japan*)

Dietary condition is influential to the development of brain function. Up to date, there have been many epidemiological studies on harmful aspects of malnutrition on the brain. However, detailed underlying molecular mechanism has not been fully investigated yet in the harmful events. Here, we investigated the effect of maternal diet on the brain development of of the offspring. Adult female mice were fed either a normal diet (ND) or a high-fat diet (HFD) before mating and throughout pregnancy and lactation. After weaning, both offspring were fed with normal diet. We found that HFD offspring showed the increased lipid peroxidations in the hippocampus during the early postnatal development. HFD offspring had less BDNF protein in the hippocampus than ND offspring did. Since BDNF has crucial role in the brain function, we investigated the hippocampal morphology and spatial learning and memory of the HFD offspring. We identified that dendritic arborizations of hippocampal new neurons are impaired in the young HFD offspring. We also found that, in Barnes maze test, HFD offspring showed the impaired acquisition of spatial learning in the young but not adult period. These results indicate maternal HFD may cause lipid peroxidation and affect the early development of the brain function of the offspring. To elucidate the molecular mechanism further, we have studied how maternal diet affects dendritic spines of the offspring. In this symposium, our on-going projects on the relationship between maternal life style and the offspring development are summarized and discussed in the aspects of oxidative stress.

3S53F-3

Dynamic changes in tissue-blood flow and -oxygen level of cerebral cortex and hippocampus by acceleration in anesthetized rats

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The brain is very sensitive to cellular hypoxia, which produces rapid loss of brain function. Positive vertical acceleration (+Gz) can decrease blood flow to the retina and the brain, which results in loss of vision and consciousness (LOC). Cerebral blood flow is controlled primarily by autoregulation when under normal conditions.+Gz exposure might disturb the brain circulation, however no estimation has been reported about tissue-blood flow or tissue-oxygen level in the brain responded to+Gz exposure. We estimated the responses of tissue-blood flow (BF) and tissue-oxygen (PO_2) level to+Gz stress (+3Gz or+5Gz) in the cortex or hippocampus of anesthetized rats. Cortical or hippocampal BF and PO_2 decreased dependently on+Gz intensity. Significant difference was found in decrease of BF between the cortex and the hippocampus by+3Gz. Changes of BF were lower than PO_2 in the cortex and the hippocampus by+3 or+5Gz respectively. After+Gz exposure, recovery time to control level of PO_2 was significantly slower than that of BF in both of the hippocampus and the cortex. G-induced LOC is divided into two incapacitation periods : absolute and relative incapacitations. The late recovery of PO_2 observed in our results could explain absolute incapacitation of G-induced LOC. These results suggest that+Gz stress decreases oxygen delivery to the brain but differently dependent upon the brain areas.

Symposium 54
Physiological function of lipid dynamics in plasma- and endo-membrane

(March 29, 13 : 20-15 : 20, Room G)

3S53F-4

Recovery of energy metabolism of rat brain after ischemia-reperfusion injury : a ^{31}P-NMR study

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By phosphorous nuclear magnetic resonance (^{31}P-NMR) spectroscopy, it is possible to quantify intracellular high-energy phosphates, phosphocreatine (PCr) and γ-ATP, and to estimate intracellular pH in rat brain slices superfused with artificial cerebrospinal fluid (ACSF). Since ^{31}P-NMR is a noninvasive measurement, it is possible to observe status of energy metabolism in the same specimen repeatedly. We also evaluate radical scavenging activity of possible neuroprotectants by electron spin resonance (ESR) spectroscopy.

Brain slices, incubated in well-oxygenated ACSF at 27.5℃, were subject to ischemia-reperfusion injury (IRI) by halting perfusion for 1 hour followed by reperfusion for 2 hours. Recovery of PCr relative to preischemia was used as an index of metabolic recovery. Recovery of PCr after IRI was significantly better when brain slices were superfused with ACSF containing glycolytic substrate derivatives such as ethylpyruvate or fructose-1,6-bisphosphate, but no neuroprotective effect was observed in neuron-rich slices pretreated with fluorocitrate, a selective glial poison. Some radical scavengers were also neuroprotective from IRI. For example, ^{31}P-NMR demonstrated better metabolic recovery after IRI when brain was pretreated with CV-159, a novel Ca^{2+}/calmodulin antagonist with radical scavenging activity assessed by ESR. We call our research project "spin resonance analyses" since both of our methodologies utilize "spin" ; ^{31}P-NMR counts spin of ^{31}P nuclei and ESR spin of electron.

3S54G-1

How Excited To Talk With PI? : Lesson From Voltage-Sensing Phosphatase

Okamura, Yasushi (*Grad. Sch. Med. Osaka Univ., Japan*)

Gene coding voltage-sensing phosphatase, VSP, is highly conserved in genomes from Coelenterata to human. VSP consists of two functional modules ; the N-terminal voltage sensor domain and the C-terminal phosphatase. Two modules are self-contained and the isolated voltage sensor domain shows charge movements upon alteration of membrane voltage, and the isolated phosphatase region dephosphorylates phosphoinositides. Since VSP has the ability of coupling from electrical signal to lipid enzyme, thus linking between biophysical and biochemical aspects of biological membranes ; voltage and lipid. How two modules are coupled to each other in translating electrical signal into lipid signal is an important issue both in membrane physiology and protein science. Studying mechanisms of VSP also requires integration of different disciplines such as electrophysiology, cell imaging, phosphoinositide signals and structural biology. The X-ray structure of the enzyme region of VSP has recently been resolved. VSPs also serve as useful materials as found in experiments of heterologously altering phosphoinositide level and voltage-probe to visualize membrane potential in specific cells. We will summarize recent understandings of coupling mechanisms between the voltage sensor domain and phosphatase region, and introduce our recent findings of molecular mechanisms.

3S54G-2

Class II PI3 kinase C2α has an essential role in angiogenesis and vascular homeostasis through regulating endosomal trafficking

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Phosphatidylinositol 3-kinase (PI3K) family regulates diverse cellular functions. Although class I PI3Ks and class III Vps34 are well-characterized, the physiological roles of PI3K class IIα (C2α), which is localized in intracellular vesicles and exclusively produces PtdIns (3) P, remain largely unknown. Global C2α-null mice and endothelial cell (EC)-specific C2α conditional KO mice showed embryonic lethality due to defects in sprouting angiogenesis and vascular maturation. In cultured ECs, siRNA-mediated knockdown of C2α resulted in decreased PtdIns (3) P-enriched endosomes and impaired endosomal trafficking. C2α knockdown also impaired cell signaling including VEGF receptor-2 internalization and RhoA activation on endosomes, but not Akt and ERK. Consequently, endosomal delivery of VE-cadherin to EC junctions was disturbed, leading to defects in VE-cadherin transport and assembly, cell migration, barrier integrity, and tube formation. These effects of C2α knockdown were C2α-specific because they were not mimicked by knockdown of other PI3K isoforms. C2α haploinsufficient mice were alive, but exhibited defective postnatal angiogenesis and vascular barrier integrity with greatly augmented susceptibility to anaphylaxis and a higher incidence of dissecting aortic aneurysm formation on angiotensin-II infusion. Thus, C2α plays a crucial role in vascular formation and barrier integrity.

3S54G-3

Critical role of PtdIns3P turn over in autophagy regulation

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Autophagy is intracellular degradation system well conserved throughout the eukaryotes. In addition to its classically defined role in intracellular homeostasis, recently it becomes apparent that it plays critical roles in a variety of important cellular physiological phenomena such as neurodegenerative diseases. Therefore its artificial regulation is attracted as a target of pharmacological researches. We have been studying the mechanism how autophagy is regulated. We have reported several mechanism regarding the involvement of PtdIns3P in autophagy (Gene Cells 2008, Nat Cell Biol. 2009, Traffic 2010, J Cell Biol 2010). During autophagy, membrane structures are dynamically rearranged. Phosphatidylinositol 3-phosphate (PtdIns3P) and specifically the phosphoinositide (PI) 3-kinase complex play important roles in this process. We have shown that PI 3-phosphatase has been shown to be important in initiating autophagy in mammalian cells, its role during autophagosome formation is still unclear. In addition, we uncovered another role of PI 3-phosphatase in autophagy. In a PI 3-phosphatase double yeast mutant, ymr1 sjl3, autophagy was severely affected under starvation conditions. Biochemical and ultra-structural analyses revealed that autophagosome formation was defective. The number of punctuate structures containing fluorescently labeled aminopeptidase I, Atg1, Atg8 and static Atg9 puncta were increased in ymr1 sjl3 mutant cells. These results indicate that the modulation of PtdIns3P dynamics involving PI 3-phosphatases is important for autophagosome formation.

3S54G-4

A role for sphingomyelin-rich lipid domains in the accumulation of phosphatidylinositol-4,5-bisphosphate to the cleavage furrow during cytokinesis

Kobayashi, Toshihide; Abe, Mitsuhiro; Makino, Asami; Hullin-Matsuda, Francoise (*Lipid Biology Laboratory, RIKEN, Saitama, Japan*)

Cytokinesis is a crucial step in the creation of two daughter cells by the formation and ingression of the cleavage furrow. Here, we show that sphingomyelin (SM), one of the major sphingolipids in mammalian cells, is required for the localization of phosphatidylinositol-4,5-bisphosphate (PIP (2)) to the cleavage furrow during cytokinesis. Real-time observation with a labeled SM-specific protein, lysenin, revealed that SM is concentrated in the outer leaflet of the furrow at the time of cytokinesis. Superresolution fluorescence microscopy analysis indicates a transbilayer colocalization between the SM-rich domains in the outer leaflet and PIP (2)-rich domains in the inner leaflet of the plasma membrane. The depletion of SM disperses PIP (2) and inhibits the recruitment of the small GTPase RhoA to the cleavage furrow, leading to abnormal cytokinesis. These results suggest that the formation of SM-rich domains is required for the accumulation of PIP (2) to the cleavage furrow, which is a prerequisite for the proper translocation of RhoA and the progression of cytokinesis.

3S54G-5

Kinetic analysis of receptor-operating TRPC channel accelerated by phospholipase C activity

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Subfamily of human expressed TRPC channels (TRPC3/6/7) are activated by diacylglycerol (DAG), a phospholipase C (PLC) product of phosphatidylinositol 4,5-bisphosphate [PI (4,5) P_2]. From recent our observation, the depletion of PI (4,5) P_2 by-itself leads to the channel inhibition even in the presence of DAG (Itsuki et al., 2012), but largely unknown yet correlation of PI (4,5) P_2-DAG signal proceeded by PLC activation and TRPC channels activities. Here, to study kinetics relation of DAG-sensitive TRPC channels expressing in smooth muscle cells and PLC activity, TRPC6 or C7 currents evoked by carbachol or vasopressin are simultaneously detected with PI (4,5) P_2 by using FRET sensor. By plotting both the channel activation and inactivation kinetics with the decaying of PI (4,5) P_2, close kinetic correlation is observed between TRPC currents and PLC activity. Furthermore, to elucidate mechanistic insight of TRPC channels, we develop a novel self-limiting regulation model which is linked with PLC activity. By using this model, we successfully simulate both the activity of TRPC6/7 channels and PI (4,5) P_2 dynamics in silico. Hence, these data indicate that self-limiting regulation coupled to PI (4,5) P_2-PLC-DAG signalling is the pivotal mechanism underlying receptor-operated TRPC channel activities.

3S54G-6

Relative contributions of PI(4)P pools of the plasma membrane and the Golgi for maintaining the PI(4,5)P_2 of the plasma membrane

Dickson, Eamonn James; Jensen, Jill Bodily; Hille, Bertil (*Department of Physiology and Biophysics, University of Washington, Seattle, USA*)

The minor plasma membrane (PM) lipid phosphatidylinositol 4,5-bisphosphate (PI (4,5) P_2) is required for KCNQ2/3 channel activity. To determine the precursor sources of the PM PI (4,5) P_2 pool, we selectively depleted PI (4) P pools at the PM, or the Golgi, or both using dual rapamycin-translocatable enzymes, pseudojanin (PJ), an engineered tandem of lipid 4- and 5-phosphatases (SAC1 and INPP5E). Selectively depleting PI (4) P at the PM with PJ-SAC (only SAC1 is active) results in a secondary decrease of PI (4,5) P_2 measured by KCNQ channels or by PH-PLC domains. Compared to control pseudojanin (PJ), the decrease in current with PJ-SAC is only partial (～60% vs ～95%) and slower (140-s vs 14-s). The translocation of PH-PLC is similarly partial and slow. Depleting PI (4) P instead at the Golgi with PJ-SAC also induces a partial (35%), slow (60 s) secondary decline of PM PI (4,5) P_2 measured by KCNQ channels. Depleting PI (4) P simultaneously at the Golgi and PM with PJ-SAC recruited to both membranes induces a stronger decrease of PI (4,5) P_2 measured by KCNQ channels (100-s, 75%). Recruiting the ER (which contains endogenous SAC1) towards the Golgi using rapamycin-induced dimerization, mimics the effects of depleting PI (4) P at the Golgi. In conclusion, the PM pool of PI (4,5) P_2 derives from precursor pools of PI (4) P both in the PM and in the Golgi. The decrease in PM PI (4,5) P_2 when SAC1 is active at the Golgi suggests that the Golgi contribution is on-going and does not wait until the PM is depleted. (NIH grants NS08174, GM83913).

Symposium 55
Advance in the regulation of cardiovascular system: developing concept of G-protein signaling

(March 29, 13 : 20–15 : 20, Room H)

3S55H-1

G protein-dependent and independent signaling pathways by G protein-coupled receptors

Kurose, Hitoshi (*Graduate School of Pharmaceutical Sciences, Kyushu University, Japan*)

Signaling through G protein-coupled receptors (GPCRs) is believed to be mediated by heterotrimeric G proteins. However, it is recently recognized that GPCRs activate intracellular signaling pathways through β-arrestins that is independent of G proteins. β-arrestins are known as one of the regulators of GPCR desensitization. β-arrestins bind to GPCRs phosphorylated by GPCR-kinases. The phosphorylated and β-arrestin-bound receptors then internalize via clathin-coated pit, as β-arrestins can bind clathrin and adaptin. In addition to the roles of β-arrestins in receptor regulation, β-arrestins are reported to be involved in GPCR-induced cellular signaling that is independent of G proteins. We found that β-adrenergic receptor blocker metoprolol activated β-arrestin-mediated signaling. When a β-blocker metoprolol was administered to wild type mice, it induced cardiac fibrosis. Metoprolol did not interact with G proteins, but interacted with β-arrestin 2. Metoprolol-induced fibrosis was almost abolished in β-arrestin2 knockout mice. These results suggest that GPCR induces the response in a G protein-independent but β-arrestin-dependent manner. So far, β-blockers are classified as intrinsic activity, selectivity, pharmacokinetic parameters and so on. The present finding suggests that β-arrestin-mediated signaling is another index of β-blocker.

3S55H-2

Identification and characterization of activator of G-protein signaling (AGS) proteins induced in pathophysiological models of the heart

Sato, Motohiko; Suzuki, Hiroko; Sakima, Miho; Iwase, Satoshi; Inukai, Yoko; Nishimura, Naoki; Sato, Maki; Shimizu, Yuuki (*Physiology, Aichi Medical University, Nagakute, Japan*)

The G-protein signaling system plays important roles in signal integration of physiological stimuli including hormones/neurotransmitters to maintain homeostasis of the cardiovascular system. In addition to traditional components of G-protein signaling such as G-protein coupled receptor (GPCR), heterotrimeric G-proteins and effectors, recent data indicate the existence of accessory proteins that directly regulate the activation status of G-proteins independent of GPCR. Here, we report identification and characterization of G-protein activators induced in the ischemic myocardium or the hypertrophic heart. AGS8 was an ischemia/hypoxia inducible G-protein activator, which was isolated from repetitive transient ischemic model of the rat heart. AGS8 formed a complex with Gβγ, and regulated hypoxia-induced apoptosis of cardiomycoytes by changing permeability of cell-surface connexin 43. AGS11 was identified as a Gα16-interacting protein in the hypertrophic hearts of mouse. AGS11 translocated Gα16 into the nucleus and increased transcription of tight junction protein, claudin 14, suggesting a novel mechanism of transcriptional regulation by G-protein-mediated signaling. These data indicated unexpected regulation of pathophysiological events by heterotrimeric G-proteins and G-protein activators. The discovery of G-protein activator may contribute to uncovering mechanism underlying cardiovascular disease as well as development of novel therapeutic approaches to human disease.

3S55H-3

Old knowledge but novel insight to the cardiac non-neuronal cholinergic system—The possible involvement of this system in metabolic intervention to cells—

Kakinuma, Yoshihiko[1]; Akiyama, Tsuyoshi[2]; Okazaki, Kayo[1]; Arikawa, Mikihiko[1]; Noguchi, Tatsuya[1]; Sato, Takayuki[1] (*[1]Department of Cardiovascular Control, Kochi Medical School, Nankoku, Japan; [2]Department of Cardiac Physiology, National Cardiovascular Center Research Institute, Japan*)

Our recent studies have confirmed that cardiomyocytes possess a machinery synthesizing ACh of their own, which plays a specific physiological role in the local region. Furthermore, it has been revealed that this ACh synthesis is transcriptionally activated through muscarinic receptors in cardiomyocytes, suggesting that this system is regulated in a positive feedback fashion, and furthermore, this system negatively regulates cellular energy metabolism and reduces oxygen consumption through a normoxic induction pathway of HIF-1 system. These findings disclosed by us suggest that this system plays a protective role against failing cardiomyocytes suffering unbalanced energy metabolism. However, thus far few studies have been performed regarding how this system can be activated pharmacologically or non-pharmacologically and how the manipulation of this system can affect a cardiac outcome in a pathological situation in vivo. Focusing on these issues, we have established the pathophysiological meanings of the non-neuronal cholinergic system and how this system could be beneficial in the heart. In this session, the novel physiological features of ACh and clinical implication of the cardiac non-neuronal cholinergic system modulating energy metabolism in the cardiovascular field will be discussed.

3S56I-1

Network oscillations in the limbic system : mechanisms, modulation and physiological relevance

Murakoshi, Takayuki (*Department of Biochemistry, Faculty of Medicine, Saitama Medical University, Iruma-gun, Japan*)

There is growing body of evidence that network oscillation plays essential roles in the processing of cognitive and executive tasks in the limbic system, composed of the frontal and temporal cortices as well as the subcortical structures. The oscillatory activities in the brain are heterogeneous in frequency among these areas, corresponding to behavioral states of the animal. Abnormality in the oscillation is also reported in psychiatric disorders such as schizophrenia. Physical and psychological stresses often induce or exacerbate those disorders most likely via neuronal and synaptic dysfunction within the limbic system, including the amygdala and the anterior cingulate cortex. Here, I introduce the oscillatory bursts of compound inhibitory transmissions observed in slice preparations of basolateral nuclei of the amygdala and their modulation by dopamine. Another example of the circuit oscillation in the limbic system will be presented on recordings of field potentials from superficial layers of the anterior cingulate cortex, evoked by kainate receptor activation. The oscillation characterized by composition of frequency ranges and balance between left/right hemispheres is affected by chronic ingestion of ethyl alcohol. The influence of stresses including the ethanol and restraint stress will be discussed in terms of the roles of GABAergic inhibition in the network oscillations.

Symposium 56
Rhythmic and Sustained Activity in Basal Ganglia and Limbic System

(March 29, 13 : 20–15 : 20, Room I)

3S56I-2

Slow calcium oscillations in striatum

Osanai, Makoto[1,2] (*[1]Tohoku Univ. Grad. Sch. Med., Sendai, Japan; [2]JST, CREST, Tokyo, Japan*)

Calcium ion (Ca^{2+}) is a universal intracellular messenger, and plays enormous versatile rolls in cells. Especially in a nervous system, it is well known that Ca^{2+} triggers a neurotransmitter release from the presynaptic terminal. On the other hand, an intracellular signal transduction, which depends on metabotropic receptors, causes Ca^{2+} release from the intracellular Ca^{2+} store, an endoplasmic reticulum (ER). Physiological meanings of the role of the Ca^{2+} released from ER remain less well-defined. We have found the long-lasting spontaneous calcium transients (slow Ca^{2+} oscillation), which lasted up to about 300 s, in the striatal neuron and astrocytes. The Ca^{2+} oscillations were not induced by action potentials, but induced by Ca^{2+} release from ER via IP3 receptor. Transient rate of these Ca^{2+} oscillations in neurons were reduced by an antagonist of mGluR5, thus, mGluR5-PLC-IP3 pathway might involve to the Ca^{2+} oscillation.This slow Ca^{2+} oscillation did not blocked, but the auto- and cross-correlations were modified by TTX administration. In the condition of TTX administration, the rhythmicity of the Ca^{2+} oscillations increased compared to the control condition. The number of the correlated cell pairs of the Ca^{2+} oscillations was decreased by TTX administration. These phenomena were observed only in the corticostriatal slice but not in the striatal slice. Thus, the cortical activities might contribute to the striatal slow Ca^{2+} oscillations. In the computer simulation study, we found out that the spontaneous Ca^{2+} oscillation could alter the firing rate of the medium spiny neuron via modulation of Ca^{2+}-dependent potassium channels.

3S56I-3

Growth of Sustained Firing in Rat Striatum

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The cortico-basal ganglia networks are considered to be important for reward-based action selection and learning. Although many neurophysiological studies suggested that neurons localized in the basal ganglia are involved in these processes, how the neural system reflects the reward outcome to the chosen action is poorly understood. In reward-based learning, reward signal is significantly delayed after the action occurs. To associate action with reward, the neural system needs to use the sustained neural activities. Here, we focus on the striatum, the major input structure of the basal ganglia, and show sustained firing and its time development. We employed the acute slice of the Wistar Thy-1.2 promoter ChannelRhodopsin-2 Venus Rat and LED based local photostimulation techniques. We recorded from striatal neurons by a tetrode with photostimulation of the striatum. Striatal neurons that responded to photostimulation showed residual firings after the end of 1 sec-long photostimulation. Furthermore, the onset and the offset of the sustained firings were accelerated and prolonged, respectively, during repetitive stimulations. The speed of time development of the acceleration and the prolongation strongly correlated with the frequency of the repetitive stimulations. The developed onset and offset returned to the initial state by a several minutes of intermission. These phenomena indicate that the neurons in the striatum can mediate their input sensitivity over time.

3S56I-4

Oscillation and changes in the firing activity of midbrain substantia nigra pars reticulata in response to lowered energy supply

Yamada, Katsuya (*Dept. Physiol. Hirosaki Univ. Grad. Sch. Med. Hirosaki, Japan*)

Deprivation of oxygen and glucose readily leads to cessation of brain activity and loss of consciousness in a few minutes, ultimately to death if unheeded. Thus, it would be critical for the brain to alert life-threatening lowering of oxygen and glucose level efficiently. The substantia nigra pars reticulata (SNr), known to exhibit the highest spontaneous firing in the brain, regulates motor activity by changing their firings which target diverse nuclei including ventral thalamus, superior colliculus, and pedunculopontine areas in the brain stem. We have shown that the SNr firing markedly decreases during brief hypoxic challenge in an ATP-sensitive potassium (K_{ATP}) channel-openings, but increased when extracellular glucose concentration was lowered independently of the K_{ATP}-mediated mechanism. In addition, some SNr GABAergic neurons show abrupt increases in their spontaneous firings to above 100 Hz periodically in response to lowering of extracellular glucose. Including other data, we will discuss a possible role of SNr to convey information on lowered energy state to remote motor-related nuclei.

3S56I-5 (SOI-1)

Subthalamo-pallidal interactions underlying parkinsonian neuronal oscillations in the primate basal ganglia

Tachibana, Yoshihisa[1]; Iwamuro, Hirokazu[1]; Kita, Hitoshi[2]; Takada, Masahiko[3]; Nambu, Atsushi[1] ([1]*System Neurophysiol, NIPS, Okazaki, Japan;* [2]*Anatomy and Neurobiology, Univ of Tennessee, Memphis, USA;* [3]*Systems Neurosci, Primate Research Institute, Kyoto Univ, Inuyama, Japan*)

Parkinson's disease (PD) is characterized by degeneration of nigral dopaminergic neurons, leading to psychomotor dysfunctions. Accumulated studies suggest that abnormal oscillations in the basal ganglia contribute to the expression of PD symptoms. However, the mechanism that generates abnormal oscillations in a dopamine-depleted state remains poorly understood. We addressed this question by examining basal ganglia neuronal activity in two MPTP-treated parkinsonian monkeys. We found that systemic administration of L-DOPA (dopamine precursor) diminished abnormal oscillations (8-15 Hz) in the internal pallidum (GPi) and subthalamic nucleus (STN) when PD signs were alleviated. GPi oscillations and PD signs were suppressed by silencing of the STN with infusion of muscimol. Neuronal oscillations in the STN were suppressed after intrasubthalamic microinjection of CPP (NMDA receptor antagonist) and NBQX (AMPA/kainate receptor antagonist) to block glutamatergic afferents of the STN. The STN oscillations were further eliminated by muscimol inactivation of the external pallidum (GPe) to block GPe GABAergic inputs. These results suggest that, in the dopamine-depleted state, glutamatergic inputs to the STN and reciprocal GPe-STN interconnections are both important for the generation of the oscillatory activity of STN neurons, which is subsequently transmitted to the GPi, thus contributing to the symptomatic expression of PD.

Symposium 57
Genetic, molecular and electrophysiological mechanisms underlying cardiac arrhythmias
[Collaboration Symposium with The Scandinavian Physiological Society]

(March 29, 15 : 20–17 : 20, Room A)

3S57A-1
Gender differences in cardiac repolarization and the underlying mechanisms
Kurokawa, Junko (*Dept. Bio–informational Pharmacol., MRI, Tokyo Med. Dent. Univ.*)

Regulation of cardiac ion channels by sex hormones accounts for gender-differences in susceptibility of arrhythmias associated with QT prolongation (TdP), that is : Women are at a greater risk of TdP than men in both congenital and acquired long QT syndrome. The risk of drug-induced TdP varies during the menstrual cycle suggesting that the dynamic change in levels of ovarian steroids, estradiol (E2) and progesterone (P4), cyclically influence action potential duration (APD). Although the underlying mechanism has been studied by analysis of chronic effects on cardiac ion channels, it remains unclear whether the gender-difference is entirely due to transcriptional regulations through nuclear hormone receptors. We therefore investigated acute effects of E2 and P4 on cardiac ion currents in mammalian hearts, ventricular myocytes, and cell lines. We have found that P4 produce NO from eNOS through a non-genomic pathway in the heart. NO induced by P4 up-regulates currents through *S*-nitrosylation of the α-subunit of the cardiac I_{Ks} channel regardless of soluble guanylate cyclase activation. With cAMP-stimulation, P4 suppressed L-type Ca^{2+} channel currents in a cGMP-dependent manner. Both modulations result in APD shortening. On the other hand, E2 partially suppresses I_{Kr} currents directly. These data may explain dynamic changes of arrhythmia risk in women during the menstrual cycle and around the delivery, and can be a clue to avoid the potentially lethal arrhythmias in long QT syndromes.

3S57A-2
The voltage sensor of voltage–gated K channels as a target for potential antiepileptic and antiarrhythmic drugs
Elinder, Fredrik (*Linköping University, Sweden*)

Electrical signalling in excitable cells depends on voltage-gated ion channels which open and close in response to alterations in membrane potential. The central ion-conducting pore domain is surrounded by four voltage-sensor domains (VSDs), which sense the membrane potential and confer this information to the pore domain. The fourth segment (S4) of each VSD carries several positively charged residues, and must traverse outwards through the membrane electric field to open the channel. The open-state structures of both K and Na channels are known at atomic level. We have described four closed molecular configurations of a VSD based on 20 engineered metal-ion bridges, Rosetta modelling and molecular dynamics (Henrion et al., 2012, PNAS 109 : 8552-8557). In the opening transition, positively charged amino acid residues swing out towards the lipid bilayer. Free polyunsaturated fatty acids (PUFAs) open voltage-gated K channels by targeting these charges. As an important consequence thereof, PUFAs can suppress epileptic seizures and cardiac arrhythmia. To develop new drugs to reduce cellular excitability we first developed an ion channel with extremely high sensitivity to PUFAs by altering the extracellular end of S4. Because different voltage-gated K channels have different charge profiles, this implies channel-specific PUFA effects. Secondly, we have started to screen for small molecule compounds targeting the opening step. The identified site and the pharmacological mechanism will potentially be useful in future drug design of small-molecule compounds specifically targeting neuronal and cardiac excitability.

3S57A-3
Arrhythmogenic nature of pulmonary vein cardiomyocytes
Ono, Kyoichi[1]; Okamoto, Yosuke[1]; Adachi, Takeshi[1]; Ohba, Takayoshi[1]; Takano, Makoto[2] ([1]*Department of Cell Physiology, Akita University Graduate School of Medicine, Akita, Japan;* [2]*Department of Physiology, Kurume University, Kurume, Japan*)

Pulmonary veins (PVs) have been described as an important source of atrial fibrillation (AF), and therefore PV isolation has become the cornerstone of AF ablation in clinical practice. One of the mechanisms underlying the condition is ectopic pacemaking activity. In fact, it has been reported that myocardial sleeves have the potential to generate automaticity under various conditions. This study compared the properties of cells from the rat myocardial sleeves of PVs with cells from the left atria. Isolated PV cardiomyocytes were visually identified as relatively large, rectangular-shaped cells with marked striation, in contrast to atrial cells, which were usually small and spindle- or rod-shaped. PV cardiomyocytes had a more depolarized resting membrane potential and a shorter action potential duration than left atrial myocytes. We have found that PV cardiomyocytes elicited repetitive and sustained spontaneous action potentials in response to norepinephrine (NE) via activation of both α_1- and β_1-adrenergic receptors. The NE-induced spontaneous activity was not associated with a change in membrane resistance but was preceded by a transient increase in $[Ca^{2+}]_i$, indicating that the automaticity is caused by Ca^{2+}-clock, not by ion channel clock mechanisms. The increase in $[Ca^{2+}]_i$ activated an inward Na^+/Ca^{2+} exchange current causing depolarization, and lead to firing of the action potential. Immunocytochemical studies showed that cardiomyocytes in PVs possessed an enriched T-tubule system, and that NCX and IP_3R were co-localized along T-tubules. Pharmacological experiments demonstrated that PLC or IP_3R inhibitors blocked the NE-induced automaticity. We conclude that functional coupling between NCX and IP_3R underlies NE-induced automaticity in rat PV cardiomyocytes, and that PV cardiomyocytes have distinctly unique morphological and electrophysiological features which predispose them to the development of spontaneous activity.

3S57A-4

Genetic variants in cardiac ion channels causing lone atrial fibrillation in young patients

Olesen, Søren-Peter; Olesen, Morten Salling; Liang, Bo; Schmitt, Nicole; Yuan, Lei; Jespersen, Thomas; Christophersen, Ingrid; Haunsø, Stig (*Danish Arrhythmia Research Centre, University of Copenhagen, Denmark*)

Atrial fibrillation (AF) is the most common type of cardiac arrhythmia affecting about 1% of the general population, and the incidence increases strongly with age. We assume that genetic factors may be specifically important in young AF patients and investigated a cohort of 197 patients developing lone AF before the age of 40.

In this young lone AF cohort, 10 variants of Nav1.5 were found distributed widely over the length of the protein. Nine out of 10 variants had compromised peak current, and 5 out of 5 that were studied for effects on the sustained Na current had a 3-8 fold increase. Interestingly, 7 of the probands carried a mutation previously associated with Long QT syndrome type 3, and these were also the patients showing the longest QT intervals in our study. The overlap between the diseases could indicate an increased tendency to early afterdepolarization in both atria and ventricles.

The young patients further showed a number of mutations in Kv7.1 conducting the cardiac Iks current found (3 gain- and 1 loss-of-function), and in Kv1.5 conducting the atrial Ikur current (3 gain- and 3 loss-of-function). Gain-of-function mutations in Kv1.5 have not been described before as cause of AF.

In conclusion, patients with the onset of AF at young age exhibit a high prevalence of Nav and Kv variants. The Nav variants are dominated by a decreased peak current and an increases late current, whereas both loss- and gain-of-function of the Kv channels are seen to enhance AF susceptibility.

Symposium 58
Novel molecular and cellular mechanisms for sex steroid actions in the brain

[Collaboration Symposium with Japan Neuroendocrine Society]

(March 29, 15 : 20–17 : 20, Room D)

3S58D-1

Roles of progranulin in mediating estrogen actions in the brain

Nishihara, Masugi (*Department of Veterinary Physiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo*)

Sex steroids play important roles in regulating brain functions of mammals throughout the life. During the fetal or perinatal period, sex steroids are involved in sexual differentiation of the brain, and this action is known as organization. After maturation, sex steroids induce sex-specific behavioral and endocrine patterns, the action of which is called activation. In addition, it is now well recognized that sex steroids are involved in neuroprotection by preventing neurodegeneration and facilitating neurogenesis, and thereby preserve cognitive function. We found that a growth factor progranulin (PGRN) is involved in mediating sex steroid actions on brain sexual differentiation and adult neurogenesis in rats. We then generated PGRN-deficient mice, and found that they showed a decrease in ejaculation incidence, elevation of anxiety and aggression, increase in the volume of the locus coeruleus, decrease in running-induced neurogenesis, and enhancement of neuroinflammatory responses following traumatic brain injury. All these observations are in consistent with the notion that PGRN is involved in both organizational and neuroprotective actions of sex steroids. Further, in vitro study using neural progenitor cell culture revealed that the actions of PGRN are mediated at least in part by phosphorylation of GSK3β. PGRN is one of the estrogen-inducible genes, and thus, PGRN plays an important role in mediating sex steroid actions in the brain. In addition, there may be common molecular mechanisms between these two sex steroid actions, namely organization and protection, in the brain.

3S58D-2

Identification and functional analysis of a sexually dimorphic protein in the AVPV

Ohtani-Kaneko, Ritsuko (*Department of Life Sciences, Toyo University*)

In the rat brain, the anteroventral periventricular nucleus (AVPV) has a greater number of neurons in females than in males. Sexual dimorphism in the AVPV is also observed in the number of neuronal subpopulations ; adult female rodents have 10-20 times more kisspeptin-immunoreactive (ir) neurons and 3-4 times more tyrosine hydroxylase (TH)-ir neurons than males. In this study, using proteomic analysis and gene-deficient mice, we attempted to identify proteins that regulate the number of TH-ir and/or kisspeptin-ir neurons in the AVPV. Analysis of protein expression in the AVPV on postnatal day 1 (PD1) identified collapsin response mediator protein-4 (CRMP4) as one of proteins exhibiting sexually dimorphic expression. Interestingly, sexually differential expression of CRMP4 mRNA and protein in the AVPV was not detected on PD6. Next, we used CRMP4-knockout (CRMP4-KO) mice to determine the function of CRMP4 in the AVPV. Knockout of *Crmp4* did not change the number of kisspeptin-ir neurons in the adult AVPV of both sexes. However, the number of TH-ir neurons was increased in the AVPV of adult female CRMP4-KO mice as compared with the adult female wild-type mice. During the development, no significant difference in the number of TH-ir neurons was detected between sexes or genotypes on embryonic day 15, but a female-specific increase of TH-ir neurons was observed in CRMP4-KO mice on PD1, when the sex difference was not yet apparent in wild-type mice. These results indicate that CRMP4 mediates the regulation of the number of TH-ir cells in the female AVPV.

3S58D-3

Epigenetic regulation of kisspeptin neurons mediating estrogen-feedback action on GnRH release

Tsukamura, Hiroko[1]; Tomikawa, Junko[1]; Uenoyama, Yoshihisa[1]; Maeda, Kei-ichiro[2] ([1]*Grad. Sch. Bioagricultural Sci., Nagoya Univ., Nagoya, Japan;* [2]*Dept. Vet. Med. Sci., Univ. Tokyo, Tokyo, Japan*)

Kisspeptin-GPR54 system has been well known to govern reproduction via regulating gonadotropin-releasing hormone (GnRH) release in mammals. Kisspeptin neurons located in the anteroventral periventricular nucleus (AVPV), are responsible for the estrogen-positive feedback action to induce GnRH/gonadotropin surge. The present paper focuses on the epigenetic mechanism mediating estrogen action on *Kiss1* gene expression in the brain to understand the mechanism underlying GnRH regulation. We revealed that histone of AVPV *Kiss1* promoter region was highly acetylated, and estrogen receptor (ER) α was recruited at the region by estrogen. In contrast, the histone of *Kiss1* promoter region in the arcuate nucleus (ARC), in which Kiss1 expression is down-regulated by estrogen, was deacetylated by estrogen. Inhibition of histone deacetylation upregulated *in vitro Kiss1* expression in a hypothalamic non-*Kiss1*-expressing cell line. Gene conformation analysis indicated that estrogen induced formation of a chromatin loop between *Kiss1* promoter and the 3' intergenic region. The notion was further supported by *in vivo* reporter assay with transgenic reporter mice. Taken together, estrogen might induce recruitment of ERα and histone acetylation in the AVPV *Kiss1* promoter region and consequently enhances chromatin loop formation of *Kiss1* promoter and *Kiss1* gene enhancer, resulting in an increase in AVPV-specific *Kiss1* gene expression. Thus, epigenetic regulation of the *Kiss1* gene is a part of estrogen-positive feedback mechanism to generate the GnRH/gonadotropin surge and consequently ovulation. This work was supported in part by the Research Program on Innovative Technologies for Animal Breeding, Reproduction, and Vaccine Development.

Symposium 59
Outsourcing for efficient and high quality research
[Japan Young Physiologist Associasion Symposium]

(March 29, 15 : 20–17 : 20, Room E)

3S58D-4

Possible involvement of microglia in regulation of GnRH neural functions

Fujioka, Hitomi; Funabashi, Toshiya; Akema, Tatsuo (*Department of Physiology, St. Marianna University School of Medicine, Kawasaki, Japan*)

Prostaglandins (PGs) are involved in the control of gonadotropin-releasing hormone (GnRH) secretion in the hypothalamus of various species, but details are not fully understood. For example, which cell types produce PGs are not known. The present studies were aimed (a) to clarify the role of PGs in regulating GnRH cell functions in the preoptic area (POA), (b) to identify cell types containing the cyclooxygenase (COX) isozyme responsible for producing PGs that regulates GnRH neurons, and (c) to determine the effects of sex steroids on the COX isozyme expression. In vivo studies, we found that pretreatment with COX inhibitors did not affect sex steroid-induced luteinizing hormone (LH) surge per se, but significantly reduced the number of GnRH-immunoreactive cells during the LH surge. Surprisingly COX-1 immunoreactivity in the vicinity of GnRH neurons was almost entirely localized in microglia in the POA, but not in neurons and astrocytes. COX-1 immunoreactivity in microglia was found in the POA of both ovarian steroid-primed and sesame oil-treated ovariectomized rats. Immunoreactivity of sex steroid receptors was not found in microglia in the POA. These findings suggest constitutive expression of COX-1 irrespective of steroid hormone milieu in microglia, which provide PGs that affect GnRH neuronal functions in the POA. We will also present direct electrophysiological effects of PGE2 on GnRH neurons in the POA.

Key words : cyclooxygenase-1, gonadotropin-releasing hormone, microglia, ovarian steroids

3S59E-1

Support of drug development and clinical research using imaging technology

Matsui, Hiroshi (*MICRON Inc., Tokyo, Japan*)

In this presentation, the method and example of outsourcing are introduced for the researcher who wants to use imaging technology like PET, MRI, the researcher who considers large-scale research, and the researcher who is planning the clinical trial.

3S59E-2

Custom monoclonal antibody production service by University-originated bio-tech venture

Tachibana, Taro[1,2] (*[1]Cell Engineering Corporation, Osaka, Japan; [2]Osaka City University, Osaka, Japan*)

We have established a bio-tech venture company based on the biotechnology developed in our University. Our company has provided a customized monoclonal antibody production service for researchers in Universities and life science companies. We have aimed to provide a high quality service and a money-back guarantee if not fully satisfied with customer's specific applications. I will talk about the merits and shortcomings of a customized service for researchers.

3S59E-3

An on-the-spot telecast from the scene of outsourcing life science study

Shinkuma, Tadanobu (*Hitec, Inc.*)

There are no many research institutions where it has sufficient and relevant equipments and specialists such as universites or companies. Besides when begin a new project ; all the more. Commonly, they want to minimize labor to setup a project in limited time. Then, some institutions adopt outsourcing to supply the deficiency. In this section, I express about advantage and disadvantage of outsourcing company and employee in life science.

3S59E-4

One should go to specialists for the best results : An example from behavioral phenotyping of genetically -engineered mice

Miyakawa, Tsuyoshi (*Div. of Sys. Med., ICMS, Fujita Health Univ. Toyoake, Japan*)

We have been investigating the relationships between genes and behaviors by conducting a systematic and well-defined behavioral test battery with the mice that have a mutation on a gene of interest (Powell and Miyakawa, 2006 ; Takao and Miyakawa, 2006 ; Takao et al., 2007). To date, we have subjected more than 160 different strains of genetically engineered mice to the comprehensive behavioral test battery, as a large-scale project in collaboration with 98 laboratories in Japan. Surprisingly, among them, more than 140 strains have shown at least some behavioral phenotypes, and we have successfully identified putative mice models of neuropsychiatric disorders (Miyakawa et al., PNAS, 2003 ; Arron et al., Nature, 2006 ; Yamasaki et al., Mol. Brain, 2008 ; Nakatani et al., Cell, 2009 ; Yamada et al., Nature Medicine, 2009 ; Ohno et al., Nature Neurosci., 2009 ; Koshimizu et al., Mol. Brain, 2012). Some of such mice models show striking similarities of their phenotypes to those of human patients (Takao et al., submitted) and are considered to be useful, or even essential, in elucidating the core pathophysiology of such disorders. In this symposium, I will discuss the importance of establishing collaborational network in Japanese research community, by showing examples from our own studies.

Symposium 60
Synaptic remodeling: molecular mechanisms and physiology

(March 29, 15 : 20–17 : 20, Room F)

3S60F-1

Neural circuit formation mediated by an endogenous Nogo receptor antagonist LOTUS

Takei, Kohtaro (*Division of Medical Life Sciences, Yokohama City Univ. School of Medicine, Yokohama, Japan*)

Neural circuitry formation depends on the molecular control of axonal projection during development. We identified a novel molecule for lateral olfactory tract (LOT) formation by functional screening, and named it LOT usher substance (LOTUS). We further identified Nogo receptor-1 (NgR1) as a LOTUS-binding protein, which is well known as a common receptor of myelin-derived axon growth inhibitors, such as Nogo. It has been thought that non-permissive environment for neural regeneration in the adult central nervous system is caused by NgR1. LOTUS suppresses binding of NgR1 ligands to NgR1 and their ligands-induced growth cone collapse and axon growth inhibition in vitro. A defasciculated axon bundle and increased axon branching of LOT were observed in single mutants of lotus-deficient mice, whereas normal axon bundle and decreased branching of LOT were seen in ngr1-deficent mice. The defasciculated LOT and increased branching seen in single mutants of lotus-deficient mice was disappeared in double mutants of lotus- and ngr1-deficient mice. These findings suggest that endogenous antagonism of LOTUS to NgR1 plays a crucial role in axon bundling and branching of LOT. Such antagonistic action of LOTUS to NgR1 provides new insight into neural development mechanisms and also therapeutic approaches for neural regeneration.

3S60F-2

Activity-Dependent Remodeling of Thalamocortical Axon Branching

Yamamoto, Nobuhiko (*Graduate School of Frontier Biosciences, Osaka University, Japan*)

How neuronal activity refines neuronal connectivity during development is one of the most intriguing issues in neuroscience. The thalamocortical (TC) projection is a suitable system in which to address this issue. TC axons from sensory thalamic nuclei form branching, primarily in layer 4 of the cortex, the TC recipient layer. TC axon branching is also known to be modified by neural activity, as exemplified in the eye-specific projections in higher mammals. A fundamental question is what molecular mechanisms are involved in the activity-dependent processes. Here we demonstrate that the netrin family member Netrin-4 is involved in TC axon branching by being expressed in an activity-dependent fashion and that the receptor in TC axons mediates the signal to alter the cytoskeleton changes. Moreover, we also show evidence that presynaptic structure may trigger axon branching.

3S60F-3

CaMKII serve as a gate of activity-induced structural and functional modification of hippocampal dendritic spines

Hayashi, Yasunori (*Brain Science Institute, RIKEN, Saitama, Japan*)

The size of the synapse is the major determinant of input strength. Therefore, the mechanism regulating the size can be the primary mechanism of synaptic plasticity. Here we demonstrate that Ca^{2+}/calmodulin-dependent protein kinase (CaMKII), the pivotal kinase in synaptic plasticity, mediates activity dependent structural modification of excitatory synapse through a novel activity-regulated F-actin stabilizing function, apart from well-known kinase signaling. This involves F-actin bundling ability of CaMKII which is negatively regulated by activation by Ca^{2+}/calmodulin and resultant autophosphorylation reaction on multiple serines and threonines within the F-actin binding domain. This allows unbundling of F-actin, which opens a temporary time window of ~1 min where F-actin remodeling by actin modifies such as cofilin, Arp2/3, and gelsolin can take place that leads to structural plasticity of dendritic spines. These observations make CaMKII a unique F-actin mechanism with a permissive role on structural regulation by synaptic activity, thereby acting as a gate of activity-dependent modification of synaptic structure.

3S60F-4

Hippocampal learning activates both excitatory and inhibitory synaptic transmission in CA1 neurons

Mitsushima, Dai[1,2]; Takahashi, Takuya[2] ([1]*Dept Systems Neuroscience, Yamaguchi Univ. Ube, Japan;* [2]*Dept Physiology, Yokohama City Univ. Yokohama, Japan*)

By combining HSV-mediated in vivo gene delivery with in vitro patch-clamp recordings, we previously reported that contextual learning drives GluR1-containing AMPA receptors into hippocampal CA3-CA1 synapses. More importantly, this molecular event is required for contextual learning (Mitsushima et al, PNAS 2011). To further examine the learning-dependent synaptic plasticity, we recorded miniature EPSC (mEPSC) and miniature IPSC (mIPSC) from the same CA1 neuron under the presence of TTX (0.5 μM). Although control rats (untrained, unpaired, or walk through) show small mEPSC and mIPSC amplitudes, IA trained rats show significantly higher mEPSC and mIPSC amplitudes with wide variation. To determine intrinsic trigger of the synaptic plasticity, cholinergic receptor antagonist was microinjected into the CA1 neurons 15 min before the contextual learning. Microinjection of muscarinic M_1 receptor antagonist (Prz) into the CA1 successfully blocked the learning-dependent increase in mEPSC amplitude but not mIPSC amplitude. Conversely, microinjection of nicotinic α7 receptor antagonist (Mla) successfully blocked the learning-dependent increase in mIPSC amplitude but not mEPSC amplitude. In behaving rats, bilateral microinjections of Prz or Mla into CA1 successfully block the learning. These results suggest that ACh mediates learning-induced plasticity at both excitatory and inhibitory synapses in CA1 neurons. The learning driven wide diversity of synaptic input in CA1 neurons may participate in engram of contextual memory.

Symposium 61
Membrane proteins in kidney tubules: from molecules to disease

(March 29, 15 : 20–17 : 20, Room G)

3S60F-5

Cross modal reorganization of cortical circuit

Jitsuki, Susumu; Takahashi, Takuya (*Department of Physiology, Yokohama City University, Yokohama, Japan*)

Loss of one type of sensory input can cause improved functionality of other sensory systems. Whereas this form of plasticity, cross-modal plasticity, is well-established, the molecular and cellular mechanisms underlying it are still unclear. Here we show that visual deprivation (VD) increases extracellular serotonin in the juvenile rat barrel cortex. This increase in serotonin levels facilitates synaptic strengthening at layer 4-layer 2/3 synapses within the barrel cortex. Upon VD, whisker experience leads to trafficking of the AMPA-type glutamate receptors (AMPARs) into these synapses through the activation of ERK and increased phosphorylation of AMPAR subunit GluR1 at the juvenile age when natural whisker experience no longer induces synaptic GluR1 delivery. VD thereby leads to sharpening of the functional whisker-barrel map at layer 2/3. Thus, sensory deprivation of one modality leads to serotonin release in remaining modalities, facilitates GluR1-dependent synaptic strengthening and refines cortical organization.

3S61G-1

Vasopressin V1a receptor gene and motivated behavior in mice and humans

Masuki, Shizue; Sumiyoshi, Eri; Nose, Hiroshi (*Dept. Sports Med. Sci., Shinshu Univ. Grad. Sch. Med., Matsumoto, Japan*)

Arterial pressure increases at the onset of voluntary locomotion, which is likely advantageous for starting to move smoothly by supplying blood flow to contracting muscles without delay. However, the mechanisms remain unclear. We previously reported in free-moving wild-type mice (WT) that increased cerebral activity suppressed baroreflex control of heart rate (HR), followed by voluntary locomotion at higher probability. Moreover, we recently found that the linkage between cerebral activity, baroreflex control of HR, and voluntary locomotion was tightened during enhanced food-seeking behavior after the onset of 24-h food deprivation in WT. However, these responses were abolished in vasopressin V1a receptor knockouts. Also, we found that the linkage was abolished in WT when V1a receptor antagonist was injected into the nucleus tractus solitarii. Based on the results in mice, we compared the adherence rate of interval walking training between polymorphism (rs1042615) of vasopressin V1a receptor in middle-aged and older people who had performed the training more than 29 mos. We found that walking intensity, walking time per day, and walking days per week decreased more rapidly in TT genotype men than those in other genotype men with significances after the 18 th mo of training. Thus, central V1a receptor plays an important role in starting motivated locomotion through suppression of baroreflex control of HR, contributing to pressor responses. This might help explain the lower adherence to exercise training in TT men.

3S61G-2

Metabolic acidosis caused by insufficient vasopressin V1a receptor in kidney collecting duct

Kawahara, Katsumasa; Yasuoka, Yukiko (*Dept. of Physiol., Kitasato Univ. Sch. Med., Sagamihara, Japan*)

The kidney maintains plasma pH homeostasis by excreting the net excess of acid in urine. The acid excretion and its related processes are precisely regulated in different nephron segments with different cellular mechanisms. Recently, Nonoguchi and his colleagues demonstrated that in the rat kidney collecting duct (CD) the expression levels of vasopressin V1a receptor (V1aR) mRNA and protein increased after metabolic acidosis (Tashima et al, 2001), and that the insufficient expression of V1aR in mice kidneys resulted in type 4 RTA (Izumi et al, 2011). In the present symposium, we would like to discuss the following issues : (1) A target cell of the vasopressin-V1aR axis along the nephron. (2) A lower urinary acidification in the $V1aR^{-/-}$ mice. (3) Acidosis-induced hypertrophy in intercalated cell (IC) through the CD requires activation of the V1aR axis. In normal condition, V1aR mRNA was moderately expressed in medullary thick ascending limb (MTAL) and highly in the IC through the CDs. During NH_4Cl-loading of 6 days, the V1aR mRNA was upregulated significantly ($P<0.05$) both in the TAL and the IC of CD in the inner stripe in the outer medulla (MTALis and IC of OMCDis, respectively). In parallel, cell-height of the tubule significantly ($P<0.005$) increased by 40% in the IC of OMCDis, which was completely attenuated in the $V1aR^{-/-}$ mice. Urinary excretion of NH_3/NH_4^+ was significantly lower in the $V1aR^{-/-}$ mice. These results strongly suggest that a vasopressin-V1aR axis in the IC of OMCDis plays an important role for urinary acidification, especially during metabolic acidosis.

3S61G-3

Role of claudin－2 in proximal tubule paracellular Na/Cl transport

Muto, Shigeaki (*Department of Nephrology, Jichi Medical University. Shimotsuke, Japan*)

Claudin-2 is highly expressed at tight junctions of the mouse proximal tubule, which is composed of a leaky epithelium, and reabsorbs the largest fraction of filtered NaCl and water. To investigate the role of claudin-2 in paracellular NaCl transport in this nephron segment, we generated knockout mice lacking *claudin-2* (*Cldn2*$^{-/-}$) by gene-targeted disruption. The *Cldn2*$^{-/-}$ mice displayed normal appearance, activity, growth, and behavior. Light microscopy revealed no gross histological abnormalities in the *Cldn2*$^{-/-}$ kidney. Ultrathin section and freeze-fracture replica electron microscopy revealed that, similar to those of wild types, the proximal tubules of *Cldn2*$^{-/-}$ mice were characterized by poorly developed tight junctions with one or two continuous tight junction strands. In contrast, studies in isolated, perfused S2 segments of proximal tubules showed that net transepithelial reabsorption of Na^+, Cl^- and water was significantly decreased in *Cldn2*$^{-/-}$ mice and that there was an increase in paracellular shunt resistance without affecting the apical or basolateral membrane resistances. Moreover, deletion of claudin-2 caused a loss of cation (Na^+)-selectivity, and therefore relative anion (Cl^-) selectivity in the proximal tubule paracellular pathway. With free access to water and food, fractional Na^+ and Cl^- excretions in *Cldn2*$^{-/-}$ mice were similar to those in wild types, but both were greater in *Cldn2*$^{-/-}$ mice after intravenous administration of 2% NaCl. Taken together, these findings indicate that claudin-2 constitutes leaky and cation (Na^+)-selective paracellular channels within tight junctions of mouse proximal tubules.

3S61G-4

Disease caused by defective trafficking of intercellular adhesion molecules in renal tubular epithelial cells

Ikari, Akira; Yamazaki, Yasuhiro; Yamaguchi, Masahiko; Sugatani, Junko (*Sch. Pharm. Sci., Univ. Shizuoka, Shizuoka, Japan*)

A deficiency in Mg^{2+} can cause hypertension, but little is known about the abnormal mechanism of Mg^{2+} homeostasis. Mg^{2+} is mainly reabsorbed by claudin-16 in the thick ascending limb of Henle's loop. So far, we reported claudin-16 was dephosphorylated in Dahl salt-sensitive hypertensive rats. Therefore, we examined whether the phosphorylation of claudin-16 affect transepithelial Mg^{2+} permeability using Madin-Darby canine kidney (MDCK) cells expressing FLAG-tagged claudin-16. Protein kinase A (PKA) and adenylate cyclase inhibitors reduced the phosphoserine level of claudin-16. Furthermore, PKA and adenylate cyclase inhibitors decreased transepithelial Mg^{2+} permeability. Wild type claudin-16 was associated with ZO-1, a scaffolding protein, and localized at the tight junction (TJ). In contrast, dephosphorylated claudin-16 moved from detergent-insoluble to soluble fractions and was dissociated from ZO-1. Fusion protein of claudin-16 with glutathione-S-transferase revealed that Ser217 was phosphorylated by PKA. The S217A mutant was translocated into the lysosome. The degradation of dephosphorylated claudin-16 and S217A mutant was inhibited by chloroquine, a specific lysosome inhibitor. Thus, the PKA-dependent phosphorylation of Ser217 in claudin-16 may be essential for its localization at the TJ and transepithelial Mg^{2+} transport. Recently, it was reported that pathways involved in blood pressure control were up-regulated in the kidney of claudin-16 knockout mice. We suggest that the dysfunction of claudin-16 is one cause of hypertension.

3S61G-5

Molecular Mechanisms of the Ion Selective Permeation Through the Channel

Oiki, Shigetoshi (*University of Fukui Faculty of Medical Sciences, Fukui, Japan*)

Regulation of the electrolyte and water balance in the kidney is coordinated by various types of ion-transporting membrane proteins on the epithelial cells. Among them, ion channels play fundamental roles for the transepithelial transport, and molecular mechanism of channels has been studied extensively. Based on the crystal structures of channel proteins, molecular features, such as the gating, ion permeation and the selectivity, have been studied. For the potassium channel, the crystal structure revealed a short narrow pore (selectivity filter), in which ions and water molecules permeate in single file. To understand the permeation mechanism through the selectivity filter of potassium channels, we developed a method for measuring the streaming potential that reveals the coupling ratio of ion and water flux ($CR_{w\text{-}i}$) through the pore. For HERG (human ether-a-go-go related gene) and KcsA potassium channels, the $CR_{w\text{-}i}$ value was one at high K^+ concentrations, indicating that ion and water molecules are aligned alternatively in the selectivity filter. These data on the microscopic process of ion permeation are crucial to understand the selectivity of the channel, and $CR_{w\text{-}i}$ is in fact subject to change for permeable ions such as Rb^+. In contrast, potassium channels are mostly impermeable for Na^+, but at high membrane potentials, intracellular Na^+ can permeate slightly with the mechanism called the punch-through. We will discuss the molecular mechanism of the ion selectivity based on the latest results.

3S61G-6

Single molecular fluctuation of CFTR channels observed by high speed AFM

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Cystic Fibrosis Transmembrane conductance Regulator (CFTR) chloride channel, a member of ABC transporter superfamily, gates following ATP-dependent conformational changes of the nucleotide binding domains. CFTR is expressed along the entire nephron whereas its function in renal tubule epithelial cells remains unclear. However, CFTR has been proposed as a regulator of ROMK channel that is critical for K^+ secretion in nephron. Channel function of CFTR has been mainly studied by measuring ionic current going through the pore using the patch-clamp technique, which has given us many important findings about CFTR dysfunction. On the other hand, recent advances in X-ray crystallography provide atomic-level structures for several bacterial and mammalian ABC transports. However, neither the electro-physiology nor the crystal structure can give us the information about the molecular dynamic processes of CFTR proteins.In this study, we applied the high speed atomic force microscopy (HS-AFM) to image dynamic structural changes and interactions occurring in individual CFTR molecules. The HS-AFM visualized a dimeric formation of DMM-solubilized, purified WT-CFTR molecules attached on the stage over sideways. Next we observed the solubilized CFTR molecules incorporated into the lipid bilayer expanded on the AFM stage. The CFTR molecules showed a fluctuation varied among themselves, which might be underlain by various pre-phosphorylation levels in the R-domain.

Symposium 62
Respiration during voluntary behaviors

(March 29, 15 : 20–17 : 20, Room H)

3S62H-1

The neural mechanism of vocalization – respiration mode switching in the Nucleus Parabrachialis

Arata, Akiko (*Div. of Physiome, Dept. of Physiol., Hyogo College of Medicine Nishinomiya, Japan*)

The NPB complex, consisting of the lateral nucleus parabrachialis, medial nucleus parabrachialis, and Kolliker-Fuse nucleus, is known as a respiratory modulating center. We examined how the NPB participates in the inspiratory off-switch using brainstem-spinal cord preparations obtained from 0-4-days old rats. First, the effects of NPB electrical stimulation on C4 ventral nerve inspiratory activity using hemisectioned the pons preparation were examined. The electrical stimulation induced a transient depression or termination in C4 inspiratory activity. This inhibition of C4 inspiratory activity was greatly reduced by perfusion of NMDA antagonists and the inhibition was blocked by perfusion of a $GABA_A$-antagonist. When NMDA-antagonist was microinjected into the NPB, the inhibition of C4 activity by the NPB stimulation was reduced. Inspiratory-expiratory (I-E) neurons were found in the NPB. We also recorded intracellularly Pre-inspiratory neurons (Pre-I), inspiratory neurons (Insp), expiratory neurons (Exp) in the medulla. Insp received IPSPs and Exp received EPSPs when NPB was stimulated. The NPB stimulation inhibited inspiratory neurons and excited expiratory neurons. It seems that NPB is active switching from inspiratory phase to expiratory phase. In conclusion, 1) NPB is involved in the inspiratory off-switch in neonatal brainstem-spinal cord preparations, 2) NMDA receptors within the NPB involved in I-E neurons which may be inspiratory off-switch neurons, and 3) NPB might be involved in the active phase switching from involuntary movements to voluntary movements such as vocalization.

3S62H-2

Breathing and Emotion

Homma, Ikuo; Masaoka, Yuri (*Department of Physiology, Showa University School of Medicine, Tokyo, Japan*)

Breathing is not only generated by metabolic demands, but also generated by emotions. This type of breathing is generally called the behavioral breathing, but because breathing and emotions are linked tightly, we also call this breathing which changes alongside with emotions, the emotional breathing. In our research, we showed that the respiratory rhythm increased during anticipatory anxiety and the increase of respiratory frequency was correlated with the trait anxiety scores. Source generators for emotional breathing were examined in humans using EEG/dipole tracing method. The source was located in the amygdala. We called this activity the respiratory related anxiety potential. Respiratory related activity was also observed in the amygdala in the limbic-brainstem-spinal cord preparation of new born rat. Relationships between emotions and respiratory frequencies were examined in various situations. Breathlessness occurred in subjects who were asked to observe breathlessness in another person. This empathetic breathing may be explained through the close relationship between emotion and breathing.

3S62H-3

Zen Meditation and Respiration

Arita, Hideho (*Dept. Physiol., Toho Univ. Sch. Med., Tokyo, Japan*)

To gain insight into the neurophysiological mechanisms involved in Zen meditation, we evaluated the effects of abdominal (Tanden) breathing in novices. We investigated hemodynamic changes in the prefrontal cortex (PFC), an ttention-related brain region, using 24-channel near-infrared spectroscopy during a 20-min session of Tanden breathing in 15 healthy volunteers. We found that the level of oxygenated hemoglobin in the anterior PFC was significantly increased during Tanden breathing, accompanied by a reduction in feeling of negative mood compared to before the meditation session. Electroencephalography (EEG) revealed increased alpha band activity and decreased theta band activity during Tanden breathing. EEG changes were correlated with a significant increase in whole blood serotonin (5-HT) levels. These results suggest that activation of the anterior PFC and 5-HT system may be responsible for the improvement of negative mood and EEG signal changes observed during Tanden breathing.

Symposium 63
Circadian Signalosome; Capturing Chrono-biosignal, toward Chrono-Molecular Medicine

(March 29, 15 : 20–17 : 20, Room I)

3S62H-4

Behavior and Respiration : A role of TRPA1

Kuwaki, Tomoyuki[1]; Yonemitsu, Toru[1,2]; Kanmura, Yuichi[2] ([1]*Dept. Physiology, Kagoshima Univ. Grad. Sch. Med. Dent. Sci., Kagoshima, Japan;* [2]*Dept. Anesth. Crit. Care Med., Kagoshima Univ. Grad. Sch. Med. Dent. Sci., Kagoshima, Japan*)

TRPA1 channel, a member of the transient receptor potential super family, is expressed in a subset of sensory neurons in the trigeminal, nodose, and dorsal root ganglia. At the vagal afferent nerve terminals in the airway, TRPA1 plays as irritant receptor that triggers respiratory slowing to diminish further inhalation of irritant materials. We hypothesized that TRPA1 would also be involved in detecting environmental chemicals before they reach to the lower airway. To test our hypothesis, we did place avoidance test using TRPA1 knockout (KO) mice and age matched wild-type (WT) mice. The mice were first allowed to freely explore a homemade apparatus that consists of two chambers and a connecting tube for 20 min. The number of entry times and the amount of time spent in each chamber were recorded. None of the animals had initial bias for either chamber. Then, each chamber was randomly assigned to a room in which a piece of cotton paper soaked with a test solution was placed. During a test period of 20 min, WT mice never tried to enter the chamber with formaldehyde, one of the known activators of TRPA1. KO mice entered the chamber without hesitation and even stayed there. A nasal but not systemic administration of AP18, a blocker of TRPA1, successfully blocked avoidance behavior of WT mice. These result show that TRPA1 in the upper airway triggers active behavioral avoidance while that in the lower airway triggers passive respiratory avoidance to the environmental irritants.

3S63I-1

Understanding the circadian signalosome to establish a basis for cancer chronotherapy

Ikeda, Masaaki[1,2] ([1]*Dept. of Physiology, Saitama Medical Univ., Moroyama, Japan;* [2]*Molecular Clock Project, Research Center for Genomic Medicine, Saitama Medical Univ., Moroyama, Japan*)

Chronotherapy is applied in various diseases, including cancer and neuropsychiatric, cardiovascular, allergic, and metabolic diseases. However, the molecular and cellular bases of chronotherapy are not fully understood, especially the basis for its application as cancer therapy. Topoisomerase I is a target molecule for irinotecan, a strong chemotherapy agent used to treat colon, lung, ovarian, and other cancers. We reported that the circadian expression of topoisomerase I transcription is regulated by CLOCK/BMAL1 and D-site finding factors, including DBP, HLF, TEF, and E4BP4 via the E-box and D-box, which are located in the promoter region. It has emerged that HIF transcription factors are other targets for cancer therapy, because of the recent development of targeted therapies exploiting the hypoxic tumor microenvironment. Understanding the relationship between HIFs/hypoxic signaling and clock and clock-controlled genes can help to provide evidence for the molecular basis of cancer chronotherapy. In this session, we will discuss the effect and the role of hypoxic signaling on clock and clock-controlled genes, and anti-cancer agent target factors.

3S63I-2

Development of artificial chromosome-based multi-color luciferase assay system

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Circadian rhythm research is a field in which luciferase is frequently used to monitor gene expression in real-time, because extremely long-term, quantitative monitoring of gene expression is more often required than in other types of biological research. In addition, stable cell lines and transgenic mice carrying promoter-luciferase gene cassette in the genome of host organisms have the advantage that promoter-driven bioluminescence oscillation can monitor conveniently and reproducibly. On the other hand, recent advances in luciferase technology allow us to monitor the expression of multiple genes simultaneously when luciferases are used that induce differently colored emission spectra, namely, green-emitting and red-emitting beetle luciferases that act on a single bioluminescent substrate (multi-color luciferase assay system). In general, however, generation of stable cell lines or transgenic mice carrying multiple promoter-luciferase gene cassettes need long time and complicated procedures. To overcome the technical limitation, we utilize an artificial chromosome vector in which multiple transgenes can be inserted into the vector by site-specific recombination. To verify capability of the vector, we generated fibroblast stable cell line expressing green- and red-emitting luciferases under the control of mPer2 and mBmal1 promoters, respectively. We successfully monitor longitudinal antiphasic bioluminescence oscillations, indicating the artificial chromosome vector serve as an effective tool for generating cell line and for monitoring multiple gene expressions.

3S63I-3

An RNAi screen of protein kinase genes identifies novel components of the circadian oscillator in *Drosophila*

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Eukaryotic circadian clocks use transcriptional feedback loops to drive rhythms in metabolism, physiology and behavior. CLK and CYC initiate transcription of *period* (*per*) and *timeless* (*tim*), PER and TIM accumulate in cytoplasm and translocate into the nucleus after a delay. Once in the nucleus, PER and TIM repress CLK and CYC activated transcription. As time goes by, PER and TIM are degraded, and CLK and CYC start the cycle anew. During the cycle, rhythmic phosphorylation of PER, TIM and CLK controls the timing of their subcellular localization, transcriptional activity and degradation, thereby determining the length of oscillator period and rhythmicity. Although SGG, DBT and CKII are known to phosphorylate PER or/and TIM, additional kinases are predicted to play a role in clocks. Taking advantage of transgenic RNAi libraries, we have been conducting RNAi screening for kinases that regulate circadian behavior by expressing RNAi in clock cells. Of 315 transgenic RNAi strains that target 189 known or predicted kinases tested in primary screening, we identified 45 candidate circadian kinases that cause arrhythmia, short-period and long-period phenotypes. To eliminate off-target effects of RNAi, multiple RNAi strains that target discrete portions of mRNA have been tested, and six kinases have been validated. Of these kinases, NEMO was identified as a component of the oscillator, and we provided evidence that NEMO phosphorylates CLK. Two kinase RNAi lines that produce a long period phenotype, but have no reported experimental functional analysis, are the focus of genetic and molecular characterization.

3S63I-4

Role of the circadian clock gene *Per2* in cold-induced thermogenesis in brown adipose tissue

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Adaptive thermogenesis allows mammals to resist cold by uncoupling the proton gradient from ATP synthesis in mitochondria to generate heat. Here we show that mice mutated in the clock gene *Period2* (*Per 2*) were impaired in adaptive thermogenesis. In brown adipose tissue (BAT), cold-exposure induced *Per2* via Heat shock factor1 (HSF1). Subsequently, PER2 and PPARα increased expression of the heat-generating *Uncoupling protein 1* (*Ucp1*). PER2 also augmented *Fatty acid binding protein 3* (*Fabp3*), which transports free fatty acids (FFA) to mitochondria, a process necessary to activate UCP1. Hence, reduction of *Ucp1* and *Fabp3* may cause the phenotype observed in *Per2* mutant mice, linking PER2 to the process of adaptive thermogenesis.

3S63I-5

Stabilizing Mechanism of the Molecular Clock through Regulation of CRY protein Lifetime

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In mammals, the circadian oscillators are driven by transcription-based negative feedback mechanism. The central circadian pacemaker in the hypothalamic suprachiasmatic nucleus (SCN) governs behavioral rhythms. Among the clock proteins, CRYPTOCHROMEs (CRY1 and CRY2) act as key players in the mammalian clockwork through their strong repressive activities on the E-box-mediated CLOCK-BMAL1-dependent transcription. We previously reported that CRY2 is phosphorylated at Ser557 in a circadian manner in the mouse SCN and liver. The priming phosphorylation of CRY2 at Ser557 by DYRK1A allows subsequent phosphorylation at Ser553 by GSK-3β, and the two-step phosphorylation of CRY2 leads to its proteasomal degradation (Harada et al., JBC, 2005 : Kurabayashi et al., MCB, 2010). On the other hand, CRY1 and CRY2 are ubiquitinated by FBXL3, an F-box-type ubiquitin ligase, leading CRYs to proteasomal degradation (Siepka et al., Cell, 2007 ; Busino et al., Science, 2007 ; Godinho et al., Science, 2007). We recently found that CRY proteins were ubiquitinated and, surprisingly, stabilized by another F-box-type E3 ligase FBXL21, which antagonized FBXL3 action on CRYs. Deficiency of these two F-box proteins alleviated the circadian period-lengthening phenotype of Fbxl3-knockout mice. The double knockout destabilized the central clock in the SCN and progressively perturbed rhythmicity of the circadian behaviors in constant darkness. We conclude that the antagonizing actions of two related F-box proteins on CRY proteins have a critical role for robust oscillation of the circadian clock.

3S63I-6

CK2-orchestrated circadian signalosome regulates mammalian clock system

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Circadian Systems (CS) based on molecular clocks function in the cells all over the body, involving in temporal regulation of various physiologies. Dysfunction of CS is involved in progression of diseases, such as cancer and metabolic syndrome. Therefore, Chrono-molecular medicine by artificial control of Circadian Signalosome (CIS), the rhythmic intracellular signaling system such by protein modification governs circadian physiologies, is expected to be a crucial medical strategy. About 20 years ago, we originally hypothesized that Periodically fluctuating kinase (PFK)-controlled circadian phosphorylation oscillator regulate molecular clocks, as the hub-regulator of CIS. Based on the hypothesis, we found/purified PFK, and identified as Casein kinase-2 (CK2). Moreover, we demonstrated that circadian CK2-mediated phosphorylation of BMAL1 (clock genes-transactivator) is indispensable for BMAL1 : CLOCK nuclear accumulation and consequent circadian functions. Additionally, we found pivotal BMAL1 modification for clock function ; SUMOlylation for controlling protein stability and CLOCK-mediated Acetylation for negative feed back suppression via recruitment of CRYs to BMAL1.Here, we will show data regarding CK2-mediated CIS governs these BMAL1 modification, and molecular mechanism of the circadian phosphorylation.By the way, CK2 is a critical regulator of the progression of disease, such as cancer, so highly potential target of Chrono-molecular medicine. Here, we show data regarding life protection system by stress-elicited CK2-mediated CIS. And we hope to evoke discussion about elucidating CIS toward Chrono-molecular medicine.