Poster Presentations

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Ionic Channel, Receptor (1)

1P-001

Quercetin diminishes the cAMP-stimulated Cl- secretion by blocking Na+,K+ -ATPase in epithelial cells

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Epithelial Cl- secretion plays an important role in production of driving force for water movement. The aim of the present study was to investigate the action of quercetin, a flavonoid, on Cl- secretion in epithelial A6 cells. Quercetin stimulated epithelial Cl- secretion under basal conditions, but diminished it under cAMP-stimulated conditions via modification of activity of Na+-K+-2Cl- cotransporter (NKCC) contributing to Cl- uptake across the basolateral membrane. However, we have no idea on mechanisms of quercetin action. As the Na+,K+-pump is essentially required for production of the Na+ gradient generating NKCC activity, we assessed effects of quercetin on the pump by measuring the ouabain-sensitive current in apically nystatin-treated cells. Quercetin reduced the pump current about 50%, but quercetin stimulated NKCC. Based on these observations, we speculate the following points. 1) Under basal conditions, the pump activity would be much larger than the NKCC activity. Therefore, even if quercetin inhibited the pump, the Na+ gradient would be still kept large enough for quercetin to fully show its stimulatory action on NKCC, resulting in an increase of Cl–secretion. 2) Under forskolin-stimulated conditions, the pump activity would not be much larger than the NKCC activity. Therefore, if quercetin inhibited the pump, the Na+ gradient would not be kept large enough for maintenance of the forskolin-stimulated activity of NKCC, resulting in a decrease of Cl–secretion. No COI.

1P-002

Moderate binding affinity of the Na^+/H^+ exchanger NHE1 for calcineurin is critical for downstream NFAT signaling.

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Calcineurin (CaN), a Ca^{2+}-dependent phosphatase, is a key molecule to govern pathological cardiac hypertrophy. CaN dephosphorylates a downstream transcription factor NFAT, which in turn induces hypertrophic gene expression. However, the mechanism of CaN regulation is unclear, because CaN is not activated by increase in cytosolic Ca^{2+} concentration caused by the excitation-contraction coupling in cardiomyocytes. CaN has been hypothesized to be efficiently regulated via the interaction with plasma membrane Ca^{2+}-handling proteins in the local vicinity. Recently, we have found that a pH-regulating transporter NHE1 activated the CaN-NFAT signaling, leading to cardiomyocyte hypertrophy via direct binding of CaN to the 6-residues motif (PVITID) in the cytosolic domain of NHE1. We hypothesized that local pH increase produced by NHE1 enhanced the activity of CaN bound to NHE1 via sensitizing Ca^{2+}. However, it is unknown how CaN signal is transmitted from NHE1 to NFAT. In this study, we performed the detailed mutagenesis study for CaN binding site of NHE1. Substitution of the PVITID sequence with either high (PVIVIT) or low affinity sequence (PVIAVN) both abolished the CaN-NFAT signaling. Alanine-scanning mutagenesis revealed that the original NHE1 sequence was the best for signal amplification, suggesting that the balanced affinity between NHE1 and CaN is critical for the efficient signaling. We consider that such moderate interaction is important for removal of CaN from NHE1 and rebinding to downstream target NFAT after NHE1-dependent activation. No COI.

1P-003

Stabilizing effects of G protein on the active conformation of the adenosine receptor type 1a differ depending on the type of G protein

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We recently reported that the stabilizing effects of G protein binding on the stabilization of the active conformation of the receptor differ depending on the type of receptors. In the present study, we aimed at investigating the effects of different types of G protein on the active conformation of the Gi/o-coupled adenosine type 1a receptor ($A_{1a}R$), by using a fluorescence resonance energy transfer (FRET) technique. For this purpose, examined Gαi1, Gαo and the chimeric Gαqi5 which binds to the $A_{1a}R$ and stimulates Gq signaling pathway. YFP fused at the C-tail of the $A_{1a}R$ showed the agonist-induced increases in FRET from CFP attached to the Gβ1 subunit in the presence of the Gαi1 and Gαqi5 but not of the Gαo, suggesting that the YFP-fused $A_{1a}R$ interacts with the Gαi1 and Gαqi5 but not with the Gαo. To examine the effects of the different Gα subunits on the active conformation of the $A_{1a}R$, we made functionally intact $A_{1a}R$ FRET constructs which are fused with YFP and CFP at the third intracellular loop and C-tail of the $A_{1a}R$, respectively. The FRET constructs showed slight FRET decreases upon the agonist application, which were significantly enhanced by the Gαi1 and Gαqi5 but not by the Gαo subunits. In addition, the enhancing effect of the Gαqi5 on the FRET decrease was significantly larger than that of the Gαi1. These results suggested that effects of G protein binding on the stabilization of the active conformation of the $A_{1a}R$ differ depending on the type of G protein. No COI.

1P-004

Structural rearrangements of the linker beta strands in P2X2 are coupled to the pore in a voltage dependent manner

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$P2X_2$ is an extracellular ATP gated cation channel, which shows voltage dependent gating with no canonical voltage sensor. Three intersubunit ATP binding sites are linked to pore forming transmembrane (TM) domains by β-strands. We analyzed voltage dependent structural rearrangements of the linker strands using an engineered thiol modifiable site. (1) We observed that a double mutants of D315C&I67C (at β-14 and β-1 respectively) shows increase in current 3-6 fold by reducing agent (DTT). Washout of DTT and application of Cd2+ induced current decline due to the bond formation between D315C and I67C. This effect was absent in WT or in either single point mutants. (2) Cd^{2+} induced current decline was analyzed at depolarized and hyperpolarized potentials with different pulse protocols in the presence and absence of ATP. (3) Current decline by Cd^{2+} could not be observed in the absence of ATP, but only in presence of ATP, suggesting state dependent modification. (4) In presence of ATP, Cd^{2+} modification was faster in hyperpolarized conditions than in the depolarized condition, suggesting voltage dependent structural rearrangements of the beta strands linking ATP binding site to TM domains. (5) Finally we performed the similar analyses with a pore mutant T339S which makes the channel constitutively active at all membrane potentials. With T339S Cd^{2+} modification rates were similar in depolarized and hyperpolarized conditions. Overall results suggest that structural rearrangements of the linker domains of P2X2 are coupled to the pore in a voltage dependent manner. No COI.

1P-005

Endothelin-1 induces contraction through endothelin receptor A coupled with $G_{q/11}$ in bovine ciliary muscle

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Purpose: To determine receptor type associated with the contractile effect of endothelin-1 (ET1) on bovine ciliary muscle and investigate the signalling mechanism involved.
Methods: Isometric tension was recorded in dissected ciliary muscle bundles, using a U-gauge transducer. Isolated myocytes were used for recording whole cell currents. Existence and localization of endothelin receptors A and B (ETRA and ETRB), M_3-muscarinic receptor (M_3R) and $G_{q/11}\alpha$ were examined by immunofluorescence microscopy.
Results: ET1 (1-100 nM) produced a slowly developing contractile response (τ= 58 ± 15 min; n=12) in a dose-dependent manner (K=6 ± 1 nM and h=1.1 ± 0.1; $d.f.$=15). Like contraction evoked by carbachol (CCh; 0.1–10 μM), ET1-evoked contraction was dependent on extracellular Ca^{2+}. It was completely inhibited by YM-254890 (0.5 μM), a $G_{q/11}$-inhibitor and partially also by Y-27632 (10 μM), a Rho kinase inhibitor. Unlike CCh-evoked contraction, however, ET1-evoked contraction had no rapid phasic component and was washed out slowly. ETRA antagonists dose-dependently inhibited the ET1-evoked whereas ETRB antagonists showed no effect. Under whole-cell voltage clamp ET1 weakly activated two types of Ca2+ permeable non-selective cation channel (NSCC) which are known to open upon M_3R stimulation. Immunofluorescence microscopy revealed a dense co-localization of ETRA and M_3R in the cell membrane.
Conclusion: The ET1-evoked contraction of bovine cilary muscle is mediated by ETRA linked with a $G_{q/11}$ signalling mechanism that communicates in some manner with NSCCs and Rho kinase pathway. No COI.

1P-006

Area-specific D1 dopamine receptor expression in adult mouse astrocytes

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Dopaminergic neurons in the midbrain nucleus substantia nigra pars compacta (SNc) release dopamine (DA) from their dendrites, which extend deeply into the adjacent substantia nigra pars reticulata (SNr), consisting mostly of GABAergic neurons. Indeed, real time RT-PCR and immunohistochemistry showed diffuse expression of D1 dopamine receptor (D1R) in the SNr. However, most acutely dissociated GABAergic SNr neurons showed no response to DA applied in our perforated patch experiments. Interestingly, examination of D1R-YFP transgenic mice revealed that SNr glial cells express strong D1R (i.e. YFP) signal in their process. In the present study, we compared D1R expression in acutely dissociated neurons and astrocytes in SNr, striatum, and cerebral cortex by double immunostainings. Vesicular GABA transporter (VGAT)-venus mice were used as well. From these studies, we suggest area-specific D1R expression in astrocytes. No COI.

1P-007

N-glycosylation modulates AMPA receptor channel properties

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The intracellular molecular mechanisms underlying the regulation of the AMPA receptor have been dramatically elucidated in the past few decades. In contrast, the regulation of the extracellular domain remains unclear. Here, we focused on N-glycosylation of the AMPA receptor in the extracellular domain and tried to clarify their functions by combining molecular biological and electrophysiological techniques. First, we examined the effects of PNGase F, which is a glycosidase that cleaves the N-glycosylation site at an asparagine residue, on AMPA currents in primary hippocampal cultured neurons and HEK293 cells expressing GluA1 using a whole-cell patch-clamp technique. The digestion of N-glycosylation induced the re-sensitization of AMPA currents in the both. Six putative N-glycosylation sites are located in the extracellular domain of GluA1. Next, we performed mutation studies of each N-glycosylation site of GluA1 to identify the N-glycosylation site responsible for the re-sensitization induced by PNGase F treatment. These results indicated that N401 was a critical site for the expression of re-sensitization. Finally, we analyzed the possibility of re-sensitization after PNGase F treatment using acute brain slices. Single electrical stimulation of Schaffer collateral did not show the re-sensitization in hippocampal pyramidal neurons, however, paired pulse stimulation generated the re-sensitization. No COI.

1P-008

Metabotropic glutamate receptor-dependent slow Ca^{2+} oscillations in striatal neurons and astrocytes.

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The striatum plays an important role in linking cortical activity to basal ganglia outputs. Group I metabotropic glutamate receptors (mGluRs) are expressed abundantly in the striatal projection neurons and may be a therapeutic target for Parkinson's disease. The group I mGluRs are known to modulate the intracellular Ca^{2+} signaling. To characterize Ca^{2+} signaling in striatal cells, spontaneous cytoplasmic Ca^{2+} transients were examined in acute slice preparations from transgenic mice expressing green fluorescent protein in the astrocytes. In both the putative-neurons and astrocytes of the striatum, spontaneous slow and long-lasting intracellular Ca^{2+} transients (referred to as slow Ca^{2+} oscillations), which lasted up to approximately 200 s, were found. Neither the inhibition of action potentials nor ionotropic glutamate receptors blocked the slow Ca^{2+} oscillation. Depletion of the intracellular Ca^{2+} store and the application of antagonists against inositol 1,4,5-trisphosphate (IP_3) receptors and mGluR5 blocked the slow Ca^{2+} oscillation in both putative-neurons and astrocytes. Thus, the mGluR5-IP_3 signal cascade is the primary contributor to the slow Ca^{2+} oscillation in both putative-neurons and astrocytes. The slow Ca^{2+} oscillation features multicellular synchrony, and both putative-neurons and astrocytes participate in the synchronous activity. Therefore, the mGluR5-dependent slow Ca^{2+} oscillation may involve in the neuron-glia interaction in the striatum. No COI.

1P-009

Comprehensive behavioral test battery analyses of the gene targeted mice of Prrt3, an orphan metabotropic receptor

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Prrt3 is an orphan metabotropic receptor with a long N-terminus extracellular region, and it belongs to family C which mGlu and $GABA_B$ receptors also belong to. The expression of Prrt3 mRNA in the brain such as in hippocampus is shown in the Allen Brain Atlas, but there is no information about its function so far. To approach the physiological significance of Prrt3, we raised knock out (KO) mice, using gene targeted ES cells obtained from KOMP Repository. The homozygous KO mice were small in the body size and most of them died within 1 week after birth, demonstrating the importance of the function of Prrt3. As it was not possible to obtain sufficient number of homozygous KO mice, we carried out comprehensive behavioral test battery analysis using heterozygous KO mice. By the Barnes probe test, we observed a significant decrease in the retention of spatial memory after 4 weeks interval from the completion of training. By the fear conditioning test, we also observed a significant decrease in the retention of fear memory after 4 weeks. As heterozygous KO mice of most of the metabotropic receptors in the brain do not show clear behavioral abnormalities, it is noteworthy that heterozygous KO mice of Prrt3 gene showed clear phenotype, demonstrating the critical role of Prrt3 in the brain function such as memory retention. No COI.

1P-010

Expression patterns of an orphan metabotropic receptor Prrt3 in mice

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Metabotropic receptors, also known as G-protein coupled receptors (GPCRs) are involved in major physiological processes, and they are targets for modern medicine. However, many still remains as orphan receptors, and the discovery of their ligands or their physiological functions have been awaited. Proline rich transmembrane protein 3 (Prrt3) is an orphan metabotropic receptor, and its physiological functions including its expression patterns have not been fully understood. Here, we explored the expression patterns of Prrt3 in mice using biochemical and histochemical methods. Our Western blot analysis experiments using membrane fractions of various organs from WT and homozygous Prrt3 KO mice indicated Prrt3 is abundantly expressed in the brain. In immunohistochemical analysis using fresh frozen sections of mouse brain, we observed specific expression of Prrt3 in hippocampus, thalamus and substantia nigra of WT mice. By close analysis the Prrt3 expression was observed but not in the soma. Prrt3 expression in different subcellular fractions was also analyzed using Western blot. The results showed Prrt3 expression was in the same fractions as synaptophysin and not PSD95, suggesting Prrt3 is not expressed at the postsynaptic densities. Synaptophysin is known to play a role in vesicle exocytosis, and expressed in presynaptic terminals. Taken together, we speculate Prrt3 is expressed in neurites, and possibly play roles at the presynaptic terminal in the brain. No COI.

1P-011

Voltage Sensing Mechanism in the Voltage-Gated H^+ Channel

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Voltage-gated channels are responsible for sensing membrane potential and generating electrical impulses in many organs. In the voltage sensing process, positively charged residues in the voltage sensor domain are known to sense membrane potential and move across the membrane electric filed, generating a transient 'gating current' ahead of an ionic current. However, the voltage-gated H^+ channel (Hv) does not show the gating current although it behaves as a voltage-gated channel. Molecular structure of Hv is also unique, where it has no canonical pore domain; and the voltage sensor domain bears both voltage sensing and H^+ permeation. Hence, they are considered to be inextricably associated with each other, but the details have yet to be elucidated. Here we report that the gating current ahead of the H^+ current was observed in a mutant Hv channel, and an additional mutation eliminated only the component of the H^+ current. The charge-voltage (Q-V) relationship of the gating current was shifted by changing the pHo/i gradient in the recording solutions, and the conductance-voltage relationship of the H^+ current well followed the Q-V shift. We also observed that the Q-V relationship was shifted toward the depolarization direction by application of extracellular Zn^{2+}, where the kinetics of the on-gating current and the following H^+ current activation were decelerated; but the deactivation kinetics of the gating was not. These results suggest that the voltage sensing and the H^+ permeation can be considered separately in the Hv channel, and extracellular Zn^{2+}, a physiological blocker, competes the voltage on-sensing. No COI.

1P-012

The role of conserved aromatic residues at the S4 voltage-sensor helix in the voltage-gated H^+ channel

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The voltage-gated H+ channel (Hv) is a voltage-sensor like protein consisting of four transmembrane segments (S1-S4). The functional unit is uniquely a dimer, and we recently proposed that the two voltage-sensor S4 helices formed an interaction interface in the dimeric channel. The amino acid sequence of S4 is well conserved in Hv, and uniquely, Tryptophan (Trp) at the middle of S4 is 100% conserved among species. Trp has a bulky sidechain and so should be less tolerated in positions that involved in protein–protein interactions. To understand functional significances of the seemingly unfavorable Trp, and we systematically made mutants and examined their electrophysiological properties. The mutation of Trp significantly accelerated kinetics of deactivation. Analyzing the effect of Trp introduced into the background of the mutant which has no aromatic residue in S4, we found that several Trp introduced into around the original Trp position effectively recovered the slow deactivation kinetics. Furthermore, the deceleration effect by the Trp introduction was not observed in the monomeric channel mutants. Mutation cycle analysis indicates that the two Trp in the S4 are energetically-coupled in deactivation but less coupled in activation. These results support the ideas of the S4-S4 interface in the dimeric channel and the rotational movement of S4 during the gating, and propose that steric hindrance between the opposite Trp provides the unique slow deactivation.

1P-013

Chimeric study between sea urchin and mouse orthologs of voltage-gated proton channel revealed the critical sites for the activation speed

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VSOP/Hv1 is a voltage-gated proton channel that contains a voltage sensor domain lacking a pore domain. Human and mouse orthologs of VSOP/Hv1 exhibit slower activation kinetics than other voltage-gated ion channels. Slow gating is favorable for NADPH oxidase activities in immune cells. However, molecular mechanisms of slow kinetics of VSOP/Hv1 remain to be fully elucidated. We have recently found that activation kinetics of sea urchin VSOP/Hv1 (Sp-VSOP) was more than 20-fold faster than that of mouse VSOP/Hv1 (mVSOP) upon heterologous expression in HEK293 cells. To understand molecular basis for rapid gating of Sp-VSOP, we made chimeras between mVSOP and Sp-VSOP. The activation kinetics of Sp-VSOP was decelerated when the 3th transmembrane segment (S3) and N terminal cytoplasmic region were substituted by the corresponding regions of mVSOP. Mutation in S3 in Sp-VSOP (R141H/S142H/S142F) decelerated activation kinetics. To localize a critical region within the N-terminus for the activation kinetics, putative helical structure in the N terminal region of Sp-VSOP was substituted by that of mVSOP. This chimeric construct exhibited slower activation kinetics than Sp-VSOP(WT). These results highlight S3 and the helical structure of N terminal region as critical determinants of the activation kinetics of mVSOP. Mechanistic insights into gating mechanisms will also be provided. No COI.

1P-014

Membrane topology analysis of the voltage-gated proton channel at resting state

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The voltage-gated proton channel (Hv1) is a protein that contains the voltage sensor domain but not pore domain. The voltage sensor domain plays both role in votage sensing and proton permeation. The putative fourth transmembrane segment (S4) of mouse Hv1 (mHv1) has three positively charged residues. S4 is suggested to undergo a movement during activation of the channels, causing conformational change possibly leading to formation of proton-selective conduction pathway. However, detailed mechanisms of proton permeation of Hv1 are unknown. In this study, we took an approach of biochemical cysteine scanning to define residues facing the aqueous environment of mHv1. Accessibilities of two cystene-binding molecules, N-ethylmaleimide (NEM) and 4-acetamido-4'-maleimidylstilbene-2,2'-disulfonic acid (AMS), were examined on cysteine introduced into individual sites. Only the first arginine on S4 (R1:R201) was inaccessible by NEM and AMS in mHv1, suggesting that the accessibility profile represents the resting state of mHv1. We also found that D108, a critical residue for proton selectivity, can be accessed both by NEM and AMS, supporting a model that D108 faces relatively large vestibule to which both anions and cations are accessible. In addition, R201, D181, E115 were both inaccessible by NEM and AMS, raising a possibility that R201 forms salt bridge with D181 or E115 forming a barrier together with the hydrophobic core region in resting state. These findings will provide insights into structural basis for proton permeation and gating of Hv1. No COI.

1P-015

Acceleration of the activation of the voltage-gated proton channel by the unsaturated fatty acid

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The unsaturated fatty acids are important component of the biological membranes and the precursors of mediators of inter- and intra-cellular signaling. It is well-known that the unsaturated fatty acids, including arachidonic acid (AA, 20 carbons and 4 double bonds), activated or inhibited the ion permeations of various ion channels. The voltage-gated proton channel (VSOP/Hv1) can control the proton conductance by membrane voltage and pH. It helps the production of reactive oxygen species by NADPH oxidase in immunocytes. The enhancement of the production of reactive oxygen species by AA has been reported to be accompanied by the increase of the proton currents in neutrophils, macrophages, and eosinophils. However, the detailed molecular mechanisms of actions have remained elusive. Here we report the effects of AA on mouse VSOP/Hv1 heterologously expressed in HEK293T cells by inside-out patch clamp technique. The addition of AA with rapid-perfusion system immediately increased the magnitude of the proton currents through mVSOP/Hv1 that are evoked during one second depolarizing step by 20 times. After washout of AA, the currents rapidly returned to the original current level. The analysis with sixty second depolarizing pulse showed that the activation kinetics was more than 15 times accelerated by AA. We also examined the effects of other fatty acids and constructs of mVSOP/Hv1. Based on these results, we will discuss the molecular mechanisms and the essential sites for the actions of unsaturated fatty acids on VSOP/Hv1. No COI.

1P-016

Metabolic Phenotype in Voltage-Gated Proton Channels Knockout Mice

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Voltage-sensor only protein, VSOP/Hv1, consists of the voltage-sensor domain lacking pore domain. It functions as voltage-gated proton channel (Sasaki et al, Science. 2006) and its expression is specific to microglia in mice brain. In phagocytes such as neutrophil, macrophage and microglia, VSOP has a crucial role in the production of reactive oxygen species (ROS) by compensating for charge imbalance upon electron extrusion by NADPH oxidase. In the present study, we found that female VSOP knockout mice show metabolic phenotype at the age of 6 month and over. Body weight gain and increased food intake was observed in female knockout mice, and the increase in visceral fat is more prominent than that of subcutaneous fat in VSOP knockout mice. We also found that leptin level and insulin level in blood serum are increased in 12 month old VSOP knockout mice. Recently, it has been reported that neuroinflammation in hypothalamus is closely associated with obesity, suggesting that VSOP deficiency in mice microglia facilitates the initiation of inflammation in mice hypothalamus. Our preliminary results suggest that primary cultured VSOP knockout mice microglia appears to show decreased phagocytic ability, which may be involved in the facilitation of inflammation in the brain. In the present study, the mechanism underlying the metabolic phenotype in VSOP knockout mice is discussed. No COI.

1P-017

Analysis of the role of voltage-gated proton channel Hv1/VSOP in neutrophil chemotaxis

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Neutrophil chemotactic movement is necessary for pathogen elimination and inflammatory responses and the motility is highly regulated. Chemoattractants such as N-formyl peptide fMLF, leukotriene B4 and IL-8 activate neutrophil, leading to ROS generation by NADPH oxidase. Voltage-gated proton channel Hv1/VSOP discovered in our laboratory helps ROS production through the regulation of NADPH oxidase activity. Mouse neutrophils lacking Hv1/VSOP gene exhibit less production of ROS, lower pH in cytoplasm, more depolarized membrane potential and less calcium ion influx than wild-type neutrophils when the oxidase is activated. We tested how chemotaxis behavior is affected in Hv1/VSOP deficient neutrophils. Using transwell chamber, bone marrow derived neutrophils were stimulated with fMLF and counted the number of cells migrating to fMLF-containing well. We found an abnormal chemotactic response to fMLF in Hv1/VSOP deficient neutrophils: Hv1/VSOP deficient neutrophils showed normal response to 10μM fMLF, but they responded more strongly to 1μM fMLF than wild-type neutrophils. These results may suggest that Hv1/VSOP regulates the sensitivity for chemoattractant. We are trying to identify which factor causes abnormal chemotactic response. No COI.

1P-018

Decreases in availability of voltage-gated proton channels through endocytic internalization induced by increases of intracellular pH in osteoclasts and microglia

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Voltage-gated proton channels (H^+ channels) are highly proton-selective and contribute to natural immunity. The throughput of H^+ channels is regulated primarily by trans-membrane voltage- and pH- gradients. In addition, the numbers of H^+ channels may change in response to varying cellular conditions. In this study, we investigated the role of the intracellular pH (pH_i) in regulating the availability of H^+ channels in two macrophage-lineage cells, osteoclasts and microglia. In whole-cell clamp recordings, the pH_i was elevated after exposure to NH_4Cl and returned to the control level after washout. However, the H^+ channel conductance did not recover fully when the pH_i increase was prolonged. The decreases in the H^+ channel conductances were accompanied by reductions in the cell capacitance. Exposure to NH_4Cl increased the uptake of the endocytosis marker, FM1-43, confirming that pH_i increases facilitated endocytosis. The pH_i increases induced by V-ATPases and H^+ channels, endogenous H^+-transferring mechanisms, in part facilitated endocytosis. Similar results were observed in osteoclasts and microglia, but not in COS7 cells expressing a murine H^+ channel gene. These results suggest that pH_i increases decrease the availability of the plasma membrane H^+ channels through facilitation of dynamin-dependent endocytosis in osteoclasts and microglia, and that significant numbers of H^+ channels are not in use at physiological pH_i. No COI.

1P-019

Proton permeation through the polytheonamide B channel: Single channel current recordings and a proton permeation model

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A 48-mer peptide from a marine sponge Theonella swinhoei, polytheonamide B (pTB), is highly cytotoxic against eukaryotic cells. The amino acid sequence of pTB is unprecedented, having alternative D- and L-amino acid residues throughout the 48-mer peptide. The pTB channel forms a $\beta^{6.3}$-helix similar to the gramicidin A channel, and allows permeation of monovalent cations. In this study we examined the proton conduction of the pTB channel by use of the planar lipid bilayer technique. Single-channel I-V curve in HCl solution exhibited weak inward rectification. The single-channel amplitude at 1 M HCl was ～100 pA at +200 mV, which is more than 40 times higher than that of Cs^+. This high conductance suggests that protons permeate via water chain in the channel (the Grotthuss mechanism). At low HCl concentration, the I-V curve was sub-linear at high potentials. Also, the concentration-dependency of single-channel conductance through the pTB channel (pH-log I curve) showed the slope of one. These results indicated that the proton permeation is diffusion-limited. The proton permeation through the pTB channel was modeled with the discrete-state Markov model. No COI.

1P-020

Optically detected structural change in the N-terminal region of the voltage-sensor domain

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The voltage-sensor domain (VSD) is a functional module that undergoes structural transitions in response to membrane potential changes and regulates its effectors, thereby playing a crucial role in amplifying and decoding membrane electrical signals. Ion-conductive pore and phosphoinositide phosphatase are the downstream effectors of voltage-gated channels and the voltage-sensing phosphatase, respectively. Upon transition, it is known that the VSD generally acts on the region C-terminal to S4. However, it has not been addressed directly whether the VSD induces any structural changes also in the N-terminal region of S1. Here, we report the existence of such an N-terminal effect. We used two distinct optical reporters, one based on the Forster resonance energy transfer between a pair of fluorescent proteins and the other based on fluorophore-labeled HaloTag, and studied the behavior of these reporters placed at the N-terminal end of the monomeric VSD derived from voltage-sensing phosphatase. We found both of these reporters to be affected by the VSD transition, generating voltage-dependent fluorescence readouts. We also observed that while the voltage dependencies of the N- and C-terminal effects appear tightly coupled, the local structural rearrangements reflect the way in which the VSD was loaded, demonstrating the flexible nature of the VSD. No COI.

1P-021

Regulatory role of arachidonic acid on human cardiac Kv1.5 channels

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Arachidonic acid (AA) is an important constituent of membrane phospholipids and can be liberated by activation of cellular phospholipase A_2. AA modulates a variety of cardiac ion channels via diverse mechanisms, including both direct effects by AA itself and indirect actions through AA metabolites. Human cardiac Kv1.5 channel (hKv1.5) is functionally expressed in atrial myocytes and has an important role in the regulation of atrial repolarization of action potential. The present study was designed to investigate the effect of AA on hKv1.5 wild type and mutant channels expressed in CHO cells using the whole-cell patch-clamp technique. AA directly inhibited hKv1.5 currents in a concentration-dependent manner (IC50 6.4 μM). The blocking action was found to progress with time during depolarizing voltage step, suggesting that AA is an open channel blocker. Mutations R487V and H463C (in the outer pore mouth) reduced AA action. I502A, V512A (in the S6 domain) also decreased AA effect. Interestingly, the mutations of T479A and T480A (located in the base of selectivity filter) have opposite action on AA inhibition; T479A enhanced the AA action. AA induced a similar inhibition of hKv1.5 in the presence of 4-Aminopyridine, a Kv1.5 blocker. Moreover, at alkaline (pH 8.0), AA action was markedly reduced. However, acidic pH (pH 6.4) failed to affect AA action. Our findings suggest that multiple amino acids (R487, H463, T479, T480, I502, I512) have important role in determining the channel sensitivity to AA and the alkaline situation may modify the binding sites of AA on Kv1.5 channels. No COI.

1P-022

The interaction between S4-S5 linker and C-linker regulates the slow deactivation of hERG channel

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hERG channel, a member of the voltage-gated K^+ channel family, is the main subunit of the rapidly activating delayed rectifier K^+ current (I_{Kr}) in human heart, and is well known for its very slow deactivation. It has six transmembrane segments comprising former four segments (S1-S4) forming the voltage sensor domain and latter two segments (S5-S6) forming the channel pore domain, and these two domains are linked by S4-S5 linker in cytoplasmic side. Furthermore, it has large cytoplasmic regions, including eag domain in the N-terminus and C-linker/cyclic nucleotide binding homology domain (CNBHD) in the C-terminus. These cytoplasmic regions are known to interact to regulate slow deactivation. In this study, we performed the cysteine-cysteine bridge formation experiment using a membrane-permeable oxidizing reagent $TbHO_2$ under two electrode voltage clamp in *Xenopus* oocytes. We obtained that the deactivation speed of the mutant carrying two introduced cysteines at glutamate 544 in S4-S5 linker and arginine 681 in C-linker after addition of $TbHO_2$ were slower than that before addition of $TbHO_2$. Further, after addition of $TbHO_2$, the conductance-voltage relationship of the mutant shifted negatively. This modification was significantly enhanced when cells were voltage-clamped at a holding potential of +40 mV. Taken together, the results suggested that S4-S5 linker and C-linker interact to regulate the slow deactivation of hERG channel, and this interaction changes dynamically between open and close state. No COI.

1P-023

Functional regulation of β2-adrenoceptors on IKs current of sinoatrial node cells in guinea pig

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The β1- and β2-adrenoceptors (ARs) are highly expressed in mammalian heart, with the predominance of the β1ARs subtype. The β1/β2AR ratio varies among different cardiac regions; it is high in the ventricles, relatively lower in atria and sinoatrial node (SA). Direct stimulation of sinoatrial β2AR in man has a positive chronotropic effect. The present study was designed to examine the action of β2AR stimulation on the slow component of delayed rectifier K^+ current (I_{Ks}) in SA cells. Zinterol in the presence of CGP20712A (β1AR antagonist) and isoproterenol in the presence of ICI118551 (β2AR antagonist) were used for activation of β2 and β1ARs, respectively. β2AR stimulation increased IKs elicited by 2-s depolarizing pulses in SA, atrial cells, but failed to potentiate I_{Ks} in ventricular cells. In SA cells, the action of β2AR stimulation was concentration-dependent and the maximal effect was significantly large than that of β1AR stimulation. Similar to β1AR stimulation, zinterol significantly shifted current activation to negative potentials. Zinterol-induced enhancement of I_{Ks} was markedly attenuated by application with protein kinase A (PKA) inhibitor, H89 but was not affected by pretreatment with pertussis toxin. These results demonstrate that the effect of β2AR on I_{Ks} activation is mediated through a PTX-insensitive G protein and PKA pathway. Thus, β2AR subpopulation is a relatively important mediator for I_{Ks} regulation in response to β-agonist stimulation. No COI.

1P-024

H_2O_2 regulates I_{Ks} currents of sinoatrial node cells in guinea-pig via both CaMKII activation and oxidation process

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Accumulating evidences suggest H_2O_2 regulates several cardiac ion channels, such as the enhancement on L-type Ca^{2+} current or Na^+ current in rabbit ventricular myocytes and inhibition on expressed hERG current. The slow component of the delayed rectifier K^+ current (I_{Ks}) plays an important role in repolarization process of cardiac action potential. In the present study, we examined the effects of H_2O_2 on I_{Ks} current in sinoatrial node (SA) cells isolated from guinea-pig heart. After 4~5 min of H_2O_2 perfusion, I_{Ks} current was started a rapid increase by 60.45% from control to a peak level after 10 min exposure. H_2O_2-enhanced I_{Ks} was significantly reduced after minimum the intracellular Ca^{2+} concentration $[Ca^{2+}]i$ of pipette solution (from pCa 7 to pCa 11), suggesting the involvement of Ca^{2+} in the regulation of H_2O_2 on I_{Ks} current activation. Next, we tested hypothesis that H_2O_2-enhanced I_{Ks} is partially induced by the activity of CaMKII signal pathway. KN-93, an inhibitor of CaMKII, significantly suppressed H_2O_2-induced increase in I_{Ks} (decreased to 10.8±4%, n = 6); AIP, another CaMKII inhibitor, applied from pipette solution also reduced the effect of H_2O_2 on I_{Ks} (decreased to 24.1±3%, n = 5). Finally, we investigated the possible modulation of H_2O_2 oxidation on I_{Ks} channels. H_2O_2-induced potentiation on IKs was fully reversed by per-treatment with reducing agent dithiothreitol (DTT). These findings demonstrate that H_2O_2-induced I_{Ks} increase is mediated through both CaMKII signal pathway and oxidation process. No COI.

1P-025

Ca^{2+} sensor proteins confer PIP_2 sensitivity on KCNQ1 channels

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KCNQ1 is a pore-forming subunit of cardiac K^+ current which slowly activates and controls repolarization of action potential. The subunit physiologically assembles with a Ca^{2+} sensor protein Calmodulin (CaM) at a 1:1 stoichiometry in a Ca^{2+}-independent manner. The binding of CaM is thought to stabilize a cytoplasmic domain of KCNQ1 and confer Ca^{2+} sensitivity. Although there are a number of Ca^{2+} sensors, the specificity of binding to KCNQ1 remains unclear. The binding sites are mapped in the vicinity of a site binding to PIP_2, a lipid essential for KCNQ1 activation. However, it is unclear how CaM binding associates with the PIP_2 sensitivity. To address these questions, we developed a detection system for the KCNQ1-CaM complex. We prepared GFP-fused KCNQ1 C-terminus and expressed with Ca^{2+} sensors in *E. coli*. The cell lysate was subjected to fluorescence-detection gel filtration. Using this system, we tested the specificity for the binding of KCNQ1 with 8 different Ca^{2+} sensors. Among them, Calml3, the closest relative of CaM, was an only molecule to restore the KCNQ1 folding. Next we examined the effects of Ca^{2+} sensors on the KCNQ1-PIP_2 interaction. Voltage-sensitive phosphatase was expressed with KCNQ1 and either CaM or Calml3 in *Xenopus* oocytes to control PIP_2-content by membrane potentials. In the presence of Calml3, the current-voltage relationship was shifted to right and the plateau was higher than that in the presence of CaM. This suggests that Calml3 augments the affinity of KCNQ1 to PIP_2. Therefore, Ca^{2+} sensors appear to confer the PIP_2 sensitivity on KCNQ1 via the direct interaction. No COI.

1P-026

A pair of phenylalanine residues on the S4 and S5 segments create a physical and energy barrier for the voltage sensor before opening in KCNQ1/KCNE1 channel

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In human heart, KCNQ1, a voltage gated K^+ channel, forms a complex with an auxiliary subunit KCNE1. A main role of KCNE1 is slowing the gating of KCNQ1 channel, and we and other groups previously found that KCNE1 slows down the movement of the voltage sensor domain (VSD). However, how KCNE1 changes the VSD movement remains largely unknown. Here, we identified two KCNQ1 phenylalanine residues, F232 on the S4 segment and F279 on the S5 segment, are critical for the VSD modulation by KCNE1. We mutated F232 or F279 into various amino acid residues and found that δG_0 (Gibbs free energy of channel opening at 0 mV) is dependent on the bulkiness of the side chain of the mutated residue, implying that F232 and F279 create a physical and energy barrier for the voltage sensor before channel opening. To confirm that, we next directly tracked the VSD movement by voltage clamp fluorometry. In the wild-type KCNQ1/KCNE1, the fluorescence change corresponding to the VSD movement well preceded the current activation as seen in the other voltage gated K^+ channels. On the other hand, in the F232A and F279A mutants, the VSD movement and the current activation were almost overlapped, indicating that the channel immediately enters the open state once the voltage sensor reaches the up state. These results suggest that F232 and F279 create a physical and energy barrier for the voltage sensor to overcome before the channel opening, and that this is the molecular mechanism for the slow gating in the KCNQ1/KCNE1 channels. No COI.

1P-027

Stoichiometry and biophysical properties of the Kv4-KChIP complex change depending on the expression level of KChIP.

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Kv4 is a member of voltage-gated K^+ channel family. It is expressed in neuronal dendrites and cardiac ventricular myocytes, regulating their excitability. K^+ channel interacting protein (KChIP) is an auxiliary subunit for Kv4 which is known to increase the current amplitudes, decelerate the inactivation and accelerate the recovery from inactivation of Kv4. However, the mechanism how KChIP modulates Kv4 remains largely unknown. We considered the possibility that Kv4 channel behavior is regulated by the stoichiometry of Kv4-KChIP complex. We first investigated how the amount of expressed KChIP4 changes Kv4.2 current properties with two-electrode voltage clamp and observed that the recovery from inactivation of Kv4.2 was gradually accelerated with the increase in the expressed amount of KChIP4. We next made tandem repeat constructs in which the stoichiometry of Kv4.2-KChIP4 complex was strictly controlled and compared their ion channel properties. 4:4 (Kv4.2:KChIP4) channel showed faster recovery from inactivation than 4:2 channel. We next directly counted the number of KChIP4 subunits in a single Kv4.2-KChIP4 complex by counting bleaching steps using single-molecule fluorescence imaging and observed that the stoichiometry of Kv4.2-KChIP4 complex changes depending on the expressed amount of KChIP4. Our results suggest that the stoichiometry of Kv4.2-KChIP4 complex is variable and the recovery from inactivation of Kv4.2 is controlled by the number of bound KChIP4. No COI.

1P-028

Effect of membrane lipids on the inactivation gating of the KcsA potassium channel

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Membrane lipids act as a cofactor for function of the ion channel and specific lipid molecules are indispensable for maintaining channel activities. For the KcsA potassium channel, the presence of negatively charged phospholipids such as phosphatidylglycerol (PG) in the membrane is prerequisite for the channel activity. Previously we demonstrated that the PG molecule on the inner (cytoplasmic) leaflet rendered the KcsA channel highly active. We found that the M0-helix lying on the inner membrane surface changed its configuration with a lipid-dependent manner, and modulated the activation gating. In this study we focused on the effect of lipids on the inactivation gating of the KcsA channel. As is the case for the voltage-gated channels, the KcsA channel upon cytoplasmic acidification exhibits slow inactivation after activation. It is suggested that the inactivation of the KcsA channel originates from closure of the selectivity filter that locates in the outer half of the transmembrane domain. We analyzed the inactivation rate of the KcsA channel in the varying lipid composition of the membrane by use of artificial lipid bilayer. The KcsA channel showed relatively slow inactivation in the negatively charged lipids compared to that in neutral lipids. By introducing mutations into the M0 helix, we further investigated the molecular mechanism underlying the lipid-dependency of the inactivation gating. No COI.

1P-029

Gating-coupled clustering-dispersion behavior of the KcsA potassium channel in membrane: Direct observation by atomic force microscopy

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The KcsA potassium channel is a pH-dependent channel, and the activation gate opens at acidic pH. Using atomic force microscopy (AFM), we have captured the open-gate structure of membrane-embedded KcsA channels by removing the potentially sight-obstructing cytoplasmic domain (CPD). Here, we revealed pH-dependent clustering-dispersion behavior of KcsA channels on the membrane, which occurred concurrently with the gating conformational change. At neutral pH, the closed channels formed self-assembled nanoclusters. At acidic pH, the open-gated channels were dispersed as singly-isolated channels. Pair correlation function of the channel position gave the nearest-neighbor distance of approximately 14 nm, indicating non-contacting dispersion. High-speed AFM revealed that the clustering-dispersion dynamics were reversible and completed within several minutes. These results suggest that the KcsA channel undergo conformational changes within the cluster but do not open until the channels are dispersed. The interplay between the gating conformational change of individual channels and the collective behavior of the clustering-dispersion dynamics provides insight into understanding membrane-mediated protein-protein interactions and functional cooperativity. No COI.

1P-030

Functional consequences of a gain-of-function mutation in the Kir2.1 inward rectifier K^+ channel associated with short QT syndrome

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The duration of action potential determines the refractory period of the cardiac muscle. The shortening of the action potential duration occurs as short QT interval in ECG, and increases the vulnerability to arrhythmia. This short QT syndrome (SQTS) can be caused by gain-of-function mutations in the KCNJ2 gene encoding the Kir2.1 subunit, which, together with Kir2.2 and/or Kir2.3, constitutes the cardiac strong inward rectifier K^+ channel (I_{K1}). In this study, we examined the functional consequences of the M301K mutation recently identified in a SQTS patient by performing conventional whole-cell patch-clamp experiments. As previously reported, expression of Kir2.1 bearing the M301K mutation (Kir2.1(M301K)) alone did not give rise to any measurable currents, but the co-expression of Kir2.1(M301K) with wild-type Kir2.1 showed K^+ currents with a weak inward rectification. Co-expression of Kir2.1(M301K) with Kir2.2 or Kir2.3 gave essentially the same results, supporting that the Kir2.1/ Kir2.1(M301K) heterozygote expresses I_{K1} with large outward currents accelerating the repolarization. Interestingly, in Kir2.1/Kir2.1(M301K) heteromeric channels, the sensitivity to the external K^+ (characteristic of I_{K1}) was altered, and the sensitivities to the external Ba^{2+} and Cs^+ blockage (a hallmark of the inward rectifiers) were increased. These results provide evidence that the mutation in the cytoplasmic pore region alters the external ion sensitivities of Kir2.1 channel. No COI.

1P-031

Identification of palmitoylation enzymes for the HCN2 channel.

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We have previously demonstrated that among hyperpolarization-activated cyclic nucleotide-modulated (HCN) channel family, HCN1, HCN2 and HCN4, but not HCN3, are the target of S-palmitoylation: For example, palmitic acid was covalently attached to multiple cysteine residues (C63, C69, C82, C89, C104) located in the cytoplasmic N-terminus of HCN2. S-palmitoylation reportedly regulates the trafficking and the function of various membrane proteins. As for HCN channels, an inhibitor of palmitoylation, 2-bromopalmitate reduced the current amplitude of HCN2 expressed in *Xenopus* oocytes approximately to 45% of control level. S-palmitoylation is a reversible post-translational lipid modification; palmitoylation and depalmitoylation are catalyzed by protein acyl transferases (PATs) and palmitoyl protein thioesterases (PPTs), respectively. In the present study, we aimed to identify types of these enzymes that regulate the palmitoylation of HCN2 channel. We overexpressed each 24 types of known PATs with HCN2 and found that Zdhhc3, Zdhhc9, and Zhhc17 significantly increased the palmitoylation of HCN2 proteins. In contrast, overexpression of known PPTs such as APT1, APT2, LYPLAL1 and PPT1, did not reduce the palmitoylation of HCN2, suggesting that unknown types of enzymes may be involved. Although our results demonstrated that HCN channels are the target of dynamic palmitoylation-cycle, further studies are indispensable to address whether these enzymes exert physiological roles in the signal transduction system which may regulate HCN channels. No COI.

1P-032

TMEM16F forms a Ca^{2+}-activated Cl^- channel

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Transmembrane protein 16 (TMEM16), which possesses eight putative transmembrane domains with the intracellular NH_2- and COOH-terminal tails, is thought to comprise a Cl^- channel family. Although it has been reported that TMEM16A and 16B function as Ca^{2+}-activated Cl^- channels (CaCC), the functional properties of TMEM16F have greatly been controversial. In the present study, we investigated electrophysiological and pharmacological properties of human TMEM16F expressed in HEK293T cells. In whole-cell patch-clamp recordings, application of a Ca^{2+} ionophore, ionomycin, or rise in the intracellular free Ca^{2+} concentration to 100 μM induced transient activation of membrane currents with strong outward rectification in TMEM16F-overexpressing cells but not in mock-transfected cells. Outward rectification of TMEM16F currents was not affected by an increase in the intracellular Ca^{2+} level. Replacing extracellular Cl^- with aspartate$^-$ positively shifted the reversal potential of TMEM16F currents, whereas no significant change of the reversal potential was observed by replacement of extracellular Na^+ with $NMDG^+$, showing that the TMEM16F current is Cl-selective. Consistently, TMEM16F currents were inhibited by CaCC blockers, niflumic acid and tannic acid. Anion selectivity sequence of the TMEM16F current was I^- > Br^- > Cl^- > F^-. In addition, a CaMKII inhibitor, KN-93, had no effect on Ca^{2+}-induced activation of TMEM16F channels. Our results indicate that TMEM16F forms a Ca^{2+}-activated Cl^- channel that is not regulated by CaMKII. No COI.

1P-033

Properties of persisitent Na^+ current in Kenyon cells isolated from the mushroom body of the insect brain

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Many types of excitable cells possess a non-inactivating or slowly inactivating, persistent sodium current (I_{NaP}).The I_{NaP} has been shown to be mediated by tetrodotoxin (TTX)-sensitive Na^+ channels. The aim of this study was to investigate the basic properties of voltage-dependent Na^+channel currents in intrinsic neurons called Kenyon cell within the mushroom body of insect brain. Using the perforated patch clamp recordings from the cricket Kenyon cells, we showed the presence of two different types of Na^+currents, fast transient current (I_{NaF}) and slow sustained or persistent current (I_{NaP}). I_{NaF} first appeared at -40 mV whereas I_{NaP} appeared at -50 mV. Both currents reached maximum at -20 mV. I_{NaF} completely inactivated during the period of membrane deporalization of 150 ms whereas I_{NaP} persisted for several seconds. I_{NaF} half inactivated at -46 mV, whereas I_{NaP} half inactivated at -30 mV. I_{NaF} was blocked more potently by TTX than I_{NaP}. Furthermore, I_{NaP} was blocked by 50μM Cd^{2+} whereas I_{NaF} was not. Riluzole, which has been proposed a relatively specific persistent Na^+ channel blocker inhibited both I_{NaP} and I_{NaF}. Under the current clamp condition where I_{NaF} was inhibited by low concentration of TTX or riluzole, the membrane oscillation appeared near the resting membrane potential. This membrane oscillation was disappeared by addition of Cd^{2+}, or 1μM TTX indicating that I_{NaP} plays a role in the subthreshold membrane oscillation and could be important to modulate Kenyon cell excitability. No COI.

1P-034

Functional analysis of Ci-CatSper channel in heterologous expression system

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CatSper channel, a cation channel expressed specifically in testis, is essential for male fertilization in mouse. CatSper channel is formed by four alpha subunits, CatSper1, CatSper2, CatSper3 and CatSper4, as a hetero tetramer. Each subunit has six transmembrane segments (S1–S6). S4 contains several positively charged amino acids, and the amino acid sequence of the putative pore forming region of S5–S6 is similar to that of voltage-dependent calcium channel, which suggests that CatSper is a voltage-dependent calcium channel. However, detailed characteristics about CatSper are still unclear because functional analysis using heterologous expression system has been unsuccessful. In this study, we report that the region only consisting of S1–S4 from Ci-CatSper3, corresponding to the voltage-sensor domain of other voltage-gated ion channels, shows ionic conductance at hyperpolarization. We expressed a version of Ci-CatSper3 truncated downstream of S4 in *Xenopus* oocyte, and detected activation of substantial ionic currents upon hyperpolarization. We found that the current is likely carried by various cations including sodium and calcium. Furthermore, elimination of the positive charges in S4 resulted in disappearance of the ionic current, indicating that the S1–S4 region itself has ionic permeability. This is, to our knowledge, the first report of CatSper current recordings in a heterologous expression system. No COI.

1P-035

Electric axon guidance in constant electric field culture

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In addition to well-known mechanisms of chemical guidance, growing axons in the nervous system are directed by an extracellular electric field in a process known as galvanotropism. The galvanotropic behavior of nerve cells *in vitro* was first demonstrated as long ago as 1920. However, it remains unknown whether embryonic nerve tissues generate a similar electric field in order to guide growing axons. Yamashita has revealed that an extracellular voltage gradient exists in the embryonic retina and that this gradient guides the axons of newborn retinal ganglion cells towards their targets (***BBRC*** 431: 280-283, 2013). The extracellular potential is generated by epithelial Na^+ channels (ENaC) in neuroepithelial cells. These findings indicate an important role for galvanotropism in the initial orientation of axons that extend over long distances, and provide insight into the mechanisms underlying the proper extension of developing axons in the embryonic brain. In the present study, a constant electric field culture system was developed in order to investigate the molecular mechanisms that underlie the galvanotropic behavior of growing axons of retinal ganglion cells. Retinal strips (1 mm in width) were made from embryonic day 6 chick retinas. They were embedded in Matrigel™ and cultured for 24 hours under a constant electric field (0.1–2.0 mV/mm), which was established by a negative feed back circuit and by monitoring a voltage difference between two points (15 mm apart) in the culture medium. It was found that the direction of growing axons was reversed by reversing the polarity of electric fields. No COI.

Poster Presentations
Heart, Circulation (1)

1P-036

Aortic baroreceptors play a greater role in baroreflex regulation of heart rate than carotid sinus baroreceptors in unanesthetized, decerebrate rats

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A previous study using decerebrate, arterially perfused rat reported that aortic baroreceptors predominantly contribute to baroreflex regulation of heart rate (HR), while both aortic and carotid sinus baroreceptors similarly contribute to baroreflex regulation of superior mesenteric sympathetic outflow. To reexamine this issue using unanesthetized, decerebrate rat, we used surgical denervation of bilateral aortic nerves (AnD) or carotid sinus nerves (CSnD), to identify the relative contribution of each baroafferent limb to baroreflex regulation of HR and renal sympathetic nerve activity (RSNA). Mean arterial pressure (MAP) was increased by phenylephrine to elicit baroreflex bradycardia and sympathoinhibition. Baroreflex sensitivity (BRS) was estimated from the maximal slope of the baroreflex curve between the responses in MAP and HR or RSNA. AnD reduced BRS for HR to 31 ± 5% of the control in intact condition, while CSnD reduced it to 63 ± 8%. There was a significant difference ($P < 0.01$) in the attenuated BRS for HR between AnD and CSnD. In contrast, BRS for RSNA was reduced to 50 ± 5% following AnD and similarly to 58 ± 6% following CSnD. These results indicate that aortic baroreceptors have a greater influence on the cardiomotor limb of arterial baroreflex, while signals of all four baroafferents equally contribute to the vasomotor limb of arterial baroreflex. No COI.

1P-037

Involvement of orexin system in the sympathetic nerve regulation

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Orexin is a neuropeptide secreted from hypothalamic neurons that is known to be activated during motivated behaviors and active waking. Beat-to-beat fluctuation in heart rate, a phenomenon termed HR variability, has been widely used to evaluate autonomic activity.In the present study, we examined the importance of orexin system in the regulation of sympathetic nerve system with orexin/ataxin-3 transgenic rat, which has marginal number of orexin neuron. RT-PCR analysis showed expression of orexin and orexin receptor (OX1R) in the superior cervical ganglion ganglion, while expression of another receptor (OX2R) was low. Orexin/ataxin-3 transgenic rat showed increased expression of two receptors, while expression of orexin was undetectable, suggesting compensatory increase of both receptors. Orexin A (10 nM) increased intracellular calcium in the superior cervical ganglionic neuron, suggesting involvement of orexin system in the sympathetic nerve control. In the ECG recording, orexin/ataxin-3 transgenic rats showed decreased responsiveness to β-blocker. Furthermore, they showed deteriorated R-R interval regulation, indicating involvement of orexin system in the sympathetic nerve regulation. No COI.

1P-038

The effect of orexin-A on diurnal variation in arterial pressure in rats fed high-fat diet

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The diurnal variation of arterial pressure (AP), lower AP during resting period and higher AP during active period, is observed in rats. Since orexin is a neurotransmitter to regulate wakefulness, orexin might participate in diurnal variation in AP. This variation is disappeared in rats fed high-fat diets (HFD), thus orexin control disorder is considered. Accordingly, we examined circadian relationship between orexin and AP in HFD rats. Rats were fed with normal-fat diets (NFD) or HFD for 11 weeks from postnatal day 28. We measured AP continuously for 24 hs. After measurement of AP, both orexin-A mRNA expression in hypothalamus and orexin-A in cerebrospinal fluid at 1:00, 7:00, 13:00, and 19:00 were measured. Baseline orexin-A in HFD rats was higher than that in NFD rats. The diurnal variation of orexin-A mRNA, higher in active period and lower in resting period, was observed in NFD rats. This variation was completely abolished in HFD rats. Acute injection of orexin-A into the rostral ventrrolateral medulla induced sympathoexcitation (172 ± 34%) and pressor response (36.0 ± 5.7 mmHg). These results indicate that higher and unchanging orexin-A might participate in disappearance of diurnal variation in AP in HFD rats. No COI.

1P-039

The rostral nucleus of the solitary tract neurons receive convergent inputs from the aortic depressor and lingual-trigeminal nerves.

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We examined responses of the rostral nucleus of the solitary tract (NTS) neurons to stimulation of the aortic depressor nerve (ADN) and lingual-trigeminal nerve (LTN), and also investigated whether these inputs converge on to rostral NTS neurons in urethane-chloralose anesthetized rats. The ADN stimulation significantly altered their firing rates in one-third of the rostral NTS neurons, and did not in two-third of the neurons. The LTN stimulation significantly altered their firing rates in less than half of the rostral NTS neurons, and did not in more than half of the neurons. Majority of the rostral NTS neurons were found to exhibit a pulse-related activity in the ECG/ABP-triggered correlation histograms. Moreover convergent inputs from ADN and LTN were found in less than 20% of the rostral NTS neurons. These results suggest that these neurons integrate the inputs from baroreceptor and lingual-somatic afferents. These neuronal activities may be involved in the reflex cardiovascular responses in part. No COI.

1P-040

Downregulation of prepro-orexin gene expression in the NTS of SHR may be prohypertensive

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Since the nucleus tractus solitarii (NTS) is a pivotal region for regulating the set-point of arterial pressure, we proposed a role for it in the development of neurogenic hypertension. Our previous findings suggest that the NTS of pre-hypertensive and hypertensive spontaneously hypertensive rats (SHRs) exhibits abnormal inflammatory condition compared to the normotensive WKY rats (Gouraud et al. J Hypertens. 2011, Gouraud et al. Auton Neurosci. 2011). Whether this chronic condition affects the neuronal activity and plasticity in the NTS remains unknown. To unveil the neuronal functions in the NTS of SHRs, we investigated the expression of neurotrophin transcripts in both young and adult SHRs. Real-time quantitative PCR revealed that the expression of Hcrt (hypocretin, transcript for prepro-orexin that forms orexin-A/hypocretin-1 and orexin-B/hypocretin-2) is altered in the NTS of both adult and young SHRs. Hcrt transcript was also found decreased in the hypothalamus of SHR but not in the cortex nor the medulla oblongata. We have also found that orexin-A peptide microinjected into the NTS of anesthetized SHRs significantly decreased arterial pressure and the depressor effects were larger in SHRs than WKY rats, suggesting that its down-regulation in the NTS may contribute to hypertension in the SHRs. These profiles may be involved in the impairment of the neuronal circuitry regulating cardiovascular autonomic activity during the development of SHR. Supported by KAKENHI (25870639). No COI.

1P-041

Cardiac vagal control in a knock-in mouse of dilated cardiomyopathy with a troponin mutation

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Aims: To investigate the cardiac vagal control functions in a knock-in mouse model of dilated cardiomyopathy (DCM) with δK210 which develops cardiac enlargement, heart failure, and frequent sudden cardiac death like DCM patients. **Methods:** Microdialysis technique was applied to the left ventricular myocardium of anesthetized mice and myocardial interstitial acetylcholine (ACh) levels were measured by HPLC as an index of ACh release from cardiac vagal nerve endings. The effects of electrical stimulation of cervical vagal nerves at 5 and 10 Hz or α2-adrenergic stimulation by medetomidine (0.1mg/kg, i.v.) on myocardial interstitial ACh levels were examined in wild-type (WT) mice and δK210 DCM mice. **Results:** Electrical vagal nerve stimulation decreased heart rate (HR) and increased myocardial interstitial ACh levels both in WT and DCM mice and the responses of HR and myocardial interstitial ACh levels were not different between WT and DCM mice. In contrast, administration of medetomidine decreased HR and increased myocardial interstitial ACh levels both in WT and DCM mice, but the responses of HR and myocardial interstitial ACh levels were significantly suppressed in DCM mice compared to those in WT mice. **Conclusions:** In a knock-in mouse of DCM, peripheral cardiac vagal control functions including ACh release from vagal nerve endings and postsynaptic response were preserved, but central vagal activation through α2-adrenergic receptor was impaired. No COI.

1P-042

Characteristics of neural regulated vasoconstriction on guinea pig hepatic vein

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It has been reported that the increase of resistance in hepatic vessels regulates the blood circulation in the body through the venous return. As the cellular mechanisms under such a vasoconstriction have not been well understood, we examined the neuronal controls of contraction on the guinea-pig hepatic veins. The transmural nerve stimulation (TNS) evoked contraction on the vessels. As the frequency of the electrical stimulation increased (10–100 Hz), the amplitude and the duration of the contraction were increased. These contractions were absent in presence of Tetrodotoxin (3 μM). Phentolamin (3 μM) inhibited the initial phase of the contraction. In addition, propranorol (3 μM) increased the remaining contraction. Guanethidine (10 μM) also inhibited the initial phase of the contraction. In contrast, atropine (1 μM) shortened the duration of the TNS-evoked contraction. The remaining contraction is abolished in presence of 10 μM guanethidine. 1–10 μM phenylephrine evoked vasoconstriction in a dose response manner. Isoproterenol (3 μM) evoked the relaxation of phenylephrine-contracted vessels. Y-27632 inhibited both the TNS- and phenylephrine-evoked contraction. These results suggest that the adrenergic and cholinergic nerves regulate the contraction on the hepatic vein. No COI.

1P-043

Central serotoninergic system may be involved in mechanisms underlying anti-hypertensive effect of exercise therapy

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We discussed the central mechanism of anti-hypertensive effect of exercise therapy with a focus on the nucleus tractus solitarii (NTS), which plays an important role in regulating cardiovascular homeostasis. We identified that gene expression of the serotonin 1A (5-HT1A) receptor, but not other subtypes, in the NTS was significantly decreased after long-term daily wheel running in hypertensive rats (SHR). We also found that serotonin microinjected into the NTS of anesthetized SHR significantly decreased arterial pressure (AP). Moreover, to identify cardiovascular control by the serotonergic system in conscious animals, we microinjected a conjugate of an anti-serotonin transporter (SERT) antibody and saporin (anti-SERT-SAP) into the NTS to kill NTS-projecting serotonergic neurons. We found that AP measured in freely moving rats significantly increased after anti-SERT-SAP injections into the NTS, and confirmed that serotoneregic neurons in the dorsal raphe nuclei were reduced by anti-SERT-SAP injections. These results strongly demonstrate that the raphe nuclei-NTS pathway decreases the basal level of AP. 5-HT2A receptors in the NTS are presumably involved in the cardiovascular responses (Laguzzi R. Cell Mol Neurobiol 2003). Since 5-HT1A receptors counteract 5-HT2A receptors action, we now hypothesized that down-regulation of 5-HT1A receptors in the NTS may be involved in the central mechanism of anti-hypertensive effect of exercise therapy. This study was supported by the JSPS (24500793). No COI.

1P-044

Renal and lumbar sympathetic nerve activity in response to repeated obstructive apnea over 4 days in rats

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The aim of the present study was to examine responses of renal (RSNA) and lumbar sympathetic nerve activity (LSNA) to repeated obstructive apnea over 4 days. Wistar male rats were chronically instrumented for measurements of RSNA, LSNA, and oxygen saturation of the brain tissue, and a tracheal balloon for induction of apnea was implanted. At least 3 days after the surgery, the rats were subjected to obstructive apnea by inflating the tracheal balloon for 40 seconds. This process was repeated 6 times/hour for 5 hours/day over a 4-day period. The onset of the obstructive apnea increased RSNA, LSNA, and arterial pressure, while those recovered to the pre-obstruction levels after the cessation of obstructive apnea. The basal level of RSNA increased gradually and significantly throughout the 4-day experimental period. The magnitude of the LSNA increase was less than that of RSNA. The basal levels of arterial pressure and heart rate remained within the normal ranges throughout the experimental period. These data suggest that repeated obstructive apnea activates RSNA in a cumulative manner, which may in part trigger the development of hypertension observed in sleep apnea syndrome. No COI.

1P-045

Responses of renal and lumbar sympathetic nerve activity to chronic intermittent hypoxia in conscious rats

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The aim of the present study was to examine responses of renal (RSNA) and lumbar sympathetic nerve activity (LSNA) to chronic intermittent hypoxia in conscious rats. Wistar male rats were chronically instrumented with bipolar electrode for measurements of RSNA and LSNA and a telemeter for measurement of arterial pressure. At least a week after the surgery, rats were exposed to 20 cycles of normoxia and hypoxia (5% O2 at nadir) per hour, 8hour a day for 2 weeks. The sham group were subject to alternating cycles of air under identical experimental conditions in parallel. When rats were exposed to the hypoxia, RSNA, LSNA, arterial increased while heat rate tended to decrease. However, basal levels of RSNA, LSNA, arterial pressure, and heart remained unchanged throughout the 2-weeks experimental period. It is therefore we failed to observe any significant changes in RSNA and LSNA in response to chronic intermittent hypoxia over the 2-weeks period in rats. No COI.

1P-046

Frequency-dependent effects of heel raising maneuver on orthostatic cardiovascular responses

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We investigated the effects of muscle pump function on cardiovascular autonomic responses by heel raising maneuver (HRM) with four different frequencies of 6 (HRM_6), 7.5 ($HRM_{7.5}$), 10 (HRM_{10}), and 15 (HRM_{15}) cycles/min. The R-R interval (RRI) and beat-to-beat blood pressure (BP) were acquired in healthy subjects (8 males and 5 females, aged 25.0±9.6). From the continuous BP measurement, stroke volume (SV) was calculated by a pulse-contour method. Cardiac output (CO) and total peripheral resistance (TPR) were derived as SV times heart rate and mean BP divided by CO, respectively. Using spectral analysis, RRI and systolic blood pressure (SBP) variability were estimated for the low- (LF) and high-frequency (HF) bands. Compared with the control condition, HRM led to increase in the mean SV during standing. Shortening of RRI during quiet standing was suppressed by HRM except for HRM_{15}. The increase in CO and decrease in TPR were observed in HRM_{10} and HRM_{15}. HRM_6 induced increases in LF powers of RRI and SBP, while increase in HF power of RRI was observed only in HRM_{10}. These results indicate that there is frequency-dependent effect of HRM on cardiovascular autonomic responses to upright posture. Our results contribute to determining adequate frequencies of muscle contraction that displays favorable hemodynamic effect of muscle pump function during orthostatic stress. No COI.

1P-047

Changes in prefrontal oxygenation associated with the Stroop interference task in elderly subjects

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Aging may lead to cognitive decline, while the underlying mechanism is not fully understood. It has been suggested that the prefrontal cortex exerts a significant influence on the cognitive function. The age-related changes in the prefrontal cortex activity may play a role in generation of cognitive decline in elderly subjects. To address this issue, the prefrontal oxygenation during a consecutive cognitive task (Stroop color word test; SCWT, 100 trials/session) was examined with a two-channel near-infrared spectroscopy (NIRS) in young (23 ± 1 yrs) and older subjects (62 ± 1 yrs). SCWT required the subjects to answer the displayed color of an incongruent color word as quickly as possible. The concentration of oxygenated-hemoglobin (Oxy-Hb) increased significantly in the bilateral prefrontal areas during SCWT in both groups, while the extent of increments was larger in young subjects. The Oxy-Hb response was comparable between the right and left hemisphere in young group, while the Oxy-Hb response in older group was markedly blunted on the left side. Although the number of errors was comparable between the two groups, the duration for SCWT became longer in elderly people. These results suggest that the decrease in the regional blood flow of the prefrontal cortex, especially on the left side, may lead to lower outcome of the cognitive task. To examine the responses among various cortical areas, a study using a multichannel NIRS has been conducted and the results will be discussed. No COI.

1P-048

Effect of exhaustive exercise on the increase response to visual stimulation in posterior cerebral artery blood flow

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Neurovascular coupling (NVC) is assessed as an increase response to visual stimulation in the blood flow velocity in the posterior cerebral artery (PCAv) (Aaslid 1987). To investigate whether an expected change in PCAv accompanied with exhaustive exercise modifies the response in PCAv to visual stimulation, we measured the PCAv by transcranial Doppler ultrasound flowmetry during rest and exercise in 13 healthy males while they performed a leg-cycle exercise at 75% of maximal heat rate until exhaustion. NVC was estimated as the relative change in PCAv from the mean value obtained during 20 s of eye closing to the peak value obtained during 40 s of visual stimulation involving looking at a reversed checkerboard. The conductance index (CI) of the PCA was calculated by dividing PCAv by the mean arterial pressure (MAP). At exhaustion, PCAv significantly decreased by -4±1% (mean±SE) with a decrease in $PaCO_2$ by -3±1%. PCAv response to visual stimulation significantly decreased from 16±1 to 11±1%. Compared to the resting baseline, exhaustive exercise significantly decreased the increase response in MAP to visual stimulation from 5±2 to 0±1 mmHg. The CI response was not significantly changed. These results suggest that exhaustive exercise decreases the PCAv, and the magnitude of the response in PCAv to visual stimulation. (Supported by Kozuki Foundation) No COI.

1P-049

Activation of NTS histamine H_1 receptors modulates the gain of baroreflex control of heart rate in rats.

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HA-immunoreactive neurons are found exclusively in the tuberomammillary nucleus and known to project to many areas of the brain, including the nucleus tractus solitarii (NTS), plays an important role in regulating cardiovascular homeostasis. We have previously shown that activation of histamine H_1 receptors in the NTS increased arterial pressure (AP) and heart rate (HR), and attenuated the cardiac baroreflex gain. In the present study, we examined whether H_1 receptors in the NTS can affect the reflex gain independently of increases in the basal AP and HR. Moreover, effects of endogenous histamine on the reflex gain were examined in urethane-anaesthetized Wistar rats. After 2-Pyridylethylamine (25 nmol), a histamine receptor H_1 agonist, was unilaterally microinjected into the NTS, the gain of the cardiac baroreflex was significantly attenuated without any changes in the set points of AP and HR. After bilateral microinjections of H_1-receptor-specific antagonist (cetirizine hydrochloride) into the NTS (100 pmol), the basal AP and HR, and the baroreflex gain were not changed from their control values. These findings suggest that NTS histamine may modulate cardiovascular system via activation of H_1 receptors in the arousal stage as a high level of activity of histaminergic neurons thorough H_1 receptors is seen in the arousal states, and we further confirmed that activation of H_1 receptors in the NTS modulates cardiovascular functions. No COI.

1P-050

Role of T-type Ca2+ channel expression in cardiomyocytes exposed to hypoxic condition in the heart

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T-type Ca^{2+} channel currents (I_{CaT}) are involved in automatic activity in cardiac cells. The $Ca_V3.1$ and $Ca_V3.2$ isoforms are responsible for I_{CaT} in normal atrial and nodal myocytes but not in ventricular myocytes. In pathological condition, both isoforms are functionally expressed in ventricular cardiomyocytes. However, a direct relation between hypoxic stress and T-type Ca^{2+} channel regulation is not known. We investigated molecular mechanisms for transcriptional regulation of the T-type Ca^{2+} channel ($Ca_V3.1$ and $Ca_V3.2$) in adult and neonatal rat cardiomyocytes exposed to hypoxia. Male Wistar rats (10 wk) were subjected to normoxia (20% O_2) or hypoxia (8% O_2) for 3 h. mRNA expression of the T-type Ca^{2+} channel and transcription factors (Sp1, Id2, Csx/Nkx2.5, NRSF) were assessed by real-time PCR. Down-regulation of $Ca_V3.1$ mRNA in hypoxic cardiomyocytes was accompanied by an increase in transcriptional repressor Id2 through the activation of hypoxia-inducible factor-1α (Hif-1α). On the other hand, expression of $Ca_V3.2$, NRSF, and Csx/Nkx2.5 mRNA was not changed. Hypoxic stimulation (1% O_2, 6–24 h) decreased the beat rate of cardiomyocytes associated with reduction of the $Ca_V3.1$ isoform. These results suggest a novel signal pathway for the pathological expression of $Ca_V3.1$ in cardiomyocytes depending on the transcription factors Hif-1α and Id2. No COI.

1P-051

Overexpression of SERCA or sarcolipin does not alter Ca^{2+} induced Ca^{2+} release and Ca^{2+} leak in mouse myocardium

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Sarcoplasmic reticulum (SR) plays an important role in cardiac excitation-contraction coupling. Recently, decreased Ca^{2+} uptake into SR by SR Ca^{2+}-ATPase (SERCA) has been reported to decrease cardiac contraction in heart failure. However, the relationship between Ca^{2+} uptake and SR functions [maximal Ca^{2+} content, Ca^{2+} induced Ca^{2+} release (CICR) and Ca^{2+} leak] has not been fully investigated. We investigated the relationship between Ca^{2+} uptake into SR by SERCA and other SR functions using genetically manipulated mice hearts. We used saponin-treated thin trabeculae obtained from mice hearts with overexpression of SERCA (SERCA-TG) or sarcolipin (SLN-TG). Ca^{2+} content was measured by releasing all Ca^{2+} from SR by caffeine (50 mM) after loading Ca^{2+} into SR in the presence of ATP (4 mM) and various concentrations of Ca^{2+} for various durations. CICR and Ca^{2+} leak were estimated by measuring the remaining Ca^{2+} in SR after treating the Ca^{2+}-loaded SR with the solutions of various Ca^{2+} concentrations (relaxing solution at pCa 20 for Ca^{2+} leak). Ca^{2+} uptake rate was faster in SERCA-TG and slower in SLN-TG than that in each non-transgenic mouse (NTG). Maximal Ca^{2+} content, Ca^{2+} leak and CICR in SERCA-TG, SLN-TG and each NTG were almost identical. The results suggest the modulation of Ca^{2+} uptake rate does not alter maximal Ca^{2+} content, CICR and Ca^{2+} leak at steady state. No COI.

1P-052

Role of mitochondria on stretch-induced increase in calcium spark rate.

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We have previously reported that myocardial diastolic stretch induces an increase in Ca^{2+} spark rate. It has been reported that diastolic stretch rapidly produces reactive oxygen species (ROS) via NADPH oxidase (NOX), and oxidates ryanodine receptors to give rise to an increase in Ca^{2+} spark rate. Mitochondria are well-known as another source of ROS via electron transport chain in ATP production process. In the present study, we investigated the involvement of mitochondrial ROS production in stretch-induced acute increase in Ca^{2+} spark rate in mouse ventricular myocytes. Isolated mouse ventricular myocytes were exposed to 10% axial stretch using computer-controlled piezo-manipulated carbon fibers, attached to both cell ends. Diastolic spark rate, ROS production and mitochondrial membrane potential were studied using Fluo-4, DCF and TMRE-loaded cells, respectively. Axial stretch significantly increased Ca^{2+} spark rate (n = 19) and ROS production (n = 16). Applying 5 μM mitochondrial metabolic uncoupler FCCP in the presence of 5 μM oligomycin (to prevent ATP depletion) blunted the both response. Stretch significantly hyperpolarized mitochondrial membrane potential.The present results suggest that myocardial stretch increases not only NOX2-derived ROS but also mitochondrial ROS, and both are involved in stretch-induced increase in Ca^{2+} spark rate. Stretch-induced mitochondrial hyperpolarization suggests that stretch enhances electron transport system, leads to increase in mitochondrial ROS production. No COI.

1P-053

$Ca_V1.3$ is involved in I_{st} in mouse SAN cells

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The sustained inward Na^+ current (I_{st}) has been put forward as a potential inward current driving the pacemaker depolarization. However, unknown molecular correlates for I_{st} limits the understanding of its significance in the SAN pacemaker activity. Previous studies have revealed close similarity between I_{st} and I_{CaL} in their pharmacological properties, suggesting that these distinct currents share a common molecular determinant. To directly address this hypothesis, the present study employed two genetically modified mouse models. Patch-clamp recordings of I_{st} in mouse SAN cells were characterized by an activation on depolarization in the pacemaker potential range, little inactivation during depolarizing pulses, Na^+-permeability and sensitivity to various classes of I_{CaL} modulators. In knock-out mice with disruption of $Ca_V1.3$ Ca^{2+} channel subunit, I_{CaL} was significantly reduced and its voltage sensitivity for activation was shifted to positive direction, which was accompanied by complete loss of I_{st}. On the other hand, in knock-in mice carrying a point mutation that renders $Ca_V1.2$ diminished affinity for binding of DHPs, a considerable fraction of I_{CaL} exhibited resistance to the inhibition by nifedipine, whereas I_{st} retained the DHP sensitivity comparable to that in wild-type. These findings clearly show that $Ca_V1.3$ subunit is involved in the generation of not only I_{CaL} but also I_{st}. No COI.

1P-054

Quantifying contributions of individual ion transport mechanisms to electric activities by using cardiac myocyte models.

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The computer models of cellular function is developed by integrating empirical experimental equations (ordinary differential equation), which describe activities of constitutive molecules. The cell model should be firstly validated by reproducing various experimental findings. Then, it is essential to analyze mathematical behavior of the model to clarify the principle of cellular function, as well as the stability of the system. Our group developed the method 'lead potential analysis' to determine quantitative contribution of each ion channels and transporters to the electric activities of the cell model, such as the pacemaker depolarization in the sino-atrial node, the ventricular repolarization and abnormal behavior. In the present study, we further revised the method and demonstrate results of applying the method to representative ventricular cell models. No COI.

1P-055

C-terminal helix D of KCNQ1 contributes to normal I_{Ks} channel function

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Mutations of *KCNQ1* which encodes the *α* subunit of the slow delayed rectifier K^+ (I_{Ks}) channel may cause cardiac repolarization abnormality and severe arrhythmia. We found that a patient with a mild QT interval prolongation carries KCNQ1 with a mutation (A590T) in the helix D, a region suggested to be important for subunit multimerization and channel trafficking. The mutation at the next position (G589D) has been reported to disrupt the interaction of KCNQ1 with Yotiao (AKAP9), which is responsible for cAMP-dependent I_{Ks} modulation necessary for normal channel gating. We examined whether and how the A590T and G589D mutations affect the molecular and electrophysiological properties of I_{Ks} channel in HEK-293T cells.
Immunoblot analysis showed that both the mutant KCNQ1 could multimerize by themselves or with wild-type subunit. Immunoprecipitation analysis showed that both the mutant KCNQ1 could form complexes with Yotiao and KCNE1. Patch-clamp analysis showed that an intracellularly applied cAMP analog augmented both I_{Ks} mediated by A590T mutant channel and wild-type channel to similar extents. These results indicate that KCNQ1 helix D plays important roles in the functional expression of I_{Ks} channel via novel mechanisms independent of interaction with Yotiao. No COI.

1P-056

Testosterone abbreviates QT intervals and up-regulates KvLQT1 channel in cardiomyocytes through genomic pathway

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After adolescence, men have shorter QT intervals compares with these in women. It is postulated that QT intervals are modulated by sex hormones. Although it has been recognized that testosterone activates delayed rectifier potassium current in cardiomyocytes by non-genome action through NOS, the long-term actions of testosterone on ion channels are not known. Then, we investigate the role of testosterone focusing on its electrophysiological long-term action in rats. After administration of testosterone to castrated male rats, QT intervals were gradually shortened, and reached significant difference a week later (-12±4%). Expression of KvLQT1 channel was increased by 43% in mRNA level, and by 10% in protein level. However, expression of ERG, Kir2.1, Kv4.3, Kv4.2, MiRP1, minK, and KChIP2 was unchanged. The similar up-regulation of KvLQT1 channel by testosterone was also confirmed by female rats' cardiomyocytes. This results suggest that testosterone modifies QT intervals through a genome pathway, which may responsible for the difference between men and women in arrhythmogenic substates. No COI.

1P-057

Effects of ibudilast on the GIRK channel in isolated atrial myocyte and atrial fibrillation induced by vagal nerve stimulation in rat

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Ibudilast is known as a non-selective phosphodiesterase inhibitor, and has been used for treatment of bronchial asthma or cerebral contraction. In an isolated atrial myocyte under voltage clamp, ibudilast non-competitively and reversibly inhibited acetylcholine (ACh)-induced G-protein-activated inwardly rectifying K^+ (GIRK) current. Furthermore, ibudilast also suppressed GIRK channel current elicited by intracellular application of GTPγS, a nonhydrolyzable analogue of GTP. Although intracellular application of ibudilast did not inhibit the ACh-induced GIRK current, extracellular application markedly inhibited it in the same cell. We further examined the effects of ibudilast on the sinus rate and atrial fibrillation in anesthetized rats. Electrical stimulation of bilateral cervical vagal nerves frequency-dependently decreased the sinus rate, and atrial fibrillation was induced by burst pacing of the left atrium during vagal stimulation. Injection of intravenous ibudilast significantly recovered the decreases in the sinus rate in response to vagal stimulation, and suppressed the onset of atrial fibrillation except one of 8 rats during vagal stimulation. These results suggest that the ibudilast is effective for prevention or treatment of atrial fibrillation by inhibiting the current of GIRK channels. No COI.

1P-058

The Effect of Nicorandil on Na/Ca Exchange current in Cardiac Myocytes

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It was previously reported that nicorandil, a K(ATP) channel opener may exert antiarrhythmic actions by abolishing triggered activity (Lathrop et al., 1990). The triggered activity induced by delayed after-depolarization (DADs) is one of the mechanisms of ventricular arrhythmias associated with intracellular Ca^{2+} overload. In this study we examined the effect of nicorandil on Na/Ca exchange current (I_{NCX}) in isolated guinea pig ventricular myocytes using the whole-cell patch clamp technique. Nicorandil enhanced I_{NCX} in a concentration-dependent manner. The EC_{50} values of nicorandil were 15.0 μM and 8.7 μM for the outward and inward components of I_{NCX}, respectively. 8-Br-cGMP, a membrane permeable analog of cGMP also enhanced the I_{NCX} in a concentration-dependent manner. ODQ, a guanylate cyclase inhibitor (10 μM) almost completely abolished the effects of both nicorandil and 8-Br-cGMP on I_{NCX}. DADs induced by electrical stimulation with ouabain disappeared in the presence of 100 μM nicorandil. We concluded that nicorandil enhances the function of Na/Ca exchanger (NCX) via guanylate cyclase, and this may partially contributed to the cardioprotection of nicorandil by accelerating Ca^{2+} exit via NCX. No COI.

1P-059

Induced automaticity in ventricular myocytes from transgenic mice overexpressing HCN2

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HCN channels are expressed in the ventricle of fetal hearts ,and are silenced during development. These channels are re-expressed in the hypertrophied heart, and have been suggested to underlie arrhythmogenesis. To test this hypothesis, we generated and analyzed transgenic mice overexpressing HCN2 specifically in their hearts (HCN2-Tg). Action potentials (APs) and membrane currents were recorded using whole-cell patch clamp method. In all of HCN2-Tg myocytes, 0.3 μM isoproterenol (ISO) significantly depolarized the resting membrane potential (RMP). In 77 % of HCN2-Tg myocytes, ISO induced spontaneous action potentials (SAPs). In the rest of HCN2-Tg myocytes, the late repolarization phase of evoked APs was significantly slower than in WT myocytes. Analysis of membrane currents and time-differential of APs revealed that these differences are attributable to HCN2 tail current. When the RMP was hyperpolarized in 3 mM K^+ bathing solution, the RMP in HCN2-Tg was more depolarized than in WT myocytes, but this difference was not observed in the presence of the HCN blocker, ivabradine. Moreover, the RMP of HCN2-Tg was unstable under hypokalemic condition, and SAPs was induced in 57% of HCN2-Tg myocyte. These findings suggest overexpression of HCN2 increases the likelihood of arrhythmia, particularly under pathological conditions such as excessive β adrenergic stimulation or hypokalemia. No COI.

1P-060

Self-beating atypically-shaped cardiomyocytes (ACMs), a novel subpopulation of heart cells, originate from cardiac progenitor cells expressing prion protein (PrP) in adult mouse heart

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Atypically-shaped cardiomyocytes (ACMs) are a new subpopulation of spontaneously beating heart cells with a peculiar morphology identified within a culture of cardiac myocyte-depleted fraction cells obtained from adult mouse cardiac ventricles. ACMs grow in size and start beating within ~3 days culture without appreciable proliferation or expression of stem cell marker proteins, but stay in the heart until elderly stages. It has been recently reported that prion protein (PrP) serves as a surface marker for isolating cardiomyogenic progenitors from murine embryonic stem cells. The present study examined the localization of original ACMs in mouse heart using PrP as a cell marker. ACMs were found to express PrP mostly in the plasma membrane even in the very early stage of culture. ACMs could be isolated by flow cytometry, though the yield was very low. Immunohistochemical analyses revealed that the cells co-expressing PrP and cardiac troponin T (cTnT) existed in the interstitial spaces among ventricular myocytes. These cells were observed as solitary or clustered cells. The results suggest that the PrP(+)/cTnT(+) cells identified in the mouse cardiac ventricles are the origins of ACMs resident as cardiac progenitor cells in the post natal mouse heart. No COI.

1P-061

Identification of a novel type of possible cardiac progenitor cells co-expressing prion protein (PrP) and cardiac troponin T (cTnT) in adult human heart

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The present study examined to seek a novel type of cardiac progenitor cells in adult human heart. We have recently identified a new population of heart cells in adult mouse cardiac ventricles that spontaneously develop into beating cardiomyocytes with a peculiar morphology, defined as atypically-shaped cardiomyocytes (ACMs). ACMs did not appreciably proliferate or express stem cell marker proteins, but survive the long-term post-natal development of cardiac ventricles. Prion protein (PrP) has been reported to serve as a surface marker for isolating cardiomyogenic progenitors from murine embryonic stem cells, and we have found that mouse ACMs strongly express PrP mostly in the plasma membrane even in the very early stage of culture. To investigate whether such PrP-positive ACM-like cells are resident in human heart, immunohistochemical analyses were performed in human cardiac ventricular tissues fixed within 2~3 hr after death selected from 5 patients (2 male, 3 female; aged 41–86 years old) who died of cancer or respiratory failure. We found that small cells co-expressing PrP and cardiac troponin T (cTnT) existed in the interstitial space among ventricular myocytes in all human tissues tested. These cells did not express hematopoietic stem cell marker CD45. The results suggest the possibility that these PrP/cTnT-positive cells survive during life as cardiac progenitor cells in human heart. No COI.

1P-062

Mathematical analysis of end systolic force length relation of a linear approximated cardiac cell contraction model

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To elucidate the behavior of Emax, which is the slope of the end systolic pressure volume relation (ESPVR), it is useful to use simulation models. This study adopts the cardiovascular hemodynamics model consists of a cardiac contraction model, Negroni Lascano 1996 (NL96) model, a circulation model, and a left ventricular geometry model to relate the NL96 model with the circulation model. The simulation model gives a good reproduction of the high linearity of ESPVR. To investigate the parameters that affect Emax, a linear approximated NL96 model was employed for the mathematical analysis. With this model, analytically derived end systolic force length relation (ESFLR) showed that the slope of ESFLR is proportional to the rate parameter of cross-bridge attachment to a thin filament site and the coefficient of overlap function that represents the overlap ratio of actin and myosin to the sarcomere length. Furthermore, it was revealed that the slope is inversely proportional to the rate parameter of cross-bridge detachment to a thin filament site. These results were verified by the numeric simulation results of the original model. No COI.

1P-063

The effect of blood contact surface area during cardiopulmonary bypass—Biological body evaluation in a rat model—

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Extracorporeal life support, such as the cardiopulmonary bypass (CPB), preserve the patient's life by providing adequate oxygen supply and blood flow to vital organs. However, previous studies have suggested that the interaction of blood and large artificial surface contributes to inflammatory response during CPB. As a result of series of chain reactions, the numerous powerful inflammatory mediators, including hormones and autacoids, are formed and released. We hypothesized that small CPB circuit which reduces priming volume and blood contact surface area attenuates the systemic inflammatory response with a reduction of inflammatory cytokine levels and organ tissue damage during CPB. Rats were divided into the high priming volume CPB (priming volume: 15 ml, surface area: 0.044 m^2) group and the low priming volume CPB (priming volume: 7 ml, surface area: 0.036 m^2) group. CPB pump flow was maintained at 80 ml/kg/min. Blood samples were collected before, and 60 min and 120 min after initiation of CPB. Pro-inflammatory markers such as (TNF-*α*) and biochemical markers (LDH, ALT, AST) were significantly elevated in the high priming volume CPB group compared with the low priming volume CPB group at 60 min. At 120 min, however, none of the markers was statistically different between the 2 groups. These data suggested that in addtion to the blood contact surface area factor, the CPB exposure duration is also an important factor for causing the systemic inflammatory response. No COI.

1P-064

Effects of capsaicin treatment on rat left ventricular mechanical work and energetics in comparison with hyperthermia

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We previously reported that the effects of hyperthermia (42°C) on left ventricular (LV) mechanical work and energetics using the excised, cross-circulated rat heart model. We now investigated the effects of capsaicin (a TRPV1 agonist) on LV mechanical work and energetics. We analyzed the LV end-systolic pressure-volume relation (ESPVR) and the linear relation between the myocardial oxygen consumption per beat (VO_2) and systolic pressure-volume area (PVA; a total mechanical energy per beat) in isovolumically contracting rat hearts during infusion of capsaicin (1–40 ng/ml) under 300-bpm pacing. In capsaicin treated-hearts, LV ESPVR shifted downward from the control ESPVR like in hyperthermic-hearts. The slope of VO_2-PVA relation was not significantly different. However, the VO_2 intercept that is composed of each VO_2 fraction consumed in excitation-contraction (E-C) coupling (mainly consumed for calcium handling) and for basal metabolism, was decreased in capsaicin treated-hearts differently from that in hyperthermic-hearts. In hyperthermic-hearts, the VO_2 intercept was not changed because of decreased E-C coupling VO_2 and increased basal metabolic VO_2. Western blotting analysis showed that the protein levels of SERCA2, phospholamban (PLB) and phosphorylated PLB were not changed. These results indicated that capsaicin decreased the LV contractility like hyperthermia due to the down-regulation of the calcium handling in E-C coupling. No COI.

1P-065

Optical mapping study of the condition for circus movement of the excitatory waves in the rat isolated atrium preparation

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Using the optical mapping methods, we have studied the spatiotemporal pattern of the electrical activitie in the isolated rat auricular preparation. Each preparation was stained with a fast merocyanine-rhodanine voltage-sensitive dye (NK2761). Using a multi-element (16 × 16) photodiode array, we assessed optically the spread of excitation by timing the feet of optical action potentials. We tried to evoke the event of the tachyarrythmia (tachycardia-like excitation) with the circus movement of excitatory waves (re-entry) by applying tetanus stimulation under several conditions. As reported previously, events of tcachycardia-like-excitation are observed in the preparation with an anatomical obstacle. In the Ca^{2+} over loaded condition, the re-entry of excitatory waves was also observed. In this study, we have newly found the tcachycardia-like-excitation with re-entry of the excitatory waves by applying ouabain or ryanodine. In both conditions, the blocked areas through which excitation could not propagate appeared transiently during the formation of the re-entry path. The blocked area without anatomical obstacles also appeared in the center of the re-entry path (singular area). All these chemical procedures seem to disturb the intracellular Ca^{2+} dynamics, supporting our hypothesis that the tcachycardia-like-excitation with anomalous patterns of the excitation spread including the circus movement of the excitatory waves is induced by the inhomogenous increase of intracellular Ca^{2+} concentration. No COI.

1P-066

High-speed live imaging of single sarcomeres in the mouse heart *in vivo*.

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To explore the molecular mechanisms of cardiac muscle contraction under physiologic conditions, we directly imaged the motions of single sarcomeres in the beating heart *in vivo* at high spatial and temporal resolution. The sarcomeric Z-lines were labeled with *α*-actinin-AcGFP via infection of recombinant adenoviruses, and sarcomere lengths were measured using a spinning disk confocal microscope at ~100 fps (resolution in XY plane, ~20 nm). We successfully imaged sarcomeric motions, simultaneously with electrocardiogram and left ventricular pressure. Our analysis revealed that there exists a positive correlation between sarcomere length and left ventricular pressure (i.e., the Frank-Starling law of the heart). Likewise, Ca^{2+} waves / transients were observed in cardiomyocytes of the isolated heart. At the meeting, we will discuss how cardiac excitation-contraction coupling is organized *in vivo*. No COI.

1P-067

Simultaneous observation of single sarcomere length and local calcium in rat neonatal cardiomyocytes via expression of cameleon-Nano in Z-discs

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In cardiac muscle, contraction is regulated by micromolar concentrations of Ca^{2+} released from the sarcoplasmic reticulum (SR) (i.e., the Ca^{2+}-induced Ca^{2+} release mechanism), resulting in the binding of Ca^{2+} to troponin C and subsequent formation of cross-bridges. In order to enhance our understanding of cardiac excitation-contraction coupling, we in the present study developed a novel experimental system for simultaneous measurement of intracellular Ca^{2+} and single sarcomere length via expression of yellow cameleon-Nano (i.e., FRET-based ultrasensitive Ca^{2+} indicator) fused to the C-terminus of α-actinin in Z-discs in primary-cultured rat neonatal cardiomyocytes. The fluorescence emission ratio (i.e., YFP/CFP) of the expressed fusion protein varied in response to a change in $[Ca^{2+}]i$. Under a dual-view microscopy, we measured local Ca^{2+} changes by imaging YFP/CFP and analyzed single sarcomere length by detecting the distance between the YFP fluorescence profiles. Accordingly, we found that sarcomere length varied in response to a change in YFP/CFP in various regions of the myocyte. These results suggest that the present experimental system with yellow cameleon-Nano is useful for the simultaneous imaging of local Ca^{2+} and single sarcomere length in cardiomyocytes. No COI.

1P-068

The developmental changes in the contractile proteins of rat embryonic hearts around the period of the appearance of the heartbeat

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Wistar rat heart begins to contract at embryonic day 9.99-10.13, before the appearance of the linear heart tube, and the beginning of the calcium transient precedes the initiation of contraction. In order to investigate the relationship between the calcium transient and contraction, we tried to detect the developmental change in the contractile protein amount of individual embryonic heart by high sensitivity 3-step Western blotting method. We already reported that myosin regulatory light chain of embryonic heart increased around the period of the appearance of the heartbeat. In this study, we showed the developmental increase in troponin I during this period. This result indicated that the developmental increase in troponin I also involves in the initiation of contraction. No COI.

1P-069

Preliminary analysis of cell cycle regulation in cardiomyocytes.

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Early in the development, embryonic cardiomyocytes (CMs) actively proliferate. But this activity is lost shortly after birth in mammals, the mechanisms of which are unknown. At birth, CMs are exposed to dramatic environmental changes, including (1) the abrupt elevation of oxygen tension by the onset of breathing (PaO_2: 20mmHg in fetuses to 100mmHg in adults), (2) the temporary starvation due to the loss of maternal blood supply, and (3) drastic hemodynamic changes. Here we focused on the effect of elevated O_2 tension on CM proliferation. Fetal CMs at embryonic (E) day 17 were isolated from C57BL/6 mice, with keeping low O_2 conditions (3%) during isolation. The exposure of these CMs to atmospheric O_2 (20%), that mimics birth, inhibited cell proliferation which was evaluated both by direct cell count and the expression of cell cycle marker Ki67. This suggests that increased O_2 exposure to CMs at birth is the fundamental signal to initiate the downstream gene programs for cell cycle exit, and low O_2 milieu may be essential for fetal CMs to actively proliferate. To identify the critical genes to mediate the O_2-dependent cell cycle exit, we performed microarray analysis using two different set of samples. The first was the CMs from fetus (E16) vs neonate (postnatal day 2–3). The second was the isolated fetal CMs cultured under low (3%) vs normal (20%) O_2 conditions. While the former covers the whole effect of complicated changes at birth, the latter dissects the effect of O_2 changes alone. We are now in the step to select the critical genes, and validate the functions of each gene in knock-down experiments. No COI.

1P-070

Submembranous di-phosphorylation of myosin light chain and actin fiber formation play a critical role in thrombin-induced endothelial barrier disruption

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Background: The phosphorylation of myosin light chain (MLC) is a fundamental mechanism for endothelial barrier disruption. MLC is phosphorylated at S19 and T18. Any differential role of mono- and di-phosphorylation of MLC (MLC-P and MLC-PP) was investigated. **Methods**: The trans-endothelial electrical resistance (TER) was monitored as an indication of barrier function in porcine aortic endothelial cells. The levels of MLC-P and MLC-PP were quantified with Phos-tag SDS-PAGE. MLC The MLC mutants were expressed by using adenoviral vector. **Results**: Thrombin induced a decrease in TER with an increase in MLC-P from 24% to 35% and MLC-PP from 2% to 35%. MLC-P localized in peri-nuclear region, while MLC-PP localized at submembranous region, where actin fiber formation was also induced. Rho kinase inhibitors (H1152 and Y27632) inhibited thrombin-induced decrease in TER and submembranous MLC-PP and actin fiber formation. The expression of exogenous MLC substituted 85% of endogenous MLC. In the cells expressing MLC^{wt}, thrombin induced submembranous MLC-PP and decreased TER. The expression of $MLC^{T18A+S19A}$ abolished thrombin-induced MLC phosphorylation and actin fiber formation, and a decrease in TER. MLC^{T18A} or MLC^{S19A} abolished MLC-PP, but had modest effect on TER. **Conclusions**: MLC-PP and actin fiber formation in the submembranous region play a critical role in thrombin-induced barrier disruption. MLC-P is sufficient for barrier disruption. Inhibition of MLC-PP is required for barrier protection. No COI.

1P-071

Cardiovascular function of marine mussels monitored by MRI

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In addition to NO, H_2S and CO are nominated as EDRF. However, toxicity of H_2S and CO make difficult to analyse their effects. Creatures in the deep sea, such as *Bathymodiolus japonicus*, live in a high H_2S environment. Therefore, these mussels must be useful for testing effects of H_2S on cardiovascular function. *Mytilus galloprovincials* were used for set up MRI experiments for analysing cardiovascular function. The heart rate could be measured using the motion ghost of the anterior artery or the branchial vessels (1.1 Hz, 23°C). Cardiac cycle was imaged by retrospectively self-gated fast low angle shot sequence. The end-diastolic, end-systolic and stroke volumes were 50%, 21% and 29% of the heart volume, respectively. Flow of haemolymph in the heart and vessels were measured by phase-contrast MRI. The haemolymph returns to the auricle via the anterior oblique vein, and passed through the auriculo-ventricular valve. The streams from the right and left valves may merge together at the posterior end of the ventricle, then they are skewed towards the anterior direction by the inner curvature of the ventricle, and flow in the ventral side of the ventricle to the anterior aorta. These vortices in the haemolymph stream may account for the high ejection fraction (58%) of the heart, even with a single outlet. Now, we step forward to analyse the cardiovascular function of *Bathymodiolus japonicus*. No COI.

1P-072

How cardiac system evolved in multicellular organisms

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How cardiac system evolved in multicellular organisms Hiroshi Shimizu National Institute of GeneticsCardiac system plays a role in circulation of blood through vascular system and transport of nutrients and waste materials. Nkx-2.5 orthologues are expressed in the cardiac mesoderm of animals that have heart. Interestingly, however, this gene is found also in primitive animals that have no heart. The tissue that expresses Nkx-2.5 orthologues shows various kinds of pumping movement. In nematodes, it is expressed in the pharynx. In hydra, a member of class hydrozoa of phylum cnidaria, it is expressed in the peduncle of the animal. Since the pumping plays a role in circulation of gastric fluid in hydra, we previously proposed that the cardiac function appeared even in the most primitive animal phylum. However, a problem remained unsolved. Since hydrozoa is the latest class of Cnidaria, it remained unclear how the situation is in primitive class Anthozoa that includes sea anemone and corals. To solve this problem, we extended our behavioral analysis to two members of Anthozoa, first sessile sea anemone (Actiniaria) and second burrowing sea anemone (Nematostella vectensis). We found that in Anthozoa the pumping movement of Nkx-2.5 orthologue expressing tissue plays a role as the generator of hydropressure. We also obtained information that in spiders the heart pumping plays two roles, first as the organ for circulatory function and second as the generator of hydropressure. From this information, we propose a scenario that Nkx-2.5 related pumping started as the pressure generator and gradually developed circulatory function with evolution. No COI.

1P-073

Age-related effects of dexmedetomidine, an alpha-2 agonist, on coronary vasoactivity and cardiac function in guinea-pig hearts

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Dexmedetomidine is a potent and selective $\alpha 2$ agonist. It has been reported that, although the substance does not appear to have any direct effects on the myocardial contractility, it sometimes causes a decrease in heart rate and a dose-dependent decrease in arterial blood pressure. We hypothesized that effects of dexmedetomidine may differ depending on postnatal ages. Hearts from young (<3 wk) and adult (>6 wk) guinea-pigs were isolated and mounted on a Langendorff apparatus, and a saline-filled balloon was inserted into the left ventricle. Coronary perfusion pressure (CPP) and the left ventricular pressure (LVP) were continuously monitored and the electro-field stimulation (EFS) was applied to stimulate sympathetic nerve terminals. Dexmedetomidine almost completely inhibited the increase of LVP induced by EFS in both young and adult hearts. On the other hand, the effect on the coronary artery resistance to dexmedetomidine altered during postnatal development. Dexmedetomidine decreased CPP at all concentrations in young hearts, whereas it increased CPP at concentrations > 10 nM in adult hearts. The increase in CPP in adult hearts was inhibited by prazosin, an $\alpha 1$ antagonist. The present findings suggest that $\alpha 1$ adrenoceptor is involved in the increase in CPP in adult hearts in addition to $\alpha 2$ adrenoceptor. Aging-associated alteration of α adrenoceptor subtypes might be linked to cardiodepressant effects of dexmedetomidine. No COI.

1P-074

Lactate impaired excitation-contraction coupling in the atrial myocardium

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‹Background›The effect of the change in metabolic status on excitation-contraction (EC) coupling in the atrial myocardium is poorly understood. Although the myocardium mostly utilizes fatty acid as an energy source, we have reported that metabolic substrate (eq lactate) can be used for energy production and that the metabolomic profile is different between the atria and the ventricles. Therefore, we aimed to investigate the effect of lactate exposure on EC coupling in the atrial myocardium. ‹Methods›We micro-injected aequorin into superficial cells of the left atrium isolated from mice (C57/BL6, 15 ~ 17 weeks of age), and simultaneously measured intracellular Ca^{2+} concentration and tension (1 Hz at 36°C). We added lactate (~10 mM) into HEPES-Tyrode solution (pH was adjusted at 7.4). ‹Results and Conclusion›Lactate at a concentration of 10 mM significantly decreased peak tension (61.7±6.0%, n=3. $p < 0.05$) and peak Ca^{2+} concentration (78.8±4.8%, n=3. $p < 0.05$). Since previous studies reported that lactate decreases peak tension, but not peak Ca^{2+} concentration in the ventricular myocytes, our results suggested that the atrium has different characteristic of EC coupling from the ventricles in response to an increase in lactate, of which condition is often observed in myocardial ischemia. Simultaneous measurement of tension and intracellular Ca^{2+} concentration in the atrial myocardium can be useful analysis of the atrial physiological property. No COI.

1P-075

DNA microarray profiling identified cardiac fibrosis-related genes following pressure overloaded hypertrophy

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It is important to identify a molecular inducer of fibrosis as a therapeutic target for heart failure. A rat model of cardiac hypertrophy and fibrosis was generated by pulmonary artery banding (PAB). Histological analyses with Masson Trichrome stain on short axis section of right ventricular papillary muscles identified that they could be clearly divided into the interstitial fibrosis group (PABF+) and the non-fibrotic, but hypertrophic group (PABF-), in comparison with the sham-operated control (Sham). Tension in PABF+ was significantly smaller than that in Sham and PABF-. We comprehensively analyzed the mRNA expression of 29215 known rat genes in the right ventricle by using GeneChip Rat Gene 1.0 ST Array (Affymetrix) to compare a gene expression profile among Sham, PABF-, and PABF+ (n=3 each). We found that the expression levels of some genes that have not been recognized as a fibrosis-related molecule were significantly higher in PABF+ than those in Sham and PABF-. Among them, we confirmed that fibroblast growth factor 23 (FGF23) [Sham; 1.0±0.4, PABF-; 0.7±0.3, PABF+; 8.5±2.0] and neural adhesion molecule 1 (NCAM1) [Sham; 1.0±0.1, PABF-; 1.7±0.7, PABF+; 18.5±2.9] in PABF+ were significantly higher than those in Sham and PABF- by RT-PCR (n=6 each). These data suggest that FGF23 and NCAM1 are critical molecular inducers of cardiac fibrosis following pressure overloaded hypertrophy. No COI.

1P-076

Heart rate response to electrical muscle stimulation in the ovariectomized rats

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Our previous study has shown that the pressor responses to electrical stimulation of the muscle are enhanced in ovariectomized (OVX) rats, and that acute administration of 17beta-estradiol relieves the responses. The present study aimed to investigate whether heart rate responses to the same muscle stimulation are also augmented in the OVX rats, and whether acute administration of 17beta-estradiol influences the responses. Experiments were performed in urethane anesthetized, artificially ventilated rats. Heart rate was measured with a pulse rate tachometer, which was triggered by systolic blood pressure waves. Electrical muscle stimulation was delivered to the hindlimb muscle for 30 s at a frequency of 80 Hz with an intensity of 1.5 mA. 17beta-estradiol was administered intravenously and the effect was examined for 135 min after the onset of the administration. Responses of heart rate were augmented in the OVX rats, compared with the sham-operated rats. Furthermore, 17beta-estradiol significantly reduced the responses of heart rate between 45–135 min after the onset of administration. The present results demonstrate that the responses of heart rate to electrical muscle stimulation were exaggerated in the OVX rats and the responses can be relieved by the acute treatment with 17beta-estradiol. No COI.

1P-077

Evidence for cholinergic vasodilatation in skeletal muscle during exercise in humans

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To test the hypothesis that central command evokes sympathetic cholinergic vasodilatation in skeletal muscle during exercise, we have attempted 1) to examine whether blood flows in contracting and non-contracting arm muscles increase at the early period of one-armed cranking, 2) to examine whether blood flows in the bilateral muscles increase during mental imagery of the one-armed cranking, and 3) to identify whether atropine blunts the increased flow responses. Ten subjects performed voluntary one-armed cranking (at 20–35% of maximal voluntary effort) and mental imagery of the exercise for 1 min. The relative concentrations of oxygenated- hemoglobin (Oxy-Hb) in bilateral biceps brachii muscles were measured as an index of muscle tissue blood flow with near-infrared spectroscopy. Both one-armed cranking with the right arm and mental imagery of the exercise produced substantial increases in the Oxy-Hb of the bilateral muscles, which were blunted by atropine (10μg/kg iv). The present findings suggest that central command evokes cholinergic vasodilatation equally in both contracting and non-contracting arm muscles during exercise. No COI.

Poster Presentations
Neuron, Synapse (1)

1P-078

Modeling of the neocortical layer 5 pyramidal cell to predict the mechanisms of the slow oscillation

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The slow oscillations of electroencephalogram (EEG) or the local field potential (LFP) were frequently observed during slow-wave sleep. It is thought that the synchronous membrane potential fluctuation between UP- and DOWN-state in the pyramidal neurons generates the slow oscillations of EEG or LFP. Recent studies showed that slow-wave sleep might be related to memory consolidation. Therefore, the attempt to reveal the mechanisms of the slow oscillation should help to elucidate the memory formation. There are hypotheses that slow oscillation is shaped by Ca^{2+} dependent K^+ channel, and that recurrent excitation regulated by inhibitory networks. However, the detailed mechanisms remain unclear. It is difficult to clarify the mechanisms involved in the slow oscillation only by electrophysiological experiments. Thus, we conducted the computer simulation study to propose the mechanisms of the slow oscillation, using NEURON simulator. For this aim, we developed the model cell. The following ion channel models were created and incorporated to the model cell; voltage dependent Na^+ channels, voltage dependent K^+ channels, voltage activated Ca^{2+} channels, Ca^{2+} dependent K^+ channels, HCN channel, existing in the pyramidal cells at cortical layer 5. We also created and incorporated the mechanisms related to the Ca^{2+} dynamics. Using this model cell, we reproduced the currents of the ion channels and the firing properties of the real pyramidal cell. We will discuss the mechanisms concerning the slow oscillation with this model cell. No COI.

1P-079

Relationship between membrane properties and functional differentiation of neurons in the area postrema.

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The area postrema is one of the circumventricular organs that lack a blood-brain barrier. Previously we reported two major cell types classified on the basis of presence or absence of displaying the hyperpolarization-activated cation current (Ih). A correspondence of each type of cells to the physiological functions, such as the regulation of food intake and triggering of nausea and/or emesis, still have remained to be unexplained. To determine the functions of each type of cells, we performed the study using a patch-clamp method in the brain slices and a behavioral analysis of nausea. The responses to cholecystokinin (CCK-8) and amylin, which is well know as a satiety hormone, were recorded from the electrophysiologically-identified each type of neurons. We also investigated the effects of ZD7288, a selective Ih blocker, on the acquisition of conditioned taste aversion (CTA), in order to evaluate the changes in the excitability of cells expressing Ih on the induction of nausea and/or emesis. All cells responded to amylin and the majority of cells responded to CCK-8 were found in cells not expressing Ih. The acquisition of CTA was significantly suppressed by the i.p. injection of ZD7288, indicating the suppression of apomorphine-induced nausea and/or emesis. These findings indicate that cells not expressing Ih play a role for the regulation of food intake, while cells expressing Ih for the induction of nausea and/or emesis. We suggest the functional differentiation of each type of neurons. No COI.

1P-080

Optogenetic activation of parabrachio-amygdaloid pathway in the nociceptive amygdala

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A large majority of neurons in the superficial layer of the dorsal horn project to the lateral parabrachial nucleus (LPB) rather than to the thalamus. The LPB neurons then project to the capsular part of the central amygdala (CeC). This non-thalamocortical pathway plays pivotal role in the nociception-emotion link. The LPB-CeC transmission is potentiated in various pain models, as evidenced by electrical stimulation of the afferent fibers in the amygdala slices. However, due to the technical limitations, it has been unclear whether EPSCs recorded in slices resulted from monosynaptic direct glutamatergic connections arising from the LPB and not from glutamatergic fibers of non-LPB origin. To address this issue, we expressed channelrhodopsin (ChR2) in the LPB neurons and triggered glutamate release from their axon terminals in the CeC in coronal amygdala slices from adult rats. 5-6 weeks after the transfection of virus vectors for synapsin-driven ChR2(H134R)-YFP expression, blue light pulses (1–5 ms) robustly triggered EPSCs that were abolished by CNQX and TTX. This EPSC was followed by large and long-lasting IPSCs, which were abolished by picrotoxin or CNQX, suggesting a feed forward regulation of CeC neuron excitability by LPB. Moreover, all types of CeC neurons as classified by their firing patterns exhibited monosynaptic excitatory responses to light stimulation. These results suggest that nociceptive inputs from LPB affect the excitability of the central amygdala network by modulating a large population of CeC neurons. No COI.

1P-081

Modulation of fear memory by dietary polyunsaturated fatty acids via cannabinoid receptors

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Though the underlying mechanism remains unknown, several studies have suggested benefits of n-3 long-chain polyunsaturated fatty acid (PUFA) for patients with anxiety disorders. Elevated fear is thought to contribute to the pathogenesis of particular anxiety disorders. However, there are no studies on the effects of dietary PUFA on conditioned fear memory in experimental animals. The aim of this study was to evaluate whether the dietary ω3 to ω6 PUFA (3/6) ratio influences fear memory. For this purpose, the effects of various dietary 3/6 ratios on fear memory were examined in mice using contextual fear conditioning, and the effects of these diets on central synaptic transmission were examined to elucidate the mechanism of action of PUFA. We found that fear memory correlated negatively with dietary and brain 3/6 ratios in mice. The low fear memory in mice fed a high 3/6 ratio diet was increased by the cannabinoid CB1 receptor antagonist, reaching a level seen in mice fed a low 3/6 ratio diet. CB1 receptor-mediated synaptic plasticity was facilitated in pyramidal neurons of the basolateral nucleus of the amygdala (BLA) in mice fed a high 3/6 ratio diet. The agonist-sensitivity of CB1 receptor was enhanced in the BLA of mice fed a high 3/6 ratio diet, and similar enhancement was induced by pharmacological expulsion of cholesterol from membrane in mice fed a low 3/6 ratio diet. These results suggest that the ratio of ω3 to ω6 PUFA is a factor regulating fear memory via cannabinoid CB1 receptors. No COI.

1P-082

Contextual memory encoding but not retrieval generates wide diversity of post-synaptic current in hippocampal CA1 neurons.

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The hippocampus plays a central role in contextual learning and memory. Since the learning strengthens both excitatory and inhibitory CA1 synapses, each CA1 neuron shows high diversity of post-synaptic currents (Mitsushima et al., *Nature Commun*, in press). In the present study, to examine whether encoding or retrieval of memory strengthens the CA1 synapses, we analyzed miniature excitatory and inhibitory post-synaptic currents (mEPSC and mIPSC) in IA-trained rats before or after the memory retention test. As a learning model, we employed inhibitory avoidance (IA) task, and acute brain slices were prepared for patch clamp analysis. Untrained rats showed relatively small mEPSC and mIPSC amplitudes with low diversity of post-synaptic currents. Conversely, both IA-trained rats before and after memory retention test showed higher the amplitudes with wide diversity, suggesting that memory encoding rather than retrieval enhances the amplitudes to generate diversity. Moreover, bath treatment of CNQX (an AMPA receptor antagonist, 10 μM) consistently blocked the mEPSC responses. In contrast, bath treatment of bicuculline methiodide (a $GABA_A$ receptor antagonist, 10 μM) consistently blocked the mIPSC responses. These results suggest that contextual memory encoding rather than retrieval drives synaptic delivery of AMPA receptors and $GABA_A$ receptors in CA1 neurons. The electrophysiological diversity in each CA1 neuron might encode experienced context in rat hippocampus. No COI.

1P-083

Activity-dependent repression of spontaneous inhibitory synaptic current in rat hippocampus

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$GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs) modify excitatory synaptic transmission through modulation of activity of principal neurons. Comparing with the plasticity of excitatory synapses, the inhibitory synaptic changes have not been well characterized. Alteration in efficacy of $GABA_A$ergic transmission after postsynaptic depolarization has been reported by several investigators, but both facilitation and inhibition were observed, and mechanisms of the plasticity have not been elucidated. In the present experiments, effect of the repetitive postsynaptic depolarization on spontaneous IPSCs was determined in acute slices of rat hippocampus. Whole cell patch-clamp recording was performed to record $GABA_A$ergic IPSCs from neonatal CA3 neurons. Repetitive depolarization of postsynaptic neurons alone did not cause marked alteration of the spontaneous IPSCs. Effect of simultaneous activation of presynaptic and postsynaptic neurons on the IPSCs was then determined. Activation of presynaptic neurons induced transient facilitation of the sIPSCs, but succeeding repetitive postsynaptic depolarization transiently reduced the frequency of the sIPSCs. Though application of a metabotropic glutamate receptor agonist could not replace the presynaptic activation, the IPSC inhibition caused by the sequential pre- and post-synaptic stimulation was suppressed by specific antagonists of the metabotropic glutamate receptors. These results suggest that postsynaptic depolarization and facilitation of synaptical release of glutamate transiently inhibit the sIPSCs. No COI.

1P-084

Inhibitory effects of 5-HT at excitatory synapses in the dentate granule cells.

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The dentate gyrus is a gateway for neuronal transmission in the hippocampal formation. Granule cells are primary neurons in the dentate gyrus, and receive excitatory inputs mainly from the entorhinal cortex via perforant-path. Serotonergic fibers from the raphe nuclei distribute into the hippocampus, and previous studies show that 5-HT modulates the activity of GABAergic interneurons in the dentate gyrus, and thereby, affects the excitatory transmission between the perforant-path and granule cells. A possible direct modulation of the excitatory synapses is, however, not well examined.In the present study, we examined actions of 5-HT on the excitatory synapses in granule cells under the condition in which GABAergic inputs were blocked by picrotoxin. 5-HT (1 μM) decreased the input resistance (IR) of granule cells around 50 % and fastened the decay of EPSP evoked by stimulation of the perforant-path. Computer simulation showed that such reduction of IR decreases EPSPs around 30 % at most. We, however, observed more than 50 % reduction of EPSPs in some preparations. Moreover, synaptic transmission failure was often increased by 5-HT, suggesting that 5-HT reduces release probability of the synapses. All of these effects were blocked by a 5-HT1A receptor antagonist, WAY100635. Mossy cells in the hilus of hippocampus also make excitatory connections to the granule cells. However, EPSPs evoked by selective stimulation of mossy cell axons were not decreased by 5-HT. These results suggest that 5-HT induces pathway-specific modulation of excitatory inputs to the dentate granule cells. No COI.

1P-085

Pairs of stimuli modulate synaptic plasticity in hippocampal CA1 area

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The hippocampus plays a key role in memory. Hippocampal CA1 neurons receive inputs via a trisynaptic pathway. Entorhinal cortex sends the information to the dentate gyrus (DG) via the peforant pathway (PP). The DG transmits the information to area CA3 via the mossy fibers. CA3 subsequently sends processed information to stratum radiatum (SR) in area CA1 via Schaffer collateral (SC) pathway. CA1 also has direct excitatory connections with entorhinal cortex via the PP. These direct inputs synapse on distal pyramidal neuron dendrites in stratum lacunosum moleculare (SLM). The function of the direct PP inputs is not well understood, although recent research indicates that these inputs have important effects on CA1 pyramidal cells. The trisynaptic path has a longer delay time so that information arising from entorhinal cortex arrives at SLM 10–20 ms prior to the arrival of information at SR in CA1. In this study, the effects of interactions between PP and SC inputs on field EPSP (fEPSP) in CA1 area in brain slice preparation of rat are investigated. We recorded population spike amplitude (PSA) in CA1 pyramidal cell and slope of fEPSP at SR in CA1. The results indicate that synaptic plasticity is modulated by different pairing intervals. In addition to this, the property of synaptic plasticity is different between PSA and fEPSP when the same stimuli were applied. No COI.

1P-086

Developmental changes in localization of the presynaptic cell-adhesion molecule neurexin-1β during hippocampal inhibitory synapse formation

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Synapse organizing molecules, neurexins (NXNs) and neuroligins, are implicated in synapse formation, maturation and maintenance. It is unclear, however, how inhibitory synapses are formed and modified during development and synaptic plasticity. Thus, we first devised a molecular probe for visualizing inhibitory synaptic contact sites. For this purpose, we constructed a cDNA for NXN-1β of which C-terminal was tagged with mCherry (NXN-1β-mCherry). When transfected into hippocampal neurons under dissociated culture, NXN-1β-mCherry was specifically accumulated in axon varicosities and co-localized with endogenous NXN-1β. Therefore, NXN-1β-mCherry could be selectively transported to synaptic sites and serve as a reliable probe for visualizing the inhibitory presynaptic site under living conditions. We then examined developmental changes in NXN-1β localization at axon terminals. The use of VGAT-Venus mice allowed us to easily identify axon terminals of hippocampal inhibitory interneurons under culture. At an early culture stage (10 DIV), discernible clusters of NXN-1β-mCherry were not present in varicosities of Venus-positive inhibitory axons, whereas NXN-1β-mCherry clusters could be detected at later culture stages (17 and 24 DIV). Thus, the observations show that NXN-1β begins to accumulate in presynaptic varicosities after inhibitory synapse formation, and suggest that this presynaptic cell-adhesion molecule plays a role in maturation and maintenance of inhibitory synapses. No COI.

1P-087

Suppression of GABAergic depolarization of hippocampal mossy fibers by bumetanide

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Intracellular chloride concentration in neurons is regulated mainly by chloride extruding K-Cl cotransporter KCC2 and chloride uptaking Na-K-Cl cotransporter NKCC1. Activation of presynaptic $GABA_A$ receptors by muscimol depolarizes mossy fibers, possibly due to high chloride concentration within the terminals and the axons. Here we examined the effect of bumetanide, a blocker of NKCC1, on muscimol-induced enhancement of excitability of mossy fibers. Hippocampal slices were obtained from C57BL6J mice of 12-17 days old. Antidromic population spikes (ASs) were elicited by stimulation at the stratum lucidum in the CA3 region, and were recorded from the granule cell layer of the dentate gyrus. Application of $GABA_A$ receptor agonist muscimol (1 μM) increased the amplitude of ASs (to 123 ± 6.7 % of control, n = 7), possibly due to recruitment of subthreshold fibers by depolarization of mossy fibers. Bumetanide at 10 μM reduced the effect of musimol on ASs (to 106 ± 2.7 % of control, n = 7; $P < 0.05$). We also used a focal perfusion system to the stimulation site to localize drug application. Again, 1 μM muscimol increased ASs (to 129 ± 6.6 % of control, n = 6) and 10 μM bumetanide suppressed the effect of muscimol (to 116 ± 6.7 % of control, n = 6; $P < 0.05$). These results showed that bumetanide-sensitive transporter NKCC1 is expressed at the mossy fiber terminals and axons, and is contributed, at least partly, to higher intracellular chloride concentrations in the presynaptic compartments. No COI.

1P-088

The effects of propofol on inhibitory postsynaptic currents evoked with paired-pulse stimulation in CA1 pyramidal cells and dentate gyrus granule cells of rat hippocampal slices

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We have reported that propofol, one of the most popular intravenous anesthetic agents, effects in GABA-mediated inhibitory synaptic transmission differently in CA1 pyramidal cells (CA1-PC) and dentate gyrus granule cells (DG-GC). Propofol has increased the amplitudes and prolonged the time constant of IPSC only in CA1-PC but not DG-GC. In this study, we examined the effects of propofol, on the inhibitory postsynaptic currents (IPSC) evoked with paired-pulse stimulation in CA1-PC and DG-GC in rat hippocampal slices.

The monosynaptic IPSC were evoked by electrical stimulation at the 400 msec interstimulus interval every 20 sec of GABAergic interneurons and recorded from CA1-PC and DG-GC by whole cell patch-clamp technique. The effects of specific concentrations of 10μM propofol on the IPSC in CA1-PC and DG-GC were examined at -100 mV membrane potentials.We recorded 50 individual traces in each cell and paired pulse ratio (PPR) of individual traces was calculated by dividing the amplitude of the second response (A2) by the first (A1), the mean (A2/A1). Propofol was observed to significantly increase the PPR in CA1-PC but not in DG-GC. The data was also analyzed with the mean A2/mean A1 in each cell. The mean A2/mean A1 was also increased in CA1-PC but not in DG-GC. The data suggests that propofol may change GABA-mediated inhibitory synaptic transmission presynaptically in only CA1-PC. No COI.

1P-089

Decreased expression of syntaxin 1A after experimental febrile seizure in mice hippocampal CA1 area

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Febrile seizures (FS) are the most common types of convulsions in infants and young children. However, it is not clear whether FS result in long-term sequences such as temporal lobe epilepsy (TLE). The hippocampus of TLE patients is characterized by mossy fiber sprouting, which is also induced by the experimental FS. The experimental FS elicited that the pup (postnatal days 14–15) exposed to a regulated heated air stream coming from a hair dryer placed 50 cm above them. In addition, mossy fiber sprouting is thought to result in neuronal hyperexcitability. To elucidate pathological mechanisms for mossy fiber sprouting induced by the FS, we investigated the effects of the FS on the expression level of syntaxin 1A (STX1A), which is known to be involved in neurite elongation and sprouting. On postnatal days 14-15, ICR mice were subjected to the FS. After the FS, we isolated mRNA from the hippocampal CA1 area and performed real-time RT-PCR analysis. The expression level of STX1A decreased 8h, 24h, and 3w after the FS. It is suggested that the FS-induced decrease in expression level of STX1A might have a causal relation to an increase in mossy fiber sprouting observed in TLE. We are now investigating whether the experimental FS leads to an enhanced mossy fiber sprouting in the hippocampus by means of Timm staining methods. No COI.

1P-090

The critical time window for dopamine actions on the dendritic spines of nucleus accumbens.

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In behaving animals, effective reinforcement occurs only when rewards come after an event within a few second. A phasic activity of dopamine neurons may work as a positive reinforcer, enabling animals to learn the event from its consequence. It has, however, not been clarified whether dopamine actions on neuronal circuits are selective to the critical time window for the reinforcement. In order to examine the temporal characteristics of dopamine actions on neuronal circuits, we investigated the excitatory synapses on dendritic spines of medium spiny neurons (MSNs) in nucleus accumbens, a center of positive emotion receiving dense dopaminergic innervations. We induced spine enlargement by two-photon uncaging of caged glutamates paired with action potentials in D1 MSNs of acute slice from young adult mice, in combination with optogenetic stimulation of dopaminergic fibers from ventral tegmental area. We found that dopamine greatly potentiated the enlargement of spines shortly (within 1.5 s) after glutamate uncaging, whereas stimulation of dopamine fibers either before or 5 seconds after glutamate uncaging did not. Our results also suggested that the critical time window for dopamine actions was shaped by Ca^{2+}-dependent activation of adenylate cyclase I and efficient degradation of cAMP by phosphodiesterase 10A in thin dendrites of MSNs. Thus, the spine structural plasticity of MSNs can be responsible for the critical time window for reward actions of dopamine. No COI.

1P-091

Spatiotemporal analysis of firing prolongation in the corticostriatal network

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We found that striatal neurons have a time integrative property, showing prolonged firings after repetitive cortical optogenetic stimulation and its residual effect after stimulation intermission. In addition to the time properties of the prolongation, we measured spatial properties of the prolonged firings in the corticostriatal slice by using a newly developed optogenetic mapping lighting system with Digital Micromirror Device (DMD) which can accurately control both the lighting pattern and timing. We employed the acute slice of the Wistar Thy-1.2 promoter ChannelRhodopsin-2 Venus Rat. Striatal neuronal firings were recorded by a tetrode with photostimulation of the cortex and the corpus callosum. Striatal neurons that responded to repetitive photostimulations with 5 seconds interval showed residual firings for a few seconds after the end of the 1 sec-long photostimulation. After 5 times repetitive photostimulations, responses to the other area of photostimulation were increased or decreased. That is, repetitive photostimulations had a significant effect on the subject neuron's "receptive field"—groupings of corticostriatal input fibers. For each striatal neuron the field either shrank or expanded. No COI.

1P-092

What does chronic methamphetamine treatment alter the activity of the medium spiny neurons in the striatum?

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The striatum is a major nucleus of the basal ganglia, and divided into two compartments, the striosomes and the matrix. These compartments are distinguishable by time of neurogenesis, cytochemical markers, and input-output circuits. Recent studies suggest that imbalances between striosome and matrix functions might contribute to basal ganglia-related disorders. Stereotypy is a pattern of behavior abnormalities, which can be induced by chronic methamphetamine (METH) treatment. In this study, we examined whether the effects of chronic METH treatment on the physiological activity of striatal neurons differ between each of the striatal compartments. To visually identify the striosomes, we used a TH-GFP transgenic mouse strain expressing GFP in a compartment-specific manner. METH (5 mg/kg) or saline was injected intraperitoneally twice daily for 5 consecutive days. Gradually, enhanced stereotyped behaviors were induced by the METH treatment. We measured stereotypy scores after the injection in the morning. After withdrawal of 2–7 days, each mouse received a challenge with METH or saline, and then we compared c-Fos expression between in the striatal compartments. We also made whole-cell recordings from medium spiny neurons in slice preparation taken from the mice treated with METH or saline. Miniature excitatory and inhibitory postsynaptic currents were compared to explore the imbalanced activity of the striatal compartments. These studies would help explain the roles of the cooperation in striosome- and matrix-based basal ganglia circuits. No COI.

1P-093

Serotonin-induced inhibition of glutamatergic transmission onto rat basal forebrain cholinergic neurons

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Whole-cell patch-clamp study was carried out to elucidate modulatory roles of serotonin in the excitatory transmission onto cholinergic neurons in the basal forebrain (BF) of the rat. BF cholinergic neurons were identified with staining by Cy3-192-IgG. EPSCs were evoked under the condition where $GABA_A$-, glycine- and NMDA receptor mediated current components were pharmacologically blocked. Bath application of serotonin at 10 or 30 μM inhibited the EPSCs by 30–40 %. (R)-(+)-8-OH-DPAT, a 5-HT_{1A} receptor agonists (30 μM), or CP93129, a 5-HT_{1B} receptor agonist also showed inhibitory effects on the evoked EPSCs to similar extent compared with the effect of serotonin at the same concentration. These results suggest that serotonin inhibits non-NMDA glutamatergic transmission onto BF cholinergic neurons via $5HT_{1A}$ and 5-HTIB receptors. No COI.

1P-094

A long term HFS on STN induced LTP of GABAergic IPSC onto SNr GABA neurons evoked by stimulation on the internal capsule.

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A high frequency stimulation on the subthalamic nucleus (STN-HFS: constant current pulse, amplitude; 50–500 μA, frequency; 125 Hz, duration; 100 μs for 20 min) was applied in the rat brain slice preparations. During STN-HFS, the substantia nigra pars reticulata (SNr) GABA neuron was kept in the current clamp mode. STN-HFS induced a LTP in the GABAergic IPSC which was evoked by an electrical stimulation on an internal capsule, including a putative direct pathway, in a half of neuron tested (9 out of 17 neurons). At 120 minutes after STN-HFS, the normalized amplitude increased to 1.492 ± 0.185 (mean ± S.E.M.). Under voltage clamp mode at the holding potential of -70 mV during STN-HFS, HFS did not induce the LTP of IPSC (n = 5). In the solution with dopamine receptor antagonists (1 μM SCH23390 and 1 μM Sulpiride), STN-HFS induced LTP in all neurons (n = 8; 2.048 ± 0.336). In the solution with 50 μM AP-5 (NMDA receptor antagonist), STN-HFS induced LTP only one neuron out of 6. With 10mM BAPTA in the electrode solution, LTP was induced also only one cell out of 6. A paired pulse ratios before and 120 min after STN-HFS are not significantly different (n = 6, before: 1.581 ± 0.126, after: 1.356 ± 0.054, p = 0.123) in the neurons LTP induced. These results suggest that the post-synaptic mechanism induces the IPSC-LTP by STN-HFS in SNr GABA neurons. On the other hand, the inward synaptic current decreased in its amplitude for long term like LTD. This synaptic plasticity might be one reason why the deep brain stimulation has the beneficial effects on the neuropsychiatric disorders. No COI.

1P-095

Intracellular distribution of AMPA receptors internalized upon LTD in cerebellar Purkinje cell

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As for molecular mechanism of long-term depression (LTD) at parallel fiber - Purkinje cell (PC) synapse, PKC-mediated destabilization of GluA2 and following endocytic elimination of AMPA receptors from the synaptic membrane has been proposed as mechanism of LTD. However, estimated enhancement of destabilization was not sufficient to explain physiological magnitude of LTD. A data-driven kinetic model of AMPA receptor trafficking predicted decrease in total recycling pool size of AMPA receptors at LTD. Actually, density of AMPA receptors in the dendritic spine of cultured rat Purkinje cell was reduced at LTD induced by chemical stimulation (High K^+ and Glu). However, whether this decrease in AMPA receptor-density in spine was caused by proteolysis or translocation was not clear. Here, we examined the effect of epoxomicin, a blocker of proteasome, and found that it did not affect decrease in AMPA receptor density in spine at chemical LTD. To confirm translocation of AMPA receptors, surface expressed GluA2 was tagged by monoclonal antibody, then, LTD-inducing chemical stimulation was applied and cells were fixed and stained. Surface expressed GluA2 was actually translocated in dendritic shaft, and its density in spine was very low. These results also support decrease in AMPA-receptor density in spine was caused by translocation of them from the spine to the shaft. No COI.

1P-096

Novel form of depolarization-induced synaptic plasticity of GABAergic transmission in the Purkinje cells

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It is well known that several forms of depolarization-induced synaptic plasticity of GABAergic transmission occur in the cerebellar Purkinje cells (PCs) such as rebound potentiation, depolarization-induced suppression of inhibition and depolarization-induced potentiation of inhibition. In this study, we found another form of depolarization-induced plasticity of GABAergic transmission in PCs. We performed the whole cell voltage-clamp recording with low Cl^- pipette solution and recorded electrical stimulation evoked IPSC (eIPSC) from young rat cerebellar slices. The direction of eIPSC changed from outward to inward immediately after PC depolarization. Thereafter, the amplitude of eIPSC was depressed (Depolarization-induced Depression of Inhibition: DDI) for more than 20 min. Biophysical and pharmacological studies revealed that reversal potential of eIPSC was positively shifted by CaMKII-dependent activation of both calcium-activated chloride channels and cation-chloride cotransporters. These data indicated that DDI is induced by postsynaptic mechanism. Further, we examined the physiological roles of DDI at PC synapses in relation to instructive stimulation of climbing fiber. Approximately 50% of climbing fiber stimulation-induced depolarization of PC decreased the impact of exogenous GABA-mediated inhibition of spontaneous spike activity. These results suggested that DDI has a synergic effect of excitatory transmission. This novel form of DDI may provide another regulatory mechanism of PCs in the neuronal computation of the cerebellar cortex. No COI.

1P-097

Roles of developmental changes of GABAergic synaptic transmission on rat deep cerebellar nuclei neurons

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Activity of the deep cerebellar nuclei (DCN) takes an important role in outputting processed information from the cerebellum. Our previous studies report that 5-HT shows the inhibitory effect on GABAergic transmission: presynaptically inhibiting the release of GABA. On the other hand, 5-HT acts as an excitatory modulator: postsynaptically eliciting slow inward current. In this study, we examined a developmental changes of both the synaptic properties and modulatory effects of 5-HT on GABAergic synapses using rat cerebellar slices. At a younger stage (~ P12), IPSCs had slower kinetics and higher susceptibility to the 5-HT-induced inhibitory action than those at an older stage (~ P21). On the contrary, the postsynaptic modulatory action showed no developmental changes. Moreover, we found that the release probability of GABA was decreased with development (or age). However, the extent of 5-HT-induced inhibitory action was not associated with the release probability. As for the developmental change in postsynaptic synaptic properties, we found that the kinetics of IPSCs was largely determined by α_1 subunit expression in postsynaptic GABAA receptors. In summary, 5-HT released onto DCN may pre- and postsynaptically play regulatory roles in both the membrane excitability and the gain control of inhibitory synapses during development. Especially, at the younger stage, these modulatory effects of 5-HT would be important to control the excitability and to form the normal cerebellar function in the adult. No COI.

1P-098

Effect of inhibitory input in corticospinal synapse plasticity

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Neuronal plasticity is generally active only in young age during a short time window so called critical period (CP), after which is closed, loss of plasticity limits major remodeling of neuronal circuits and will never be re-opened throughout life. Molecules that may alter such CNS plasticity, however, still remains poorly understood. Because GluN2B (2B) show much longer time course with larger Ca influx than GluN2A (2A), developmental shift of 2B to 2A is hypothesized to be essential for regulating this time window. In this study, using in vitro model of corticospinal projection system, we tried to identify mechanism that closes the CP. In order to selectively activate the corticospinal axons, we infected the cortical slices with AAV-EYFP-tagged Channelrhodopsin (ChR2) and stimulated with LED light (465 nm). 2B that is known to shift to 2A during development declined markedly toward the end of CP in the spinal cord. In 2A knockout mice, which express 2B in high level even after the end of the CP, showed extension of the CP. Partial reduction of inhibitory input by strychnine (0.2 μM), which markedly enhanced Ca influx through 2B channels, also extended the CP. Our findings indicate that loss of 2B subunit plays an essential role in closing the CP and that inhibitory input has an important modulatory effect. Furthermore 2B may be the molecule that reactivates the synaptic plasticity even in more matured stage in this system. No COI.

1P-099

Closure of the critical period in developmental corticospinal plasticity is determined by the decline of synaptic expression levels of GluN2B-NMDA receptors: Biochemical and live imaging study

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It is known that NMDA receptor (NMDAR) subunit composition is shifted from GluN2B to 2A during development. In vitro mouse model of corticospinal (CS) projection with functional synapses, CS axons innervated entire spinal cord at an early developmental stage and then regressed mostly from ventral side in a GluN2B-dependent manner. There is critical period (CP, 6–11 DIV) for this activity-dependent synapse elimination with axonal regression. In this study, to examine the role of synaptic expression levels of GluN2B (synaptic GluN2B) in this regression, we employed live imaging of developmental dynamics of EYFP tagged Channelrhodopsin (ChR) -labeled CS axons. When axonal regression was blocked by APV application during the CP, and thereafter APV was removed, the CS axons on the ventral side were no longer regressed in wild type mice (WT), while those were eliminated in GluN2A knockout mice (2AKO). At the end of the CP, synaptic GluN2B in 2AKO spinal cords remained higher levels comparable to those in WT during the CP. Furthermore, even after the CP was closed, upregulating synaptic GluN2B by proBDNF application induced the axonal regression in WT. Together with these findings, the closure of the CP in this developmental plasticity in the form of regression of CS axonal projection is determined by the decline of the synaptic GluN2B. No COI.

1P-100

The critical period in corticospinal plasticity was re-opened by upregulation of GluN2B: Electrophysiological and optical recording study

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In *in vitro* system of corticospinal (CS) projection using slice co-cultures of mice cerebral cortex and spinal cord (SpC), the CS synapses are once formed throughout SpC, and then eliminated from ventral side during early development. This elimination occurs only from 6 to 11 days *in vitro* (DIV), which is dependent on NMDA receptor (R) and its subunit, GluN2B (2B). This term was called critical period (CP) in this system. Because of developmental shift of NMDAR subunit from 2B to GluN2A (2A), it is assumed that decline of 2B may determine the closing of the CP. We studied mechanisms that close the CP by electrophysiological and optical recordings. CS axons were stimulated at the deep layer of cerebral cortex. Spatial distribution of CS synapses was studied by optical recordings of CS-EPSPs (optEPSPs) with voltage-sensitive dye. Whole cell recordings were made to measure 2B component of CS-EPSCs. To see whether the CP is closed or not, we first applied APV until 11 DIV to block the synapse elimination and thereafter APV was removed. We found that optEPSPs decreased on the ventral side in 2A knockout (KO) mice after the end of the CP. 2B currents in 2AKO at 11 DIV were equivalent WT in early development. ProBDNF which are known to increase 2B, produced reduction of optEPSPs on the ventral side after the end of the CP; i.e., the CP was re-opened. These suggest 2B decline is crucially important for closing the CP in the CS synapse elimination. No COI.

1P-101

Quantitative analysis of postnatal development of corticospinal axons projecting to the spinal gray matter (C7) in the mouse

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Rodent corticospinal (CS) axons postnatally show dynamic changes of axon and synapse distributions in the spinal cord. This study addresses postnatal development of CS axons in the spinal gray matter at the cervical enlargement segment C7, which is involved in control of forelimb movement. To visualize every CS axon, we used transgenic mice, referred to as CST-YFP mice, in which the CS axons express yellow fluorescent protein (YFP). Distribution of the CS axons in the gray matter at C7 and its change during postnatal development were investigated with a laser scanning confocal microscope. GFP-immunoreactive axons ran through the ventralmost of the dorsal column where the CS tract is located in rodents and enter the gray matter. At postnatal day 7 (P7), fine CS axons extended radially and reached the margin of the gray matter. Small fusiform/granular varicosities were evenly spaced along the CS axons. As development proceeds, distribution of CS axons becomes segregated to four regions. The gray matter received dense projection of the CS axons dorsomedially and laterally, and moderate projections ventromedially, and light projections ventrolaterally. Quantification of CS axons running ventrolaterally showed an increase in number from P7 to P14. Densities of CS axons peaked at P14, and then declined thereafter. These results, together with previous results, suggest that a population of the CS neurons eliminate synapses after innervating infant gray matter. No COI.

1P-102

Homeostatic maintenance of the large-scale depolarization wave in the developing central nervous system

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Widely correlated spontaneous activity in the developing CNS is transiently expressed and is considered to play a fundamental role in neural circuit formation. The depolarization wave, which spreads over a long distance along the neuraxis, is an example of this activity. Although the wave is typically initiated in the spinal cord in intact preparations, spontaneous discharges have also been detected in the isolated brainstem. Although this suggests that the brainstem has the ability to generate spontaneous activity, but is paced by a caudal rhythm generator of higher excitability, a number of questions remains. Does brainstem activity simply appear as a passive consequence, or does any active change occur in the brainstem network to compensate for this activity? If the latter is the case, does this compensation occur equally at different developmental stages? Where is the new rhythm generator? To answer these questions, we optically analyzed spatio-temporal patterns of activity detected from the brainstem before and after transection at the obex. The results revealed that the wave was homeostatically maintained, which was characterized by an increase in excitability and/or the number of neurons recruited to the wave. The wave was more easily maintained in younger embryos. Furthermore, we demonstrated that the ability of brainstem neurons to perform such an active compensation was not lost even at the stage when the wave was no longer observed in the intact brainstem. No COI.

1P-103

Are motoneurons "star-shaped"?

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It is generally believed that dendritic morphology of spinal motoneuron (MN) is "star-shaped". However, the analysis of their dendritic pattern at an early stage has not been done. Thus we investigated the shape of rat cervical MNs in an early developmental age (P6–8). The MNs were identified by retrograde labeling with cholera toxin B subunit conjugated with Alexa Fluor 488 which was injected into the forelimb or trunk (pectral or serratus anterior muscles) muscle groups. Following a 48-hour survival period, transverse slices of the cervical cord were prepared. Whole cell recordings were made from retrogradely-labeled fluorescence-positive MNs. Neurobiotin was injected from the recording pipettes which were visualized with Texas Red-avidin. Double-labeled neurons were imaged with two-photon laser scanning microscope and traced with Neurolucida system. The MNs received monosynaptic responses to the stimulation of corticospinal tract were limited to those innervating forelimb muscles. The dendrite in these MNs showed preferred orientation for dorsomedial direction toward the dorsal column where the CST is located in rodents. The dendrites of MNs innervating pectoral and serratus anterior muscles showed bipolar orientations along boundary between the gray and white matter. These observations mean that the dendritic pattern of most MNs is greatly deviated from a typical "star-shaped" pattern at least at an early developmental stage. No COI.

1P-104

Transection of the whisker sensory nerve reorganizes topographical wiring of afferent fibers in the whisker sensory thalamus of mice

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A mature relay neuron in the whisker sensory thalamus (VPm) typically receives only one sensory afferent fiber, which originates from the whisker-representing brain stem nucleus (PrV2). Recently, we have demonstrated that transection of the whisker sensory nerve recruits multiple afferent fibers onto a relay neuron. However, origins of newly recruited fibers (PrV2 or not) and potential competition between newly recruited and preexisting fibers remain unexplored. Therefore, we here generated the Krox20-Cre;Ai14 transgenic mice in which PrV2-origin fibers are specifically labeled with tdTomato (tdT). Using the mice, PrV2-origin and non-PrV2-origin fiber terminals in the VPm were analyzed as tdT(+) and tdT(-) VGluT2-immunoreactive puncta, respectively. After the transection, tdT(+) puncta significantly decreased compared with that in the sham group (4.8 ± 1.5 vs. 6.1 ± 1.2, mean ± s.d, $\times 10^{-3}/mm^2$), whereas tdT(-) puncta increased (0.3 ± 0.2 vs. 0.1 ± 0.1). Densities of total puncta did not change. In addition, the size of tdT(+) puncta significantly decreased after the transection (2.0 ± 0.2 vs. 2.3 ± 0.2 μm^2). These results indicate considerable retraction and weakening of PrV2-origin (whisker-related) fibers and invasion of non-PrV2-origin fibers in the whisker sensory thalamus after the transection. No COI.

1P-105

Cortical feedback activity regulates connection pattern of afferent lemniscal synapses in the somatosensory thalamus.

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Plenty of studies revealed synaptic/molecular mechanisms for initial formation and neuronal activity-dependent refinement of synaptic connections in early development. However, mechanisms for subsequent maintenance of once established circuits in matured brain are not fully understood. In the sensory thalamus (VPm and dLGN), the sensory afferent synapses (lemniscal and retinogeniculate (RG) synapses) onto a thalamic neuron are maintained in an experience-dependent manner after developmental synapse elimination. A thalamic neuron receives two kinds of excitatory inputs from afferents and massive feedback corticothalamic (CT) inputs. Thus, the functional linkage between the two inputs may underlie the maintenance of thalamic afferent synapses. Recently we found that type 1 metabotropic glutamate receptor (mGluR1) plays a critical role in the experience-dependent maintenance of RG synapses (Narushima et al., in preparation). Because mGluR1 densely expressed at postsynaptic site of the feedback CT synapses, we hypothesized that cortical activity regulates maintenance of afferent synapses after maturation. Here we report that pharmacological manipulation of cortical activity triggered remodeling of lemniscal synapses. The results support the idea that CT feedback inputs heterosynaptically regulates maintenance of matured mono-innervation of afferent synapses in the sensory thalamus. No COI.

1P-106

A novel approach to evaluate the anesthetic depth with somatosensory evoked potential elicited by double stimulation

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Evoked potentials have been used in many studies as an indicator of the neural activity to the sensory input. We observed a novel phenomenon that a ratio of two evoked potentials elicited by double sensory stimulation dynamically changes in response to the depth of anesthesia. Small screw electrodes set up for evoked potential measurement in male SD rat skull under sevoflurane anesthesia. Two consecutive electrical stimulations were applied to the upper limb, and an amplitude ratio of the first response (R1) and second response (R2) were calculated (R2/R1). While the inter-stimulus intervals are long enough, R2/R1 was stable regardless of the anesthetic depth. On the other hand, when the inter-stimulus intervals are short (300–500ms), R2/R1 dynamically decrease in response to the depth of anesthesia. These results suggest that the R2/R1 may be a novel indicator for evaluating the anesthetic depth. No COI.

1P-107

Mechanisms of synaptic transmission mediated TRPM1 channels between retinal rod bipolar and AII amacrine cells

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In the mammalian retina, synaptic transmission from rods to rod bipolar cells is mediated by the metabotropic receptor mGluR6. In the dark, rods release glutamate by which rod bipolar cells hyperpolarize; conversely, in response to light, reduced release of glutamate from rods opens a non-selective cation channel, namely a TRPM1 channel, and depolarizes the rod bipolar cells, from which glutamate is released to post-synaptic AII amacrine cells (AII cells). We found that an inter-event interval and amplitude of EPSCs observed in AII cells dramatically increased with the temperature increase and that the current-time integral of the EPSCs at 35 °C was about 3-fold greater than that at 20 °C. The temperature dependent EPSCs should reflect glutamate input from rod bipolar cells because the EPSCs were blocked by CNQX or L-AP4 (an agonist of mGluR6) regardless of the temperature, but not strychnine and bicuculline, and the reversal potential was near 0 mV. Membrane potential of rod bipolar cells depolarized at 22 mV/10 °C using whole-cell patch-clamp recordings. Although the temperature dependent depolarization was not decreased by L-AP4, it was depressed using perforated patch-clamp recordings. The temperature dependent EPSCs in AII cells were not observed in TRPM1 KO mice. These results suggest that glutamate release from rod bipolar cells via mGluR6-TRPM1 cascade is temperature dependent and that both adequate temperature and intracellular ligand might necessary for regulation of the open probability of the TRPM1 channel. No COI.

1P-108

"Electrophysiological Effects of IL-1β, IL-6 and TNF-α on Thalamic Dorsal Lateral Geniculate Nucleus Relay Neurons"

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Major inflammatory cytokines like Interleukine 1β, Interleukine 6 and Tumor Necrosis Factorα play important roles in the development of neuropsychiatric conditions. We present here how electrophysiological properties of thalamic relay neurons are affected by high levels of IL-1β, IL-6 and TNF-α. Patch-clamp recordings of thalamic relay neurons from brain slices freshly dissected from mice were performed. IL-1β (1.4nM) induced a hyperpolarization of resting potential (-64.1±1.35 (IL-1β) vs. -61.3±1.28 (control) mV, $p<0.05$), a decrease in normalized Ih amplitude (0.74±0.05 vs. 0.87±0.04, $p<0.05$), a decrease in normalized action potential threshold (1.08±0.03 vs. 0.96±0.03, $p<0.01$), a decrease in normalized membrane resistance (Rm, 0.80±0.05 vs. 0.97±0.03, $p<0.01$), a decrease in spike numbers in bursts (5.0±0.57 vs. 7.2±0.49, $p<0.01$) and increased normalized burst latency (1.09±0.06 vs. 0.95±0.02, $p<0.05$). IL-6 (1nM) induced a decrease in normalized Ih amplitude (0.73±0.05 vs. 0.87±0.04, $p<0.05$), a decrease in normalized Rm (0.67±0.09 vs. 0.97±0.03, $p<0.01$) and an increase in normalized burst latency (1.36±0.16 vs. 1.06±0.06, $p<0.05$). TNF-α (1.2nM) induced no change in the evaluated parameters. These results show that IL-1β, IL-6, but not TNF-α may play important roles in electrophysiological modulation on thalamic relay neurons. Altered neuronal behavior promoted by these cytokines could be a possible mechanism behind conditions where sensory processing and consciousness are affected, such as in delirium. No COI.

1P-109

Cholinergic gain control in the primary visual cortex of awake rats

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We recently found two important roles of Acetylcholine (ACh) in visual functions. First, ACh administered to V1 caused a response gain control in anesthetized rats. The responses to various stimulus contrast were facilitated or suppressed in proportion to the magnitude of the control responses, by which the shapes of original contrast-response functions were unchanged. Second, the systemic administration of a cholinesterase inhibitor, donepezil (DP), improved the contrast detectability in behaving rats. However, the detectability improvement cannot be simply due to the response gain control in V1 because 1) DP acts at other areas than V1, 2) anesthetic may influence ACh's actions. To clarify those points, we performed extracellular recordings from V1 of head-fixed rats under awake and anesthetic conditions and compared the ACh's modulatory effects. DP was topically administered onto V1. DP predominantly caused response gain control in both physiological conditions but it also evoked decrease of contrast gain in awake rats, suggesting a high responsiveness to low contrast stimulus. Therefore, ACh-evoked two types of gain control within V1 seem to be one of the reasons for DP-induced detectability improvement of awake rats. All experimental protocols were approved by the Research Ethics Committee of Osaka University, and all procedures were carried out in compliance with the policies and regulations of the guidelines approved by the Animal Care Committee of the Osaka University Medical School. No COI.

1P-110

Impaired visual working memory in mice with reduced clusters of protocadherins

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Clustered protocadherins (cPcdhs) are neurospecific cell adhesion molecules characterized with multiple clusters. Of the 12 clusters in cPcdh*α*, cPcdh*α*1,12 mice have only cPcdh*α*1 and cPcdh*α*12, so that cPcdh*α* diversity is reduced in these mice. However, no apparent abnormality has been found in these mice. In the present study, we report that visual working memory is impaired in cPcdh*α*1,12 mice. First, we tested working memory regarding spatial locations of visual cues using a T-maze. Although cPcdh*α*1,12 mice showed a good performance in a visually-guided T-maze task, the performance in a memory-guided T-maze task was significantly worse than that in wild-type mice ($P<0.01$). We developed an M-maze equipped with a display for testing shape-recognition working memory. In a control task, a cue shape A or B was presented at center of the display, and the choice shapes A and B were presented at either branch of the M-maze with overlapped timing. If mice selected the branch with the different choice shape presented as the cue shape, they obtained a reward. Both wild-type mice and cPcdh*α*1,12 mice showed a good performance with a success rate >85%. In a working memory task, the delay period between the presentation of the cue shape and the choice shapes was set at 20 s. In this working memory task, the performance in cPcdh*α*1,12 was significantly worse than that in wild-type mice ($P<0.003$). These results indicate that cPcdh*α* diversity plays an essential role in visual working memory. No COI.

1P-111

Frequency dependent integration of excitatory synapses in the dendrites of auditory coincidence detector neurons of birds.

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Difference of sound arrival time between two ears (interaural time difference, ITD) is a major cue for sound source localization. In birds, neurons in nucleus laminaris (NL) are the coincidence detector of bilateral synaptic inputs and involved in processing of ITDs. Importantly, NL neurons are tuned to a specific frequency of sound (characteristic frequency, CF) and have several functional specializations depending on CF. In particular, the length of dendrites varies along the frequency axis and neurons in low-CF region (low-CF neurons) have dendrites 7–20 times longer than higher CF neurons have. However, the functional roles of CF specific variation of dendritic length for the ITD processing still remain elusive. In this study, we analyzed the distribution of glutamate receptors along the NL dendrites with the focal uncaging of MNI-glutamate in the chicken brain slices. We found that the density of glutamate receptors was heterogeneous in the low-CF neurons and increased toward the distal end of dendrites. On the other hand, this tendency was not observed in high-CF neurons. In the low-CF neurons, the properties of focal-evoked mEPSCs showed no clear differences between the proximal and distal dendrites, suggesting that the type and density of receptors under the synaptic release site is similar along the dendrites. We will further examine the integration process at the focal dendrites to address the functional roles of dendrites in the NL neurons. No COI.

1P-112

Tonotopic variation of inhibitory synaptic transmission in nucleus magnocellularis of chick

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Neurons in avian nucleus magnocellularis (NM) generate action potentials in response to excitatory synaptic inputs from the auditory nerve, and are involved in a relay of auditory timing information to higher auditory center. NM neurons are tuned to a specific frequency of sound and arranged tonotopically within the nucleus. It is known that temporal precision of auditory nerve activity differs along the tonotopic axis, and it decreases toward lower tuning frequency. To compensate for this variation, NM neurons differ in the number and size of excitatory inputs along the tonotopic axis; high-frequency neurons receive a single large input, while low-frequency neurons converge multiple small inputs to generate a spike. However, it remains unknown whether NM neurons also vary in their inhibitory inputs in a manner dependent on tuning frequency. In this study, we studied characteristics of miniature inhibitory postsynaptic current (mIPSC) in NM neurons along the tonotopic axis, with whole-cell patch-clamp techniques in brain slices of posthatch chickens. What we found was that mIPSC showed higher frequency in low-frequency neurons than in high-frequency neurons, without any differences in the amplitude and time course. This may suggest that low-frequency neurons receive a larger number of inhibitory projections compared with high-frequency neurons. We will further record unitary IPSC to confirm this idea, and discuss possible roles of this abundance of inhibitory projections in the temporal coding of low-frequency NM neurons. No COI.

1P-113

Homeostatic regulation of potassium channels in avian auditory neurons

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Appropriate adjustment of neuronal activity is crucial for maintenance and performance of neural circuits. Axon initial segment (AIS) is accumulated with voltage-gated Na^+ (Nav) channels and is the site of spike initiation in neurons. We previously showed that deprivation of auditory inputs increased the expression of Nav channels at the AIS and augmented excitability of neurons in avian cochlear nucleus. However, how auditory deprivation affects other ion channels, such as voltage-gated K^+ (Kv) channels, remains unknown. In this study, we addressed this issue with immunohistochemistry and electrophysiology, and found that auditory deprivation changes the expressions of Kv channels at the AIS in a subtype-specific manner; Kv1.1 decreased, while Kv7.2 increased, showing a complementary change in their expressions. Interestingly, auditory deprivation caused a negative shift of spike threshold without changes in the resting membrane potential. Kv1.1 has low threshold and rapid kinetics for activation and strongly inhibits firing, while Kv7.2 has slow kinetics and contributes to set the resting potential. These indicate that the decrease of Kv1.1 enhanced excitability of neurons but it was balanced with the increase of Kv7.2 at the rest. Thus, Kv1.1 and Kv7.2 work cooperatively at the AIS, and maintain excitability and resting potential of neurons during deprivation of afferent inputs in the cochlear nucleus. No COI.

1P-114

Detrended fluctuation analysis reveals temporal patterns of spontaneous oscillatory activity in the olfactory center of the land slug

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Spontaneous oscillation of activity in olfactory system is commonly observed in various species and is thought to be important for odor-information processing. The terrestrial slug Limax valentianus has a highly developed olfactory center, the procerebrum (PC), in which exhibits oscillation of local field potential (LFP) at 0.7 Hz. Here, we investigated the statistical property of spontaneous oscillation in the PC over an extended timescale. To analyze the autocorrelation of the time series of interspike intervals, we performed a detrended fluctuation analysis, which is a scaling analysis technique used to provide a quantitative parameter (scaling exponent, α). The fluctuation of spike timing of the PC possessed a long-term correlation at timescales larger than 10^2 spikes (α = 1.21). The application of cychloheximide, an inhibitor of protein synthesis, induced a decrease in the scaling exponent α at the larger time window. This result indicates that the fluctuations of spike timings are characterized by a dynamics of mRNA translation in the PC. Furthermore, to investigate the contribution of innervations to spontaneous oscillation, we measured the LFP following the tentacle amputation. In the scaling region for small n ($n < 10^2$ spikes), the scaling exponent α decreased from 0.73 to 0.58, indicating that the fluctuations of spike timings are uncorrelated under this experimental condition. These results suggest that the innervations to PC maintain the intrinsic dynamics. No COI.

1P-115

Cholinergic system regulates oscillatory activity of the olfactory center in the slug

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Laminar structure of synchronous oscillatory activity is common in the olfactory system of both vertebrates and invertebrates. In the terrestrial slugs, periodic oscillation is recorded from the surface of the laminar structure of procerebrum (PC) and its frequency changes are suggested to encode the olfactory information and memory. Acetylcholine (ACh) is known to increase the frequency of the local field potential (LFP) oscillation in the PC, and is one of the candidates of the neurotransmitters that are involved in such higher cognitive functions. In the present study, we thus examined what role cholinergic system plays in the PC oscillatory network. First, the acetylcholinesterase (AChE) inhibitor enhanced the excitatory effect of ACh, and furthermore, AChE alone increased frequency of the LFP oscillation in the PC. Second, the mRNAs for nicotinic ACh receptors were expressed with narrow distribution of the PC. Nicotine induced the inward currents in some PC neurons and its estimated reversal potential was -55 mV. From the calculated chloride Nernst potential of -56 mV, this nicotine-evoked inward current is primarily carried by Cl^-. These results suggest that ACh can function as an excitatory transmitter for PC neurons present the PC via mainly nicotinic ACh receptors activation. Furthermore, the olfactory tentacle ablation enhanced the nicotine-induced increase in LFP frequency of the PC. This result additionally suggests the presence of feedforward inhibition in the cholinergic afferents from the olfactory tentacle to the PC. No COI.

1P-116

Spontaneous excitatory transmission enhancement and outward current produced by carbacrol in rat spinal substantia gelatinosa neurons

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Although the oral administration of an essential oil component carvacrol produces antinociception, cellular mechanisms for this action have not yet been examined. We examined the action of carvacrol on glutamatergic spontaneous excitatory transmission by applying the whole-cell patch-clamp technique to substantia gelatinosa (SG) neurons in adult rat spinal cord slices. Carvacrol superfused for 2 min produced either increase in the frequency of spontaneous EPSC with a minimal increase in the amplitude or outward current at -70 mV. The frequency increase and outward current had the EC_{50} values of 0.69 mM and 0.55 mM, respectively. The former action was inhibited by a TRPA1 antagonist HC-030031 but not a TRPV1 antagonist capsazepine, while the latter action was unaffected by the antagonists. Current-voltage relationship of the outward current indicated an involvement of a change in the membrane permeability of K^+. The outward current was inhibited in 10 mM-K^+ but not low-Cl^- and K^+-channel blockers (TEA and Ba^{2+})-containing Krebs solution. In conclusion, carvacrol increased the spontaneous release of L-glutamate onto SG neurons from nerve terminals by activating TRPA1 but not TRPV1 channels. Carvacrol also produced membrane hyperpolarization, which was mediated by TEA- and Ba^{2+}-insensitive K^+ channels, in SG neurons without TRPV1 and TRPA1 activation. It is suggested that the hyperpolarizing effect of carvacrol could contribute to its antinociceptive effect. No COI.

1P-117

Cellular mechanisms for inward currents produced by oxytocin in adult rat spinal substantia gelatinosa neurons

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We have previously reported that oxytocin produces an inward current at -70 mV by activating oxytocin receptors, resulting in the enhancement of GABAergic and glycinergic spontaneous inhibitory transmisison in spinal substantia gelatinosa (SG) neurons, a cellular mechanism for antinociception produced by intrathecally-administered oxytocin. The present study examined cellular mechanisms for the oxytocin-induced inward current by applying the whole-cell patch-clamp technique to the SG neurons of adult rat spinal cord slices. The oxytocin current was inhibited by a phospholipase C inhibitor U-73122, an IP_3-induced Ca^{2+}-release inhibitor 2-aminoethoxydiphenyl borate. On the other hand, a Ca^{2+}-induced Ca^{2+}-release inhibitor dantrolene, a protein kinase C inhibitor chelerythrine, and dibutyryl cyclic-AMP did not affect the oxytocin activity. In 31 % of neurons exhibiting oxytocin activity, a net of oxytocin current, estimated from a difference between current-voltage relationships in the absence and presence of this peptide, reversed at around the equilibrium potential for K^+. The other neurons did not show such a reversal, and the net oxytocin current was inward at negative potentials. The oxytocin current was depressed in peak amplitude in high-K^+ and low-Na^+ solution. It is concluded that the oxytocin-induced inward current in SG neurons is due to a change in membrane permeabilities to K^+ and/or Na^+, which is possibly mediated by phospholipase C and IP^3-induced Ca^{2+} release. No COI.

1P-118

Optical survey of initial expression of synaptic function in the embryonic chick trigeminal sensory nucleus

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We examined initial expression of synaptic function in the embryonic chick trigeminal nucleus using voltage-sensitive dye recording. Brainstem preparations with three trigeminal nerve afferents, the ophthalmic (V_1), maxillary (V_2) and mandibular (V_3) nerves, were dissected from 5.5- to 6.5-day old chick embryos. In our previous study (Sato et al., 1999), we detected slow signals corresponding to glutamatagic EPSPs and identified the principal sensory nucleus of the trigeminal nerve (Pr5), spinal sensory nucleus of the trigeminal nerve (Sp5) and trigeminal motor nucleus (Mo5). In this study, we examined effects of removing Mg^{2+} from physiological solution, which enhanced NMDA receptor function in the sensory nuclei. In 6.5-day old embryos, the slow signal was observed in the Pr5 and Sp5 only when the V_1 nerve was stimulated, whereas it appeared in Mg^{2+}-free solution with every nerve stimulation. In 6-day old embryos, the slow signal was observed only in the Sp5 with the V_1 nerve stimulation, and the appearance of synaptic function in Mg^{2+}-free solution varied depending on nerves and preparations. In 5.5-day old embryos, synaptic function was not detected even when external Mg^{2+} was removed. These results indicate that the initial expression of synaptic function in the trigeminal system is earlier than previously considered, and that the developmental organization of synaptic function is different between the three trigeminal nerves and between the two sensory nuclei. No COI.

1P-119

Attempts to record voltage sensitive FRET signal and extracellular spike current from a neuron using a photometric patch electrode

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How neurons generate spikes as a consequence of synaptic inputs is an important issue to understand the flow of neuronal information, particularly in a deep brain tissue where most of neural activities are out of the reach of modern electrophysiological or optical recording techniques such as whole cell patch recording or two photon microscopy. We have established a new photometric patch electrode (PME) recording method that delivers and collects light from a target neuron by using a patch electrode as a light guide, which enabled us to obtain fluorescent and electrical signals from a neuron. By using this method in a deep brain tissue of living chicks we have so far succeeded to record Ca2+ sensitive Oregon Green BAPTA-1 fluorescence simultaneously with the field current in response to sound stimulus from inferior colliculus and Field-L (avian auditory cortex). We are applying the PME to monitor voltage signals from a chick auditory brainstem neuron in slices by using DiO-DPA FRET technology that was recently reported as a fast and sensitive membrane potential indicator. We are interested in applying this technique eventually to auditory nuclei in vivo. The PME recording technique in combination with the voltage sensitive FRET recording may contribute to investigate the process of synaptic integration as FRET responses and the neuronal output as cell attached extracellular field responses, which allows us to understand the flow of neuronal information in a single cell level in deep brain tissues. No COI.

1P-120

Generation of OXTR-IRES-Cre knock-in mice

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The oxytocin receptor (OXTR) and ligand oxytocin (OXT) regulate reproductive function (i.e. parturition and milk ejection), socio-sexual behaviors and so on. We reported that OXTR-deficient mice exhibited pervasive social deficits (1) and so on. Successively, we generated OXTR-Venus knock-in mice to locate and analyze the neurons expressing OXTR by visualizing neurons expressing OXTR (2). With them, we detected the expression of OXTR on GABAergic neurons, serotonergic neurons and so on. In the present study, we newly generated OXTRcDNA-HA-IRES-Cre knock-in mice, to express both OXTR and Cre recombinase under the control of OXTR promoter, and to restrictedly modify the neurons expressing OXTR. As in the previous study, we found an impaired phenotype in a part of their social behaviors with heterogonous OXTR KO mice (haplo insufficiency) (3), to avoid such effect caused by inactivation of one allele of OXTR gene with insertion of the other gene, and further to facilitate analysis of subcellular localization of OXTR, HA-tag-modified mouse OXTR cDNA, and following IRES element were placed at the upstream of Cre sequence in a knock-in vector. This line is also useful for monosynaptic analysis of neural circuit, optogenetic analysis of neurons expressing OXTR, and so on, by combination with our new AAV vector system.1. Takayanagi,Y. et al., Proc Natl Acad Sci USA 102: 16096 (2005)2. Yoshida,M. et al., J.Neuroscience 29:2259 (2009)3. Sala,M. et al., J Neuroendoclinol (2012) No COI.

1P-121

Development of human iPSCs-derived neurons

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Differentiated neurons induced from human induced pluripotent stem cells (hiPSCs) are expected to be a tool for developing a new method of treatment for various neurological diseases. However, the detail developmental properties of hiPSCs-derived neurons have not yet been known. In this study, we analyzed neuronal development of hiPSC-derived neurons (iCell neurons, Cellular Dynamics International) focusing on their early developmental stages compared with rat cultured neurons. In 2 days in vitro (DIV) culture, we observed three different stages of rat neurons, which were stages 1, 2 and 3 in developmental classification proposed by Dotti. In contrast, we observed only hiPSCs-derived neurons of stage 1 and 2. This suggests that difference of the maturation speed between human and rat neurons has already appeared at stage 3. Moreover, to test whether there are differences in effect on pharmacological agents for F-actin and transcription, we treated hiPSCs-derived neurons and rat neurons with Cytocharasin D (CytoD) and HDAC-inhibitor valproic acid (VPA). The CytoD treatment caused translocations of drebrin and F-actin from the transitional zone to the distal edge of growth cone in both iCell and rat neurons. The VPA promoted neurite outgrowth in iCell neurons longer than in rat neurons. These data suggest that the development of iCell neurons shows differences at developmental speed and reagent reactivity. No COI.

Poster Presentations
Sensory Function (1)

1P-122

Effects of anion permeability of GABAA and GABAC receptors on surround response polarity in bipolar cells of the mouse retina

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In the mouse retina, surround responses in ON- and OFF- bipolar cells are regulated by GABA-mediated chloride (Cl^-) current in dendrities. An opposite polarity of surround responses between ON- and OFF-bipolar cells is explained by the hypothesis that reversal potential of Cl^- (E_{Cl}) in ON-bipolar cells is higher than that of OFF-bipolar cells. The immunohistochemichal findings, electrophysiological data and imaging of the intracellular Cl^- concentration support the hypothesis. However, the reversal of response polarity between ON- and OFF-bipolar cells is not completely elucidated by these findings. In the present study, we examined whether the presence of bicarbonate ion (HCO_3^-) shifts E_{Cl} in $GABA_A$ and GABA $_C$receptors by means of patch clamp techniques, since distribution of $GABA_A$ and $GABA_C$ receptors can contribute to the formation of surround responses if they have different E_{Cl}. We found that the reversal potential of $GABA_A$ and $GABA_C$ receptors without extracellular HCO_3^- were -5±3.5 mV (n=6) and -3.8±4.8 mV (n=5), respectively. The reversal potential of $GABA_A$ and $GABA_C$ receptors with extracellular HCO_3^- (24 mM) were -4.5±5.7 mV (n=6) and -2.6±3.4 mV (n=5), respectively. These results rule out the possibility that the distribution of GABA receptors subtypes with different HCO_3^- permeability in bipolar cells contributes to the formation of an opposite polarity of surround responses between ON- and OFF-bipolar cells. No COI.

1P-123

Mechanism of receptive field generation in early visual pathway: simultaneous recording of retinal ganglion cells and lateral geniculate cells

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Retino-geniculate transmission has been thought to be relatively simple because both retinal ganglion cells (RGCs) and relay cells of the lateral geniculate nucleus (LGN) have circular receptive fields (RFs). However, we have recently reported that RF structure of cat LGN neurons is rather elliptical than circular, giving them moderate orientation sensitivity (Suematsu et al., 2012; Naito et al., 2013). To clarify the connection rule for generating LGN RFs, we simultaneously recorded pairs of single-unit activities of RGCs and LGN neurons, which showed functional monosynaptic connections with cross-correlation analysis.

We found that 1) spatial RF structure of both RGCs and LGN neurons were comparatively elliptical, 2) spatial RF structures of the pairs with same-response-sign were often overlapped and similarly-oriented, 3) most of the pairs with opposite-response-sign exhibited RF structures which were spatially displaced and independently-oriented, and 4) the temporal RF structures of the RGCs were tightly correlated with those of their target LGN neurons.

These results indicate that RF structure of LGN neuron is mainly inherited from that of a primary-projecting RGC, and that stimulus feature selectivity of LGN neurons is sharpened by more convergent inputs from multiple RGCs than previously conceived. No COI.

1P-124

Trigeminal interpolaris/ caudalis transition neurons mediate ocular blood flow responses evoked by bright light in the rat

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Abnormal sensitivity to bright light can cause discomfort or pain and evoked parasympathetic reflexes such as pupillary constriction, lacrimation and vsodilation in the choroid. The mechanisms underlying abnormal sensitivity to light remain elusive; however, it has long been proposed that trigeminal sensory nerves play a significant role. Trigeminal sensory nerves that supply the eye project to two spatially distinct regions of the lower trigeminal brainstem nuclear complex, subnucleus interpolaris/caudalis (Vi/Vc) transition as well as the caudalis/upper cervical junction (Vc/C1) regions. Recently we reported that light-evoked Vi/Vc and Vc/C1 neural activity involved increased parasympathetic outflow consistent with a vascular-linked mechanism with in the eye. However, since little is known about underlying neural circuitry for reflex choroidal blood flow (CBF) responses to bright light stimuli, CBF was measured in a separate group of male rats after synaptic blockade of Vi/Vc or Vc/C1 regions. Bright light (20k lux)-increased CBF was markedly inhibited (>80%) by 10min after CoCl2 injection into the Vi/Vc region, wheres blockade at the Vc/C1 had no effect (<10%).These results support the hypothesis that light responsive neurons at the Vi/Vc transition region are critical for ocular reflex function such as change of intra ocular blood flow. No COI.

1P-125

Orexin-A inhibits ocular-responsive trigeminal subnucleus caudalis neurons in the rat

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Orexin-A (OxA) is synthesized exclusively in the hypothalamus and is associated with homeostatic regulation and pain modulation. Previously we reported that increased posterior hypothalamus outflow reduced light-evoked trigeminal subnucleus caudalis/upper cervical (Vc/C1) neural activity and reflex lacrimation. To determine if OxA contributed to posterior hypothalamus modulation of ocular nociception, OxA was applied to the dorsal brainstem surface while recording from ocular-responsive neurons in superficial laminae at the Vc/C1 region. Vc/C1 ocular units were activated by exposure to bright light or ocular surface application of hypertonic saline. OxA markedly reduced both light- and NaCl-evoked neural activity. Co-application of the orexin-1 receptor antagonist, SB334867, reversed OxA-mediated inhibition of ocular-evoked activity. OxA also reduced the high threshold convergent cutaneous receptive field area of ocular units. OxA did not alter the spontaneous activity of Vc/C1 neurons or resting mean arterial pressure. Local application of SB334867 alone had no effect. Co-application of OxA and the orexin-2 receptor antagonist did not prevent OxA-mediated inhibition. These results suggested that OxA acted through orexin-1 receptors to modulate somatosensory input from the ocular surface and deep tissues of the eye to neurons in superficial laminae of the medullary dorsal horn. Acknowledgements: Univ. of Minnesota, Prof. D.A. Bereiter. This work was supported by a Grant from NIH (EY021447). No COI.

1P-126

The effect of Menthol, Capsaicin and AITC on the thermal response of corneal primary afferent neurons

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Previous studies have found that cold cells innervating the cornea are sensitive to the ocular fluid status of the corneal surface and may be responsible for the regulation of basal tear production. In the present study, we examined the effect of the TRPM8 agonist menthol, the TRPV1 agonist capsaicin, and the TRPA1 agonist allyl isothiocyanate (AITC) on cold-evoked responses in these corneal primary afferent neurons. Extracellular, single-unit recordings were performed in urethane-chloralose anesthetized rats. Electrodes positioned in the trigeminal ganglion were used to isolate and characterize cold-sensitive corneal neurons. At low concentrations, Menthol increased the spontaneous and enhanced the cold-evoked response. On the contrary, a high concentration of menthol suppressed the cold cell activity. Capsaicin and AITC increased the spontaneous activity and suppressed the cold-evoked response. These results indicated that most of the cold cell has not only TRPM8 channel but also TRPV1 and TRPA1channel. Menthol, though actions at TRPM8, sensitizes own TRPM8 channels. Capsaicin, though actions at TRPV1, and AITC, though actions at TRPA1 may suppress TRPM8 channel directly or by other intracellular mechanisms. Understanding of the mechanisms of these desensitization of TRPM8 channel might be useful for knowing Dry eye syndrome. No COI.

1P-127

Tyrosine hydroxylase immunoreactive amacrine cells in the gerbil retina

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We have found two kinds of TH-IR amacrine cells (type A and type B). The two types were clearly different in the shape of somata and stratification of TH-IR dendrites in the inner plexiform layer (IPL) at P7. Type-A TH-IR cells had monostratified dendrites extending in the outermost layer of the IPL, while type-B cells had dendrites extending in the middle of IPL. At P7, type-A somata were observed in the inner part of the inner nuclear layer (INL), its dendrites extended into the outer part of the IPL. Type-B somata were located in the inner part of the newly formed INL. Its dendrite was observed to spread in the middle of the IPL. At P28, type-A somata became spherical and significantly larger, and more thickly stained, located in the vicinity of outermost layer of the IPL. The dendrites of type B extended in the middle of the IPL, where the point-like layer was observed. In adult type A, densely stained, round-shaped and large somata were located in the innermost part of the INL, and their dendrites with varicosities extended into the outermost part of the IPL. Adult type-B somata, on the other hand, were stained weakly, with their thin dendrites penetrating the outer part of the IPL. These results suggest that two kinds of dopaminergic amacrine cells have different developmental properties in the developing gerbil retina. Eye opening is an important period for the maturation of dopaminergic amacrine cells and for the maturation of the IPL. No COI.

1P-128

Whisker-guided visual map shifts and formation of ocular dominance column-like structures in mice

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Mice navigate nearby space using their vision and whiskers. Therefore, young mice must learn to integrate these heterogeneous inputs, although the mechanisms are unknown. We have previously reported that cortical depression was induced by spatial misalignment between visual and whisker inputs in the primary visual cortex (V1) of young mice that had worn a monocular prism goggle. Cortical depression in V1 alone do not eliminate the visuotactile spatial misalignment. However, partial depression of the visual responses with spatial eccentricity and the resulting map shifts may alleviate the spatial misalignment. To test this possibility, we investigated the location of visual responses elicited by LED stimuli placed between 0° and 100° at 20° intervals in control mice and mice that had worn the prism goggle. We found that uniform medial shifts of cortical responses in V1 of mice that had worn the prism goggle. So far, ocular dominance column has not been found in V1 of mice. However, the uniform medial shifts of visual responses suggests a possibility that ocular dominance column-like structures could be formed in the binocular region of V1 after prism wearing. As expected, the visual responses elicited by LED stimuli placed at 0° via each eye were clearly separated. These results indicate that visuotactile misalignment between whisker and visual inputs could induce visual map shifts and formation of ocular-dominance-like structures in mice. No COI.

1P-129

Pupillary reaction and eye movement during looking Benham's illusion figures with rotation or movement

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Recent years, study of illusion has been mainly carried out again in the field of psychology. In some of optical illusion, there is figure is called Benham's disk. In spite of drawn by black and white in the figure, we feel the color at 3–6 round/s of rotation speed. However, there is little research on pupil reaction and eye movement about Benham's disk. Therefore, we examined about these when we are looking at rotating figure. In addition to rotating of Benham's disk, we used a rectangular figure drawn same Benham's disk pattern but moves horizontally. The head was fixed by the fixation device of forehead and chin. Eye was taken with an infrared CCD camera, the eye movement of the center of gravity and pupil area were measured and analyzed. As a result, the area of the pupil changed whether color is visible or not. Pupil area was larger in the rotation speed of colors can be seen compared to fast rotation speed at 8 round/s. These occurred regardless of the direction of rotation and movement speed in Benham's figures. Further, it was found that in the movement of rectangular figure at 1 round/s the repeated smooth pursuit movement and saccade were observed. These decreased in the movement begin to see the color. It is considered that these are caused because color figure is recognized as a still image. Therefore, it is concluded that color of Benham's illusion figure is recognized as a still image and in the case of rotation and movement speed at not too fast. No COI.

Poster Presentations
Behavior Science, Biorhythm

1P-130

Cooperative coding of unstationary images by multiple subtypes of retinal ganglion cells

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There are many subtypes of ganglion cells in the retina, and it is assumed that each subtype may detect a specific visual feature of the retinal image. However, it is not clear whether each subtype processes visual information independently or cooperatively. Applying the multi-electrode array to the isolated goldfish retina, we recorded spike discharges from multiple ganglion cells. Dynamic light stimuli were projected onto the retina to mimic the retinal image motion. The stimulus was composed of a bright square target and a surrounding large background with or without random dots. The target and the background was jittered or rapidly moved separately or together. Based on the receptive field profile estimated by the spike-triggered average, we classified ganglion cells into six subtypes (Fast/Slow, transient/medium/sustained). We found that the Fast-transient cells fired synchronously and anticipatory when the target rapidly moved together with the background with random dots. Under this stimulus condition, cross-correlation analysis of spike trains revealed that functional connectivity was established among the Fast-transient cells and other specific subtypes. The functional connectivity was not fixed but changed depending on the light stimulus pattern. These results suggest that multiple subtypes of ganglion cells may send visual information cooperatively to the brain, and that the Fast-transient cells may play a key role among the subtypes. No COI.

1P-131

Age-dependent relationships between heart rate variability and body acceleration in free-moving human

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The aim of this study is to clarify effects of aging on relationships between heart rate variability (HRV) and body acceleration of the free-moving human. R-R intervals and body acceleration were recorded every one minutes from 65 adults, including 18 young (20 to 39 years), 26 middle (40 to 59 years), and 21 elderly subjects (60 years or more) for 24 hours during their free-moving days. For HRV analysis, high-frequency (HF; 0.15–0.4 Hz) and low-frequency (LF; 0.04–0.15 Hz) components of HRV were calculated by using MemCalc, a time series analysis technique that combines a non-linear least square method with maximum entropy method. The lag between time-series of HRV and physical acceleration was determined by the cross-correlation analysis. For three to four hours before night sleep and after wake-up, the percent ratios of the subjects with zero lag between physical acceleration and HRV (both LF/HF and HF/TF) were significantly decreased in an age-dependent manner. After wake-up, the ratios of the subjects with zero lag tended to be lowered in all generations. These results suggested that the coordination between physical activity and autonomic nervous system was diminished with the aging. No COI.

1P-132

Circadian desynchronization disrupts heart mitochondrial metabolism in mice

Hashimoto, Izumi; Kohsaka, Akira; Das, Partha; Nakao, Tomomi; Gouraud, Sabine S; Waki, Hidefumi; Maeda, Masanobu(*Dept Physiol, Wakayama Med Univ, Wakayama, Japan*)

Continuous shifting of the sleep-wake cycle, often observed in shift work, is associated with increased cardiovascular morbidity and mortality. However, molecular mechanisms underlying the detrimental effects of shifting in the daily physiological rhythm on cardiac function remain unknown. To elucidate a molecular link between the circadian clock and cardiac function, we used mice which developed cardiac hypertrophy by phenylephrine infusion, and maintained these animals on a 12-h phase shift in the light-dark (LD) cycle for 18 days. We found that chronic exposure to the LD shift protocol decreased cardiac function in mice. In addition, the reduced cardiac function led by LD shifting was accompanied by alteration in cardiac mitochondrial metabolism. Diurnal rhythms of expression of genes encoding key components of mitochondrial oxidative metabolism were abolished in mice maintained on the LD shift protocol. More importantly, disrupting physiological rhythmicity induced by LD shifting resulted in a significant decrease in enzymatic activity of mitochondrial complex I in heart. Together, our results suggest that chronic desynchronization of the daily physiological rhythm with external LD cycles may have adverse effects on heart function through alteration in mitochondrial energy metabolism. No COI.

1P-133

Changes in the state of wakefulness in the locus coeruleus noradrenergic neurons-ablated mice

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The noradrenergic (NA) neurons in the locus coeruleus (LC) are crucial components in the sleep-waking mechanisms. To explore the roles of these neurons, we specifically ablated them with immunotoxin(IT)-mediated cell targeting in mice, and assessed sleep-waking behaviors and arousal responses induced by auditory stimuli. The cortical electroencephalogram (EEG) and the neck electromyogram (EMG) were continuously recorded from one week before to two weeks after the IT injection in the freely-moving condition. During both the light periods and dark periods, there were no differences in the total amounts of wakefulness (W), light slow-wave sleep (SWS), deep SWS and paradoxical sleep between the NA-LC ablated (Ab) mice and normal mice. However, from 5 to 8 days after the injection, the duration of each bout of W decreased and the number (frequency) of W increased in the Ab mice. In response to the sound stimuli (45 or 55 dB, 200ms) applied during sleep, the occurrence probability of EEG desynchronization, the sign of W, was unchanged, while that of transient EMG activity with short latency was increased in the Ab mice. These results suggest that the NA-LC neurons have a role in maintaining or stabilizing the state of W and exert inhibitory influences to the auditory reflex pathway in the brainstem. No COI.

1P-134

Optical imaging of spatially isolated solitary neuron of suprachiasmatic nucleus using micloisland culture method

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A master circadian clock in mammals is located in the hypothalamic suprachiasmatic nucleus (SCN) which is composed of multiple, single-neuron circadian oscillator cells. Recent studies have suggested that these neurons are heterogeneous in not only cytochemical but also their oscillatory properties. In this study, we examined circadian properties of the solitary SCN neuron on small spots using dissociated culture of SCN neurons. Culture dish were prepared by opening a hole in the bottom of a 35 mm petri dish and attaching ITO (Indium Tin Oxide) coated glass slide. A collagen layer was formed on the ITO surface, and above it, agarose thin layer was made. Collagen islands were formed by an infrared laser beam which melted agarose thin layer. This method enables us to make small spots of any size at any density. SCN neurons were derived from transgenic mice carrying a bioluminescent reporter for *Per1* or PER2. Bioluminescence from each single cell was measured by an EMCCD camera every hour for at least 5 days. We succeeded to demonstrate that spatially dissociated solitary SCN neurons exhibit circadian rhythms of *Per1* expression or PER2. We also detected circadian oscillation of clock gene/protein of glial cells on islands with/without SCN neurons. These results suggest that each of single SCN neurons exhibits cell-autonomous circadian oscillation. Further, the rhythmic activity of glial cells may affect circadian expression of clock gene/protein in each SCN neurons, or vice versa. No COI.

1P-135

High plating density is necessary for robust AVP releasing rhythm of SCN cells in culture

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In mammals, circadian rhythms are driven by a pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Recordings of neuronal firing of dissociated SCN cells suggest that single SCN cells are competent circadian oscillators. The SCN cells also show clear circadian rhythm of arginine vasopressin (AVP) release in cell culture when they are plated at high density. We examined the effect of plating density on AVP releasing rhythm in culture. The same numbers of the cells were plated on different size of area in a well of cloning plates. When cells were plated in large area, AVP-releasing rhythm was attenuated. Both the amount of AVP release and amplitude of the rhythm depended on the plating density. Co-culture with cortex cells could not restore the loss of rhythmicity in low-density culture. These results suggest that cell-cell contact of SCN cells is necessary for robust AVP releasing rhythm. No COI.

1P-136

Involvement of gap junction in kainic acid-induced neuronal oscillations in anterior cingulated cortex

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Neuronal oscillation is a prominent form of rhythmic activity occurring in the brain. Fast neuronal oscillations (30–100Hz) are frequently observed in the thalamo-cortical structure during wakefulness and attentive behavior. Abnormalities in these oscillations in the anterior cingulated cortex (ACC), a medial part of the prefrontal cortex, might underlie neuropsychiatric illnesses such as schizophrenia. In the current study, to investigate the role of gap junctions in the neuronal oscillation in the ACC, we developed an ***in vitro*** model of neuronal oscillation in a slice preparation including ACC from mice, and examined if the gap-junctional communication is involved in the generation mechanisms of the oscillation. We evoked oscillation by perfusing 50μM kainic acid (KA) for 10 seconds. Oscillation activity was evaluated by power-spectral density analysis. Gap junction antagonist (150μM 18β-glycyrrhetinic acid, 100μM carbenoxolone, 100μM octanol) was administered by perfusion for 30 min before KA activation. These gap junction antagonists markedly decreased oscillation power evoked by kainic acid. These results suggest that gap junctions contributes to generation of the neuronal oscillation in the ACC. No COI.

1P-137

Neuronal activity of the insular cortex modulates approach behavior to food in mice

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The insular cortex is known as sensory cortex that integrates multiple modalities such as visceral, gustatory and olfactory inputs and plays an important role for representing bodily state. It has been reported that neurons in the insular cortex respond to reward-predictive cues, however it remains unknown how the anticipatory activity shapes the animals reward-motivated behaviors. Here, we investigate the involvement of insula in the reward approach behavior in mice. Using a classic Pavlovian paradigm, adult mice were trained to form an association between the sensory cues and subsequent delivery of a highly palatable food pellet. Local pharmacological inactivation of the insula significantly reduced reward-approach behavior, suggesting a modulatory function of the insula. Single unit recording from behaving mice showed significant changes of firing activity during cue-period in about 30% of recorded neurons in same area of insula. To determine if insular activity is specifically important during the cue-presentation period predicting the subsequent reward delivery, we employed optogenetic techniques. Using halorhodopsin, we inhibited insular activity selectively during the cue period. Insula inhibition significantly reduced approach behavior during the cue period. These data suggest that activity of the insular cortex during the reward-predictive cue contributes and modulates the expression of reward-approach behavior. No COI.

1P-138

Conditional knockout of the orexin 2 receptor gene specifically in GABAergic neuron reduce wakefulness

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Orexin neurons project almost all brain regions with especially dense projections being seen in monoaminergic and cholinergic nuclei involved in the regulation of sleep/wakefulness. There are orexin 1 (OX1R) and 2 receptor (OX2R) expressed in these neuron, and also GABAergic neurons are likely to function as local inhibitory interneurons and distributed in monoaminergic neuron and excited by OX1R and OX2R. However precise role of orexin receptor mediated GABAergic neuron activity in sleep/wake regulation is unclear. To address this, we generated conditional OX2R knockout mice especially in GABAergic neuron, and analyze sleep/wakefulness states of these mice monitored by simultaneous EEG/EMG recordings. We found that these mice showed significant decrease of wakefulness time, accompanied by increased NREM sleep. Also, these mice exhibited significant decrease in episode duration of wakefulness in dark period. These results suggest that GABAergic neuron mediated by OX2R play an important role in maintaining wakefulness in dark period. No COI.

1P-139

Comparison of firing properties of sleep-related neurons in the mesopontine tegmentum and amygdala

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The masopontine tegmental area has a crucial role in regulation of REM sleep. A large population of neurons in this area including laterodorsal tegmental nucleus (LDT) discharge specifically during REM sleep (PS active neurons) or discharge highly both during REM sleep and waking. On the other hand, the amygdala, a center of emotion during waking, also contains considerable number of PS active neurons. Although these two areas contain similar type of neurons (PS active neurons), functional significance of the PS active neurons in these two areas in regulation of REM sleep has never been compared. In the present study, single neuronal activity under sleep waking cycles was recorded from the mesopontine tegmental area and the amygdala in head-restrained, non-anesthetized rats and firing properties of the neurons in these two areas were compared. In the mesopontine tegmental area, some of the PS active neurons started to increase firing before the onset of REM sleep and continued to fire in tonic fashion during REM sleep, while the PS active neurons in the amygdala showed phasic discharge intermittently during REM sleep, and increase in firing started after the onset of REM sleep. Some neurons in the mesopontine tegmental area have a correlation with eye movement during REM sleep, while none of the amygdala neurons have such correlation. Both in the LDT and amygdala some neurons have a close correlation with blood pressure fluctuation during REM sleep. Roles of these two areas in relation of REM sleep would be discussed. No COI.

1P-140

Influences of stress on sleep profiles and blood pressure fluctuation during REM sleep

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The stress during waking has some influences on sleep. During REM sleep, fluctuation of autonomic nervous system including that of blood pressure, heart rate or respiration occurs and it is highly probable that such events have some relations with the stress during waking. In the present experiment, to elucidate the influence of stress on sleep profiles we examined the effect of stress on sleep-wake cycles and on blood pressure fluctuation during REM sleep. Male rats in a shock chamber were given, every one minute from 16:00 to 17:30, 10 seconds tone of 10 KHz (15 times), which was associated with electric shock (0.7 mA, 1 sec), and of 500 Hz (75 times) without shock. After the shock, rats were moved to the recording chamber to record sleep-wake profiles and blood pressure from 20:00 to 8:00 (dark period), then from 8:00 to 16:00 (light period). During dark period, electric shock induced 72% increase of the amount of REM sleep ($p<0.01$), 20% increase of light slow wave sleep ($p<0.05$), and 7% decrease of wakefulness ($p<0.01$) comparing with control animals, which were given no shock in the shock chamber. During light period, amount of REM sleep increased to 17% ($p<0.05$). After the electric shock, phasic decrease of blood pressure occurred more frequently comparing with control animals ($p<0.01$), while increase of blood pressure had no difference comparing with control animals. These results suggest that the electric shock stress associated with sound has a facilitatory role on REM sleep and has some influences on blood pressure fluctuation during REM sleep. No COI.

1P-141

Relationship between sleep quality and menstrual cycles in healthy women

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The present study aimed to clarify the relationship between menstrual cycle and sleep quality in healthy women. Sleep quality during the menstrual cycles of six women (age range, 19–28 years) was measured for about one month. Basal body temperature was measured while still in bed in the morning, and sleep was assessed using a mat-type device. Basal body temperatures during the ovarian follicular, the corpus luteum, and the menses phases of the menstrual cycle were 36.44 ± 0.15°C, 36.73 ± 0.25°C, and 36.53 ± 0.19°C, respectively ($p= 0.00$).The total amount of sleep per night was 300.48 ± 109.17, 302.83 ± 74.70, and 276.23 ± 77.023 min, during the three phases, respectively, and the amount of time spent in REM sleep was 62.62 ± 33.20, 59.30 ± 14.27, and 43.63 ± 20.76 min, respectively ($p = 0.05$). The amount of time spent being awake and in both light and deep sleep did not significantly differ among the three phases. However, the amount of time spent in REM sleep significantly differed ($p = 0.04$), indicating that quality of sleep is affected by the menstrual cycle in healthy women. No COI.

1P-142

The role of ghrelin in entrainment of circadian rhythms to scheduled daily feeding in CS mice

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The circadian clock in CS mice entrains to restricted feeding schedule (RF). Behavioral rhythm and clock gene rhythms in the suprachiasmatic nucleus (SCN, a central oscillator in the circadian clock system) of CS mice show clear entrainment to RF (food entrainment). To investigate the mechanisms of food entrainment in CS mice, present study was designed to clarify the role of ghrelin, which is a peptide closely related to feeding behavior, in the food entrainment in CS mice. Plasma ghrelin concentration was measured at 6 time points while behavioral rhythm was entrained to RF. Plasma ghrelin concentration levels under RF in CS mice were significantly increased prior to feeding time, while there were no rhythmic changes in ad lib feeding mice. The changes in freerunning behavioral rhythm under continuous darkness (DD) were measured when ghrelin was periodically administered. Periodic ghrelin administration induced no changes in freerunning behavioral rhythms. These results indicate that ghrelin is not a major cue for food entrainment of circadian clock in the CS mice. The present results suggest that ghrelin might have a role in the food-entrainable oscillator which regulates food anticipatory activity under RF. No COI.

1P-143

Effect of feeding patern on sleep depth

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We investigated whether different feeding rhythm affects sleep/wake regulation. Three groups of C57BL/6J mice were given lab chows freely during dark period (ZT12-24, Control group), first half of dark period (ZT12-18; Morning group), or last half of dark period (ZT18-24, Evening group) for 2 weeks respectively. Off-line sleep scoring was done on the computer screen by visual assessment of the electroenchphalogram (EEG) and EMG activities, thereby distinguishing phases of wake, rapid eye movement (REM) and non-REM (NREM) sleep. The EEG delta and theta frequency band was set at 0.5–4.0Hz and 4.0–7.8Hz, respectively. In this study, power density of the EEG delta (ratio of delta to theta) during NREM sleep was used as a parameter of sleep pressure (slow-wave activity, SWA). Mice in Evening group showed lower SWA than that of other 2 groups. On the other hand, an amount of NREM, REM and wake in 3 groups did not change. We observed higher monoamine concentrations, which activates wake system, in cerebral cortex in Evening group. We also found increased mRNA expression of orexin in hypothalamus in Evening group. These results indicate that feeding only in the last half of dark period alters sleep homeostasis. This effect may be partly involved in increase of orexin expression in hypothalamus and elevated monoamine concentration in cerebral cortex. No COI.

1P-144

The effect of feeding condition and cold ambient temperature to synchronization on the rhythms of body temperature and sleep-wake pattern

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Aim Homeothermic animals have synchronized circadian rhythms of body temperature and sleep-wake pattern. Fasting and extreme ambient condition modulate body temperature and sleep-wake pattern; however, we do not know how the relationship of the two rhythms is affected in such conditions. Therefore, the present study aimed to clarify the relationship during fasting and cold in mice.
Methods Male ICR mice (age, 2–4 m) housed at 27°C with a lighting cycle of 12:12 h (lights-on, 7 am) were used. Using a radio transmitter device, body temperature (T_b), EEG , EMG and spontaneous activity was obtained. Mice were exposed to 20°C or 27°C for 30-h with or without food deprivation.
Results T_b decreased at fasting at 27°C by 1.0± 0.2°C and 0.9 ± 0.2°C in the light and dark phases compared with feeding at 27°C, respectively. At 20°C exposure, the reductions were disappeared (0.1 ± 0.1°C and 0.1 ± 0.1°C); however augmented at fasting condition (1.5 ± 0.2°C and 2.2 ± 0.2°C). Fasting condition at 27°C, total sleep time did not change; however, both feeding and fasting at 20°C cold condition, total sleep time decreased in both phases. In all conditions, ultradian rhythms of the body temperature in the light phase were well correlated to wake-sleep pattern.
Conclusion Cold and fasting modulates both rhythms of sleep and T_b, but did not affect synchronized ultradian rhythms of T_b and wake-sleep. No COI.

1P-145

5HT1A receptors in orexin neurons play an important role in regulation of sleep/wake behavior

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Orexin A and orexin B are lateral hypothalamic neuropeptides. A series of studies have suggested that orexin-deficiency causes narcolepsy in humans and other mammalian species, highlighting roles of this hypothalamic neuropeptide in the regulation of sleep and wakefulness. Orexins were shown to have a strong excitatory influence on serotonergic neurons in the raphe nuclei through both orexin 1 and orexin 2 receptors. Conversely, orexin neurons receive abundant input from the serotonergic neurons in the raphe nuclei. We also found serotonin potently inhibited orexin neurons through 5HT1A receptors, implying the negative feedback regulation. This linkage might play an important role in the regulation of sleep/wakefulness. To evaluate this hypothesis, we generated mice in which orexin neurons specifically lack expression of 5HT1A receptors utilizing Cre-loxP mediated deletion of 5HT1A gene. Histological studies showed specific disruption of 5HT1A receptor expression in orexin neurons. We examined sleep/wake characteristics of these mice, and found that these mice exhibited several abnormality in sleep/wake architecture, including severe fragmentation of sleep states. This observation suggests that serotonergic inhibitory regulation of orexin neurons play an important role in normal maintenance of sleep/wake behavior. No COI.

1P-146

Serotonin is possibly involved in the anorexigenic and anti-depressant effects of estrogen in rats

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Estrogen attenuates food intake and enhances c-Fos expression in the suprachiasmatic nucleus (SCN) specifically during the light phase in ovariectomized rats. In addition to the anorexigenic effect, estrogen reportedly has antidepressant effects. Serotonin is one of the agents regulating circadian rhythm responding to light and controlling food intake, and serotonin deficiency is a pathogenesis of depression. To test the hypothesis that serotonin mediates anorexigenic and antidepressant effects of estrogen, we examined the effect of selective serotonin reuptake inhibitor, fluoxetine (FLX) administration on food intake, depression-like behavior and c-Fos expression in the SCN. Rats were ovariectomized and implanted either with estradiol (E2) or cholesterol (Veh) containing silicon tubing and after surgical recovery, were administrated with FLX or saline at the beginning of the light phase and the dark phase for 10 days. FLX attenuated food intake specifically during the light phase in the both groups, and reduced depression-like behaviors in forced swim test in the Veh-group (on 7th day of administration of FLX), and increased c-Fos like immunoreactive cells in the SCN specifically during the light phase only in the Veh-group. These suggest that serotonin is a candidate mediating the anorexigenic and antidepressant effects of estrogen in a photo stimulation dependent manner. No COI.

1P-147

Prolonged Bioluminescence Monitoring in Mouse *ex vivo* Bone Culture Revealed Persistent Circadian Rhythms in Articular and Epiphyseal Cartilages

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The bone is a metabolically active organ which undergoes repeated remodeling cycles of bone resorption and formation. In this study, we revealed a robust and extremely long-lasting circadian rhythm in ex vivo culture maintained for over nine months from the femoral bone of a $PERIOD2^{Luciferase}$ mouse. Furthermore, we also identified robust circadian clocks in flat bones. High- or low-magnification real-time bioluminescence microscopic imaging revealed that the robust circadian rhythms emanated from the articular cartilage and the epiphyseal cartilage within the growth plate of juvenile animals. Stimulation by forskolin or dexamethasone treatment caused type 0 phase resetting, indicating canonical entraining properties of the bone clock. Together, our findings from long-term *ex vivo* culture revealed that "tissue-autonomous" circadian rhythm in the articular cartilage and the growth plate of femoral bone functions for several months even in an organ culture condition, and provided a useful in vitro assay system investigating the role of the biological clock in bone formation or development. No COI.

1P-148

Stability of whole-body rhythmic movement is enhanced by self-vocalization

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It has been reported that human rhythmic movement are stabilized by being entrained to rhythmic auditory cue. In previous research, rhythmic auditory cues have often been generated by external device such as a metronome. On the other hand, we can generate rhythmic auditory cue by self-vocalization. Using self-vocalization instead of external auditory cue, in principle, a variety of rhythmic movement can be stabilized by being entrained to self-voice. Therefore, we investigated whether entrainment occurs between self-vocalization and whole-body rhythmic movement, and the stability of whole-body movement. The voice rate ranged from 80 to 180 beats per minutes (bpm) in steps of 50 bpm, and in one trial one voice rate was produced. Whole-body rhythmic movements were two kinds of knee bending movement: knee-flexion-on-the-voice and knee-extension-on-the-voice. At higher voice rate, entrainment between knee-flexion and vocalization was observed. The variability of knee movement interval under the vocalization coordination was less than under the non-vocalization. We revealed the entrainment between vocalization and whole-body movement, and the stability of whole-body movement caused by self-vocalization. These findings indicate that auditory-motor entrainment occurs regardless of the sources of auditory information (i.e., external or internal), suggesting that the neural pathway underlying the entrainment between rhythmic movement and external auditory cue can also be activated by self-vocalization. No COI.

1P-149

Acetaldehyde induces thirst sensation in hangover

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In hangover, people experience heavy thirst. The metabolite of EtOH acetaldehyde (ACD) has never been considered to be a thirst inducing factor in hangover. Recent studies show that ACD activates mast cells and elicits renin release. We hypothesized that ACD is a factor inducing thirst sensation in hangover. Male Wistar rats were used in the present study. EtOH significantly increased water intake. Coadministration of the ACD dehydrogenase inhibitor cyanamde with EtOH increased both water and salt intake further and earlier. ACD with cyanamide more rapidly elicited water and salt intake. Urination was less found in the early stage even in the administration of ACD and cyanamide. When allowed to drink water and salt solution, urine volume was increased only after drinking, suggesting that urination is not a main trigger for initiation of drinking behavior. The elicited water and salt intake were suppressed by intraperitoneal and intracerebroventricular injections of AT1 antagonist candesartan. The drinking behavior was also suppressed by the mast cell membrane inhibitors cromolyn and doxantrazole. Immunohistochemical study showed that EtOH (and ACD) increased the number of c-Fos immunopositive neurons in the brain regions of thirst center. Taken together, thirst sensation in hangover of alcohol may be induced through renin secretion from mast cells and AT1 receptor activation of neurons in the thirst center of the brain in rats. No COI.

1P-150

Orexin receptors are involved in establishing cued and contextual fear memory.

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Orexins are neuropeptides which play important roles in regulating sleep/wakefulness, energy homeostasis, feeding behavior, and reward system. To know whether orexin receptor 1 (OX1R) and 2 (OX2R) are implicated in establishing cued and contextual fear memory, we examined phenotypes of orexin receptor double knockout mice (OXRD-KO), and prepro-orexin knockout mice (pOXKO) using cued and contextual fear conditioning test. We found that OXRDKO and pOXKO mice showed much lower freezing time than that of OX1R and OX2R single knockout mice in both conditioning and test period of cued and contextual fear. These data suggest that cued and contextual fear memory require OX1R and OX2R function. To further decipher the mechanisms by which orexin receptors contribute to the fear memory consolidation, we examined freezing behavior of C57BL6J mice in cued and contextual fear conditioning with pharmacological blockade of orexin receptors. We injected suvorexant 1hour before the test period of cued and contextual fear conditioning. Acute blockade of orexin receptors using suvorexant reduced freezing behavior in test period of cued and contextual fear conditioning to levels comparable to that of OXRDKO and pOXKO. These observations suggest that orexin signaling play an important role in cued and contextual fear memory consolidation after the training period. No COI.

1P-151

Circadian rhythms and the effect of glucocorticoids on clock gene Period2 expression in mouse nasal mucosa

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The symptoms of allergic rhinitis (AR) show marked circadian changes, becoming worse in the early morning. Yet, no information is so far available as to the clock mechanisms in the nasal mucosa. Intranasal application of glucocorticoids is widely used as topical treatment of AR without knowing the effects on the nasal clock. In the present experiment, we investigated the clock functions in the mouse nasal mucosa and the effects of Dexamethasone (DEX).

We cultured nasal mucosa of adult male PER2::LUC knock-in mice and measured bioluminescence levels more than 2 weeks. In addition, 100nM DEX was applied for 2 hours on the 5th day of culture at 4 different circadian phases.

Mouse nasal mucosa exhibited a robust circadian rhythm in PER2 levels with the peak in the early subjective night. DEX phase-dependently phase shifted circadian rhythms of nasal mucosa, with the maximal phase delay at subjective dusk and the maximal advances at middle of subjective night. Our results demonstrated the autonomic circadian rhythms in the nasal mucosa for the first time. Since the phase-shifts due solely to DEX were observed at the phase when the serum glucocorticoids level is relatively high, the results suggest that endogenous glucocorticoids control the peripheral oscillator of mouse nasal mucosa. No COI.

1P-152

Effect of mental task on taste perception and preference, and its relationship with daily eating behavior in young females.

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It was reported that restrained eaters who intentionally restrict food intake to maintain or lose weight consumed more energy under stressful conditions. We tested the hypothesis that taste perception and preference were affected by both mental task and daily eating behavior. Twenty-seven females (18–22 yr, BMI 20.3 ± 0.3) participated in this study. Individual eating behavior was assessed by the Japanese version of the Dutch Eating Behavior Questionnaire (DEBQ). The subjects performed Stroop color-word test (CWT) as a mental task. Before and after the CWT, sweet taste recognition threshold was measured using different concentration of sucrose solutions (0.2–100 mM), and perceived intensity of sweet taste of and preference for 200 mM sucrose solution, perceived stress and appetite were evaluated using visual analog scale. These measurements were also performed before and after resting as control trial. The CWT increased perceived stress and appetite. Sweet taste recognition threshold was decreased and perceived intensity of sweet taste was increased after the resting, but these were not changed in the CWT trial. Sweet taste preference did not change in both trials. Individual change in the sweet taste preference from before to after the CWT was negatively correlated with the score of the restraint eating scale of the DEBQ. These results suggested that sweet taste perception and preference could be modulated by both mental task and daily eating behavior. No COI.

1P-153

Genetic differences in glutamate appetite in mice depend on vagus-mediated postingestive effects of glutamate

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L-glutamate (Glu) is widely used as a flavor supplement (as monosodium Glu, MSG). Glu elicits umami taste sensation, and ingested Glu evokes multiple physiological responses. Therefore, ingestive behavior towards Glu can be influenced by both its sensory and postingestive effects. The purpose of this study was to understand the mechanism of Glu appetite. In our survey of 28 inbred mouse strains, we found that mice from the C57BL/6ByJ and 129P3/J strains had large differences in voluntary MSG consumption. To develop a better model for genetic and physiological studies of Glu appetite, we had intercrossed these strains and then used selective breeding to produce mouse strains with high and low MSG intake (MSG-H and MSG-L, respectively). After 14 generations of selective breeding, MSG-H mice drink 6 times more 300 mM MSG than MSG-L mice. We used MSG-H and MSG-L mice to examine 1) neural taste responses to amino acids and other tastants, 2) behavioral responses to MSG in brief-access tests of naïve and MSG-exposed mice, 3) blood glucose after MSG gavage, 4) effect of vagotomy on MSG consumption in preference tests, and 5) flavor preferences conditioned by oral MSG. We found that strain differences in MSG intake most likely depend on postingestive effects mediated by the vagus nerve. These data support the role of the vagus afferent nerve pathway in Glu appetite. No COI.

1P-154

Calming responses during maternal carrying in human infants and mouse pups: comparative and ontogenic analyses

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Mother-infant bonding is the earliest and most critical social relationship of mammalian infants. To promote this bond, infants have innate behaviors to seek maternal proximity. However, the physiological mechanisms underlying these infant behaviors remain largely undefined. Here we show a novel set of infant cooperative responses during maternal carrying. Infants carried by a walking mother immediately stopped voluntary movement and crying and exhibited a rapid heart rate decrease, compared with those held by a sitting mother. Furthermore, we identified similar responses in mouse pups as defined by immobility and diminished ultrasonic vocalizations and heart rate. Using mouse pups, we found the upstream and downstream neural systems regulating the calming response. Somatosensory and proprioceptive input signaling are required for induction, and parasympathetic and cerebellar functions mediate cardiac and motor output, respectively. Ontogenic analyses revealed that the calming response occurred within a specific postnatal time window. In addition, pups showed an increased pain tolerance during the calming response. The loss of the calming response hindered maternal rescue of pups, suggesting a functional significance for this response. These findings collectively indicate that the infant calming response is a coordinated set of central, motor, and cardiac regulations and is a conserved component of mammalian mother-infant interactions. No COI.

1P-155

CIN85 regulates child-rearing behaviors through dopamine-prolactin signaling in the brain

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Cbl-interacting protein of 85 kDa (CIN85) is a scaffold/multi-adaptor protein implicated in the regulation of receptor endocytosis and the cellular cytoskeleton. Recently, we reported that mice deficient of CIN85 expression show hyperactive phenotypes. As a molecular explanation of this phenotype, the absence of striatal CIN85 causes decreased dopamine receptor endocytosis in striatal neurons in response to dopamine stimulation.We show here another phenotype besides the hyperactivity of CIN85 knockout (KO) mice that of maternal neglect to her newborns. Even though there is no difference in the number of live births from CIN85 KO homozygote, heterozygote and wild-type mothers, respectively, almost all pups born to CIN85 KO homozygote mothers have died within two days of birth. This could be explained by the fact that CIN85 KO mothers showed significantly decreased arched-back nursing, a kind of maternal behavior, and newborn pups from CIN85 KO mothers were often found scattered within the bedding. Importantly, when measuring the plasma levels of prolactin (PRL), we detected significantly decreased PRL levels in CIN85 KO mice compared to heterozygote and wild-type mice. PRL injection in CIN85 KO mice could however partially rescue the defect in maternal behavior of the next generation. Our findings indicate an important role of CIN85 in the regulation of the dopamine-PRL system in the brain and provide new insight into a molecular explanation for maternal behavior. No COI.

1P-156

Pathophysiological basis of maternal neglect in Cacna1a mutant rats

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Cacna1a gene encodes the P/Q-type voltage-gated calcium channel pore-forming α1 subunit. Groggy rat (GRY/Idr) with M251K missense mutation in *Cacna1a* gene has absence epilepsy and cerebellar ataxia. We noticed that groggy mother rats often failed to raise up pups. However, problems of maternal behaviors in *Cacna1a* mutant rat were largely unknown. We aim to clarify the pathophysiological basis of maternal neglect and attachment disorder in groggy rats. We compared maternal behaviors and the attachment behaviors of the pups with wild type, heterozygous, and homozygous rats. Assessment of the maternal behaviors includes nest building, retrieving the pups to the nest and time in licking the pups clean. To evaluate the attachment behaviors of the pups, ultrasonic vocalizations at 50 kHz in infant rats at 9 days was measured by UltraVox (Noldus). Ultrasonic vocalizations are recorded when an infant rat is isolated from mother and the second isolation. Female homozygous GRY/Idr rats showed failure of the nest building, decreased number of the survival of pups, prolonged retrieval latency and short licking duration. Infant heterozygous GRY/Idr rats exhibited significantly lower frequency of the vocalization in the first and the second isolation from mother as compared to wild type. Our results suggested that *Cacna1a* mutation may related to insufficient maternal behaviors and attachment disorder in pups. No COI.

Poster Presentations
Neurochemistry

1P-157

Neurodevelopmental disorder like behaviors in *Cacna1a* mutant rats

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Voltage-gated calcium channel, *CACNA1A* mutations have linked to absence seizures, cerebellar ataxia, learning disability, and hemiplegic migraine in human. Rats with M251K missense mutation in *Cacna1a* (GRY/Idr) also have absence seizures and cerebellar ataxia. In this study, we aim to elucidate that whether GRY/Idr rats have neurodevelopmental disorder like behaviors and how its pathological mechanism. To assess neurodevelopmental disorder like behaviors, we performed open field test, elevated plus maze, Barnes maze test, three chamber test and novel object test. Heterozygous rats with *Cacna1a* mutation (GRYw/+) and wild type rats (WT) were examined from 5 to 9 weeks of age. We confirmed there was no difference of locomotor activity between GRYw/+ and WT in open field test. Histological assessment was undertaken at 8 weeks of age. Striatum, nucleus accumbens, midbrain and frontal cortex regions were dissected and regional concentration of 5-HT were measured by high-performance liquid chromatography. GRYw/+ rats showed less anxious-like behavior, learning disability and cognitive inflexibility with statistical significance. These results agreed with symptom in part of neurodevelopmental disorders which was observed in patients with *CACNA1A* mutations. The concentration of 5-HT of some regions in homozygous GRY +/+ rats were significantly lower than those of WT. Neurodevelopmental disorder like behaviors in GRY rats can be caused by low level of 5-HT in the brain. No COI.

1P-158

Upregulation of leukemia inhibitory factor (LIF) during zebrafish optic nerve regeneration

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Fish retinal ganglion cells (RGCs) can regenerate their axons following optic nerve injury, whereas mammalian RGCs fail to survive. Leukemia inhibitory factor (LIF), which had been originally discovered as a differentiation factor of myeloid cells, has been now recognized as having versatile functions such as an effect on axonal regeneration. These findings encouraged us to investigate the function of LIF on zebrafish optic nerve regeneration. LIF mRNA expression was studied by RT-PCR and *in situ* hybridization, and revealed to be upregulated in 1-5 days post-injury (dpi) in the RGCs. Immunohistochemistry and western blotting against LIF also revealed the increase of LIF protein at 3 dpi in the RGCs. Next we investigated the activation of STAT3, the downstream molecule of LIF. As expected, STAT3 was activated in 3-5 dpi in the RGCs. To determine whether the activation of STAT3 was directly evoked by LIF, we employed a LIF-specific morpholino to knockdown the expression of LIF in the RGCs. As a result of LIF knockdown, STAT3 activation was cancelled, implying that LIF does activate STAT3 pathway after injury. In addition, LIF knockdown impaired neurite sprouting from retinal explants *in vitro*, decreased GAP-43 expression, narrowed the optic tract width and undermined the visual function recovery after nerve injury *in vivo*. All told, LIF is an indispensable factor for establishment of optic nerve regeneration in adult zebrafish. No COI.

1P-159

The functional study of Neuroglobin under oxidative stress in mouse retina.

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Neuroglobin (Ngb) was identified as a new member of heme proteins which is expressed at high concentrations in central nervous system neurons. Recent studies have shown that mammalian Ngb is involved in neuroprotection under various oxidative stress, such as ischemia and reperfusion insults. In RGC-5 cells, murine retinal precursor cells, overexpression of Ngb enhanced cell viability in culture compared to mock control under hypoxic stress conditions. Addition of 100–500 μM cinnamic acid, a potent inducer of Ngb, increased cell viability more than two-folds under hypoxic conditions. These data suggest that Ngb promotes neuronal protection and cell survival under oxidative stress. Following mouse optic nerve injury, Ngb clearly decreased from retinal ganglion cells (RGCs) within several days and most of RGCs underwent apoptosis. In contrast to mouse, zebrafish (zf) RGCs can survive after optic nerve injury and Ngb significantly increased in damaged zf retina. To investigate the role of Ngb, we made chimeric ZHHH Ngb protein in which module M1 of human Ngb is replaced by that of zebrafish. The addition of ZHHH Ngb protein induced neurite outgrowth in RGC-5 cell cultures. These data suggest that Ngb promotes cell survival and neurite outgrowth *in vitro*. No COI.

1P-160

Functional characterization of Ftsj1, a X-linked mental retardation-related gene

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Genetic mutations in X chromosome-linked genes have been associated with mental retardation (XLMR). Recently, linkage analyses performed in Belgian, Chinese and Japanese families have identified FTSJ1 gene as a novel candidate gene. FTSJ1 shares homology with a bacterial 23S rRNA methyltransferase Ftsj. However, the molecular function of FTSJ1 and its pathological relevance in mental retardation have remained unknown. To understand the molecular mechanism of XLMR, we have generated FTSJ1 knockout (FTSJ1 KO) mice, and investigated the neurophysiological impacts. While the FTSJ1 KO mouse developed normally, we observed a decreased protein synthesis level in MEF cells derived from KO mice. There was a marked dysregulation of synaptic proteins including glutamate receptors and signaling molecules. Furthermore, there was an increase of immature dentric spines in cortical, striatal and hippocampal neurons. There results suggest that loss of FTSJ1 might have a profound impact on the neuronal activity due to dysregulation of protein synthesis in neurons. No COI.

1P-161

Mechanical hyperalgesia induced by repeated cold stress in SHRSP5/Dmcr rats: PCR-based cDNA subtraction analysis for peripheral expression changes in pain related genes

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Repeated cold stress (RCS) is known to transiently induce autonomic imbalance associated with hypotension and hyperalgesia. In this study, we investigated the effects of RCS on the thresholds for cutaneous mechanical pain responses and on peripheral expression of "pain related genes" in SHRSP5/Dmcr (Arteriolipidosis-prone, AL) rats. AL rat is derived from stroke-prone spontaneously hypertensive rats, therefore it is considered to be sympathicotonia. RCS was given by changing the environmental temperature from 24 to 4°C at 30 min intervals during the day and 4°C at night for 2 days, and then 24°C during the day and 4°C at night for 2 days. At immediately post-RCS, the mechanical threshold was significantly decreased ($p<0.001$ vs control by Student's t-test). This hyperalgesia is thought to be caused in part by hypofunction of descending pain inhibitory systems, we confirmed increased expression of corticotropin-releasing hormone in the hypothalamus ($p<0.001$). To determine the gene expression changes accompanied by RCS in dorsal root ganglion cells (DRGs), we performed PCR-based cDNA subtraction of mRNAs from DRGs. We detected up-regulation of Tac 1 (substance P) and down-regulation of S100a10 (annexin 2 light), CatB (cathepsin B), and Fstl1 (follistatin-like 1). At least some of these genes in DRG may play key roles to mechanical hyperalgesia induced by RCS. [Research funds: KAKENHI 23590724] No COI.

1P-162

Effects of environmental enrichment on a sensory-motor cortex in mice

Inoue, Mai[1]; Odagawa, Maya[1]; Honma, Chihiro[1]; Yamada, Kazuyuki[1]; Akagi, Takumi[1]; Suzuki, Takayuki[1]; Murayama, Masanori[1,2]([1]*BSI, RIKEN, Saitama, Japan,* [2]*Tokyo Tech, Tokyo, Japan*)

Environmental enrichment (EE) is known to cause neuroplastic changes in lower-order cortices (e.g., the primary sensory area, S1) of mice. Mice raised in EE conditions (EE-mice) also show increased social ability, as observed in a social interaction test. Because sociality is largely based on higher brain functions, the increased sociality of EE-mice suggests that EE induces neuroplastic changes not only in lower- but also in higher-order cortices. Although some cellular mechanisms have been proposed for EE-induced neuroplastic changes in lower-order areas, the mechanisms underlying EE-induced changes in higher-order areas are unknown. Our aim was to examine the effects of EE on neuroplasticity in a lower- and a higher-order cortex. Here, we specifically interested in a reciprocally connected sensory-motor circuit, S1 and a secondary motor area, M2. To understand effects of EE of the S1-M2 circuit, we raised 2-week-old mice in either EE condition or normal environment (NE) for 3 weeks, and observed cortical activity evoked by hindpaw stimulation using a voltage-sensitive dye. Neuronal activity in M2 was significantly increased in EE-mice than in NE-mice, suggesting plasticity in the M2 under EE conditions. To test this hypothesis, we measured the numbers of neurons and synapses in EE- and NE-mice using immunohistochemistry and transmission electron microscopy, respectively. Here, we report the differences in various physiological and anatomical parameters between EE- and NE-mice. No COI.

1P-163

The neuroprotective effects of a novel nucleoside analogue (2Cl-C.OXT-A) on intracerebral hemorrhage

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2Cl-C.OXT-A (COA-Cl) is a novel synthesized nucleoside analogue with the molecular weight of284. It is soluble, stable and easy to synthesize. It has been reported that COA-Cl enhancesangiogenesis in human umbilical endothelial cells (HUVEC). The previous study in our lab showedthe neuroprotective effects of COA-Cl on ischemia stroke by reducing infarct volume and attenuating the behavioral deficits. The purpose of the present study is to evaluate the neuroprotective effect of COA-Cl on intracerebral hemorrhage (ICH), another common type of stroke, and investigate the potential mechanism of action. Sprague-Dawley (SD) rats were used for this study, and the ICH models were performed by the injection of autologous blood into the right basal ganglia. COA-Cl was injected intracerebroventricularly 10min after ICH with a dosage at 30ug/kg. We examined brain edema 1day after ICH by water content test, and evaluated the behavioral deficits using forelimb placing and corner turn test. COA-Cl significantly reduced the brain edema 1day after ICH and improved the behavioral deficits in the acute phase of ICH. Our reults indicated that COA-Cl may have a neuroprotective effect on ICH, and it may provide a new therapeutic approach for treating hemorrhagic stroke. No COI.

1P-164

Quantification of brain dopamine and amino acids with high resolution mass spectrometry using novel stable isotope labeled reagent.

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We invented a pyrylium-based labeling reagent (PyII) for mass spectrometry. This agent interacts with primary amines, such as catecholamine, amino acid and lysine residue in peptides. Even number of stable isotope 13C between 0 and 12 are replaced in the PyII. Thus there are seven kinds of PyII reagent of different mass, we are able to analyze seven samples simultaneously. After labeling individually, each sample is pooled and assayed by nano LCMS system. Though labeled amines derived from different samples have different mass by 2.006 dalton, these labeled amine share same chromatographic properties, allowing both amine identification and quantification to be derived from the same MS spectrum. The principal advantage of PyII over other labeling agent for mass spectrometric analysis is that seven samples can be analyzed simultaneously and that PyII and labeled amine are quite stable in water, thereby allowing for biological application.In this study, using the combination PyII with nano LCMS system, dopamine and GABA are analyzed in nano litter volume of section from rat brain. After fixing the rat brain by microwave, 30 micrometer-thick section were made by frozen microtome. Five hundred micrometer square of tissues, i.e. 7.5 nl, for dopamine analysis and 100 micrometer square, i.e. 0.3 nl, for GABA analysis were dismounted by laser microdissection. After labeled with PyII reagent, dopamine and GABA in the tissue were quantified and mapped in the brain atlas. No COI.

1P-165

Relevance of RISE (Repetitive LTP-Induced Synaptic Enhancement) to memory consolidation in vivo.

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Memory is consolidated after acquisition, by the structural changes of neurons including formation of new synapses. In the stable slice cultures of the rodent hippocampus, we previously reported that the repeated induction of LTP led to a long-lasting synaptic enhancement coupled with new synapse formation. We named this phenomenon RISE. Assuming RISE as an in vitro representation of memory consolidation in the brain in vivo, we are analyzing it to reveal cellular mechanisms underlying the consolidation. In this study we tried to obtain support for this assumption. We found that the Ca^{2+}-permeable AMPA receptors (CP-AMPARs) are transiently expressed during RISE development and the blockade of CP-AMPAR abolished RISE establishment. Making use of these facts, we administered a CP-AMPAR blocker (JSTX; Joro-Spider Toxin) to the mice during contextual fear conditioning. Intracerebroventicular injection of JSTX before or in the day of conditioning showed no effect in the mice's freezing response as assayed 1d after the injection. However, the injection 1d after conditioning significantly interfered with the freezing response as assayed 1d after injection. These results support the assumption mentioned above that RISE is relevant to the memory consolidation in vivo. No COI.

1P-166

Differential role of kinase activity of Ca^{2+}/calmodulin-dependent protein kinase IIα in hippocampus- and amygdala-dependent memory

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Ca^{2+}/calmodulin-dependent protein kinase IIα (CaMKIIα) is one of the most abundant protein kinases in the central nervous system, and is thought to be a key mediator for hippocampal synaptic plasticity. To examine whether CaMKIIα is similarly involved in amygdala-dependent memory, just as in hippocampus-dependent memory, we made use of our kinase-dead knock-in mouse of CaMKIIα (CaMKIIα-KI) in fear conditioning, in which contextual memory is both hippocampus- and amygdala-dependent, whereas cued memory is amygdala-dependent, but not hippocampus-dependent. After receiving one pairing of tone and footshock, wild-type (WT) mice showed distinct contextual (context-dependent) and cued (tone-dependent) fear memory. On the other hand, homozygous CaMKIIα-KI mice showed no contextual memory, but cued memory was formed to a certain extent. When intense training was performed, CaMKIIα-KI mice showed increased fear not only in the conditioning chamber, but also in a completely different chamber, indicating that they could not discriminate contextual difference, while WT mice could. Cued memory was evident in CaMKIIα-KI mice, although to a lesser extent than in WT mice. Combined with biochemical analyses, these results indicate that kinase activity of CaMKIIα is differentially involved in hippocampus- and amygdala-dependent memory.

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Poster Presentations
Autonomic Nervous Systems (1)

1P-167

Roles of GABAergic and glutamatergic neurons in the tachycardia caused by activating forebrain beta adrenoceptors

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We previously reported that beta adrenoceptor (B-ADR) activation in different brain regions elevates heart rate (HR), at least in part, by potentiating sympathetic activity. However, it is not clear how activation of B-ADRs may excite the cerebral mechanism involved in stimulating sympathetic neurons in the spinal cord. This study aimed to clarify roles of GABAergic and glutamatergic (Glu) neurons in the tachycardia due to stimulation of B-ADRs in the anteroventral third ventricular region (AV3V), because earlier data suggest pivotal contribution of those neurons in controlling autonomic function. Experiments were performed in conscious rats, monitoring both cardiovascular and plasma variables. The following results were obtained: 1) Stimulation of Glu receptors by AV3V applications of agonists caused the tachycardia capable of being inhibited by those of antagonists. 2) AV3V applications of a GABA agonist (Mus) did not alter HR, whereas those of its antagonist evoked tachycardia capable of being blocked by preapplications of Mus or Glu antagonists. 3) AV3V injections of a B-ADR agonist isoproterenol caused tachycardia and rises in plasma noradrenaline, without affecting blood pressure. Its tachycardic action was inhibited by AV3V injections of Mus or a Glu antagonist or systemic infusions with hexamethonium. These results suggest that activation of AV3V B-ADRs may cause tachycardia through inhibition of local GABAergic neurons and excitation of Glu neurons. No COI.

1P-168

Study of the re-activated endogenous microglia for treatment of chronic spinal cord injury.

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After spinal cord injury (SCI), inflammatory cells and microglia play major roles on maintaining local circumstances by secreting several growth factors and by removing cell debris. After 1 week, microglial activation, however, was reduced, and lost the activity for regeneration. In this study, we investigate weather p38 MAP kinase (p38) could re-activate resting microglia to remodel spinal cord structure after chronic SCI. Stimulated microglia obtained from rat perinatal spinal cord were stimulated by kinase-active (p38a) and kinase-dead (p38d) recombinant p38 protein. After 16 h, Glial cell-line derived neurotrophic factor (GDNF) and Vascular endothelial growth factor (VEGF) RNA expression wad elevated by addition of p38a, but not by p38d. Enhancement of protein expression of GDNF and VEGF and phagocytic activity of microglia are also enhanced by the treatment of p38a, but not by p38d. Furthermore, continuous p38 injection enhances CD68 and Iba1 expression in the adult rat spinal cord slice culture. These results suggest that resting microglia during chronic SCI can also be re-activated by p38a protein as an extracellular stimulator. No COI.

1P-169

Erythropoietin released from astrocyte protects the oligodendrocyte precursor cell against hypoxic and reoxygenation injury

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The hypoxia responsive cytokine erythropoietin (EPO) provides neuroprotective effects in the damaged brain. The purpose of the present study is to evaluate the EPO/EPO-receptor (EPOR) system between astrocyte and oligodendrocyte precursor cell (OPC) under hypoxia. We report elevated EPO mRNA levels and protein release in astrocytes following hypoxic stimulation by RT-PCR and ELISA. Furthermore, EPOR expressions were detected in OPCs by RT-PCR and cell staining. To evaluate the protective effect of EPO from astrocyte to OPC, EPO/EPOR signaling was blocked by EPO siRNA or EPOR siRNA gene silencing. The suppression of EPO production in astrocytes by EPO siRNA decreased the protection to OPC against hypoxic stress. OPC with EPOR siRNA had less cell survival after hypoxic/reoxygenation injury. It suggested that EPO/EPOR signaling from astrocyte to OPC could prevent OPC damage under hypoxic/reoxygenation condition. Our present finding of the interaction between astrocytes and OPCs may propose the new therapeutic approach to OPCs against cellular stress and injury. No COI.

1P-170

IGF-1 and phosphorylated S6 protein increase in upper motor neurons in zebrafish brain after spinal cord injury.

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Fish can regenerate its motor axons and regain locomotive function after spinal cord injury (SCI). Motor neurons of adult zebrafish can be classified into two: (1) upper motor neurons in the brainstem and (2) lower motor neurons in the spinal cord. Upper motor neurons are known to survive via Akt/ Bcl-2 pathway after SCI, but the mechanism of axonal elongation is not well known. In fish optic nerve regeneration, we have previously reported that insulin-like growth factor-1 (IGF-1) induced neurite outgrowth and survival through Akt/ Bcl-2 pathway. Therefore, we investigated the time course of expression of IGF-1 in the upper motor neurons following SCI. IGF-1 increased at 1–3 days post-injury (dpi). To find whether the mammalian target of rapamycin (mTOR) pathway, known as a downstream molecule of IGF-1 and a key to axonal elongation, is activated in axotomized upper motor neurons, we performed an immunohistochemistry of phospho-S6 (p-S6) protein, a resultant molecule of mTOR pathway activation.The p-S6 is upregulated after SCI. Furthermore, Temusirolimus, the mTOR inhibitor, inhibited the axonal elongation of upper motor neurons in culture. These data indicate the axonal elongation of upper motor neurons induced by IGF-1 and mTOR pathway. No COI.

1P-171

Western blot analysis of tau phosphorylation on closely-located phosphorylation sites.

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The immunoreactivity of AT8 had been known to express in the brains of the early stage of Alzheimer's disease patients. AT8 is used to visualize phosphorylated tau proteins in the neurofibrillary tangles. The epitopes of AT8 consist of multiple phosphorylation of adjacent Ser/ Thr motifs, Ser199, Ser202, and Thr205. However the modulatory mechanisms of AT8 epitopes have not been clarified. We are showing phosphorylation mechanism for the AT8 specific epitopes, especially in C-terminal domain of Ser/Thr sites in vitro kinase reaction by the multiple combinations of CDK5-35 or CDK5-p25, or, GSK3beta, sequential or simultaneous applications. Phosphorylation was analyzed by Western blot (AT8, anti-phospho Ser202 tau antibody (pS202), pT205, pS199, and pS199/202) and two dimensional phosphopeptide maps. Even CDK5 and GSK3beta share their activity on phosphorylatable sites, phosphorylation of AT8 epitopes was regulated by CDK5-p25 and the particular combinations of CDK5-p35 and GSK3beta. On the other hand, we found protective regulation of AT8 epitopes. We will also show the discrepancy of Western blot signal by anti phospho-tau antibody, which recognizes a singly phosphorylated site. No COI.

1P-172

HPC-1/STX1A and STX1B contribute to regulation of social behavior

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Two types of syntaxin1 isoforms, HPC-1/syntaxin1A (STX1A) and syntaxin1B (STX1B), are expressed in neurons and regulate vesicle exocytosis as t-SNAREs. Previously, we generated STX1A gene knockout mice and STX1B gene knockout mice. Interestingly, STX1A null mutant mice (KO) normally developed, whereas STX1B KO were born alive but dead within 14 days after birth. The studies for neuropsychological properties revealed that STX1A KO showed autistic-like behavioral abnormalities (Fujiwara et al 2010) and STX1B heterozygous mutant mice (HT) showed schizophrenia-like behavioral abnormalities (Fujiwara et al 2012).
In this study, we focused on the social behavior both in STX1A KO and STX1B HT. We observed that STX1A KO exhibited impairment of social discrimination (ISD), and STX1B HT showed social avoidance behavior (SA) which is not observed in WT mice. In STX1A KO, ISD was improved by administration of oxytocin or DA transporter inhibitor but not by desipramine. In contrast, SA in STX1B HT was improved by desipramine but not by oxytocin. These observations indicate that STX1A and STX1B differently contribute to regulation of social behavior. Additionally, in WT, we observed that chronic social defeat stress decreased both of STX1A and STX1B protein expression and caused abnormal social behavior. These results suggest that decrease of STX1A and/or STX1B gene expression in CNS may induce abnormal social behavior. No COI.

1P-173

GTP cyclohydrolase 1 and its regulatory protein (GFRP) in the rat brain stem

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Tetrahydrobiopterin (BH_4) is a cofactor required for the biosynthesis of several important neurotransmitters such as the catecholamines, serotonin and nitric oxide. The enzyme GTP cyclohydrolase 1 (GCH1) is the first and rate-limiting enzyme in the metabolic pathway of BH_4. *In vitro*, the GCH1 feedback regulatory protein (GFRP) may mediates the feedback inhibition of GCH1 activity by BH_4. Here, we investigated the physiological function of GFRP *in vivo* in the rat brain stem. The distribution of GFRP and GCH1 were examined by immunohistochemistry using home-made antisera on paraformaldehyde-fixed sections. Interaction between GFRP and GCH1 was examined using 2D-SDS-PAGE and proximity ligation assay, while activity of the BH_4 metabolic pathway was monitored using HLPC-ECD-UV. Our preliminary results indicated that *in vivo*, GFRP and GCH1 are localized in serotoninergic and catecholaminergic neurons. Expression level of the two proteins, however, seems to vary greatly in function of the neuronal type. Moreover interactions between GFRP and GCH1 as well as the modulation of BH_4 level appear to be different depending on the neuronal structure studied. Those results suggest that the regulation of BH_4 biosynthesis by GFRP occurs differently in function of the structure and neuronal type. No COI.

Poster Presentations
Muscle Physiology (1)

1P-175

Sarcomere length nanometry in cardiomyocytes expressing α-actinin-AcGFP in Z-discs

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In cardiac muscle, a change in sarcomere length (SL) by ~100 nm causes a dramatic change in contractility (i.e., the Frank-Starling mechanism), indicating the need for the simultaneous measurement of SL and intracellular $[Ca^{2+}]_i$ in cardiomyocytes at high spatial and temporal resolution. To accurately analyze the motions of individual sarcomeres with nanometer precision during excitation-contraction coupling, we in the present study applied nanometry techniques to primary-cultured rat neonatal cardiomyocytes. First, we developed an experimental system for simultaneous nano-scale analysis of single sarcomere dynamics and $[Ca^{2+}]_i$ changes via expression of AcGFP in Z-discs. We found that following treatment with ionomycin, myocytes exhibited spontaneous sarcomeric oscillations (Cell-SPOC) at partial activation with blockage of sarcoplasmic reticulum functions, and the waveform properties were indistinguishable from those obtained under electric field stimulation. Finally, we interpreted the present experimental findings in the framework of our mathematical model of spontaneous sarcomeric oscillations. The present experimental system has a broad range of application possibilities for unveiling single sarcomere dynamics during excitation-contraction coupling in cardiomyocytes under various conditions. No COI.

1P-174

Roles of Epac in masseter muscle hypertrophy induced by chronic stimulation of β_2-adrenoceptor

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To elucidate the roles of Epac (exchange protein directly activated by cAMP) in the pathogenesis of muscle hypertrophy and slow-to-fast fiber-type transition induced by clenbuterol (CB), a β_2-adrenoceptor (β_2-AR) agonist, we examined the effects of chronic CB treatment (i.p., 2mg/kg/day for 3 weeks) on muscle mass, fiber diameter and myosin heavy chain (MyHC) composition in masseter muscle (the principal jaw closer in rodents) of wild-type controls (WT) and Epac1 knockout mice (KO). Masseter mass and muscle fiber diameter were significantly increased by CB treatment in WT while not in KO. On the other hand, CB treatment significantly increased the proportion of MyHC IIb at the expense of that of MyHC IId/x in both WT and KO, indicating that disruption of Epac1 did not affect the MyHC transition towards faster isoforms. In addition, we examined whether Epac mediated CB-induced hypertrophy through Akt/mTOR pathway, a possible downstream pathway influencing muscle phenotype. Phosphorylation levels of Akt and its downstream targets, P70S6K and 4EBP, was significantly increased by CB treatment in WT, but the increase was abrogated in KO. These results suggest that chronic stimulation of β_2-AR induces masseter muscle hypertrophy through Epac/Akt/mTOR pathway. No COI.

1P-176

Imaging of sarcomere dynamics in neonatal rat cardiomyocytes expressing F-actin-containing stress fiber-like structures

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In the present study, we investigated whether sarcomeric dynamics is influenced by the development of F-actin-containing stress fiber-like structures in neonatal rat cardiomyocytes. Ventricular myocytes were isolated from 1-day-old Wistar rats, and cultured on collagen-coated glass bottom dishes. Stress fiber-like structures developed when myocytes were cultured for three days in the presence of basic fibroblast growth factor (FGF-2, 1.4 nM). Likewise, stress fiber-like structures developed when myocytes were cultured in the presence of the actomyosin inhibitor N-benzyl-p-toluenesulphonamide (BTS, 20 μM). The magnitude of sarcomeric contractions did not significantly change upon treatment with FGF-2 or BTS.We hereby conclude that in neonatal cardiomyocytes, 1) intracellular F-actin-containing stress fiber-like structures develop with the suppression of actomyosin interaction, and 2) the stress fiber-like structures do not significantly alter sarcomere dynamics. No COI.

1P-177

The time course of Fyn and ROK activation in the signal transduction of abnormal vascular smooth muscle contraction

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Rho-kinase (ROK)-mediated Ca^{2+}-sensitization of vascular smooth muscle (VSM) plays a critical role for abnormal VSM contractions such as vasospasm. Previously we identified sphingosylphosphorylcholine (SPC) as a novel molecule to induce the ROK-mediated Ca^{2+}-sensitization leading to abnormal VSM contractions. Furthermore, we also identified Fyn, a member of Src family tyrosine kinase (Src-TKs), as a novel signaling molecule for abnormal VSM contraction meditating the SPC-induced ROK activation. In the present study, we analyzed the time course of Fyn and ROK activation in human coronary artery smooth muscle cells. To distinguish the activation of Fyn from that of other member of Src-TKs, Fyn was immunoprecipitated and its activation was analyzed by western blot analysis to detect its autophosphorylation of Y420. ROK activation was analyzed by the phosphorylation of its target molecule myosin phosphatase targeting subunit 1. The result showed that Fyn was activated after 5 min of SPC stimulation whereas no remarkable activation of c-Src, another member of Src-TKs, was detected. After 30 min of SPC stimulation, the Fyn activity was back to its basal level. In contrast, ROK activation was detected after 5 min of SPC stimulation and sustained even after 30 min. These results show that the activation of Fyn was temporal whereas that of ROK was sustained after SPC stimulation in the signal transduction of abnormal VSM contraction. No COI.

1P-178

Stretch speed-dependent muscle damage, functional loss, and mechanical hyperalgesia after lengthening contraction in rats

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Muscle damage, functional deficits, and pain are often elicited by exercise, especially after lengthening contraction (LC). Here we examined whether stretch speed of LC could be a determinant leading to these changes. Under isoflurane anesthesia, LC was repeatedly loaded to the rat ankle extensor muscles at a different stretch speed (i.e. angular velocities of 50, 100, 200, or 400 deg./s) over a fixed stretch range of motion (i.e. 90 deg.). The number of muscle fibers labeled with Evans-blue dye, a marker of muscle damage or increased membrane permeability of cells, increased as the angular velocity of LC was increased. The number of muscle fibers with the large cross sectional area was selectively and significantly decreased three days after LC (200 deg./s). Isometric torque of dorsiflexion measured two days after LC decreased as the angular velocity of LC was increased. Muscular mechanical hyperalgesia after LC was augmented in an angular velocity-dependent manner while repetitive stretch of the muscle alone without contraction had no effect on the nociceptive threshold. These results indicate that the faster angular velocity of muscle stretch during LC was a critical determinant leading to increased membrane permeability, decreased function, and facilitated mechanical hyperalgesia in the muscle. These findings could be useful when prescribing exercise for athletes, elderly people, and patients. No COI.

1P-179

Influence of the Connection between Skeletal Muscles on Muscle Shortening Velocity

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The present study was investigated to know the influence of the connection between skeletal extensor muscles by fasciae in knee joint on the shortening velocity. We measured the force - velocity relationship, *in vivo*, in whole muscle preparations in knee extensor, triceps femoris muscle (TFM), of the frog, *Rana catesbeiana*. TFM consists of three muscles, rectus femoris (RFM), vastus medialis (VMM) and vastus lateralis (VLM). Frogs were anesthetized by injecting urethane intraperitoneally. Then all the branches of sciatic nerve except for the one innervating VMM and VLM were cut. The active isotonic velocity of VMM and VLM was measured at various steps of load at 20 ± 0.5 °C. Experiments were performed on three different kinds of preparations; in one preparation, surface of TFM was totally covered with fascia (NF), in the second one, hamstrings was removed from TFM, and in the third one, RFM was removed from NF. The maximum shortening velocity in NF was the fastest among three conditions. And the output of power in NF was also the largest. The these results indicate that connection between TFM and hamstrings and that between RFM and the other in TFM have remarkable influence on muscle shortening velocity and muscle power output, suggesting that one of the functional roles of connection between muscles is to produce characteristic contractile properties of muscles. No COI.

1P-180

Attenuating effects of β_2-agonist clenbuterol on cast-immobilization induced atrophy of skeletal muscle fibers in rats: histochemical analyses

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Using histochemical methods, we investigated whether daily administration(dose=1mg/kg body weight/day) of clenbuterol (CLE) prevented cast-immobilization (IMM)-induced atrophy of skeletal muscle fibers. Adult male Sprague-Dawley rats were divided into control (CON), CLE, IMM, and IMM±CLE groups, and maintained for 9 days. The extensor digitorum longus (EDL) and soleus (SOL) muscles were isolated and analyzed histochemically after ATPase staining. EDL and SOL muscle weights in the IMM group were lower than those in the CON group. The analyses of cross-sectional area in muscles revealed that EDL muscle atrophy was limited to type II fibers; SOL muscle atrophy was mediated by type I and type II fibers. In the IMM+CLE group, IMM-induced atrophy was attenuated in the EDL but not in the SOL muscle. The attenuating effect of CLE was observed in type II fibers. These results suggest that CLE attenuates IMM-induced atrophy of type II fibers in the fast-twitch EDL but not in the slow-twitch SOL muscle. No COI.

1P-181

Evidence for a new type of rigor actin-myosin linkages in skinned skeletal muscle fibers

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It is generally believed that myosin heads (M) bind with actin filaments (A) to exert power stroke, associated with reaction AM· ADP· Pi to AM + ADP + Pi. This scheme indicates that, at the end of power stroke, myosin heads take the form of rigor or rigor-like actin-myosin linkages AM. X-ray diffraction studies on contracting skeletal muscle, however, can not detect such rigor linkages. To settle the problem, we first fully activated single skinned skeletal muscle fibers in contracting solution (pCa,4), put the fibers into high-Ca (pCa,4) or low-Ca (pCa,>9) rigor solution, and then the fibers were subjected to a series of release-stretch cycles (amplitude, 5% of fiber slack length) at various times after transfer of the fibers into rigor solution. Rather unexpectedly, the fibers exhibited small but distinct tension recovery following the initial tension drop coincident with applied release. The extent of the tension recovery increased markedly by lowering temperature from 20 to 10°C, and decreased in the presence of EDTA (5–10mM). The tension recovery gradually decreased with time in rigor solution, but was still observable for at least 90min. ADP (up to 10mM) had no appreciable effect on the tension recovery. These results indicate the presence of two different kinds of rigor actin-myosin linkages in rigor fibers; one with, and the other without, active tension response. This suggests that myosin heads in contracting muscle do not pass through rigor configuration. No COI.

1P-182

ATP release from the rat skeletal muscle differs among muscle contraction forms

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Delayed onset muscle soreness (DOMS) is induced by muscular lengthening contraction (LC), but by neither muscular shortening contraction (SC) nor stretch. Adenosine triphosphate (ATP) is well known to be released from excised muscle and would serve as a trigger for releasing bradykinin-like substance that stimulates upregulation of nerve growth factor, which induces mechanical hyperalgesia in DOMS. We investigated whether there is any difference in ATP release from the skeletal muscle among LC, SC and stretch. We measured ATP release from the extensor digitorum longus (EDL) muscle in vitro. The muscle-peroneal nerve preparation was put in a small chamber and superfused. In LC and SC group, the nerve was electrically stimulated for 1 s to induce muscle contraction and followed by 3 s rest period. This pattern was repeated for a total of 10 min. The current was set at three times the twitch threshold. The stimulus parameter to induce tetanic contraction was a frequency of 50 Hz with pulse duration of 1 ms. In LC group, the muscle was stretched by 2 mm from natural length during the contraction period. In stretch group, the muscle was stretched without contraction. The superfusate was sampled at -6, -3, 0 (start of electrical stimulation), 0.5, 1, 1.5, 2, 5 and 10 min. The ATP concentration was measured using the luciferin-luciferase method.The largest amount of ATP was released by LC. The peak was around 1 min after LC was started and then gradually decreased despite the existence of muscle contraction. Meanwhile, ATP release was not increased by stretch. No COI.

1P-183

Deficiency of Heat Shock Transcription Factor 1 Up-Regulates Interleukins in Regenerating Skeletal Muscle in Mice

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Skeletal muscle has a greater regenerative potential. During inflammatory response following muscle trauma, interleukin-6 (IL-6) and IL-1β are up-regulated. IL-6 and IL-1β are potentially mitogenic for myoblasts, as well as inhibitors of myogenic differentiation. Heat shock transcription factor 1 (HSF1) suppresses inflammatory genes. However, the interaction between HSF1 and inflammatory genes in skeletal muscle cells remain unclear. The purpose of this study was to investigate a physiological role of HSF1 gene on skeletal muscle regeneration. Necrosis-regeneration cycle was initiated by the injection of cardiotoxin into the left soleus muscle of male HSF1-null and wild-type mice. Slower regeneration was observed in HSF1-null mice, compared with wild-type mice. Up-regulations of IL-6 and IL-1β mRNAs were enhanced in HSF1-null mice. HSF1-deficiency-associated partial depression of skeletal muscle regeneration might be attributed to up-regulation of proinflammatory cytokines. This study was supported, in part, by JSPS KAKENHI Grants Numbers 22240071, 24650411, 24650407 and the Promotion and Mutual Aid Corporation for Private Schools of Japan. No COI.

1P-184

Screening of SESTD1 C-terminus binding proteins in skeletal muscles

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SESTD1 is a novel 79kDa protein that consists of SEC14 domain, three spectrin repeats and unique sequences. Some research suggests that SESTD1 is involved in the planar cell polarity pathway during mammalian embryonic development and the other research suggests that SESTD1 regulates transient receptor potential channels in smooth muscle. SESTD1 is also found in another tissues but is not analyzed. In this research, we investigated localization and binding proteins of SESTD1 in striated muscles. Western blot experiments using anti-SESTD1 monoclonal antibody elucidated that SESTD1 is not cardiac muscle protein but skeletal muscle protein. Immunofluorescence microscopy using that monoclonal antibody revealed that SESDT1 is localized around Z-line of sarcomere in skeletal muscles. To search the binding proteins of SESTD1 in skeletal muscle, we carried out the yeast two-hybrid screening using SESTD1 C-terminus as bait and muscle cDNA libraries as preys. Several proteins, located in Z-lines of sarcomere, such as N-RAP and Desmin were obtained as candidates for SESTD1 C-terminus binding proteins. These results indicate that SESTD1 is the novel Z-line protein of the sarcomere in skeletal muscle. We are currently investigating the role of SESTD1 in myofibrillogenesis. No COI.

1P-185

Interaction between water and proteins in skeletal muscle

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Magnetic resonance (MR) images reflect not only water content, but also water states in tissue. In skeletal muscle, MR distinguishes five water states whose localization has been clarified taking advantage of well-organized crystalline structure of sarcomere. Detailed nature of each water state is, however, not clarified yet.

Interaction between water molecules and macromolecules such as proteins is considered to restrict their mutual motional freedom to render characteristic state to the clusters of water molecules. From this, it follows that an increase in thermal molecular motion with temperature, water and macromolecules would store entropic free-energy that can be partly liberated in the process of muscle contraction. With this view in mind, we observed heat capacity of skeletal muscle (prepared from sartorius muscle of *Rana Catesbeiana*) with gradual increase in temperature using differential scanning calorimetry (DSC). The frozen preparation of skinned muscle showed at least two muscle-specific endothermic changes at -50℃ and -25℃. Furthermore, the integrated heat capacity, up to physiological temperature in which muscle contract from -80℃, of the fresh specimen was 10 J(~0.2mmol ATP-hydrolysis) larger per 1g specimen than that of denatured specimen. With this abundant energy, role of water state as an energy reservoir is suggested. No COI.

1P-186

Effects of heat stress on mTOR signaling and HSP expression in rat skeletal muscle

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Heat stress is well known to increase the expression of heat shock protein (HSP) in skeletal muscle. HSP plays an important role in chaperoning nascent peptides during translation. Meanwhile, the mammalian target of rapamycin (mTOR) signaling pathway has a key role in stimulating translation initiation. The purpose of the present study was to investigate the effects of heat stress on mTOR signaling pathway in rat skeletal muscle. Wistar male rats (14 wk-old) were randomly assigned into two groups: sedentary control (SC; n=4) and heat stressed group (HS; n=24). After overnight fasting, a leg of rat in HS group was immersed in a hot water (43℃) for 30 min under anesthesia and the gastrocnemius muscles in both legs were removed at immediately (0min, n=6), 30 min (n=6), 60min (n=6) and 24 hours (24h, n=6) after the heat stress. In SC group, 4E-BP1 and S6K1 phosphorylation, and HSP72 expression in white portion of gastrocnemius muscle were significantly lower than those of red portion. Heat stress significantly increased Akt, mTOR, 4E-BP1, S6K1 and eIF2α phosphorylation at 0 min in red portion of the muscle, whereas these phosphorylation significantly increased at 0-60 min in white portion. HSP72 expression in red portion did not change, whereas heat stress significantly increased HSP72 expression at 24 h in white portion. These results suggest that heat stress increases mTOR signaling and HSP expression in rat skeletal muscle, which may be affected by muscle fiber type. No COI.

1P-187

Localization of L-type calcium channels in skeletal muscle is regulated by binding of the C-terminus of CaV1.1 subunits with junctophilins.

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In skeletal myocytes, $Ca_V1.1$ L-type calcium channels (LTCC) form a functional complex with ryanodine receptors at junctional membrane (JM) where the sarcolemma is closely apposed to sarcoplasmic reticulum membranes. Junctophilins (JP) are known to stabilize the JM complex by bridging the sarcolemma and sarcoplasmic reticulum. Although we previously showed that knockdown of JPs inhibited the proper JM-targeting and function of LTCC in skeletal myocytes, the molecular mechanisms underlying these phenomena is unclear. In this study, we investigated the interaction of LTCC and JPs with biochemical techniques. Co-immunoprecipitation study showed that $Ca_V1.1$ interacted with JP1 and JP2 in mouse skeletal muscle. Pull down assay with GST-fusion proteins bearing cytosolic regions of $Ca_V1.1$ indicated that the C-terminus of $Ca_V1.1$ specifically interacted with JP1 and JP2. Pull down assay with GST-fusion proteins bearing several partial fragments of the C-terminus of $Ca_V1.1$ revealed that amino acid residues between 1545 and1606 in the proximal C-terminus are necessary for the binding of $Ca_V1.1$ and JPs. This JP binding region of $Ca_V1.1$ includes the amino acid stretch corresponding to the JM-targeting motif of $Ca_V1.2$ identified previously, suggesting that interaction of this part of $Ca_V1.1$ and JPs is important for the proper localization of LTCC. No COI.

1P-188

Neuromuscular transmission in the guinea-pig prostate

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Sympathetic neurogenic contractions contribute to an increased dynamic prostate smooth muscle tone often seen in benign prostatic enlargement. Blockade of electrical field stimulation with α_1 adrenoceptor antagonists is only partial. A secondary component is speculated to be purinergic in origin; therefore, this study examines the excitatory junction potentials (EJPs) elicited during electrical field stimulation to determine the contribution of purinergic signalling towards neurogenic contractions in the prostate. α_1 adrenoceptor inhibitor phentolamine (1μM) reduced the amplitude of EJPs to 61% of control (n = 4, $P<0.05$) while also reducing the E_{max} of FRCs to 56.7% (n = 5, $P<0.01$) indicating part of the sympathetic neuromuscular transmission is mediated by adrenergic nerves. EJPs (n = 4) were suppressed by α-β methylene ATP (10μM) suggesting P2X receptors also contribute to the EJPs and the E_{max} of FRCs were reduced by 44.5% (n = 5, $P<0.05$). Although PPADS (5μM), a $P2X_1$ receptor subtype inhibitor, reduced the amplitude of EJPs by 64.8% (n = 4, $P<0.01$), a combination with phentolamine (1μM) was required to abolish EJPs. It is apparent a large component of the neurogenic response in the prostate is mediated by purinergic signalling, whereby nerve-mediated ATP release likely acts in conjunction with α_1 adrenoceptors. This may provide a novel basis for improved therapeutical treatment of an enhanced prostate tone. No COI.

1P-190

The STZ-induced diabetes influences protein expression of the sarcoplasmic reticulum in the fast-twitch muscle fiber

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In diabetes mellitus (DIA), force production and fatigue-resistant in skeletal muscle is impaired. We demonstrated that diabetes impairs intracellular Ca^{2+} ($[Ca^{2+}]_i$) homeostasis and sarcoplasmic reticulum (SR) function in rat skeletal muscle (Eshima et al. AJP, 2013). However, it is unclear whether these functional decline is muscle fiber type specific. We tested the hypothesis that attenuation of calcium-reguratory protein depends on muscle fiber type (slow-twitch vs. fast twitch fiber) in DIA. Adult male Wistar rats were divided randomly into diabetic (DIA: Streptozotocin i.p.) and healthy (CONT) groups. Four weeks later animals were anesthetized and extensor digitorum longus (EDL; fast-twitch) and soleus (SOL; slow-twitch) were isolated. We examined muscle fiber type compositions (Type I, IIa, IIb and IIx) and cross-sectional area by immunohistochemical staining, and calcium-regulatory protein levels (Ca^{2+} release; RyR, Ca^{2+} uptake; SERCA1 and SERCA2) by western blot. In EDL, muscle fiber type switching (-42.2%, IIb), atrophy(-13.7%) and up-regulation of SERCA1 and SERCA2 (+38.7% and +54.7%, respectively) were observed compared with CONT ($P < 0.05$). In contrast, RyR protein level in DIA was lower compared with CONT ($P < 0.05$). These series of alteration was not observed in SOL. The present investigation demonstrates the STZ-induced diabetes causes metabolic and morphologic changes for fast-twitch fibers. Also the reciprocal adaptations of the SR function was found such as impairment of Ca^{2+} release and increased SR Ca^{2+} transport activity. No COI.

1P-191

Manual therapy and heating stimulation modulate mitochondrial activity following a lengthening contraction of rat's gastrocnemius muscle

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Physical therapies (e.g., manual therapy by compression of the muscle and heating stimulation) have been used as an effective intervention for muscular pain. Although we reported that these interventions ameliorated mechanical hyperalgesia induced by the lengthening contraction (LC) of rat's gastrocnemius muscle, the mechanisms of analgesic effect remain unclear. The aim of this study was to assess mitochondrial activity as a key component of muscle metabolism. Six week-old male SD rats at the beginning were used. Following adequate handling, LC was applied to the left gastrocnemius muscle. Just after LC, heating stimulation was applied to exercised muscle for 20 min by heating pack (42 °C). One day after LC, manual therapy with intermittent compression was applied to the left gastrocnemius muscle of the awake rats for 10 min. A Western blot analysis revealed that expression of cytochrome c oxidase subunit IV, indicator of mitochondrial activity, gradually increased following the lengthening contraction, but significantly suppressed by heating or manual therapy as same level as non-LC control. The results indicate that physical therapy could modulate mitochondrial activity following LC. No COI.

1P-192

Force-inhibiting effect of phosphatase inhibitors on bovine ciliary muscle

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Ciliary muscle is a smooth muscle characterized by a rapid response to muscarinic receptor stimulation and sustained contraction. In general, regulation of smooth muscle contraction involves protein phosphorylation, such as myosin and other regulatory proteins. However, little is known about the role of protein phosphorylation in ciliary muscle contraction. In our previous study, okadaic acid, a potent PP2A inhibitor, attenuated taenia cecum contraction, but not ciliary muscle contraction. To address the role of protein phosphorylation in the ciliary muscle contraction, we examined the effects of selective PP2A inhibitors on bovine ciliary muscle and guinea pig taenia cecum.
Methods: Muscle strips were contracted with ionomycin, and isometric tension was recorded. Various concentrations of phosphatase inhibitors were administered to contracted or relaxed muscle strips.
Results: Low concentration of okadaic acid and Fostriecin impaired ionomycin-induced contraction in taenia cecum, but not in ciliary muscle. Rubratoxin impaired contraction both in taenia cecum and ciliary muscle.
Conclusion: In this study, we showed that 1) some protein phosphatase inhibitors had different effects on taenia cecum and ciliary muscle, 2) force-inhibiting effects were different among the examined PP2A inhibitors. These results may be attributable to the differences in the specificity of PP2A inhibitors. If so, in ciliary muscle, unlike other smooth muscles, force-inhibiting effect of okadaic acid may be masked by activating contractile-response through inhibition of another phosphatase. No COI.

1P-193

Role of T-type Ca^{2+} channel in generating spontaneous Ca^{2+} transients in pericytes of the guinea-pig stomach

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Objective: Spontaneous Ca^{2+} transients of interstitial cells in various smooth muscle tissues primarily rely on periodic Ca^{2+} release from sarco-endoplasmic reticulum. A growing body of evidences indicates that voltage-dependent Ca^{2+} channels also contribute to the generation and/or synchrony of Ca^{2+} transients. **Methods:** In the myenteric layer of the guinea-pig gastric antrum, spontaneous Ca^{2+} transients in different cell populations were visualized using fluo-8 fluorescence Ca^{2+} imaging. **Results:** An extensive network of interstitial cells of Cajal (ICC) generated synchronous spontaneous Ca^{2+} transients. Besides ICC Ca^{2+} transients, pericytes also exhibited spontaneous Ca^{2+} transients that were developed almost simultaneously along the length of the microvasculature. ICC and pericytes Ca^{2+} transients were not affected by nidefipine (3 μM). Pericytes Ca^{2+} transients were prevented by cyclopiazonic acid (10 μM), caffeine (1 mM), Ni^{2+} (10 μM), mibefradil (10 μM) or nominally Ca^{2+} free solution but not tetracaine (100 μM). Blockade of T-type Ca^{2+} channels with Ni^{2+} or mibefradil abolished the initial component of ICC Ca^{2+} transients, but failed to disrupt synchrony, although a significant delay in the propagation of Ca^{2+} transients was evident. **Conclusions:** Functional coupling between cytosolic Ca^{2+} oscillator and T-type Ca^{2+} channels appears to play a critical role in the generation of pericytes Ca^{2+} transients, while the role of T-type Ca^{2+} channels in maintaining intercellular coupling in ICC network could be replaced by other ionic conductance. No COI.

1P-194

Epithelium of seminal vesicle modulates neural activity dependent contraction and originates stretch-sensitive contraction

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Seminal vesicle (SV), a male accessory sex gland, has been known to contract and expel their secretion into the ejaculatory duct in response to the sympathetic excitation. As novel contractile mechanisms of SV, we have previously suggested that the epithelium of SV originates a stretch-sensitive contraction (SSC) and depresses noradrenaline-induced contraction. In the present study, we examined the influence of epithelium on the contraction induced by neural excitation of SV wall. A reversed ring preparation (RRP; ~4 mm long) was dissected from SV of guinea pig and stretched by elevation of a force transducer to measure its isometric contraction. The magnitude of the contraction evoked by electrical field stimuli (EFS-C) was increased in a frequency-dependent manner (5~100 Hz). Blockade of neural excitation by tetrodotoxin (1 μM) abolished EFS-C but failed to affect SSC. EFS-C was due to excitation of adrenergic and cholinergic nerves, because it was depressed by application of phentolamine, an α-adrenoceptor blocker, and further diminished by an additional application of atropine. Removal of epithelium from RRP, which abolished SSC, significantly enhanced the maximum amplitude of EFS-C (2.86±0.50 g, n=6) compared with that of epithelium-intact RRP (1.47±0.20 g, n=6). We consider that the epithelium of SV plays an unexpected regulatory role on SV contraction, although the mechanism remains unsolved. No COI.

Poster Presentations
Oral Physiology (1)

1P-195

Comparative study of hyperalgesia in hindlimb- or ankle-immobilization rat model

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It is important to establish the characterization of pain measurement in animal model for elucidation of hyperalgesia mechanism and its treatments. In this study, we measured the thermal and mechanical stimulation sensitivities in hindlimb(H)- or ankle(A)- immobilization (IM) rat models and were compared. In H-IM, right hind limb was immobilized by cast, and in A-IM, only the right ankle was immobilized with an external fixation. In both groups, hyperalgesia and body weight were measured every once a week after immobilization for 4 weeks. The thermal sensitivity was measured by planter test and the mechanical sensitivity was measured by von Frey test. The planter test data showed % of right/left hindlimb escape time (second). Von Frey test data showed % of right (g)/left (g) hindlimb: pain threshold. Two weeks after IM, in planter test, the escape time decreased to 69.9% in H-IM and 83% in A-IM, respectively. In von Frey test, the pain threshold significantly decreased to 58% in H-IM and 21% in A-IM after 2 weeks IM, respectively (H-IM vs A-IM, $P<0.05$). The body weight decreased in H-IM (-7.5 g), but A-IM increased as same as intact model (88 g) 4 weeks after IM. These results suggest that A-IM make good use of hyperalgesia model, because A-IM has low pain threshold and less stress. No COI.

1P-196

Interaction of sympathoadrenal system and cervical sympathetic nerves mediated by adrenergic and NPY receptors on the hemodynamics in the jaw muscle

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Sympathoexcitation is known to cause changes in either increase or decrease in the masseter muscle blood flow (MBF). Although the reason for the differences in the effects is unclear, the interaction between neural and humoral mechanisms of MBF regulation may be important for the difference because sympathoexcitation induces activation of both sympathoadrenal system releasing circulating adrenaline and cervical sympathetic nerves including noradrenaline and NPY. However, it is unclear whether there is an interaction between these systems in the MBF regulation. We explored this question by investigating the effects of electrical stimulation of splanchnic nerve (SPLN) on the MBF for either intact or sectioning of superior cervical sympathetic trunk (CST) and adrenergic and NPY receptor antagonists (BIBP 3226) on the responses in anesthetized rats. The SPLN stimulation caused a significant MBF increase through β_2-adrenoceptors in the animals with intact CST. Sectioning of the CST changed the MBF increase to decrease by SPLN stimulation. The MBF increase evoked by SPLN stimulation was almost abolished by phentolamine. Although BIBP 3226 alone had no significant effect on the MBF increase, BIBP 3226 in combination with phentolamine caused a significant MBF increase evoked by SPLN stimulation. These indicate that cervical sympathetic nerves are involved in the MBF increase mediated by sympathoadrenal system, and suggest that α-adrenoceptors and NPY receptors contribute to MBF modulation during sympathoexcitation. No COI.

1P-197

Functional expression of glutamate receptors in mouse odontoblasts

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Odontoblasts are cells that receive various stimulation applied to the dentin surface. As sensory receptor cells, they drive the sensory-transduction sequence and form "reactionary dentin". In addition to various types of membrane receptors expressed in odontoblasts, such as bradykinin, ATP, and muscarinic-cholinergic receptors, the expression of glutamate receptors has recently been reported to be localized on the odontoblasts membrane. However, their functional role and detailed properties remain to be clarified. Glutamate receptors are divided into two groups; ionotropic glutamate receptors (iGluRs: NMDA and AMPA/KA receptors) and metabotropic glutamate receptors (mGluRs which are subdivided into Group I, II, and III). In the present study, we investigated the functional expression of these glutamate receptors by measuring intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) using fura-2 fluorescence and mRNA expression using real time RT-PCR. PCR analysis revealed the expression of all mGluR and NMDA receptor subtype mRNAs, with an abundant expression of group III mGluR. Application of monosodium glutamate (10 nM–100 μM) induced a transient and dose-dependent increase in ($[Ca^{2+}]_i$). In the presence and absence of extracellular Ca^{2+}, the selective agonists of Group I, II, and III mGluRs increased ($[Ca^{2+}]_i$), while a similar increase in ($[Ca^{2+}]_i$) was not observed after activation of NMDA receptors in the absence of extracellular Ca^{2+}. These results suggested that odontoblasts express Group I, II, and III mGluRs as well as NMDA receptors. No COI.

1P-198

Activation of $P2Y_{12}$-BK receptor coupling disarms the cAMP-mediated inhibitory effect on ryanodine receptor channel-induced Ca^{2+} release in trigeminal ganglion neurons

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Purinergic $P2Y_{12}$ receptors ($P2Y_{12}$) are classified as $G_{i/o}$-protein-coupled receptors. The neuro pathological roles of $P2Y_{12}$ in trigeminal ganglion (TG) neurons remain to be fully elucidated. We investigated $P2Y_{12}$ by immunofluorescence analysis and measurement of intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) in primary cultured rat TG neurons. We observed intense immunoreactivity for $P2Y_{12}$ in TG somata, dendrites, and axons, showing, co-localization with pan-neuronal marker, neurofilament 200, isolectin B4, and substance P. In the presence or absence of extracellular Ca^{2+} ($[Ca^{2+}]_o$), application of a $P2Y_{1,12,13}$ agonist (2-MeS-ADP) and bradykinin (BK) transiently increased $[Ca^{2+}]_i$. In the absence of $[Ca^{2+}]_o$, the 2-MeS-ADP-induced increase in $[Ca^{2+}]_i$ was reduced by applying inhibitors of the sarcoplasmic reticulum Ca^{2+}-ATPase and the ryanodine receptor channel. Application of an adenylate cyclase inhibitor transiently increased $[Ca^{2+}]_i$. Increases in $[Ca^{2+}]_i$ caused by 2-MeS-ADP were inhibited by a phosphodiesterase inhibitor. Both 2-MeS-ADP-induced and BK-induced increases in $[Ca^{2+}]_i$ were significantly inhibited by selective $P2Y_{12}$ antagonists in the presence of $[Ca^{2+}]_o$. These results show that $P2Y_{12}$ are distributed A-, non-peptidergic C- and peptidergic C-neurons. Activation of $P2Y_{12}$ induces the release of Ca^{2+} from Ca^{2+} stores, triggered by a decrease in internal cAMP levels. $P2Y_{12}$-BK receptor coupling play an important role in driving nociceptive function by disarming cAMP-induced inhibition of Ca^{2+} mobilization. No COI.

1P-199

Luminal entry of fluorescent dye is driven by solvent drag due to paracellular fluid secretion in the submandibular gland.

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Primary saliva is a mixture of fluid secreted through paracellular route and transcellular route. By luminal dye-dilution in the isolated acini, the transcellular fluid secretion was estimated as 25μl/g-min and the paracellular fluid secretion as 60μl/g-min during sustained stimulation (2002). In the present study, using the perfused submandibular gland we examined whether fluorescent dye enters the intercellular canaliculi by solvent drag. The gland was surgically isolated from the Wistar rat under anesthesia, and perfused arterially on the stage of confocal microscope. (5 Live, Zeiss). When the fluid secretion was induced by α1-adrenergic stimulation with phenylephrine, the fluorescence of intracellular canaliculi increased dose-dependently. Combined stimulation with phenylephrine and xamoterol (β1 agonist) increased the fluorescence more than only phenylephrine. Single stimulation with isoproterenol (1 μM) decreased the fluorescence intensity in the canaliculi, suggesting lowering fluid secretion through pressure-dependent paracellular route. The present findings lead the conclusion that we could observe entry of fluorescent dye from intercellular space to luminal space by pressure-dependent paracellular route by solvent-drag, and that the fluid secretion through pressure-independent route is still open for further studies. No COI.

1P-200

Effect of Danshen on paracellular fluid secretion in the isolated rat submandibular gland

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Danshen (DS) induces a salivary fluid secretion without any other sectretagogues in isolated and arterially perfused rat submandibular gland (SMG). The secretory effect was not blocked by atropine, an inhibitor of muscarinic receptors, or by phentolamine, an inhibitor of alpha-adrenergic receptor. The salivary fluid secretion by DS includes tanscellular fluid secretion because the blockers of Na/K-ATPase (ouabain) and Na/K/2Cl-co transporter (bumetanide) decreased DS-induced fluid secretion. In the present study, we examined whether DS influences the paracellular fluid secretion, by measuring secretion of Lucifer yellow (LY), a fluorescent dye with ca 500 mw in the perfusate. Then the secreted saliva was collected along time course during DS stimulation. Because the collected saliva only by DS was too small to be measured, LY secretion by DS plus carbamylcholine (CCh) was measured. The time course of LY secretion was different from only by CCh. The time course of salivary fluid secretion was in parallel with secretion of fluorescent dyes, and showed an initial slow increase followed by a slow decrease. The present observation suggested that DS could control junctional fluid passage with large molecule through the paracellular route. No COI.

1P-201

Sex hormone-regulated expression of microRNAs in mouse submandibular glands: a putative Bio-Marker of DHT-dependent diseases

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Mouse submandibular glands (SMGs) have sexual dimorphism *via* androgen receptor. MicroRNAs (miRNAs) are small non-coding RNAs that play key roles in the regulation of gene expression. We examined effects of sex hormones on the expression pattern of miRNAs in mouse SMGs.

SMGs of ICR mice were investigated for miRNAs and 42 miRNAs were identified. Expression patterns of these miRNAs in mice with various hormonal treatments were analyzed by quantitative real-time PCR.

Among of the 42 miRNAs, miR-21a and miR-143 were much abundant and miR-141 slightly abundant in male mouse SMGs. Castration caused remarkable decrease in the expression of these three miRNAs. DHT administration to the castrated animals greatly increased miR-21a and miR-141 but slightly miR-143, resulting the miR-141 expression surpassed the miR-143 expression. These effects of DHT on castrated mice were similar to those of DHT on female mice. On the other hand, DHT administration to normal males increased the miR-21a, miR-141 and miR143 expression with almost the same ratio, suggesting that miR-21a and miR-141 were mainly regulated by DHT while miR-143 expression was dependent on testosterone. A hormonal condition of castrated animals that were administrated DHT seemed to be similar to that of partial androgen deficiency in aging males (PADAM). The change in expression patterns of miR141 and miR143 was expected to apply for diagnosis of benign prostatic hyperplasia (BPH) and androgenetic alopecia (AGA). No COI.

1P-202

Expression of nestin in injured salivary gland

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Objective: It is known that salivary gland has regenerative ability. But the origin of regenerated salivary gland cells is still unclear. Our goal is to identify the precursor cell of regenerated acinar cells. We have previously reported that tissue injuries induce de-differentiation of cells, which expressed a neural stem cell marker, nestin, in vitro. In this study, we examined whether nestin is expressed in undifferentiated salivary gland in vivo. Methods: We performed unilateral parotid duct ligation of mouse and extracted parotid gland at 4, 7, 10 days after ligation. We compared expression of nestin with control by immunoblot analysis, HE staining and immunohistochemical staining. Next, we removed the clip at 7 days after ligation, and extracted parotid gland in 14 days later. Results: By immunoblot analysis, expression level of nestin was significantly increased at 4 and 7 days in the ligation side compared to the control. Morphologically, the atrophy of acinar cells was observed in the ligation side. In the atrophied parotid gland, nestin-positive cells were detected. The parotid gland that has been released from duct ligation showed histological similarity to the control. Conclusion: Because nestin was expressed in the salivary gland that changed to an undifferentiated condition by tissue injury, it was suggested that nestin can be used as a precursor marker in salivary gland. No COI.

1P-203

Localization and function of V-ATPase in salivary glands of mouse—analysis of the a3 subunit knockout mouse—

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Vacuolar H^+-ATPase (V-ATPase) is localized in intracellular membranes of organelle such as the vacuole, lysosome, Golgi apparatus, synaptic vesicle, and is known to acidify intracellular compartments as well as pump H^+ across the plasma membrane. V-ATPase is composed of two large domains, a cytosolic (V_1) domain, consist of A-H subunits complex, and a transmembrane (V_o) domain, consist of a, c, c', c", and d subunits complex. We previously reported that a2, a3, d1, B2, C1, E2 subunit isoforms are commonly expressed in the major salivary glands, and B2 subunit isoform is localized in the duct cells of each salivary glands. In this study, we have further analized subcellular localization of the B2 subunit isoform using a structured illumination microscopy (Nikon). Immunofluorecence was found in apical cell membrane and cytoplasm of striated parotid ductal cells. In ductal cells of the sublingual and submandibular glands, immunofluorecence was found in the basolateral region of cytoplasm. By analyzing phenotypes of the knockout mouse of the a3 subunit isoform, there was little saliva production and the size of salivary glands was smaller than that in the wild type mice. Intraoral salivary pHs tend to be slightly acidified. These results suggest that V-ATPases play a critical role for salivary ductal function, such as ion transports involved in epithelial electrolyte absorption, anion secretion and modification of pH. No COI.

1P-204

The localization of parasympathetic vasodilator fibers related to blood flow dynamics in rat salivary gland.

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Objective: The parasympathetic blood flow increase was reported in rat salivary glands. However, the localization of vasodilator fibers in salivary glands are not clear. The sublingual gland (SLG) have mainly mucous acini, while submandibular gland (SMG) have a mixture of serous and mucous acini. This suggests that the hemodynamic mechanism of both glands may be different. In this study, we analyzed parasympathetic blood flow increase of rat SLG and SMG by laser speckle imaging (LSI) technique to examine localization of parasympathetic vasodilator fibers.

Methods: The urethane anesthetized rats paralysed by pancuronium bromide were artificially ventilated. The cervical vagi and cervical sympathetic trunk were cut in the neck. The hemodynamics of SLG and SMG were analyzed by LSI when the lingual nerve (LN) was electrically stimulated.

Results & Discussion: The basal blood flow was higher in the SLG than SMG. The regional differences of blood flow increase were found among SLG and some parts of SMG during LN stimulation. These increases were completely inhibited by hexamethonium (10 mg/kg i.v.). Although these increases were inhibited by atropine (0.1 mg/kg i. v.), inhibitory effect was stronger in SMG than SLG. There was no significant difference in blood flow increase of each gland by intravenous administration of acetylcholine (0.01~1 mg/kg i.v.). These indicate that parasympathetic blood flow increase of SMG is mainly evoked by cholinergic fibers and that of SLG is evoked by both cholinergic and non-cholinergic fibers. No COI.

1P-205

Salivary functions change in experimental periodontitis model rats

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This study was designed to investigate the mechanism of salivary dysfunction in an experimental periodontitis rat model and to examine the improvements in salivary secretion following treatment of the experimental periodontitis. In the periodontitis model, which included a unilateral ligature for 4 weeks around the second upper molar, several salivary functions were investigated. Changes in the salivary function were evaluated 4 weeks after removal of the ligature in some rats. The periodontitis model showed significant reductions in the weight of the bilateral major salivary glands and pilocarpine-induced salivary secretion. The model also showed an increase in the number of apoptotic cells in bilateral salivary glands. According to Ca^{2+} imaging and Western blotting, there were no differences in the muscarine-induced intracellular Ca^{2+} mobilization in acinar cells or in the M3 receptor and AQP5 expression levels in the salivary glands between the sham and the periodontitis model. Following removal of the ligature, differences in the weights of salivary glands and pilocarpine-induced salivary secretion between the sham and the periodontitis model animals were not found.These results suggest that experimental periodontitis leads to hyposalivation and that relief from it improves salivary function. No COI.

1P-206

Maintenance of secretory proteins during maturation of rat parotid secretory granules

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The diameter of secretory granules (SGs) in rat parotid acinar cells is about 1 μm, and therefore they can be clearly observed. This is an advantage in investigation of granule maturation. The maturation process of SGs comprises enlargement, increase in density, vesicle transport, and remodeling of membrane. However, the maintenance of secretory proteins in SGs during granule maturation is poorly understood. β-adrenergic agonist isoproterenol was injected into rats to induce the exocytosis of SGs from their parotid glands, which were removed 2 hrs after the injection. The isolated acini, which had no SGs, were then incubated with Lucifer Yellow (LY) dye as a tracer for 3 hrs. The localization of LY into newly-formed SGs was estimated by the following methods: (1) the co-localization with amylase by confocal laser microscopy, (2) the secretion of LY upon stimulation, (3) the detection of fluorescence from purified SGs, and (4) the intracellular localization by electron microscopy. After 2 days cell culture, newformed SGs became larger. In addition, co-localization of fluorescence with amylase was observed. Although the fusion and budding of small vesicles were contributed to process of granule maturation, LY stayed in SGs even after the maturation. These results suggest that the secretory proteins which have no transport signal such as LY are retained into SGs during the granule maturation in parotid acinar cells. No COI.

1P-207

A new relation between visceral sensation and nizatidine, a gastric-acid inhibitor

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Nizatidine collaterally increases salivary secretion in patients with gastroesophageal reflux disease (GERD). However, it is not clear whether nizatidine affects central regulation of salivation. In the present study, we hypothesized that intracerebroventricular (the fourth ventricle) administration of nizatidine would collaterally enhance salivary secretion related to the vagus nerve, focusing on the central effects of nizatidine. We aimed to clarify induction of salivation by central nizatidine and / or afferents of the vagus nerve in rats. Male Wistar rats were used for experiments. We performed real-time detection of salivary secretion from submandibular gland. Vagus nerve-induced salivary secretion was measured for 3 min following intracerebroventricular administration of nizatidine. In the results, 1) the vagus nerve stimulation increased salivary secretion, 2) nizatidine enhanced salivary secretion induced by the vagus nerve stimulation. Total salivary secretion was significantly increased by nizatidine in a dose-dependent manner. Our findings suggest that central nizatidine enhances salivary secretion induced by the vagus nerve stimulation in rats. No COI.

1P-208

Cytotoxicity of low-dose povidone-iodine and its effects on rat oral mucosa

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Povidone-iodine (PVP-I) is as complex of polyvinylpyrrolidone and iodine developed as an oral cavity gargle. It works as a broad spectrum microbicide against bacteria and virus based on oxidizing effect. PVP-I exposure causes nephropathy and iododerma as previously reported, however, there is few quantitative studies. The aim of this study is to evaluate the dose- and time- dependence of PVP-I toxicity against HeLa cells and rat oral mucosa.

We found growth inhibition at 10^{-2}μM, increased apoptosis at 10^{0}μM, blister-like cytoplasm at 10^{1}μM, and increased necrosis at 10^{2}μM after 2-day exposure with PVP-I. As clinically used concentration of PVP-I is 10^{3}–10^{4}μM, relatively low concentration of PVP-I may be able to damage cells with long-time exposure. Ion composition of extracellular solution had relationship with PVP-I effect.

We also found thinner epithelium in rat oral mucosa after 1-day exposure with 10^{-1}μM of PVP-I, and it had many apoptotic cells. Our study suggests that risks and benefits of PVP-I application for sterilization and anti-cancer agent should be reconsidered. No COI.

1P-209

The mechanism of oral ulcer-induced pain hypersensitivity in rats.

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Oral ulcers induce pain during food consumption and speaking. However, because no animal assay measures intraoral pain-induced behaviors, pain mechanisms in oral ulcers are poorly understood. In this study, we investigated pain sensitivities to chemical and mechanical stimulations in oral ulcers using our newly developed behavioral assays in conscious rats. Treatment with acetic acid in the labial fornix region of the inferior incisors developed obvious oral ulcers, showing severe infiltration of inflammatory cells and epidermolysis in histology. Grooming-like behaviors following the administration of capsaicin and citric acid solutions were significantly enhanced by oral ulcers. The head withdrawal threshold to mechanical stimulation to oral mucosa was significantly decreased in oral ulcers. In immunofluorescence, oral ulcers produced a high infiltration of substances compared to the healthy oral mucosa, without changes in the expression levels of TRPV1 and ASIC3 in trigeminal ganglion. The mechanical allodynia induced by oral ulcers was not significantly inhibited by indomethacin injection. These results suggest that oral ulcers induce pain hypersensitivity to chemical and mechanical stimulations, most likely due to tissue damage. No COI.

1P-210

Difference in modulation of jaw-opening reflex between working and balancing side during fictive mastication in rabbits

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The aim of present study is to investigate the modulation of jaw-opening reflex (JOR) responses evoked by innocuous stimulation during chewing, and assess the extent of suppression between working and balancing sides.EMG activity of the digastric muscle was simultaneously recorded with movements of the incisor point of the mandible during fictive mastication induced by electrical stimulation to the cortical masticatory area of anesthetized rabbits. The jaw movement signal was used to deliver the stimuli to the inferior alveolar nerve (IAN) at given jaw positions as follows: 1) halfway of jaw closing phase, 2) end of the closing phase (ECP), 3) midpoint of the occlusal phase, 4) the maximum jaw opened position. The JOR amplitude at ECP was most suppressed (17.9% of the control) compared with the other positions, and the significant difference exists between the working side and balancing side on ECP. Because the mandibular position at SOP was exactly just before contacting of the opposing molars, it is conceivable that such JOR suppression depending on the mastication side at ECP does not result from afferent information from the IAN induced by teeth contact that is unique to the working side during occlusal phase of mastication. It may be concluded that the stronger JOR suppression on the working side at ECP could be advantageous to develop stronger biting force on the working at which the low threshold mechanoreceptor in the periodontal ligament is inevitably activated by teeth contact. No COI.

1P-211

Spatial profile of neural excitation in rat somatosensory cortex evoked by electrical stimulation of tooth pulps: an optical imaging study

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Nociception is processed in the several regions in the cerebral cortex including the somatosensory cortex (SS). The topographical representation in the SS is well identified, however, it is still an open issue how the nociception of the molar tooth pulps is processed in the SS. This issue is critical to understand the mechanism of tooth pain, because patients with tooth pain often claim that they cannot identify the diseased tooth. To examine the precise regions responding to the electrical stimulation of the maxillary and mandibular molar tooth pulps, we performed the optical imaging technique with a voltage sensitive dye, which enables us to visualize neural excitability in a macroscopic manner. Electrical stimulation of the molar tooth pulps evoked neural excitation in the two parts of the ventral part of the SS: (1) the rostroventral part of the SS adjacent to the tongue region, and (2) the ventral part of the SS around the middle cerebral artery. The former SS region responded to both the maxillary and mandibular molar tooth pulp stimuli. On the other hand, the later SS region could be further divided into rostral and caudal subregions, which responded to the maxillary and mandibular tooth pulp stimulation, respectively. These results suggest that a part of the SS responds to both the maxillary and mandibular tooth pulps, and this might be a reason for difficulty in idenfying a diseased tooth. No COI.

1P-212

Involvement of the red nucleus in the suppression induced by stimulation of the raphe magnus nucleus on the jaw-opening reflex

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We found that stimulation of the red nucleus (RN) modulated the low- and high-threshold afferent-evoked jaw-opening reflexes (JORs). It has been reported that the RN receives projection fibers from the raphe magnus nucleus (RMg), and stimulation of the RMg inhibited the nociceptive JOR. The present study aimed to investigate the effects of RMg stimulation between low- and high-threshold afferent-evoked JORs, and whether RMg-induced modulation of the JOR is affected by electric lesion of the RN. The experiments were performed on anesthetized rats. The test stimulation was applied to the inferior alveolar nerve to evoke the JOR. The stimulus intensity was either 1.2 (low threshold) or 4.0 (high threshold) times the threshold. The electromyograms were recorded from the digastric muscles. The conditioning stimulation was applied to the RMg. The control JOR responses were recorded as well as the modulation induced by stimulation of the RMg. The RN lesion was made by the passage of electric current. The effect of the RMg stimulation on the JOR was tested at the termination of lesion. Stimulation of the RMg modulated the low-threshold afferent-evoked JOR. Stimulation of the RMg significantly suppressed the high-threshold afferent-evoked JOR. Electrically induced lesions of the RN reduced the RMg-induced suppression of the JOR evoked by the high-threshold afferents. These results suggest that the suppressive effect of RMg stimulation on the JOR is mediated in part by a relay in the RN. No COI.

Poster Presentations
Endocrinology (1)

1P-213

Involvement of a neurosteroid 7α-hydroxypregnenolone in the expression of courtship behavior of the male newt

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A neurosteroid, 7α-hydroxypregnenolone, has recently been found to enhance locomotor activity in the male newt, *Cynops pyrrhogaster*. Here, we show that this neurosteroid is also involved in enhancing the expression of courtship behavior. Intracerebroventricular (ICV) injection of 7α -hydroxypregnenolone enhanced courtship behavior dose-dependently in the sexually undeveloped males that had been pretreated with prolactin and gonadotropin, which is known to bring the males to a sexually developed state. In the males receiving no prior injections of these hormones, however, the neurosteroid did not elicit the behavior. ICV administration of ketoconazole, an inhibitor of the steroidogenic enzyme cytochrome P450s, suppressed the spontaneously occurring courtship behavior in sexually active males. Supplementation with 7α-hydroxypregnenolone reversed the effect of ketoconazole in these animals. It was also demonstrated that the effect of the neurosteroid on the courtship behavior was blocked by a dopamine D2-like, but not by a D1-like, receptor antagonist. These results indicate that endogenous 7α-hydroxypregnenolone enhances the expression of the male courtship behavior through a dopaminergic system mediated by a D2-like receptor in the brain. No COI.

Poster Presentations
Kidney, Urination

1P-214

H^+ secretion across the distal tubule in perfused bullfrog kidney

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In this study, we examined the H^+ transport in the dilution segment (distal tubule) by measuring transepithelial potential (TP) with pH in the tubular fluid (pH_{TF}) in perfused bullfrog kidney with double-barreled ion-selective microelectrodes. Futhermore, the same experiments were conducted in the proximal tubule. Decreasing pH in the peritubular perfusion solution from 7.7 to 6.7 by decreasing HCO_3^- concentration produced the increase in the TP from ~+10 mV to ~+15 mV with triphasic change in pH_{TF} (initial slight increase, second decrease, followed by the increase in in the in the distal tubule). On the other hand, decreasing pH in the peritubular perfusion solution produced the increase in the TP by 2 mV with biphasic change in pH_{TF} (an initial slight increase followed by the decrease in pH_{TF}). The peritubular perfusion with the acid solution produced the decrease in cytosolic pH in proximal tubule cell with depolarization of peritubular membrane potential. 10^{-6} M bafilomycin caused the initial decrease followed by the increase in pH_{TF} in both distal and proximal tubule. These results indicate that H^+ secretion system primarily depends on the same mechanisms, such as Na^+/H^+ exchanger or H^+-ATPase in the luminal membrane and electrogenic HCO_3^- transporter in the peritubular membrane, in both the proximal and the distal tubule, probably except H^+ extrusion mechanism in the luminal membrane in the distal tubule. No COI.

1P-215

Urinary Bladder Mucosal Responses to Chronic Ischemia

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Chronic bladder ischemia (CBI) has been shown to lead to bladder dysfunctions. We investigated the expression of various cellular proteins within the urothelium (UT) and lamina propria (LP) following CBI in the rat urinary bladder.

Urinary bladders were removed from adult SD rats that underwent a balloon endothelial injury of the iliac arteries. We examined distributions of LP-vimentin-immunoreactive (IR) cells and UT-connexins (Cx26, Cx43); and additional proteins involved in UT barrier and sensory functions.

CBI was associated with significant increases in vimentin-IR cells and increased expression of the gap-junction proteins Cx26 and Cx43 as compared to sham control. CBI also resulted in changes in expression levels of junctional markers (ZO-1) and NGF as well as norepinephrine. Our findings reveal that CBI alters a number of proteins within the urothelium and underlying lamina propria. These proteins are involved in remodeling, repair as well as intercellular communication. In addition, the increased expression of vimentin-IR cells also suggests that changes in cell-cell interactions could play a role in CBI-induced changes in bladder activity. No COI.

1P-216

New methods for in vivo study of rapid homeostatic response of the kidney to calcium reduction in the mouse.

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Calcium (Ca) homeostasis of the body fluid is well known to be strictly regulated by two hormonal systems, i.e., parathroid hormone and vitamin D systems. The kidney is also associated with the Ca homeostasis by active resorption of Ca at the distal convoluted tubule, however, its autonomous contribution to the Ca homeostatic response, especially to the acute Ca decrease, is unclear. To elucidate the acute, dynamic regulation of Ca homeostasis by the kidney, I developed a technique to collect blood simultaneously from both the renal artery and vein in the anesthetized mouse. This technique enabled differential measurement of Ca concentration of the input-output of a kidney, by using i-Stat (free Ca concentration) and/or DriChem (total Ca concentration) systems. In addition, for rapid lowering of Ca concentration, I used a novel "dilution method". In this method, the intravenous injection of calcium-free perfusate lowers Ca concentration to the aimed level and the dilution rate can be confirmed by measuring hematocrit. As the result, Ca concentration in the output vein kept higher than that in the input artery of the kidney at all periods after lowering Ca. This suggests the kidny autonomously elevates Ca concentration in response to rapid drop. No COI.

1P-217

Effect of a hydrogen-enriched solution on the albumin redox of hemodialysis patients.

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Elevated oxidative stress (OS) is associated with severe cardiovascular disease and premature death among patients treated with hemodialysis (HD). OS is enhanced by contact between blood and dialysis membranes during HD sessions. This study aimed to clarify whether hydrogen (H2), which is a known antioxidant, is capable of suppressing increased OS induced during HD sessions.

Eight patients on regular HD treatment were studied. Two HD sessions were performed in a cross-over design trial using standard (S-HD) and hydrogen-enriched solutions (H2-HD). Blood samples were obtained from the inlet and outlet of the dialyzer during HD to determine changes in plasma levels of glutathione, hydrogen peroxide, and albumin redox state as a marker of OS. Comparison of inlet and outlet blood revealed significant decreases in total glutathione and reduced glutathione, as well as significant increases in hydrogen peroxide in both HD treatments. However, the mean proportion of reversibly oxidized albumin in outlet serum was significantly lower than in inlet serum following the H2-HD session, whereas no significant changes were found in S-HD, suggesting that intra-dialyzer OS is reduced by H2-HD.

In conclusion, the application of H2-enriched solutions could ameliorate OS during HD. No COI.

Poster Presentations
Ionic Channel, Receptor (2)

2P-001

Identification of amino acid residues involved in the TRPA1 inhibition by utilizing species specific difference

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Transient receptor potential ankyrin A1 (TRPA1) receptor is a member of TRP superfamily and an excitatory nonselective cation channel. An increasing body of evidence suggests that TRPA1 acts as a nociceptive receptor for various chemicals and physical stimuli. Many TRPA1 antagonists have been developed and some of them function as analgesic agents. A previous study showed that AP18, a mammalian TRPA1 antagonist, did not inhibit heterologously expressed western clawed frog TRPA1 (fTRPA1). Here, I show that fTRPA1 is also insensitive to A967079 (A96), one of the most potent mammalian TRPA1 antagonist which is structurally similar to AP18. In a heterologous expression system with Xenopus oocytes, A96 failed to inhibit fTRPA1 activation elicited by TRPA1 agonists. Mutant channel analyses revealed that specific two amino acid residues located within the fifth transmembrane domain were involved in the inhibitory action of A96. Same amino acids were previously reported to be involved in the AP18 inhibition. These findings are based on species differences in sensitivity of TRPA1 antagonists provide novel insights for structural-function relationship of TRPA1 and useful information in the search for new analgesic medicines targeted for TRPA1. No COI.

2P-002

Heat and AITC activate green anole TRPA1 in a membrane-delimited manner

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Transient receptor potential ankyrin 1 (TRPA1) is a Ca^{2+}-permeable non-selective cation channel that is activated by various noxious stimuli. TRPA1 was initially identified as a potential mediator of noxious cold stimuli in mammalian nociceptive sensory neurons while TRPA1s from non-mammalian vertebrates (snakes, green anole lizards and frogs) were recently reported to be activated by heat, but not cold stimulus. In this study, we examined properties for TRPA1 channel of green anole (gaTRPA1) relating to thermal and chemical stimulation in whole-cell and single-channel recordings. Heat and AITC activate gaTRPA1 both solely and synergistically in whole-cell level. In the absence of extracellular Ca^{2+} very small currents were observed upon heat stimulation, while current sizes and desensitization were similar both in the presence and absence of extracellular Ca^{2+} upon AITC stimulation. gaTRPA1 channels were also activated by heat and AITC in excised membrane patches with an inside-out configuration. By comparing the kinetics of heat- and AITC-evoked single-channel currents, we defined similarities and differences of gaTRPA1 channel responses to heat and AITC. We observed similar current-voltage relationship and unitary amplitutdes for heat- and AITC-evoked currents while we found that heat-activated currents showed shorter durations of both open- and closed-times. Our results suggest that the gaTRPA1 channel is directly activated by heat and chemical stimuli. No COI.

2P-003

Evolution of vertebrate TRPA1 channel as O_2 sensor

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The fluctuations in atmospheric oxygen (O_2) level have driven the evolution of organisms. Approximately 2 billion years ago, when O_2 was geologically accumulated after the evolution of oxygenic photosynthesis in cyanobacteria, mitochondrion was ingested in the progenitor cell of Eukaryota, enabling the reduction of O_2 toxicity as well as the efficient production of ATP. Approximately 200 million years ago, when O_2 was geologically reduced presumably by the global volcanic action, ancestor of mammalian acquired viviparity, enhancing O_2 supply to their fetus. Furthermore, higher organisms have acquired the sensing systems of low-O_2 (hypoxia) and high-O_2 (hyperoxia) to secure energy production while diminishing the risk of O_2 toxicity. However, it remains unclear when animals acquired the O_2 sensing systems in the process of evolution. Our group has previously shown that mouse and human TRPA1 channel detects O_2 availability in vagus. In this study, we explored the O_2 sensitivity of TRPA1 channels in different species (amphibia, avian, and marsupial). Genetic analysis showed that xenopus, chicken, and wallaby TRPA1 did not obtain the prolyl residue responsible for hypoxia sensing in human TRPA1, whereas vivipara obtained the prolyl residue on TRPA1. Electrophysiological measurements revealed that chicken TRPA1 failed to respond to hypoxia. Thus, it is highly possible that TRPA1 acquired O_2 sensing mechanisms around the same time as the acquirement of viviparity function, suggesting that hypoxic environment approximately 200 million years ago have driven the acquirement of O_2 sensing mechanisms as well as that of viviparity function. No COI.

2P-004

Model Simulation of PLC-operated TRPC Currents Regulating by PI(4,5)P_2-DAG Signaling

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Transient receptor potential classical/canonical (TRPC) 3, C6 and C7 are non-selective cation channels, playing key roles in cardiovascular, gastrointestinal, renal and nervous system physiology. We have recently reported that the depletion of phosphatidylinositol 4,5-bisphosphate [PI(4,5)P_2] inhibits TRPC channel activity, irrespective to the presence of the channels activator 'diacylglycerol(DAG)'. However, the complex regulation by PI(4,5)P_2 and DAG to the PLC-coupled receptor TRPC currents is difficult to address how the respective regulators differentially contribute to these channels. To aid an intuitive understanding of this complex regulation, we here built a PLC-coupled TRPC channel model consisting of PI-turnover metabolism, channel gating, and behavior of PI(4,5)P_2 sensor proteins (CFP/YFP fused PH-domain). Respective sections of this model were made based on the enzymatic reaction, the ligand-gated channels, and protein-ligand isotherms, respectively. By accordance of the simultaneous measurements of lipid dynamics / TRPC currents and the model simulation, our results demonstrated that the strength of PLC activity is critical to the appearances of TRPC currents, such as the current amplitudes, the total ionic influx and the peak time through the various changes of PI(4,5)P_2 and DAG. No COI.

2P-005

Abnormal receptor-response and mechanosensitivity of mutant TRPC6 channels associated with familial focal segmental glomerulosclerosis (FSGS) - involvement of cytoskeleton and podocin

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Several mutations of transient receptor potential cation channel 6 (TRPC6) in human podocytes are known to cause the inherent forms of glomerosclerosis, FSGS. We investigated the functional impacts of murine TRPC6 FSGS mutations near its N-terminal ankyrin repeats (G108S, P111Q, N124S, M131T, N142S, R174Q) in heterologous expression system with Ca^{2+} imaging and patch clamp techniques. The 111Q, 131T and 142S mutants showed enhanced receptor responses to carbachol. In contrast, except for some of 131T mutants being highly mechanosensitive, all FSGS mutants showed diminished mechanical responses to 2,4,6-trinitrophenol which is normal in wild-type TRPC6. The enhanced mechanical response of 131T mutant as well as normal mechanosensitivity of wild-type were abolished by disrupting actin cytoskeleton with cytochalasin D. In coimmunoprecipitation experiments, simultaneous application of receptor stimulation and mechanical stress enhanced the physical interaction of actin with TRPC6 protein, which was variably affected by FSGS mutations. Co-expression of TRPC6 with podocin, a slit diaphragm protein, suppressed both receptor and mechanical responses. These observations indicate that FSGS mutations near N-terminal ankyrin repeat domains alter the degree of receptor activation of TRPC6, and its mechanosensitivity and interaction with actin. Such differences may partly explain the etiology of FSGS in which degeneration of podocytes leads to disruption of renal filtration barrier. No COI.

2P-006

Redox signal-mediated sensitization of Transient Receptor Potential Melastatin 2 (TRPM2) to temperatures affects insulin secretion from the pancreatic β-cells

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Pancreatic β-cell has a unique function coupling glucose sensing and metabolism to insulin secretion, a vital process in energy homeostasis. In the pancreatic β-cell, reactive oxygen species (ROS) including H_2O_2 are generated concomitantly with the glycolytic and respiratory metabolism. Interestingly, expression levels of antioxidant enzymes are extremely low in the pancreatic β-cells compared with other tissues. This raises the possibility that ROS produced incidental to blood glucose elevation could serve signaling functions in glucose-induced insulin secretion from the pancreatic β-cells.

TRPM2 is a Ca^{2+}-permeable cation channel and expressed in various tissues including brain, spleen, kidney and pancreatic β-cells where TRPM2 is surrounded by body temperatures. We have previously found an activation mechanism of TRPM2 whereby its heat-evoked response is dynamically elevated by H_2O_2, termed "sensitization". The regulatory mechanism by H_2O_2 is thought to enable TRPM2 to activate at body temperature by lowering temperature threshold from supraphysiological to physiological levels.

In WT pancreatic β-cells, H_2O_2 enhanced not only heat-evoked $[Ca^{2+}]_i$-increase but also glucose-induced $[Ca^{2+}]_i$-oscillation at 37°C. However, these effects of H_2O_2 were not observed in TRPM2KO cells. Because an oscillatory increase in $[Ca^{2+}]_i$ is a key feature in glucose-induced insulin secretion from the pancreatic β-cells, we will show the difference in insulin secretion between WT and TRPM2KO pancreatic islets. No COI.

2P-007

Involvement of TRPM6/TRPM7 heterotetramers in maternal-fetal calcium transport

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Serum Ca^{2+} level in fetus should be finely maintained in order to sustain the development and function of the brain, muscle and other tissues. In parallel, during the last trimester, fetus develops bones to survive after birth. However, the molecular components as well as the mechanism of regulation of the maternal-fetal Ca^{2+} transport have not been fully understood. In this study, we identified TRPM6, a Ca^{2+} permeable cation channel as a candidate apical Ca^{2+} entry pathway for maternal-fetal Ca^{2+} transport. TRPM6 mRNA was densely localized in the intraplacental yolk sac, which was reported to be important for maternal-fetal Ca^{2+} transport in rodents, and its mRNA level significantly increased during the last 4 days of pregnancy, which coincided with fetal bone formation in mice. Moreover, in primary mouse trophoblasts, using Ca^{2+}-imaging and patch-clamp methods we observed an intracellular Ca^{2+} increase and current with charecteristics of TRPM6/TRPM7 heterotetramer. These currents were also observed in human TRPM6c-transfected HEK293 cells which express human TRPM7 endogenously. However, these currents were not observed in mock- or mouse TRPM6-transfected HEK293 cells. These results suggest that TRPM6 is involved in the maternal-fetal Ca^{2+} transport, forming tetramers with TRPM7 in placental trophoblasts. No COI.

2P-008

TRPM7 is highly expressed in the odontoblasts

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Transient receptor potential (TRP) ion channel family is well known to play a role in a sensor such as temperature, osmotic pressure, and redox status. Among TRP channel family, TRPM7 has a unique structure organization that contains a TRP channel domain with 6 transmembrane segments fused to an atypical serine-threonine kinase domain at its C terminus. Genetic deletion of TRPM7 in model systems demonstrates that this channel is critical for cellular growth and embryonic development. In this study, we found that TRPM7 is highly expressed in odontoblasts in the dental pulp by in situ hybridization of mouse embryo. Quantitative real-time PCR analysis revealed that expression of TRPM7 in the tooth was remarkably higher than any other tissue of adult mouse. We also confirmed that TRPM7 protein is expressed in odontoblasts by immunohistochemistry. To investigate the physical function of TRPM7 in odontoblasts, we examined TRPM7 channel activities using rat odontoblast cell line. These results suggested that higher expression of TRPM7 play as an important role in odontoblasts. No COI.

2P-009

TRPM7 kinase activity is not necessary for oxidative stress-induced current inhibition.

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TRPM7 is a Ca^{2+}/Mg^{2+} permeable non-selective cation channel which contains a kinase domain at its carboxyl terminal. We previously demonstrated that TRPM7 current was inhibited by oxidative stress. In the present study, we explored the mechanisms for redox sensing by introducing mutations in TRPM7. Tetracycline-inducible HEK cell lines for stably expressing wild type (WT), kinase domain deleted (deltaK), M1596A (MA), and K1645R (KR) murine TRPM7 were established. Whole-cell recordings in WT revealed that TRPM7 current was inhibited by oxidative stress induced by hydrogen peroxide (H_2O_2, 500 μM) in a $[Mg^{2+}]_i$ dependent manner (2.8±2.7%, 26.3±1.4% or 82.0±5.7% inhibition under 0, 7.9 or 232 μM $[Mg^{2+}]_i$ conditions, respectively). H_2O_2 inhibited 24.3±4.2% of deltaK current in the absence of intracellular Mg^{2+}. However, Mg^{2+} dependent inhibition couldn't be examined, since deltaK current exhibited rundown with Mg^{2+}-containing intracellular solutions. M1596 is a corresponding residue of M1755 of TRPM6 which has been reported to be oxidized by H_2O_2 and involved in the current inhibition. While mutation of M1755 to alanine made TRPM6 resistant to H_2O_2, TRPM7 MA mutant remained sensitive to H_2O_2. Moreover kinase inactive KR mutant exhibited the current inhibition by H_2O_2 similar to WT (12.0±1.5%, 50.5±10.2% or 81.8±2.3% inhibition under 0, 7.9 or 232 μM $[Mg^{2+}]_i$ conditions, respectively). It is concluded that M1596 and K1645 are not indispensable for inhibiting TRPM7 current in response to oxidative stress. No COI.

2P-010

Investigation the effect of palmitoylation on TRP channels

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S-palmitoylation is a common reversible post-translational modification of many proteins. It has been shown to control protein surface expression, spatial organization, protein-protein interactions, and functional activity. Dysregulation of S-palmitoylation has been reported to be associated with a large number of diseases, such as schizophrenia, mental retardation and cancer. Recently, some ion channels, such as AMPA, NMDA-type glutamate receptors, potassium and sodium channels have been proven to be regulated and influenced by palmitoylation. TRP channels are non-selective cation channels. They are expressed in most tissues and cell types, are activated by a wide range of stimuli and play important roles. Many studies have revealed that the TRP channels are important for sensations such as taste, pain, and temperature, both in the peripheral and central nervous systems. Defects and abnormalities in TRP channels are associated with many diseases such as pain disorder, polycystic kidney disease and cancer. This study is designed to clarify whether and how TRP channels are regulated by palmitoylation. In addition, this study also examines whether the abnormal palmitoylation state of TRP channels are associated with any diseases in our body. No COI.

2P-011

Analysis of the propofol-induced mammal TRP channel activation.

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Propofol (2,6-diisopropylphenol) is one of the intravenous anesthetic drugs and commonly used in clinical field for general anesthesia. Additionally, it causes an intense pain upon injection. To understand the mechanism of propofol-induced pain, we focused on transient receptor potential (TRP) receptors vanilloid 1 (TRPV1) and ankyrin 1 (TRPA1), which are important ion channels for pain sensation. We performed calcium-imagings and whole-cell patch-clamp recordings by using mouse dorsal root ganglion (DRG) cells prepared from wild-type (Wt) and knockout (KO) mice. In the calcium imaging study, there was no difference in percentage of the propofol-responsive DRG cells isolated from Wt, TRPV1KO and TRPA1KO mice. Propofol-induced calcium influx were still observed in the DRG cells from TRPV1/A1 double KO mice. Picrotoxin, an antagonist of $GABA_A$ receptors, completely inhibited the propofol-induced calcium influx observed in the DRG cells from TRPV1/A1 double KO mice. In the whole-cell patch-clamp study, propofol caused inward currents in the DRG cells from TRPV1/A1 double KO mice and they also responded to the application of GABA. The results indicate that propofol might cause calcium influx into DRG cells partially through $GABA_A$ receptors. No COI.

2P-012

Inhibition of the compound action potentials of frog sciatic nerves by aroma-oil compounds having various effects including TRP activation

Ohtsubo, Sena; Matsushita, Akitomo; Fujita, Tsugumi; Jiang, Chang-Yu; Xu, Zhi-Hao; Kumamoto, Eiichi(*Dept. Physiol., Saga Med. Sch., Saga, Japan*)

We have previously reported that capsaicin, menthol and allyl isothiocyanate, which activate TRPV1, TRPM8 and TRPA1, respectively, inhibit fast-conducting compound action potentials (CAPs) recorded from the frog sciatic nerve without TRP activation. The present study examined how aroma-oil compounds having various actions including TRP activation affect frog sciatic nerve CAPs by using the air-gap method. Citral, which activated TRPV1, TRPM8, TRPA1 and TRPV3, attenuated CAP peak amplitudes with the half-maximal inhibitory concentration (IC_{50}) value of 0.48 mM; this action was resistant to a non-selective TRP antagonist ruthenium red. Camphor, a TRPV1 and TRPV3 agonist, also reduced CAP peak amplitudes (by 30 % at 5 mM) in a manner insensitive to ruthenium red. Lavender-oil compounds, linalyl acetate and linalool, reduced CAP peak amplitudes with the IC_{50} values of 0.49 and 1.65 mM, respectively. Citronellal, L-bornyl acetate and rose oxide reduced CAP peak amplitudes with the IC_{50} values of 0.50, 0.65 and 2.0 mM, respectively, while myrcene at a high concentration such as 5 mM hardly reduced CAP peak amplitudes. An efficacy sequence of the aroma-oil compounds for the CAP inhibitions was aldehydes (citral, citronellal) > esters (linaryl acetate, L-bornyl acetate) > alcohols (linalool) > oxides (rose oxide) >> hydrocarbons (myrcene). It is suggested that aroma-oil compounds inhibit nerve conduction in a manner specific to their chemical structures without TRP activation. No COI.

2P-013

Interaction among crude medicines contained in daikencyuto in frog sciatic nerve compound action potential inhibition

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We have previously reported that traditional Japanese medicines have an ability to reduce the peak amplitude of frog fast-conducting compound action potential (CAP) and that daikencyuto (DTK) is the most effective in inhibiting CAPs among various kinds of the medicine. DKT is composed of three kinds of crude medicine, ginseng, Japanese pepper and processed ginger. The present study was undertaken to know whether their crude medicines inhibit CAPs and if so whether there is an interaction among their inhibitory actions. The experiments were performed by applying the air-gap method to the frog sciatic nerve. When each of the crude medicines at 2 mg/ml was tested, Japanese pepper and processed ginger reduced CAP peak amplitude by 70 % and 30 %, respectively, while ginseng radix hardly affected CAPs. The inhibitory action of Japanese pepper had a half-maximal inhibitory concentration (IC_{50}) value of 0.77 mg/ml. Ginseng radix (0.6 mg/ml), which had no effect on CAPs, unaffected the inhibitory action on CAPs of processed ginger in a range of 0.2 to 2 mg/ml, but had a tendency to enhance the inhibition of CAPs by low (< 0.5 mg/ml) but not high (> 0.5 mg/ml) concentrations of Japanese pepper. Processed ginger (1 mg/ml) also had a tendency to increase CAP inhibition by Japanese pepper at low but not high concentrations. It is suggested that there is an interaction in nerve conduction inhibition among crude medicines contained in DKT in a manner dependent on their concentrations. No COI.

2P-014

Molecular basis of voltage-dependent inactivation of TRPP3 channels

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We previously found that transient receptor potential polycystin 3 (TRPP3)-expressing HEK293T cells exhibit distinct tail currents induced by repolarization after depolarization, suggesting that TRPP3 channels at an open state can transit to an inactivated state in response to membrane depolarization. However it has not been clarified molecular basis of this transition. In some Kv channels, N-type and C-type inactivation mechanisms have been reported: the pore is occluded by movement of intracellular N-terminus in the N-type mechanism, and the peripheral structure of a selective filter is altered in the C-type mechanism. To investigate the inactivation mechanism of TRPP3 channels, we constructed several mutants: ΔN, Δ2-90 amino acid residues; R534A; R534K; R534Q; 4A, NANR(531-534)-AAAA; 3A, NANR(531-534)-AAAR). In the ΔN mutant, the tail currents were still observed by repolarization after depolarization. On the other hand, no significant tail currents were observed in the R534A, R534Q and 4A mutants, while the currents were retained in the R534K and 3A mutants. These results suggest that the intracellular N-terminus is not involved in the TRPP3 inactivation, and that positive charge of the outer pore residue R534 is essential for the voltage-dependent inactivation of TRPP3 channels. No COI.

2P-015

Molecular basis for the species difference of TRPV1 channel property related to temperature adaptation in *Xenopus* species

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Our aim is to clarify the molecular mechanism of temperature adaptation especially focusing on evolutionary changes of temperature sensation among different species. For this purpose, we focused on two species of clawed frog (*Xenopus tropicalis* and *Xenopus laevis*). The optimal temperature range of *X. laevis* is 16–22°C, while that of *X. tropicalis* is 24–28°C. We first compared the thermal behavioral responses and found that *X. laevis* is much more sensitive to heat stimulation than *X. tropicalis*. Thus, we cloned TRPV1, which serves as a heat receptor in a wide variety of vertebrates, from both species and compared the channel properties. TRPV1 channel property differed between the two species in that *X. laevis* TRPV1 exhibited almost full activity from the first heat stimulation, while *X. tropicalis* TRPV1 exhibited only a partial activity in the first heat stimulation and the activity gradually increased with repeated heat stimulation. By characterizing TRPV1 chimeras and point mutant channels, we found that the three amino acid substitutions in TRPV1 between *X. laevis* and *X. tropicalis* are involved in the species differences of channel property. In conclusion, we identified the functional change of a temperature receptor related to temperature adaptation and also clarified the molecular basis for the differences of the temperature receptor function. No COI.

2P-016

Alanin scan analysis in the pore region of TRPV1 channel

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TRP channels can sense and are activated by diverse environmental stimuli such as changes in temperature, membrane voltage, mechanical force and internal or external chemical ligands. However it is not known that why TRP channels could be activated by such various stimuli. We would like to reveal the molecular basis for the TRP channel gating. Are there any common mechanisms to TRP channel gating? TRP channels appear to have many bulky amino acid residues in pore regions compare to Kv channels. We studied the role of the bulky residues in TRPV1 channel function. We made series of alanin replacement mutants in S5 and S6 of TRPV1. TRPV1 and their mutants were expressed in HEK293 cells and patch clamp recordings were performed to evaluate the change of gating properties. In addition, we examined the sensitivity of mutant channels to capsaicin. No COI.

2P-017

Activation of TRPV2 may inhibit the differentiation of mouse brown adipocytes

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Transient receptor potential vanilloid 2 (TRPV2) is a Ca^{2+}-permeable non-selective cation channel, which has been reported to be sensitive to temperature, mechanical force and some chemicals. TRPV2 plays vital roles in the regulation of various cellular functions. However, the molecular identity and function of TRPV2 remains unexplored in mouse brown adipocytes. This study aimed to clarify the expression of TRPV2 and its function in brown adipocytes. We found that TRPV2 mRNA and protein are expressed in brown adipocytes. Electrophysiological results demonstrated that functional TRPV2 is predominantly observed in brown adipocytes. Moreover, the expression of TRPV2 was dramatically increased during the differentiation of brown adipocytes. Non-selective TRPV2 agonists, 2-Aminoethoxydiphenyl borate (2APB) and lysophosphatidylcholine (LPC) inhibited the differentiation of brown adipocytes with a dose-dependent manner and the inhibition was rescued by a TRPV2 selective antagonist, SKF96365. In addition, mechanical stimulation, which activates TRPV2, also strength-dependently inhibited the differentiation of brown adipocytes and the effect was recovered by SKF96365. Thus we conclude that TRPV2 might be involved in the regulation of brown adipocyte differentiation. No COI.

2P-018

Transient receptor potential vanilloid 4 (TRPV4) as a mechanotransducer in the mouse esophagus

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Mechanical sensation from the esophagus is conveyed to the CNS via vagal afferents that have special terminal structures in the esophageal wall called intraganglionic laminar endings (IGLEs) and express purinergic receptors. The exact mechanism that mediates this mechanotransduction is still largely unknown. Transient receptor potential channel vanilloid 4 (TRPV4) can detect various stimuli such as warm temperature, stretch and some chemicals. Its expression and function in the esophagus was not studied. Here, we show structural and functional TRPV4 expression in mouse esophagus and its involvement in ATP release.

TRPV4 mRNA and protein were detected in esophageal keratinocytes. Several TRPV4 activators, including stretch stimuli, have increased cytosolic Ca^{2+} concentrations in WT, but not in TRPV4KO keratinocytes. GSK (TRPV4 agonist) and heat stimulus evoked TRPV4-like current responses in WT, but not in TRPV4KO cells. The mRNA of a newly identified ATP transporter, VNUT and its protein were also detected in WT keratinocytes. TRPV4 activators (GSK and heat stimulus) increased ATP release from WT, but not from TRPV4KO cells. **Conclusion:** TRPV4 mediates esophageal mechanotransduction via ATP release that possibly activates purinergic receptors on IGLEs. No COI.

2P-019

Involvement of L-type Ca2+channels in postnatal neurogenesis of the rodent adult hippocampal derived stem cells

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Dentate gyrus of hippocampus is a region of well documentated neurogenesis. Neurogenesis is presumed to be regulated by L-type Ca2+ channels (LTCCs), via the mitogenic and survival signaling pathways. Here, we tested if LTCCs are involved in neurogenesis using PZ5, a neural stem cell line. We demonstrated that the proliferation rate of undifferentiated PZ5 was affected neither by the general LTCC agonist, BayK 8644, Cav1.2/1.3 specific agonist, FPL64176, nor by the LTCC antagonist, nimodipine, as compared to the control. Only Cav1.2 and Cav1.3 LTCCs subsets were presence at undifferentiated PZ5 cells. Retinoic acid induced differentiation was carried for 12-days in respective LTCC agonists and antagonist. BayK and FPL significantly increased the fraction of neuronal population (βIII-tubulin+/MAP2+), while nimodipine retarded it. We also noted an inverse pattern of oligodendrocytes fraction as compared to the neuronal fraction. The astrocytes fraction remained the same in all groups. The number of NeuN+/βIII-tubulin+, appeared significantly higher in FPL and BayK as compared to nimodipine, suggesting that early maturation in LTCC active cells. These neurons were able to invoke action potential, suggesting its functionality. It is suggested that Cav1.2 and Cav1.3 promote PZ5 differentiation into neuronal fraction, at the expense of the oligodendrocytic population. No COI.

2P-020

The lobe-specific interaction of calmodulin with the cardiac CaV1.2 channel

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To assess the contribution of lobes in the calmodulin (CaM) interaction with cardiac Cav1.2 channel, CaM was separated into 2 parts at the middle of the molecule: N-lobe and C-lobe (N-CaM and C-CaM). We examined the binding of N-CaM and C-CaM with the fragments of the putative CaM binding domains (N-terminus and CT1 in the Cav1.2 channel). We found that both N-CaM and C-CaM bound to N terminus and CT1 in a Ca^{2+}-dependent manner, while mutation of Ca^{2+} binding sites in each lobe abolished Ca^{2+}-dependence of binding. Interestingly, although N terminus just bound 1 molecule of lobe, 1 molecule of CT1 bound 3 molecules of C-CaM and 1 molecule of N-CaM, respectively. Combined with our previous studies which show that CaM interacts with Cav1.2 in 2:1 manner, our present study suggests that that one molecule of CaM may bind with both lobes bound to C-terminal of CaV1.2, the other molecule of CaM binds with only a single C-or N-lobe bound to C-terminal of CaV1.2 and another N-or C-lobe bound to N-terminal of CaV1.2. No COI.

2P-021

The role of nucleotides in regulation of cardiac Cav1.2 channel

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Our previous studies suggest that MgATP coordinates with calmodulin (CaM) in maintenance of cardiac Cav1.2 channel activity in the inside-out patches via direct interaction with channel. Since the similarity of structures among nucleotides, we speculate that other nucleotides can also induce Cav1.2 channel activity in the presence of CaM. To examine this hypothesis, we recorded the single Ca^{2+} channel current in isolated guinea pig ventricular myocytes using patch clamp technique. After the patches excised from cell membrane, 1μM CaM together with nucleotides (ATP, GTP, CTP and UTP) were applied, we found, although CaM alone had minimal effect on Cav1.2 activity, it significantly induced channel activity in the presence of either ATP, GTP, CTP or UTP. The working efficiency of nucleotides is ATP>GTP>UTP=CTP. This result suggests that the direct dynamic interaction of nucleotides and CaM are required for basic Cav1.2 activity. No COI.

2P-022

RIM proteins suppress voltage dependent inactivation (VDI) through C-terminus of Voltage-dependent calcium channel(VDCC)-α1A subunit.

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Ca^{2+} influx into the presynaptic active zones through the Voltage-dependent calcium channels (VDCCs) is important for neurotransmitter release. Our group has been demonstrated that interaction between VDCC-β subunits and RIM proteins, presynaptic active zone scaffolding proteins, enhances the neurotransmitter release via suppressing of voltage dependent inactivation (VDI) of VDCCs, and anchoring synaptic vesicles in the vicinity of VDCCs (Kiyonaka et al. 2007, Uriu et al. 2010). Recently, Sudhof's group reported that PDZ domain of RIM proteins directly interacts with the C-terminus of α1A and α1B, which are pore-forming VDCC subunits, to contribute the localization of VDCCs to presynapse (Kaeser et al. 2012). However, this contribution of the interaction between the C-terminus of α1A or α1B and RIM proteins on VDI of VDCCs is controversial. Here, in addition to the VDI suppression by β-subunits, we extend our findings by observation that RIM2α significantly enhances the suppression of the VDI in long α1A splicing isoform, which possess PDZ binding motif, compared to the VDI in short isoform of α1A, which lacks PDZ binding motif. This enhancement was observed only in RIM2α expressing cells but not in RIM3γ expressing cells, which contains VDCC-β subunits interacting region but not PDZ domain. Thus, functional coupling among RIM protein and β-subunit and C-terminus of α1A may enhances neurotransmitter release via sustaining Ca^{2+} influx through the suppression of VDI. No COI.

2P-023

Effects of amino-terminal disease-associated mutations on the RyR1 channel

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Type 1 ryanodine receptor (RyR1) is a Ca^{2+} release channel in the sarcoplasmic reticulum and plays a pivotal role in excitation-contraction coupling in skeletal muscle. RyR1 is the major target for muscle diseases, e.g., malignant hyperthermia (MH) and central core disease (CCD). To date, over 200 mutations have been identified in the RyR1 gene of MH and CCD patients. It is widely believed that MH and CCD mutations cause hyperactivation of the Ca^{2+}-induced Ca^{2+} release (CICR), resulting in abnormal Ca^{2+} homeostasis in skeletal muscle. CICR shows biphasic Ca^{2+} dependence, thus the activity can be determined by three parameters: sensitivity to activating Ca^{2+}, sensitivity to inactivating Ca^{2+}, and the gain (i.e., peak activity). However, it remains unclear how the disease-associated mutations affects these parameters. In this study, we expressed several RyR1 channels carrying different MH/CCD mutations at amino-terminal region in HEK cells and tested their CICR by live-cell Ca^{2+} imaging and [^{3}H]ryanodine binding. Our results suggest that the amino-terminal disease-associated mutations divergently affects the parameters of CICR depending on the sites for mutation. The underlying molecular mechanism will be discussed. No COI.

2P-024

Ca^{2+} release properties of type 2 ryanodine receptor mutants associated with ventricular arrhythmia and sudden death

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Type 2 ryanodine receptor (RyR2) is a Ca^{2+} release channel in the sarcoplasmic reticulum and plays a pivotal role in excitation-contraction coupling in heart. RyR2 is the major target for arrhythmogenic diseases, i.e., catecholaminergic polymorphic ventricular tachycardia (CPVT) and arrhythmogenic right ventricular cardiomyopathy (ARVC). To date, over 150 mutations have been identified in the RyR2 gene of CPVT and ARVC patients. Although it is widely believed that CPVT and ARVC mutations cause hyperactivation of the Ca^{2+}-induced Ca^{2+} release (CICR), the reasons for the difference between the two diseases are not well known. CICR shows biphasic Ca^{2+} dependence against cytoplasmic Ca^{2+}, thus the activity can be determined by three parameters: sensitivity to activating Ca^{2+}, sensitivity to inactivating Ca^{2+}, and the gain (i.e., peak activity). In addition, CICR is also regulated by luminal Ca^{2+}; high luminal Ca^{2+} activates CICR. It remains unclear how the disease-associated mutations affect these parameters. In this study, we expressed RyR2 channels carrying several CPVT and ARVC mutations in HEK cells and evaluated their Ca^{2+} release properties by live-cell Ca^{2+} imaging and [^{3}H]ryanodine binding. Our results suggest that the disease-associated mutations divergently affects the parameters of CICR depending on the sites for mutation. The difference between the CPVT and the ARVC mutations will be discussed. No COI.

2P-025

A ventricular cell model incorpolating new Ca2+-induced Ca2+-release

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A ventricular cell model incorpolating new Ca2+-induced Ca2+-releaseDept. of Life Sciences, Graduate School of Life Sciences, Ritsumeikan Univ.Hiraku Memida, Akinori Noma.The graded Ca2+ release via RyR at the terminal cisterna of sarcoplasmic reticulum (SR) is dependent on the extent of activation of the L-type Ca2+ channel current (ICaL). This local control of Ca2+ release is still not reproduced in most of cardiac cell models developed on the desk-top computer level. This is because the Ca2+-induced Ca2+-release occurs in an all-or-none manner when a single common pool is assumed for both ICaL and RyR Ca2+ fluxes. We aimed at improving our ventricular cell model by adopting the tight coupled LCC-RyR model (CaRU) based on local control theory (Hinch et al. 2004). The original model of Hinch, however, failed to reconstruct the spontaneous Ca2+ release from SR, which has been well established in experimental studies. We have made an attempt to reconcile this difficulty by assuming a local Ca2+ accumulation near the Ca2+ releasing site. The ionic currents were also improved according to new experimental data. We will discuss relevance of our new animal model in studying mechanisms of physiological functions of the ventricular myocytes. No COI.

Poster Presentations
Heart, Circulation (2)

2P-026

Subcellular Na Channel Remodeling in Ischemic Border Zone as a Mechanism of Reentrant Ventricular Tachyarrhythmia: In Silico Study

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Previous experimental studies have reported that in the ischemic border zone (IBZ) of the infarcted ventricular myocardium, the sodium (Na) channels were redistributed within a myocyte, and that the subcellular Na channel remodeling contributed to conduction slowing. However, the impact of intracellular Na channel remodeling on the arrhythmogenicity in ischemic myocardium is unclear. We recently reported in simulations that the subcellular Na channel remodeling observed in IBZ enhanced the sensitivity of the ischemic myocytes to Na channel blockers in physiological relevant myofiber model incorporating the electric field mechanism (taking into account the intercellular cleft potentials). As a result, Na channel blockade tended to cause conduction block in the IBZ than in the non-ischemic zone (NZ). Here, we extend the simulations into the investigation of effects of the Na channel blockade in a ring-shaped myocardial model consisting of NZ and IBZ on premature ventricular contractions. Then we found that unidirectional conduction block by Na channel blockade was more likely to occur at the border between IBZ and NZ. Furthermore, we found that such unidirectional conduction block was involved in the initiation of reentrant tachyarrhythmia. Ischemia-induced subcellular Na channel remodeling might be partly responsible for the arrhythmogenic mechanism of class-I antiarrhythmic drugs in patients with old myocardial infarction. No COI.

2P-027

Inhibitory effects of verapamil on bidirectional ventricular tachycardia in anesthetized rats

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Purpose: The purpose of present study is to investigate the effect of caffeine on the occurrence of BVT and inhibitory effect of Ca^{2+} channel blocker, verapamil. **Methods :** Adult female Wister rats were anesthetized with halothane gas (1.2%), and ECG was recorded with a Biopac system MP-150. Protocol 1: We injected continuously caffeine (0.5mg/kg/min) through the right femoral vein, and then added epinephrine (10μg/kg/min) at 15 min after the start of caffeine. We observed evoked BVT and evaluated the effect of drugs using ECG measurements of RR, PR, QTc, JTp/JT and Tp-e/QT. Protocol 2: We injected verapamil (0.25 mg/kg) at five minutes before the caffeine administration, and observed ECG continuously. **Results:** In 12 animals of protocol 1, BVT was observed in ten animals. BVT was not eboked in any of 8 animals of protocol 2. The administration of caffeine elicited prolongation of QTc, reduced Tp-e/QT and increased JTp/JT. However, the pretreatment of verapamil inhibited the prolongation of QTc and JTp. **Discussion:** It has been recognized that BVT is caused by delayed after depolarization and intracellular abnormal Ca^{2+} handling. In the present study, BVT was elicited by caffeine which increases Ca^{2+} release from the sarcoplasmic reticulum, on the other hand, BVT was inhibited by the voltage-dependent Ca^{2+} channel blocker. We conclude that the influx of Ca^{2+} is one of pathogenic mechanism of BVT, and an abnormality of Ca^{2+} handling is represented in the repolarization characteristic (JTp). No COI.

2P-028

Dynamical mechanisms of early afterdepolarizations in long QT syndromes: insights from bifurcation analysis of mathematical models for human ventricular myocytes

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We theoretically investigated dynamical mechanisms of early afterdepolarizations (EADs) in long QT syndrome (LQTS) by stability and bifurcation analyses, as well as numerical simulations, using mathematical models of single human ventricular myocytes (Kurata et al, Biophys J, 2005). LQT1-3 model cells were developed by reducing the slow (IKs) or rapid (IKr) component of the delayed-rectifier K channel current (IK) (LQT1, 2) or incorporating a non-inactivating component of the Na channel current (INa) (LQT3). EADs were induced by enhancement of ICaL in the LQTS model cells. Their stability and bifurcations during increases of the L-type Ca channel current (ICaL) or IK reductions were determined by bifurcation analyses to construct bifurcation diagrams: equilibrium points (EP), limit cycles (LCs), and their stability were determined as functions of bifurcation parameters. Decreasing IK, increasing ICaL or increasing non-inactivating INa leads to EAD generation in the vicinity of Hopf bifurcation (HB) points. Further inhibition of IK yielded stable EP at depolarized potentials and quiescence. The threshold of EAD generation during IK decreases or ICaL increases was lower in the LQTS model cells than in the normal cell, which was due to the shift in HB points. EADs could be regarded as transient LCs induced via HB (EP destabilization) at depolarized potentials during the slow activation of IKs. No COI.

2P-029

Channel dysfunction in ryanodine receptor mutants evoking fatal arrhythmia

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Mutations of the human cardiac ryanodine receptor gene (*hRyR2*) mapped to 1q42-q43 are associated with catecholaminergic polymorphic ventricular tachycardia (CPVT). CPVT is a severe genetic arrhythmogenic disorder characterized by exercise- and stress- induced ventricular tachycardia manifesting as syncope and sudden death. Full length of mouse RyR2 cDNA (accession: NM-023868) cloned by Kurebayasi and Murayama, which is different from that of the Chen's group (accession: AF295105), was inserted into a tetracycline-inducible mammalian expression vector. Each mutation was introduced using the Quick Change site-directed mutagenesis kit. The stable inducible HEK293 cell lines were generated for the Wild-type or mutants. Here we examined how CPVT-associated RyR2 mutants are dysfuctioned. First, the single-channel currents from purified RyR2 proteins were activated by CPVT mutations. Second, ^{3} H-ryanodine binding from the microsomes of HEK293 expressed with RyR2 showed that the RyR2 channel activity was increased by CPVT mutations. Third, intracellular Ca^{2+} oscillations in HEK293 cells measured by single-cell Ca^{2+} imaging using fura-2 occurred more frequently in CPVT mutants than in wild-type. Thus, our CPVT mutants consistently exhibited a gain of function activities, suggesting that Ca^{2+} leak via RyR2 from cardiac sarcoplasmic reticulum is enhanced. No COI.

2P-030

Safety Mechanism of hERG-Targeting Class III Anti-arrhythmic Agents

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Potassium channels encoded by human *ether-a-go-go-related gene* (hERG) underlie the rapidly activating component of the delayed rectifying potassium current (I_{Kr}) in heart and play an important role in terminating the cardiac action potential (AP). Recently, we have experimentally demonstrated a dual effect of some anti-arrhythmic agents, such as nifekalant, on hERG channel. Besides blocking hERG channel, these compounds can facilitate its activation. To assess the clinical relevance of hERG channel modulations by compounds, we conducted simulations of cardiac AP in a Priebe-Beuckelmann model with hERG channel block and facilitation, using our experimental data of nifekalant. We found that the hERG channel block prolonged action potential duration (APD) in the block conditions both with and without facilitation. In addition, the refractory period in both conditions increased from control condition so that the ectopic cell excitation is suppressed. As in our simulation, we could observe an early afterdepolarization (EAD) when both I_{Kr} and the slowly activating component of the delayed rectifying potassium current (I_{Ks}) were blocked. Importantly, facilitation mechanism prevents hazardous prolongation of APD and the induction of EAD by accelerating the repolarization rate via an increase in I_{Kr} during prolonged phase 3. Therefore, anti-arrhythmic agents that has both block and facilitation effects on hERG channel may have a lower risk for inducing EADs and triggered activity and thus be more suitable for the treatment of arrhythmias than pure hERG blocker. No COI.

2P-031

Modeling fluid exchange at the capillary, tissue and lymphatic capillary

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Fluid exchange between blood and interstitial fluid at the capillary, and flow at the lymphatic capillary is one of the most indispensable issues for the homeostasis of the internal milieu. However, complex interdependency of the pressures involved in determination of the fluid exchange makes it difficult to tackle this issue without mathematical approach. In the present study, we developed a capillary model, which allowed us to clarify mechanisms regulating the interstitial fluid volume. The capillary is assumed to be a cylinder. The transcapillary fluid flux is calculated from the fluid pressure and colloid pressure difference between plasma and interstitial fluid. Well known 3 safety factors against edema, 1. Low tissue compliance in negative pressure range, 2. Increased lymphatic flow and 3. Protein washout by the lymph, are incorporated into the model. When the interstitial fluid pressure was within a physiological range in our model, an increase in blood pressure induced an interstitial fluid volume increase, which in turn reduced negative tissue pressure to prevent edema. The lymph flow alleviates the edema by both carrying fluid off from the tissue, and decreasing the interstitial fluid colloid pressure. From time course of the effects, it was found that the tissue compliance and the lymph flow act as a long-term and a short-term regulator of the interstitial fluid volume, respectively. Mathematical modeling of the fluid exchange at the capillary enabled us to calculate fluid convection quantitatively and estimate functional impacts of the tissue compliance and lymph flow. No COI.

2P-032

Effects of period of sinusoidal leg exercises on brachial and middle cerebral artery blood flows

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To explore the control of upper non-exercising limb and cerebral circulation during leg exercise, we measured the dynamics of brachial artery (BA) and middle cerebral artery (MCA) blood flow (BF) in response to sinusoidal work rate (WR) protocols of varying periods. Seven healthy young male subjects performed upright leg ergometer exercise with a constant WR for 30 min followed by three different periods of sinusoidal WR exercises (number of the repetitions); 6-min (3), 4-min (4), and 2-min (7) where WR fluctuated in the range of 20 W to peak WR (60 % of peak VO2). During the protocols, we measured pulmonary gas exchange, heart rate (HR), mean arterial blood pressure and stroke volume, blood velocity (BV) and cross sectional area of BA, BV of MCA, and forearm and forehead skin BF and sweating rate (SR). The variableswere fitted as $y(t)=M+A*\sin((2\pi/T)*t-\theta)$, where t: time, A/M: relative amplitude, T: period, θ: phase shift. Almost variables traced the sine wave adequately. The phase shifts and A/M of BV in MCA (phase (°): 6-min, 44+/-13, 4-min, 69+/-20, 2-min, 112+/-33; A/M (%): 6-min, 4.5+/-1.5; 4-min, 4.8+/-1.9; 2-min, 3.3+/-0.7, mean+/-SD) were similar with variables regarding O2 delivery (e.g. VO2, HR). Contrarily, the response of BF in BA displayed an anti-phase and a constant A/M (approximately 28 %) regardless of the period. Non-active limb circulatory control is differential to the main coordination of O2-delivery to active muscle. No COI.

2P-033

Spontaneous and nerve-evoked constrictions of submucosal venules in rat stomach

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Objective: Venules of the bladder and distal colon exhibit rhythmic spontaneous constrictions that are likely to prevent the stagnation of blood even during their distension. Here we examined the spontaneous and nerve-evoked activity of rat stomach microvasculature. **Methods:** Changes in diameter of microvasculature were monitored by video imaging system, while intracellular Ca^{2+} in venular smooth muscle cells (VSMCs) were visualised using fluo-8 fluorescence Ca^{2+} imaging in the submucosal preparations of rat stomach. **Results:** VSMCs showed synchronised spontaneous Ca^{2+} transients and associated venular constrictions. L-type Ca^{2+} channel blocker (1 μM nifedipine) disrupted the synchrony of Ca^{2+} transients and abolished spontaneous constrictions. The inhibitors of sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (10 μM CPA), IP_3 receptors (100 μM 2-APB) and Ca^{2+}-activated Cl^- channels (100 μM niflumic acid) abolished spontaneous constrictions. Transmural nerve stimulation immediately triggered purinergic/α-adrenergic arteriole constriction while evoking long-lasting α-adrenergic constriction of venules (1 μM phentolamine). All these responses were blocked by sympathetic nerve transmitter depletion (10 μM guanethidine). The activation of primary afferent nerves (300 nM capsaicin) dilated venules by releasing CGRP. **Conclusion:** Spontaneous constrictions in submucosal venules of the rat stomach appear to primarily depend on Ca^{2+} release from SR. Synchrony of Ca^{2+} transients amongst VSMCs may require electrical coupling through 'regenerative' L-type Ca^{2+} channel activation. No COI.

2P-034

Characteristics of spontaneous transient hyperpolarizations in vascular endothelial cells

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It has been reported that the vascular endothelial cells always release small amount of nitric oxide (NO) which is playing an important role in the basic regulation of the total peripheral resistance. As the cellular mechanisms under such a spontaneous release of NO have not been studied, we examined endothelial spontaneous activities using the endothelial layer preparations acutely dispersed from the guinea-pig mesenteric arteries. In the Ca^{2+}-imaging of these preparations, we have found that spontaneous transient increases in the $[Ca^{2+}]_i$ occur in individual cells. We then tried to examine whether such an increase in $[Ca^{2+}]_i$ induced any electrical events in the membrane. So a patch electrode was applied to a cell within the preparation and the membrane potential was observed under the current clamp condition. The membrane potential was not constant but transient hyperpolarizations occurred spontaneously. These activities were blocked by either charybdotoxin (10^{-7} M), apamin (10^{-7} M) or removal of external Ca^{2+}. Ca^{2+}-activated K^+-channels sensitive to both charybdotoxin and apamin seemed to be involved in the transient hyperpolarizations. Ca^{2+} influx might activate these channels directly or be necessary to refill the intracellular Ca^{2+} stores, the released Ca^{2+} from which might activate these channels. No COI.

2P-035

Implications of adenosine and muscarinic acetylcholine receptors on bradycardia during systemic episodic hypoxia in rats

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Pronounced bradycardia is frequently observed in patients with obstructive sleep apnea, and has been recapitulated in rodents subjected to systemic episodic hypoxia. To elucidate its mechanism, male Wistar rats previously implanted with telemetry devices to monitor arterial blood pressure (TRM54P, TR-Millar) and heart rate were exposed to acute episodic hypoxia (90-s room air / 90-s N2; 5% O2 at nadir) in a sealed chamber. Acute episodic hypoxia elicited a marked pressor response, followed by significant bradycardia, as previously reported. This was not mediated solely by a baroreflex, as α_1 adrenergic receptor blockade with prazosin (2mg/kg, ip) suppressed only pressor but not bradycardiac responses during episodic hypoxia. Under the influence of prazosin, both the non-selective muscarinic acetylcholine (ACh) receptor antagonist atropine (1 mg/kg, ip) and the selective M_2 ACh receptor antagonist AF-DX116 (0.2 mg/kg, ip) completely blocked the bradycardiac response to episodic hypoxia. Furthermore, both the non-selective adenosine receptor antagonist caffeine (50 mg/kg, ip) and the A_1-selective antagonist DPCPX (2 mg/kg, ip) significantly attenuated bradycardia. In conclusion, non-baroreflex mediated bradycardia observed during systemic episodic hypoxia is mediated not only by M_2 ACh receptor but also by adenosine A_1 receptor, suggesting that they share a common signaling mechanism to modulate heart rate in response to systemic episodic hypoxia. No COI.

2P-036

Prostaglandin E2-EP4 signaling-induced fibulin-1 may play a role in intimal thickening in the ductus arteriosus

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Intimal thickening (IT) is essential for closure of the ductus arteriosus (DA). However, molecular mechanisms of IT in the DA are not fully understood. Since we have previously reported that prostaglandin E_2 (PGE_2)-EP4 signaling promoted hyaluronan-mediated IT in the DA, we sought to uncover a novel EP4-mediated mechanism that induce IT in the DA.

Microarray analysis and quantitative RT-PCR (qRT-PCR) using smooth muscle cells of rat DA (DASMCs) on day 21 of gestation (e21; near-term) revealed that EP4 stimulation for 24 h increased fibulin-1 (273-fold, $p<0.001$, n=10). Western blotting also showed that EP4 stimulation for 72 h increased secretion of fibulin-1 from DASMCs (21-fold, $p<0.001$, n=4). Immunohistochemistry of human DA revealed that fibulin-1 was highly expressed in the area of IT. Furthermore, fibulin-1 mRNA expression was 1.6-fold higher in the rat DA than in the aorta on e21 ($p<0.005$, n=3-5), and was developmentally increased during day 19 to 21 of gestation (1.6-fold vs. e19, $p<0.005$, n=5). Messenger RNA of ADAMTS-1 which regulates proteolysis of glycoprotein by interacting with fibulin-1 was abundantly expressed in the rat DA compared with the aorta on e21 (4.7-fold, $p<0.001$, n=4-5).

In conclusion, PGE_2-EP4 signaling-induced fibulin-1 may play a role in IT formation in the DA by enhancing the activity of ADAMTS-1. No COI.

2P-037

Mechanisms of vasoconstriction induced by acetylcholine on guinea pig vena cava

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Objective: To investigate the mechanisms underlying acetylcholine (ACh)-induced contraction of the guinea pig vena cava. **Method**: Isometric tension recordings were made from ring preparations of guinea pig vena cava. In some experiments, the endothelium was mechanically removed. **Results**: ACh (1 or 10 μM) caused contractions of the vena cava, while transmural nerve stimulation or phenylephrine (10 μM) failed to contract the vessel. Nifedipine (10 μM) abolished 1 μM ACh-induced contractions and suppressed 10 μM ACh-induced contractions. In nominally Ca^{2+}-free solutions, 1 μM ACh-induced contractions were unexpectedly enhanced. 10 μM ACh-induced contraction was attenuated, albeit it was larger than that in nifedipine. L-nitro-arginine (10 μM) augmented ACh (1 or 10 μM)-induced contractions. In endothelium-denuded preparations, the removal of extracellular Ca^{2+} or nifedipine diminished ACh (1 or 10 μM)-induced contractions. However, unlike endothelium-intact preparations, ACh-induced contractions in nifedipine were similar to those in Ca^{2+}-free solutions. **Conclusion**: These results suggested that proportional contributions of Ca^{2+} influx and Ca^{2+} release from internal stores to ACh-induced contractions may be different depending on the concentrations of ACh. In addition, the endothelium appears to counteract vascular contractility by releasing nitric oxide upon ACh stimulation. No COI.

2P-038

Secretome-Based Identification of PGE2-EP4-Inducible Factors in Human Abdominal Aortic Aneurysm

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[Backgrounds] Abdominal aortic aneurysm (AAA) is a common disease in the elderly. Although the rupture of AAA is fatal, there is no effective pharmacological therapy available. We have previously reported that prostaglandinE2 (PGE_2), via its receptor EP4, exacerbates AAA by increasing secretion of Interleukin-6 and Matrix Metalloproteinase-2 (MMP-2) both in mouse models and in human AAA tissues. In the present study, by applying mass spectrometric analysis, we identified secreted proteins increased by EP4 stimulation in human AAA tissues, which are potential exacerbating factors of AAA.

[Materials & Methods] Human AAA tissues were obtained at a blood vessel prosthesis implantation with written informed consents. The tissues were incubated in serum-free DMEM followed by stimulation with EP4 agonist or PGE_2+EP4antagonist for 24h. Trypsinized proteins were labeled with iTRAQ and were analyzed by LC/MS/MS. Identified proteins were classified by GO terms and secreted proteins were extracted.

[Results] A total of 694 proteins were identified from 3 specimens among which 41 proteins were increased (>1.3-fold vs control) by EP4 agonist. These included neutrophil-related protein, extracellular matrices, proteoglycans, MMP-2, and MMP-9. Among them, PGE_2-induced MMP-2 secretion was attenuated by EP4antagonist.

[Conclusion] Mass Spectrometric Analysis identified potential exacerbating factors of AAA, rising new insights into AAA progression by PGE_2-EP4 signaling. No COI.

2P-039

Catecholamine-induced cardiac automaticity in rat pulmonary veins

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Atrial fibrillation (AF) is the most common sustained arrhythmia and its crucial origin is pulmonary vein (PV). However, the arrhythmogenic nature of PV is less clear. We revealed that norepinephrine (NE) evoked a spontaneous activity in rat PV cardiomyocytes and the responsible molecules for the arrhythmogenecity. We performed Ca^{2+} imaging, path-clamp and immunocytochemistry to characterize physiological properties of enzymatically isolated PV cardiomyocytes. When we applied NE to these cells, they showed repetitive intracellular Ca^{2+} increase accompanied by depolarization, and automatic action potentials. We observed that an oscillatory current generating the automaticity was always inward irrespective of the membrane potential, indicating the current was derived from the Na^+-Ca^{2+} exchanger (NCX). The automaticity was suppressed by blocking 1,4,5-inositol triphosphate receptor (IP3R) and the NCX and IP3R were co-localized along T-tubules. These findings suggest that a functional coupling between the NCX and IP3R causes arrhythmic excitability. Furthermore, we found a unique hyperpolarization-activated Cl^- current whose biophysical properties differed from ClC-2 current in the reaction to each intracellular Cl^-, extracellular acidification and osmotic stress. Cl^- channel blockers that attenuated the current inhibited the NE-induced automaticity. These findings suggest that the special channel may be involved in the automaticity. No COI.

2P-040

Venous identity lost in jugular segment of carotid-jugular (C-J) shunt but arterial identity strengthened in pulmonary artery in adult rats with C-J shunt

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Objectives We investigated whether vessel identity is remodeled in adult rats with C-J shunt. Methods and Results The hemodynamics of C-J shunt was characterized with the features of both carotid artery and jugular vein. EphB4 transcripts were significantly down-regulated in jugular segment of patent C-J shunt and brachiocephalic vein, whereas Ephrin-B2 transcripts were strongly induced in pulmonary artery. A decreasing trend in collagen I transcripts with a concomitant increasing trend in elastin transcripts was found in jugular segment of C-J shunt. Elastin vs. collagen ratio might be modulated. Gene expression changes evoked in the jugular segments of C-J shunt were further revealed by microarray analysis. Genes involved in vascular remodeling were highly regulated. The arterial marker Ephrin B2, and other markers of arterial identity were not induced in jugular segment. Conclusions Markers of vessel identity are not quiescent but plastic, and will be remodeled to adapt to hemodynamic changes in adults. Vessel identity remodeling was preferentially developed in original low-pressure blood vessels when exposed to high-pressure oxygenated blood flow from C-J shunt. Whether vessel identity remodeling would be implicated in structure and composition remodeling in adults deserves further exploration. No COI.

2P-041

Intimal Cushion and Elastic Fibers Degradation were shownFormation Were Less Prominent in the Chicken Ductus Arteriosus

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Background The ductus arteriosus (DA) is a fetal artery that closes after birth. Our previous studies have demonstrated that the PGE2-EP4 signal pathway promotes neointima thickening and fragmentation of the elastic fibers in the rodent DA. The avian DA also closes after birth, although the PGE2-EP4 signal pathway may not play a significant role in vascular remodeling in the avian DA, because the chick has no placenta that is a source of PGE2.We investigated whether vascular remodeling was observed in the chicken DA.Experience & Results We isolated the DA from chicken at embryonic day 16, 19, hatching and within a day after birth. The serial paraffin sections were stained with Elastica van-Gieson staining. We found the sparse and fragmented elastic fibers in the medium from embryo day 16, and impairment became more apparent with development. Intimal cushion formation was not clearly observed even after external hatching. Real-time PCR analysis revealed that the expression levels of elastin and lysyl oxidase mRNAs were significantly lower in the DA than in the aorta. In addition, genes related with the PGE2-EP4 signal pathway were not predominantly expressed in the chicken DA.ConclusionInternal cushion formation was less prominent in the chicken DA than in the mammalian DA.On the other hand,elastic fiber formation was severely impaired in the chicken DA, which was unlikely due to the PGE2-EP4 signal pathway. No COI.

2P-042

$S1P_1$ receptors are necessary, but not sufficient, for the angiogenic responses induced by a novel nucleic acid analogue COA-Cl

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COA-Cl is a recently developed nucleic acid analogue that promotes angiogenesis via the MAP kinases ERK1/2. We now show that antagonists for the $S1P_1$ receptors, W146 and VPC23019, abolish effects of COA-Cl at the levels of ERK1/2 activation and tube formation in cultured human umbilical vein endothelial cells. Genetic knockdown of $S1P_1$ with siRNA attenuates COA-Cl-elicited ERK1/2 responses. Thus, the $S1P_1$ receptors are necessary for the angiogenic actions of COA-Cl. Signaling properties of COA-Cl showed similarities with those of S1P, an endogenous $S1P_1$ ligand, which were sensitive to pertussis toxin (Gα i/o inhibitor), BAPTA-AM (calcium chelator), and PP2 (Src inhibitor). However, heterologous overexpression of $S1P_1$ in CHO-K1 cells did not promote COA-Cl-evoked ERK1/2 responses, suggesting that the receptors per se are not sufficient for the COA-Cl. In summary, results point out the $S1P_1$ receptors as a key molecule at which the novel nucleic acid analogue COA-Cl induces angiogenic responses in vascular endothelial cells. No COI.

2P-043

Tonic Rho-kinase mediated vasoconstriction contributes to coronary vasomotor tone in early diabetic rat

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Activation of RhoA/Rho-kinase (ROCK) is increasingly implicated in acute vasospasm and chronic vasoconstriction in major organ systems. We aimed to ascertain whether increased ROCK activity plays a role in the coronary dysfunction in early diabetes. Synchrotron radiation microangiography was used to determine in vivo coronary responses in diabetic (3 weeks post streptozotocin 65 mg/kg ip) and vehicle treated male SD rats. Changes in vessel number and calibre during vasodilator stimulation before and after blockade of nitric oxide synthase and cyclooxygenase were compared between rats. Diabetic rats showed no significant changes in fibrotic scores, media to lumen ratio, capillary density or baseline visible vessel number or calibre. Responses to acetylcholine were similar between groups, but the diabetic group showed more segmental constrictions during blockade, which were not completely alleviated by acetylcholine, but were alleviated by fasudil (10 mg/kg iv). Further, second order vessel branches in diabetic rats were significantly more dilated after fasudil compared to control rats. ROCK2 expression was borderline greater in diabetic hearts. Based on nitrotyrosine staining oxidative stress was not significantly elevated in diabetic rats. We conclude that tonic ROCK mediated vasoconstriction contributes to coronary vasomotor tone in early diabetes. No COI.

2P-044

Involvement of PAF, but not histamine or serotonin, in increased pulmonary vascular resistance during anaphylactic hypotension in anesthetized mice

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Systemic anaphylaxis accompanies pulmonary vasoconstriction, which may contribute to increased right heart afterload, and finally anaphylactic hypotension. Recently we reported that the systolic right ventricular pressure, measured as an indicator of pulmonary arterial pressure (PAP), did not increase during anaphylaxis in anesthetized mice (Shinomiya et at. Exp Lung Res. 2013). However, pulmonary vascular resistance (PVR) was not exactly measured in that previous study. Here it was determined by measuring directly PAP, left atrial pressure and aortic blood flow (ABF) in open-chest sensitized BALB/c mice. We also clarified the roles of platelet-activating factor (PAF), histamine and serotonin in the responses. Anaphylaxis was induced by an intravenous injection of the ovalbumin antigen. Along with the aforementioned variables, mean arterial pressure (MAP), central venous pressure, and airway pressure were continuously measured. In sensitized control mice, MAP and ABF substantially decreased following a transient increase after the antigen injection, while PAP increased at 1–3 min after antigen. PVR increased to the peak of 2.8-fold at 20 min and returned towards the baseline levels. The PAF antagonist attenuated PVR elevation, although either antagonist of PAF or histamine attenuated hypotension. In conclusion, PAF, but not histamine or serotonin, is involved in an increase in PVR during anaphylactic hypotension in anesthetized mice. No COI.

2P-045

Experimental study on usefulness of second derivative of central pressure waves in normal and heritable hypercholesterolemic rabbits

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We investigated how second derivative of central pressure waves (SDCPW) reflected cardiovascular hemodynamics in normal and Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits aged 12 months. Pressure and flow waves at the ascending aorta were simultaneously recorded using a catheter-tip transducer (3Fr) and an ultrasonic flow probe under pentobarbital anesthesia (30 mg/kg). Initial positive (a) and early negative (b) waves of SDCPW were distinct, whereas c, d and e waves were not clear in both strains. An amplitude ratio of b to a waves (b/a) was -0.71±0.06 (mean±SD) and -0.48±0.05 in the normal and KHC rabbit groups, respectively, which was significant between two rabbit strains ($p<0.001$). A surrogate index of aortic compliance (SV/PP) was significantly smaller in the KHC rabbit group (0.043±0.008) than in the age-matched control group (0.062±0.009) ($p<0.001$), where SV and PP were stroke volume and pulse pressure, respectively. The value of b/a showed weak ($r=0.571$, $p<0.05$) and strong ($r=0.819$, $p<0.001$) negative correlation with SV/PP in the normal and KHC rabbit groups, respectively. We can conclude that b/a of SDCWP was useful for estimating aortic compliance like that of second derivative of photoplethysmogram waveform. No COI.

2P-046

Significance of Hairy-related transcription factor (Hrt) in ischemia-induced angiogenesis

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Angiogenesis is a process to form new blood vessels from preexisting vessels under physiological and pathological conditions. The Notch signaling pathway plays a critical role in embryonic angiogenesis. Hairy-related transcription factor 1 (Hrt1) and Hrt2 are enriched in the cardiovascular system, and their expression is directly regulated by the Notch signal activation. Hrt1/Hrt2 compound KO mice die in utero with severe vascular abnormalities, indicating that these two Hrt genes have essential roles in embryonic vascular development. Notch signaling is also implicated in postnatal angiogenesis, but the significance of Hrt1 and Hrt2, especially that in pathological conditions, remained unclear. In a mouse model of pathological angiogenesis by hindlimb ischemia (HLI) surgery with femoral artery occlusion, the mRNA expression of Hrt1, but not Hrt2, was markedly induced in ischemic muscle. Dominant induction of Hrt1 mRNA expression was also observed by the treatment of cultured endothelial cells with VEGF, an activator of Notch signal, suggesting that Hrt1 is a principal mediator of Notch activation in endothelial cells of ischemic muscle. Blood flow recovery in Hrt1 KO ischemic hindlimb after HLI was significantly blunted compared to WT. Revascularization of ischemic hindlimb was also attenuated in Hrt1 KO mice. Accordingly, the severity of ischemic injury such as tissue necrosis and loss is much higher in Hrt1 KO mice than WT. These results suggest that Hrt1 plays important roles in regulating ischemia-induced angiogenesis. No COI.

2P-047

Identification and characterization of disease-specific activator of G-protein signaling (AGS) proteins: exploration of AGS protein in the polycystic kidney disease

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Cell signal mediated by heterotrimeric G-protein plays important roles in maintaining the homeostasis of cardiovascular system against physiological stimuli. In addition to G-protein coupled receptor (GPCR) at cell-surface, other entities can directly regulate the activation status of G-proteins, and they are involved in the development of diseases. As a part of approach to identify such proteins, we have previously identified activator of G-protein signaling 8 (AGS8) from the ischemic myocardium and AGS11 from the hypertrophic heart. Thus, AGS8 was involved in the hypoxia-mediated apoptosis of cardiomyocytes via regulation of $G\beta\gamma$ and channel protein connexin 43. AGS11, isolated from the hypertrophic heart, translocated $G\alpha$ 16 into the nucleus and increased the transcription of the claudin 14. We expanded the approach to the polycystic kidney disease to identify disease-specific AGS proteins. CDNA library, generated from the rat model with progressive cystic enlargement of the kidney, was subjected to yeast-based functional screen for receptor independent activators of $G\alpha i3$, $G\alpha s$ or $G\alpha 16$. In addition to previously reported AGS proteins, we identified two candidates for AGS protein expressing polycystic kidney disease. The roles of these proteins in the pathophysiology of polycystic kidney disease will be discussed. No COI.

2P-048

Mechanisms of cardioprotective effects of high concentration of magnesium on hypoxia/reoxygenation injury in chronic magnesium deficient rat heart are differ from normal rat heart

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Magnesium (Mg) deficiency has been reported to be associated with the development of the cardiovascular diseases, sudden death and metabolic syndrome. We reported that 1) extracellular high Mg concentration (12 mM) during hypoxia protected the heart from hypoxia/reoxygenation injury in normal rat, and its protective effect was induced not only by suppression of energy consumption, but also by accelerated opening of mitochondrial K_{ATP} channel, 2) in chronic Mg-deficient mice hearts, tolerance to hypoxia/reoxygenation injury decreased. Aim of this study was to determine whether high concentration of Mg during hypoxia could protect the hypoxia/reoxygenation injury in chronic Mg-deficient rat heart using Langendorff perfused rat heart model. Rats were fed an Mg-deficient diet for 8 weeks. The hearts underwent hypoxia for 30 min, followed by reoxygenation for 30 min. In Mg-deficient group, the recovery of cardiac function from hypoxia/reoxygenation injury was significantly worse than that of control group. When extracellular Mg concentration increased from 1.2 to 12 mM during hypoxia, in Mg-deficient group, recovery of PRP was similar to that of control group. 5-hydroxy decanoic acid (5-HD), a mitochondrial K_{ATP} channel specific blocker, partially inhibited the protective effect of high Mg in Mg-deficient group. These results suggest that cardioprotective effects of high Mg in Mg-deficient rat are not only opening the mitochondrial K_{ATP} channel but also unknown other mechanism. No COI.

2P-049

Application of 4-hydroxybenzoic acid as a trapping agent to monitor hydroxyl radical production during myocardial ischemia and reperfusion

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Background: Hydroxyl radical (OH) is involved in the myocardial ischemia/reperfusion injury. Hydroxylation of aromatic compounds has been used as an indirect marker of in vivo production of ˙OH. **Purpose**: To detect the production of ˙OH during myocardial ischemia/reperfusion by applying 4-hydroxybenzoic acid (4-HBA) as a trapping agent. **Methods**: Using microdialysis technique, dialysis probe was implanted in the left ventricular myocardium of anesthetized rats and perfused with Ringer solution containing 4-HBA (5×10^{-4} M) at 2 μl/min. Dialysates were sampled at baseline, during 30 min-coronary occlusion and after reperfusion (180 min). A sampling period was 10 min. Dialysate concentration of the 4-HBA hydroxylation product, 3,4-dihydroxybenzoic acid (3,4-DHBA) was measured using HPLC-ECD. **Results**: Dialysate 3,4-DHBA concentration was 1.5±0.3 nM at baseline. After coronary occlusion, dialysate 3,4-DHBA concentration gradually increased and reached 3.0±0.4 nM at 20-30 min after coronary occlusion. After reperfusion, dialysate 3,4-DHBA concentration further increased to 4.9±0.8 nM at 0-10 min after reperfusion and then reached the peak level (6.0±0.7 nM) at 10–20 min after reperfusion. After 20 min of reperfusion, dialysate 3,4-DHBA concentration gradually declined and almost recovered baseline level at 120 min after reperfusion. **Conclusion**: Detection of ˙OH with 4-HBA could be less complicated and more reliable than that with other aromatic compounds. No COI.

2P-050

Myocardial interstitial serotonin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) levels during ischemia-reperfusion

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Background: It has been reported that 5-HT accumulates in the heart during ischemia-reperfusion and contributes to the cardiomyocyte injury. Little information is, however, available on the in vivo 5-HT kinetics during myocardial ischemia-reperfusion.Purpose: To elucidate the in vivo 5-HT kinetics including accumulation and degradation during myocardial ischemia-reperfusion.Methods: Microdialysis technique was applied to the left ventricular myocardium of anesthetized rats, and dialysate 5-HT and its metabolite by monoamine oxidase (MAO), 5-HIAA concentrations were monitored as indices of myocardial interstitial 5-HT and 5-HIAA levels during 30 min-ischemia and 45 min- reperfusion.Results: In vehicle, 5-HT levels continued to increase during ischemia but 5-HIAA levels decreased and then recovered during ischemia. After reperfusion 5-HT levels transiently increased and then declined. 5-HIAA levels increased after reperfusion and kept high level by 45 min after reperfusion. Local administration of pargyline (1mM), a MAO inhibitor did not influence the increase in 5-HT levels and the decrease in 5-HIAA levels during ischemia. Pargyline enhanced the increase in 5-HT levels immediately after reperfusion and suppressed the increase in 5-HIAA levels after reperfusion.Conclusion: Simultaneous monitoring of interstitial 5-HT and 5-HIAA levels is useful for understanding 5-HT kinetics during myocardial ischemia-reperfusion. No COI.

Poster Presentations
Neuron, Synapse (2)

2P-051

Dynamin isoforms decode action potential firing for synaptic vesicle recycling in sympathetic neurons

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Presynaptic nerve terminals must maintain stable neurotransmission despite encountering wide fluctuations in the number and frequency of incoming action potentials (APs). Here, we examined the role of dynamin in activity sensing in the presynaptic superior cervical ganglion (SCG) neurons. Dynamin 1, 2 or 3 expressed in SCG neurons was acutely knocked down by microinjection of the siRNA. 3 days later, each dynamin expression in the soma was approximately 60% and the synaptic transmission with various AP firing pattern was impaired. Paired-pulse recordings with AP interval of 20 and 30 ms showed that knockdown (KD) of dynamin 2 or 3 reduced the amplitude of the second excitatory postsynaptic potential (EPSP) more than control. With AP interval of 50–1000 ms, each dynamin KD similarly reduced the second EPSP amplitude. EPSP recording during and after high frequency AP firing at 5, 10 or 20 Hz showed that dynamin 1, 2 KD induced severe synaptic depression during and after AP firing, whereas dynamin 3 KD induced it during AP firing. In contrast, with low frequency AP firing at 0.05 and 0.2 Hz, dynamin 3 KD reduced gradually synaptic transmission, whereas dynamin 1 or 2 KD reduced it at 0.2 Hz. After depletion of synaptic vesicles in the readily releasable pool, dynamin 1 or 2 KD delayed the fast recovery, while dynamin 2 or 3 KD delayed the slow recovery. These results suggest that the three isoforms of dynamin, an essential endocytic protein, work individually to match vesicle reuse pathways, having distinct rate and time constant with physiological AP frequencies. No COI.

2P-052

Temporal Ca^{2+} regulation of synaptic vesicle release efficacy following action potential firing at the release site

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At the presynaptic active zone, an action potential (AP) triggers Ca^{2+} influx and entered Ca^{2+} causes synaptic vesicles (SVs) exocytosis. Here, we show temporal Ca^{2+} regulation of release ready SVs (RRSVs) in the readily releasable pool (RRP) following AP firing in presynaptic sympathetic neurons in culture applying rapid-on-rate and slow-on-rate Ca^{2+} chelators. Under reduced basal release probability by membrane permeable O, O'-1,2-bis(2-aminophenoxy)ethane-N,N,N',N'- tetraacetic acid, tetraacetoxymethyl ester (BAPTA)-AM or Ethyleneglycol-bis(β-aminoethyl)- N,N,N',N'-tetraacetoxymethyl Ester (EGTA)-AM, a rapid synaptic depression within 30 ms and 30–120 ms after generation of an AP was reduced by BAPTA and EGTA, respectively. During repetitive AP firing with 50–200 ms interval, EGTA, but not BAPTA, delayed the RRP replenishment. After the RRP depletion with a train of APs firing, BAPTA delayed rapid and slow replenishment of SVs into the RRP, while EGTA delayed the slow RRP replenishment. Slow Ca^{2+} regulation supported generation and maintenance a presynaptic short-term plasticity, augmentation. Unexpectedly, a rapid Ca^{2+} regulation underlies induction of posttetanic potentiation. Together, following AP firing, the temporal change in Ca^{2+} level at release sites, in addition to spatial change in the presynaptic terminal and beneath the active zone as previous findings, controls the RRSVs release efficacy for incoming nerve signals, and underlies synaptic plasticity resulted from various firing activity. No COI.

2P-053

Myosin IIB and VI regulates synaptic vesicle trafficking at presynaptic nerve terminals.

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Accumulating evidence suggests that myosin regulates vesicle transport in neurons. We have shown that among myosin isoforms previously characterized in neurons myosin IIB is localized to presynaptic nerve terminal of cultured sympathetic neurons from the rat superior cervical ganglion. Recently, myosin VI is reported to be involved in postsynaptic vesicle endocytosis in the brain. Here we examine the function of myosin IIB and VI for trafficking of synaptic vesicles in presynaptic nerve terminals by measuring changes in neurotransmitter release by disturbing function of myosins by specific siRNAs. Synaptic transmission triggered by action potentials was inhibited by myosin IIB or VI siRNA, but not by IIA or control siRNA. Synaptic depression with high frequency AP trains was accelerated by myosin IIB or VI knockdown. Knockdown of myosin IIB or VI knockdown delayed rapid or slow recovery of synaptic vesicles in the readily releasable pool after the depletion, respectively. This trafficking step is highly sensitive to activity-dependent calcium transient, consistent with a calcium requirement for myosin activation. These results suggest that myosin IIB and VI control a discrete calcium-dependent synaptic vesicle trafficking to the readily releasable pool through distinct recycling pathways in response to presynaptic firing activity. No COI.

2P-054

Optical and electrophysiological measurements of synaptic exo-endocytosis at the rat calyx of Held synapse

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At the nerve terminal, neurotransmitter is released by the fusion of synaptic vesicles with presynaptic plasma membrane. After exocytosis, vesicles are retrieved by endocytosis and recycled for reuse. The coupling of exo- and endocytosis of synaptic vesicles is essential for the maintenance of synaptic transmission. The exo- and endocytosis have been measured by capacitance measurement, or by imaging (e.g. FM dyes, pHluorin). However, the number of studies that apply both techniques at the same time is very limited, because of the technical difficulty caused by the small size of the nerve terminal (usually around 1 μm).

The calyx of Held is a large synapse in the auditory brainstem. Because of its terminal size (~20 μm), it allows us to apply electrophysiological and optical methods and analyze presynaptic mechanisms reliably.

In this study, we simultaneously measured the turnover of fusion related synaptic vesicle proteins and plasma membrane by applying imaging method and electrophysiological recordings, respectively. We labeled synaptotagmin-2 (a Ca2+ censor for exocytosis) with an antibody coupled to pH-sensitive fluorophore cypHer5E to monitor the dynamics during exo- and endocytosis. Fluorescent changes of cypHer were measured simultaneously with membrane capacitance to compare the cycling of synaptic vesicle proteins and that of plasma membrane. By varying stimulus conditions or by applying pharmacological agents to manipulate the time course of endocytosis, we tested how the time courses of both measurements were correlated (or not correlated). No COI.

2P-055

Synaptophysin-EGFP puncta slowly move retrogradely along axons in slice culture during the phase of en passant type synaptogenesis

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One neuron makes multiple synaptic connections to postsynaptic cells, which can be implemented by en passant type synapses. Little is known about the process of en passant type synaptogenesis. One of the most important aspects of synaptogenesis is the assembly of synaptic vesicles on the presynaptic active zone. What is the entire course of events for synaptic vesicles until getting together at en passant synapses? We attempted to visualize the overall process of synaptic vesicle assembly at en passant synapses by way of our stage top CO_2 incubator system equipped to an upright confocal microscope. We cultured corticospinal slice coculture in this incubator and chased the fate of synaptophysin-EGFP (Syp-EGFP) labeled vesicles at various stages for up to 50 hours. During the early phase of 2–4 days in culture, EGFP positive vesicles appeared to form clusters to show Syp-EGFP puncta. Unexpectedly, those Syp-EGFP puncta showed very slow (less than 100 μm/hour), clearly retrograde, and long-distance movement (more than 200 μm). The movement consisted of constant-velocity moving phases and pauses. Frequency of movement declined toward 7 DIV, when active corticospinal synapses are there. At this stage, larger degree of colocalization of active zone protein Bassoon with Syp-EGFP puncta was detected. After formation of clusters, the clusters of vesicles may move slowly and retrogradely for long-distance to find the site of en passant synapses. No COI.

2P-056

Effects of synaptotagmin binding on SNARE-mediated fusion and SNARE complex formation

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SNAREs (syntaxin, SNAP25, synaptobrevin) and synaptotagmin play a central role during Ca^{2+}-dependent neurotransmitter release. Recently, we found that synaptotagmin dissociates from syntaxin by Ca^{2+}. Here, we have studied the effects of synaptotagmin binding on SNARE-mediated fusion and its complex formation. Recombinant SNAREs were expressed in E. coli, purified and reconstituted into liposomes. In lipid mixing assay, incubation with the cytosolic fragment of synaptotagmin inhibited SNARE-mediated lipid mixing in the absence of Ca^{2+}. However, this inhibition was reversed by Ca^{2+}, indicating that Ca^{2+} released the inhibitory effect of synaptotagmin from SNAREs, which triggered fusion. The SNARE complex has been proposed to assemble like a zipper, starting from the membrane-distal termini and progressing toward the membrane-proximal termini of the SNARE motifs. If synaptotagmin suppresses SNARE-mediated fusion by preventing SNARE complexes from fully zippering, SNAREs may exist in a half zippered state when bound to synaptotagamin. To determine whether such an intermediate SNARE complex exists in the presence of synaptotagmin, we employed FRET. The data indicate that binding of syntaxin and synaptobrevin proceeds to the immediately vicinity of their transmembrane domains even in the presence of synaptotagmin without Ca^{2+}. These results suggest that synaptotagmin suppresses SNARE-mediated membrane fusion through its synatxin binding before Ca^{2+} triggers without inhibiting the interaction between syntaxin and synaptobrevin SNARE motifs. No COI.

2P-057

Domain-swapped oligomerization of SNAP25 for ultrafast exocytosis at presynaptic terminals

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We analysed the molecular structure of the SNARE complex using intermolecular fluorescence resonance energy transfer (FRET) with 2-photon fluorescence lifetime imaging. We observed the domain-swapped oligomerization of SNAP25 at the presynaptic terminals of mouse cortical neurons. SNAP25 is one of the SNARE proteins at the target plasma membrane and it carries two α-helices, SN1 and SN2. We measured the intermolecular FRET ratio between Tq-SNAP25 and SN1-Venus-SN2 and observed a high binding fraction (BF: 26%) at the active zone. These data indicated the presence of a domain-swapped model, where SN1 bound to the SN2 of the other SNAP25 molecule, and formed an oligomer. In addition, we utilized SNAP25 knock-out mice and a rescue system by viral transfection so that only fluorescent-labelled SNAP25 functioned. The kinetics of Ca^{2+}-dependent exocytosis was measured using IPSC triggered by flash photolysis of caged Ca^{2+}compounds or electrical field stimulation. Transfection by wild-type FRET probes triggered IPSC with a time constant (τ) of 2 ms. Furthermore, we developed various fluorescent probes of the SNAP25 mutant with longer linker between the two α-helices (BF: 12%, IPSC delayed), or the SNAP25 mutant carrying mutations (M71A, I192A), which trigger slower membrane fusion (BF: 16%, no IPSC). C-terminal deletion mutant (7 or 9AA) triggered delayed IPSC and BF was reduced. Therefore, the domain swapping of SNAP25 is necessary for rapid neurotransmitter release. No COI.

2P-058

Unique Roles of Docking Proteins, SNAP-25 and Syntaxin, in Short-Term Synaptic Plasticity Discovered by Botulinum Neurotoxins Studies

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Tetanic enhancement and post-tetanic potentiation are conspicuous features of synapses in respect of the short-term synaptic plasticity. Since membrane impermeable protein modifying reagents altered the short-term synaptic plasticity, its system was thought to be either Ca^{2+} channels or docking proteins locating in the active zones in synaptic terminal membrane predominantly. Using frog neuromuscular preparations, we made a Copernican breakthrough by investigating the short-term synaptic plasticity from different angle: i.e. the roles of syntaxin and synaptosome-associated protein of 25 kDa (SNAP-25), plasmalemma-bound docking proteins. Botulinum neurotoxins are specific enzymes to act on the docking proteins. Truncation of SNAP-25 by Botulinum neurotoxin serotype A (BoNT-A) selectively abolished the slow potentiation, whereas cleavage of syntaxin by Botulinum neurotoxin serotype C (BoNT-C) suppressed the augmentation strongly. Further, double poisoning by BoNT-A and BoNT-C eliminated both augmentation and potentiation. Although syntaxin and SNAP-25 have been known to cooperatively act as a docking device, the results undoubtedly indicate that these exert unique temporally different late actions in the molecular processes leading to the short-term synaptic plasticity. BoNTs studies were conducted in 1997and 1998 at the Dept. Physiol., Shimane Med. Univ. No COI.

2P-059

Functional role of syntaxin 1B in the regulation of synaptic vesicle exocytosis and of the readily releasable pool at central synapses

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Two syntaxin 1 (STX1) isoforms, HPC-1/STX1A and STX1B, are co-expressed in neurons and function as neuronal t-SNAREs. However, little is known about their functional differences in synaptic transmission. STX1A null mutant mice develop normally and do not show abnormalities in fast synaptic transmission, but monoaminergic transmissions are impaired. We previously reported that STX1B null mutant mice died within 2 weeks of birth. In the present study, in order to examine functional differences between STX1A and STX1B, we analyzed the presynaptic properties of glutamatergic and GABAergic synapses in STX1B null mutant and STX1A/1B double null mutant mice. We found that the frequency of spontaneous quantal release was lower and the paired-pulse ratio of evoked postsynaptic currents was significantly greater in glutamatergic and GABAergic synapses of STX1B null neurons. Deletion of STX1B also accelerated synaptic vesicle turnover in glutamatergic synapses and decreased the size of the readily releasable pool in glutamatergic and GABAergic synapses. Moreover, STX1A/1B double null neurons showed reduced and asynchronous evoked synaptic vesicle release. Our results suggest that although STX1A and STX1B share a basic function as neuronal t-SNAREs, STX1B has an essential role in the regulation of spontaneous and evoked synaptic vesicle exocytosis in fast transmission. No COI.

2P-060

Serotonergic fiber distribution and chronic pain-induced changes of the emotional behaviors in syntaxin 1A knockout mice

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Serotonergic fibers are distributed widely throughout the brain and are involved in variety of brain functions. We have reported that mice with largely reduced serotonergic fibers demonstrate impaired performance of water maze learning and reduced prepulse inhibition. Syntaxin 1A is involved in the synaptic transmission and dysfunction of the syntraxin 1A results in abnormal brain functioning. Recent work indicated that serotonergic functions were impaired in the syntaxin 1A knockout mice. To support the idea that syntaxin 1A affects the emotional behaviors through impairing serotonergic functions, we investigated the serotonergic fiber distribution in the brain and the emotional behaviors of syntaxin 1A knockout mice. The reduction of the serotonergic fibers in the brain of knockout mice was investigated under fluorescent microscope using the BAC transgenic mouse expressing green fluorescent protein under the control of promoter of the tryptophan hydroxirase 2. We investigated the emotional behaviors of the knockout mice with arthritic hind paw. In the arthritic mice group, CFA (0.05ml) was injected into knee joint of the left hind paw under halothane anesthesia 1-2 days before behavioral tests. Ultrasonic vocalization (USV, 50 ± 4 kHz) was recorded after nociceptive stimulation to the hind paw. Arthritic knockout mice emitted USV to a greater extent compared to arthritic wild type mice. The results suggest that the knockout of syntaxin 1A affect chronic pain induced emotional behaviors through the impairment of the serotonergic system in the brain. No COI.

2P-061

Direct mechanical regulation of presynaptic functions by enlargement of dendritic spines in CA1 pyramidal neurons

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Dendritic spines of cortical excitatory synapses undergo extensive enlargement during long-term potentiation (LTP). It has been, however, still elusive whether LTP involves alterations of presynaptic functions. For example, retrograde messengers have not been identified. We therefore test the possibility that the enlargement of dendritic spines mechanically facilitates presynaptic functions. Glutamate release from presynaptic terminals on an identified spine was imaged using GCaMP6s expressed in CA1 pyramidal neurons in cultured hippocampal slices. Calcium signals from dendritic spines showed either success or failure of transmission in response to Schaffer collateral stimulation, allowing measurement of transmitter release probability (Pr). We found that Pr was tightly correlated with the spine volume in the resting-state, and markedly enhanced during artificial spine enlargement induced by blue light irradiation of photo-activatable Rac1 (PA-Rac1). Local application of hypertonic solution also increased Pr. Moreover, our SNARE-based FRET probes demonstrated immediate increases in presynaptic SNARE complex by spine enlargement. These results suggest that spine structural plasticity can regulate presynaptic functions by direct mechanical interactions in rapid and synapse specific manner. Thus, the spine enlargement may rapidly potentiate both pre and postsynaptic functions, and effectively facilitate the formation of cell assembly in cortical networks. No COI.

2P-062

Roles of Paranodal Potassium Channels in Cerebellar Purkinje Cell Axons

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Voltage-gated potassium channels are uniquely situated at and near the node of Ranvier and thought to play crucial roles in regulating action potentials (APs) in the axon. Recently we obtained results that the calcium- and voltage-activated Slo/BK potassium channel is expressed in the paranodal region of myelinated axons in cerebellar Purkinje cells (PCs). Therefore, we examined whether paranodal Slo/BK channels regulate APs in PC axons. Using mouse cerebellar slices, we recorded antidromic APs, which were evoked by electrical stimulation of PC axons in the white matter, from their soma by whole-cell current-clamp recordings. Short single stimulation caused a single AP with a delay depending on the distance of the stimulation electrode from the soma, suggesting that the APs we observed are antidromic APs. To test the role of Slo/BK channels in AP propagation, we examined the failure rate of the antidromic APs upon repetitive stimulation at 50-300 Hz. In the control, failure of the antidromic APs was observed with the stimulation at high frequencies over 100 Hz. Puff-application of a Slo/BK channel blocker near the stimulation site in the axon significantly increased the failure rate. Moreover, local axonal applications of nickel also increased the failure rate. These results suggest that paranodal Slo/BK channels impact propagation of antidromic APs, particularly at high firing rates. We will also discuss axonal calcium transients, which activate paranodal Slo/BK channels, the ionic mechanism of how Slo/BK channels regulate APs, and their potential implications in cerebellar PCs. No COI.

2P-063

Estimation of the coupling distance between Ca^{2+} channels and exocytotic sensors using 3D diffusion model

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Neurotransmitter release is evoked by Ca^{2+} entering through Ca^{2+} channels. Ca^{2+} spreads by diffusion and reaches exocytotic Ca^{2+} sensors within ~100 nm from the channel. To estimate the coupling distance and the effect of paired-pulse activation on the coupling distance, we constructed a simple model of Ca^{2+} diffusion and reaction with buffers (fixed, mobile, and exogenous), and combined it to the Ca^{2+}-dependent stochastic release model. Simulation was done in 2D- and 3D-spaces using the finite difference method with the 10 nm grid size. When there was one channel, a separate model of concentric multilayered shells was also used. In 2D simulation, the coupling distance was ~100 nm. When two Ca^{2+} channels (300 nm apart) were activated, P_r (release probability) at the midpoint was enhanced by paired pulse activation. Enhancement was clearly reduced by EGTA, without influencing P_r near the channels. These results were consistent with our previous experimental data, and suggest the microdomain signaling in paired-pulse facilitation. In 3D simulation, diffusion became the predominant determinant, and the coupling distance became shorter. The EGTA effect on P_r between the activated channels was not observed. This suggests involvement of other factors to determine the coupling distance, such as occupation of yet another endogenous buffers, hindrance of diffusion by the intracellular matrix, sustained Ca^{2+} binding to exocytotic sensors. Further scrutiny is required for a more realistic model. No COI.

2P-064

Role of glutamate transporter in inhibitory synaptic transmission

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Fast inhibitory neurotransmission in the central nervous system is mediated by GABA and glycine, which accumulate in the same presynaptic nerve terminal by vesicular inhibitory amino acid transporter (VIAAT) and are then co-released. However, the mechanisms that control the packaging of GABA+glycine into synaptic vesicles are not fully understood. In this study, we demonstrate the dynamic control of the GABA/glycine co-transmission by the neuronal glutamate transporter, using paired whole-cell patch recording from monosynaptically coupled cultured spinal cord neurons derived from VIAAT-Venus transgenic rats. Short step depolarization of presynaptic neurons evoked unitary (cell-to-cell) inhibitory postsynaptic currents (IPSCs). Under normal conditions, the fractional contribution of postsynaptic GABA or glycine receptors to the unitary IPSC did not change during a 1 hour recording. Raised extracellular glutamate levels increased the amplitude of GABAergic IPSCs by enhancing glutamate uptake and reduced glycine release. Similar effects were observed when presynaptic neurons were intracellularly perfused with glutamate. Interestingly, high-frequency trains of stimulation decreased glycinergic IPSCs more than GABAergic IPSCs. The present results suggest that the enhancement of GABA release by glutamate uptake may be advantageous for rapid vesicular refilling of the inhibitory transmitter at mixed GABA/glycinergic synapses and thus may help prevent hyperexcitability. No COI.

2P-065

Simulation analysis of K^+ buffering in astrocyte

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In response to elevation of extracellular K^+ concentration ($[K^+]_{out}$), astrocytes clear excessive extracellular K^+ to maintain proper environment for neural activity. The K^+ clearance mechanism in astrocytes takes two forms: K^+ uptake and K^+ spatial buffering. The high $[K^+]_{out}$ also induces swelling of astrocyte, which leads to edema and cell death in the brain. Here we report simulation analysis on the mechanisms of the astrocytic K^+ clearance and swelling. Astrocyte models were constructed by incorporating into a compartment model various mechanisms, such as intra/extracellular ion concentrations of Na^+, K^+ and Cl^-, cell volume, and models of Na,K-ATPase, Na-K-Cl cotransporter (NKCC), K-Cl cotransporters, inwardly rectifying K^+ (KIR) channel, passive Cl^- current, and Aquaporin channels. Simulated response of astrocyte models to high $[K^+]_{out}$ revealed significant contributions of NKCC and Na,K-ATPase to the increases of the intracellular K^+ and Cl^- concentrations, and swelling. Moreover, we show that the KIR channel localized at synaptic cleft absorbs the excessive K^+ by depolarizing equivalent potential for K^+ (E_K) above membrane potential, while the K^+ release through KIR channel localized at perivascular is enhanced by hyperpolarizing E_K and depolarizing membrane potential. Further analysis of simulated drug effects shows that K^+ uptake, K^+ release and swelling can be modulated differently by blocking each of the ion channels and transporters. Thus, by identifying their distinct roles, we here show that astrocytes can be new potential target in drug therapy for diseases accompanying high $[K^+]_{out}$. No COI.

2P-066

Manipulation of neuronal activities by non-invasive optogenetic approach to astrocytes

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How and where do focal neuronal activities spread? Functional MRI can be a tool to monitor spreading manners, however, the requirements of both time-controlled activation and reproducible outcome make the application difficult. We thus challenged to establish an experimental model that satisfies above points. Optogentics is ideal for time-controlled manipulation, but an optic fiber insertion to brain parenchyma causes an injury, which disturbs a reproducible result. The usage of light-sensitive channelrhodopsin variant, C128S, permitted successful photoactivation of cortical astrocytes by an illumination over the skull, resulting in consistent neuronal activation. We further demonstrated that a weak illumination resulted in the focal activation but a strong illumination resulted in the propagation of neuronal activity throughout the hemisphere. These data indicated an establishment of non-invasive, controllable model in neuronal activity. No COI.

2P-067

Developmental change in the nonuniform distribution of presynaptically silent synapses in single cultured hippocampal neuron

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A concept of silent synapse is widely accepted in the central nervous system. It is well known that postsynaptic silencing results from a failure of synaptic transmission despite that glutamate is released normally from presynaptic terminals. On the other hand, presynaptically silent synapse is physiologically determined as the fact that the presynaptic terminal is present but is not releasing neurotransmitters. However, precise characteristics of presynaptically silent synapses are not fully understood. In this study, presynaptically silent synapse was identified at excitatory glutamatergic synapses on single cultured hippocampal neuron at 7–9, 13–15 and 21–27 days in vitro (DIV) by double staining with a styryl dye, FM1-43 and anti-VGLUT1 antibody. Synaptic allocation was determined as a function of a given area of concentric circles from soma. We found that the fraction of presynaptically silent synapses somehow increased depending on the distance from soma. In addition to the nonuniform distribution, total percentage of presynaptically silent synapses within a single neuron was decreased during culture periods, indicating that presynaptic silencing shifts to active state in a developmental stage-dependent manner. We conclude that information processing would be finely tuned by change in the fraction of presynaptic silencing with age. No COI.

2P-068

Binomial distribution analysis of two components of short-term synaptic plasticity, facilitation and potentiation, at the frog neuromuscular junction

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To investigate the mechanism of tetanic stimulation induced enhancement of transmitter release, we analyzed two components of short-term synaptic plasticity, facilitation and potentiation, using binomial distribution with two parameters, release probability (**p**) and number of releasable synaptic vesicles (**n**) having that release probability. Frog neuromuscular junction was used as synapse preparation. Endplate potentials (EPPs) and miniature endplate potentials (MEPPs) were electrically recorded with an intracellular glass microelectrode in a low Ca^{2+} high Mg^{2+} Ringer's solution (0.50–0.75 mM Ca^{2+}, 5 mM Mg^{2+}). Facilitation was induced by 8 stimuli with interval of 25 or 30 msec. Potentiation was induced by 500 stimuli at 20 Hz and steady enhanced state was maintained by 350 stimuli with interval of 150–200 msec. The ratio of variance increase to mean increase of facilitated EPPs distribution was almost 1. However, that value of potentiated EPPs distribution was smaller than 1. These results suggests that the enhancement of transmitter release during facilitation and potentiation was caused mainly by the increase of **n** and **p**, respectively. The detailed binominal analyses of the change of two parameters, **p** and **n**, during facilitated and potentiated EPPs distributions confirmed that increase of **n** and **p** contributed largely to the enhancement of transmitter release in facilitation and potentiation, respectively. No COI.

2P-069

Effects of astaxanthin on neuronal functions

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Astaxanthin (AX) is a carotenoid pigment. It is widely distributed in nature, which is included in microalgae, crustacean, and the other marine animals. AX could eliminate reactive oxygen species (ROS), therefore it has attracted attention as a protecting agent against damage caused by oxidative stress. Several experimental evidences showed that AX provides antioxidant effects, however the direct effects of AX on neuronal cell have rarely reviewed. In this study, we investigated the effects of AX on neurite growth in cultured rat spinal neurons. Neurofilaments were identified by immunocytochemistry using anti- neurofilaments antibody (SMI-31, SMI-32), and measured neurite length. Neurons pretreated for 24 h with AX alone and subsequently treated for 72 h with AX and the ROS donor. Treatment with AX alone was not significantly effects on neurite growth, whereas it blocked against the growth inhibitory effects of the ROS donor in neurons. Furthermore, we examined the direct effects of AX on axonal transport in cultured dorsal root ganglion neurons. No COI.

Poster Presentations
Sensory Function (2)

2P-070

Cortico-cortical connections of the insular auditory field in mice

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An auditory field in the insular cortex has recently been identified. As the first step to understand the function of the insular auditory filed (IAF), here we examined the connection of IAF with other areas of the cerebral cortex in mice. First we focused on the connection between IAF and the anterior auditory field (AAF). Using voltage-sensitive dye-based optical imaging, we identified IAF and AAF by their characteristic frequency gradient. Two fluorescent tracers in distinct colors were then injected into each of the fields at frequency matched sites. After a survival period of three days, retrogradely labeled cells were found in both IAF and AAF, within the injection sites, suggesting a reciprocal, topographic connection between the two fields. Interestingly, we also found retrogradely labeled cells in sensorimotor areas of the cortex. Such connections of IAF with areas of different modalities suggest its role in linking sounds to actions. No COI.

2P-071

Quantitative frequency-position relationship in the primary auditory cortex in guinea pigs

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Orderly representation of sound frequency over space is a hallmark of the primary auditory cortex (A1). A quantitative relationship between sound frequency and cortical position is yet to be established. Here we examined this relationship in guinea pig A1 by presenting stimulus tones in a wide frequency range, and recording the evoked cortical responses with a high spatial resolution optical imaging technique. We determined three best-frequency positions as the cortical positions in A1 for each tone frequency: the onset response position, the peak amplitude position, and the maximum rise rate position of the response evoked by a tone of the frequency. A nonlinear log frequency-position relationship was found for each of the three indices, and the frequency-position relationship was always well described by a Greenwood equation, with correlation coefficients greater than 0.99. Cortical magnification factor, measured in octave/mm, was found to be a function of frequency, not a constant. Representation of frequency in A1 was more spatially even than in the cochlea. Our results establish a quantitative relationship for sound frequency and cortical position in guinea pig A1, and should find application in an array of studies including modeling of the auditory cortex. No COI.

2P-072

Spectral and temporal sensitivity of sustained and phasic neurons investigated for multiple sounds in the primary auditory cortex of awake cats

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In previous studies we have shown that the primary auditory cortex (A1) neurons of awake animals showed diversity of the response time-courses from phasic to sustained patterns during pure tone stimuli. Following studies also showed that A1 neurons have such response patterns during amplitude modulation (AM), frequency modulation (FM), and vowel sounds. It is not yet investigated whether or not a given phasic (sustained) neuron responds to any kinds of sound stimuli in the phasic (sustained) time course. In this study we investigated response time courses to various artificial sounds (pure tones, two tones, noises, click trains, AM sounds, and FM tones) and natural sounds (environmental sounds, meow, vowels, and consonants). Neurons with phasic responses to pure tones had wide frequency response field (FRF) and showed phasic responses to all kinds of sounds investigated. Comparison of peak response amplitudes among sounds showed no sound specificity. Neurons with sustained responses to pure tones had relatively narrow FRF and many neurons showed inhibitory subfields to two tones stimuli. They showed sustained responses to all sounds when the spectral frequency of the sound was located at the cell's FRF and not located at the inhibitory subfields. Comparison of mean driven rate among sounds showed no sound specificity. These results suggest that a single neuron in A1 processes similar information contained in any kinds of sounds and each neuron with different time-courses codes the different acoustic characteristics of those sounds. No COI.

2P-073

Properties as associative memory circuits in the primary auditory cortex of mice

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In the present study, we found properties of the mouse primary auditory cortex (AI) as associative memory circuits. Previously, we have reported that responses to fundamental frequency (f0) are recorded in AI of mice using flavoprotein fluorescence imaging, even when spectral energy at f0 is missing. Harmonic sounds of simultaneously presented 20 kHz and 25 kHz, which produced missing f0 perception at 5 kHz, activated the 5 kHz area, while inharmonic sounds of simultaneously presented 19 kHz and 26 kHz did not. Two-photon imaging confirmed the results obtained by using flavoprotein fluorescence imaging: single neuronal activities to 20+25 kHz sound were significantly correlated with those to 5 kHz, but had little correlation with those to 20 kHz alone or 25 kHz alone in the 5 kHz area of AI. F0 responses were not observed in the mouse strain which exhibited almost no chirps, except in the case that they were reared by normal parents with frequent chirps. In mice reared in the presence of 5+19+26 kHz, the 5 kHz area in AI responded to inharmonic sounds of 19+26 kHz. These results indicate importance of experience for producing f0 responses. Furthermore, a new hypothesis is suggested that recall of missing f0 responses from experience may be only a part of AI functions: AI may have properties as associative memory circuits. In accordance with this possibility, harmonic sounds of 4+8 kHz activated the 20 kHz area, while inharmonic sounds of 5+7 kHz did not. These results clearly indicate the properties of AI as associative memory circuits. No COI.

2P-074

Responses of auditory fields sensitive to species-specific pup calls in rats

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Rodent pups emit lower frequency calls other than isolation induced ultrasonic calls. We have reported that low frequency pup calls of rats can be divided into three groups: harmonic-type calls, noise-type calls and pulse trains. Rat pups frequently utter low frequency calls when they are with the mother, indicating that pup calls are an important factor in maintaining a close mother-pup relationship. Endogenous flavoprotein fluorescence imaging (excitation: 450–490 nm, emission: 500–550 nm) conducted in mother rats after 3 weeks of weaning period and nulliparous control rats shows that harmonic calls evoke clear fluorescence responses in the primary auditory cortex (AI). In contrast, synthetic noise-type calls evoke marked responses in the posterior part of AI and posterior auditory field (PAF). In the present study, we investigated plastic changes in auditory cortical responses to low frequency pup calls in mother rats. Fluorescence responses of AI to harmonic calls and those to artificial harmonic sounds (a fundamental and 2nd and 3rd overtones) were not significantly different in nulliparous rats. However, fluorescence responses to harmonic calls were greater than those to artificial harmonic sounds in mother rats, indicating AI of mother rats responds selectively to harmonic pup calls. Synthetic noise calls evoked stronger fluorescence responses in PAF of mother rats than nulliparous rats. These findings indicate that different groups of auditory fields are sensitive to distinct type of species-specific pup calls in rats. No COI.

2P-075

Distinctions in burst spiking between auditory cells in thalamic reticular nucleus projecting to first and higher-order thalamic nuclei

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Thalamic reticular nucleus (TRN) plays a pivotal role in gain and/or gate control of sensory inputs transmitted from thalamic nuclei to cortical areas. TRN contains two types of auditory cells projecting to the ventral (MGV) and dorsal divisions (MGD) of medial geniculate nucleus, which, as first- and higher-order thalamic nuclei, are involved in sensory processing of lemniscal and non-lemniscal systems. Previously, we reported distinctions in auditory response properties between the two types of cells (TRN-MGV and TRN-MGD cells), which were determined in anesthetized rats, using juxta-cellular recording and labeling techniques. In the present study we further examined spontaneous cell activity recorded in a state with a steady ambient noise level (< 45 dB).TRN cells exhibited single spikes and bursts. Bursts of TRN-MGV cells consisted of larger numbers of spikes with shorter inter-spike intervals as compared to those of TRN-MGD cells in both spontaneous activity and auditory response elicited by noise burst stimuli (intensity, 70–80 dB; duration, 100 ms). These distinctions in burst spiking were comparable to those observed in the two types of TRN visual cells projecting to first- and higher-order thalamic nuclei (Neuroscience, 2012). Burst spiking of TRN cells could have a significant impact on thalamic cell activity. In the loop connectivity between the cortex and thalamus TRN is highly likely to subserve differential modulations of sensory processing in lemniscal and non-lemniscal systems. No COI.

2P-076

The effect of claudin-14 on maintenance in EP and endolymphatic ionic concentrations

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We measured K^+, Na^+, Ca^{2+}, and H^+ concentrations in endolymph ($[K]_e$, $[Na]_e$, $[Ca]_e$, pH_e) with endocochlear potential (EP) in both wild type ($Cldn14^{+/+}$) mice and claudin-14 knockout ($Cldn14^{-/-}$) mice at 8 weeks of age, using double-barreled ion-selective microelectrodes. We also measured the auditory brain response (ABR) in both mice. We obtained the following results. 1) pH_e, $[K]_e$, $[Na]_e$, and $[Ca]_e$ in $Cldn14^{+/+}$ mice were 7.98, 155 mM, ~0.5 mM, ~100 nM, respectively and those in $Cldn14^{-/-}$ mice were 7.85, 154 mM, 1.2 mM, ~1 μM, respectively. Significant difference in above those values between $Cldn14^{+/+}$ and $Cldn14^{-/-}$ mice were observed in pH_e and $[Ca]_e$. EP was +102 mV in $Cldn14^{+/+}$ and +90 mV in $Cldn14^{-/-}$ mice. At more than 14 weeks of age in $Cldn14^{-/-}$ mice, EP was reduced to +30–50 mV, pH_e was decreased to 7.51, and $[Ca]_e$ was ~0.1 mM. Significant correlation between EP and $[Ca]_e$ was observed in both $Cldn14^{+/+}$ and $Cldn14^{-/-}$ mice. ABR was gradually reduced from 4 weeks to 14 weeks of age in $Cldn14^{-/-}$ mice. These findings suggest that $Cldn14^{+/+}$ mice have the EP more than 100 mV, high pH_e, and low $[Ca]_e$ and claudin-14 has small, but important effects on maintenance in EP and endolymphatic ionic concentrations. No COI.

2P-077

Interaural canal affects the sensitivity of processing the interaural time difference detected in the nucleus laminaris in the chick

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The interaural time difference (ITD) is a major cue for sound localization and is first processed in the nucleus laminaris in birds. ITD is a function of a head size of animal, thus it is extremely small in small-headed animals such as chicks. It has been suggested that ITD is enhanced through the acoustic interference of sound between two middle ear cavities through the interaural canal. However, the effect of the interaural canal has not been directly demonstrated in the ITD processing in any auditory nuclei. We therefore conducted experiments that occlude the interaural canal with an agarose gel, and examined the effects of occlusion on the neural activity in the nucleus laminaris. After the occlusion, the ITD tuning function is shifted along the ITD axis, thus the property of ITD processing was changed especially within the physiological range of ITD. These findings confirm that acoustic coupling through the interaural canal affects the sensitivity of ITD processing in the nucleus laminaris. No COI.

Poster Presentations
Autonomic Nervous System (1)

2P-078

Regulation of energy metabolism and cardiovascular function by the hypothalamic AMP-activated protein kinase

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In mammals, AMP-activated protein kinase (AMPK) is extremely essential in the intracellular signaling pathway involving active leptin receptors. We investigated the potential role of hypothalamic AMPKα2 in cardiovascular function and energy metabolism with using in vivo siRNA injection to knockdown AMPKα2 in rats. In the present study, we produce reduced hypothalamic AMPKα2 expression by 3 days injection of siRNA into the third ventricle. The AMPKα2 siRNA-treated rats had lower post-transfection body weights, whereas body weights were unchanged in control siRNA-treated rats. In addition, basal levels of mean arterial pressure of AMPKα2 siRNA-treated rats were significantly higher compared with those of control siRNA-treated rats. Basal levels of blood adrenaline, noradrenaline, glucose and leptin were similar between the two groups, but leptin effects on body weight, food intake, blood pressure, heart rate and blood FFA levels were eliminated in AMPKα2 siRNA-treated rats. Moreover, leptin-evoked enhancements of the sympathetic nerve outflows to the kidney, brown and white adipose tissues were attenuated in AMPKα2 siRNA-treated rats. These results suggest that hypothalamic AMPKα2 may be involved not only in appetite and body weight regulation but also in the regulation of circulation and energy metabolism. Especially, AMPK might function as a key molecule in the cardiovascular and lipolytic effects of leptin through the sympathetic nervous system. No COI.

2P-079

Effects of orexins on parasympathetic preganglionic neurons in the superior salivatory nucleus innervating the salivary glands

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Orexin (ORX)-A and -B are synthesized by lateral hypothalamic neurons (LH) and are implicated in the regulation of feeding and arousal. Meanwhile, neuroanatomical studies revealed that the superior salivatory nucleus (SSN) neurons innervating the submandibular and sublingual salivary glands, which is the primary parasympathetic center, receives direct projections from LH neurons. However, there are no reports that neuronal activity of SSN is influenced by ORXs. This work aimed to examining the effects of ORXs on neuronal activity and expression of orexin receptor (ORXR) subtypes (ORXR1 and 2) in rat SSN neurons. Whole-cell patch-clamp techniques were applied to SSN neurons retrogradely labeled with a fluorescent tracer from the chorda-lingual nerve. At 100 nM, ORX-A induced inward currents and elicited action potentials in most of SSN neurons. Although ORX-B induced inward currents in 40% of neurons, it hardly elicited action potentials. In the presence of an ORXR1 antagonist, SB-334867, the peak amplitude of inward currents to ORX-A were completely inhibited in about 60% of neurons. Immunohistochemical studies showed that ORXR1 and 2 expressions in SSN neurons are 53% and 40%, respectively. These data suggest that SSN neurons were mainly excited by ORX-A via ORXR1. ORXs may contribute to abundant salivary secretion during feeding. No COI.

2P-080

Immunohistochemical analysis of Nav1.9 sodium channel and transient receptor potential ankyrin 1 (TRPA1) in newborn rat large intestine enteric nervous system

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In this study, we examined whether Nav1.9 sodium channel and transient receptor potential ankyrin 1 (TRPA1) are detectable immunohistochemically in neonatal rat enteric nervous system of large intestine. We used newborn rat (a week old). After euthanasia, segments of the rat colon were removed. The tissues were washed and dehydrated in graded sucrose solutions (10, 20 and 30 %) at 4°C. The mucosa was eliminated with tweezers and the remaining tissues were frozen in liquid nitrogen. Cryostat sections were cut at 10 μm thickness, and incubated with primary antibodies directed against Nav1.9 sodium channel, TRPA1 and PGP9.5. For double immunostaining, incubations with the three combinations of the antibodies (Nav1.9 and TRPA1; Nav1.9 and PGP9.5; TRPA1 and PGP9.5) were conducted for three nights at 4°C. Then the tissues were incubated with secondary antibody (1 hr, at room temperature) and washed with PBS before mounted on slides. We detected Nav1.9 or TRPA1 expression in the PGP9.5 positive neurons, which are distributed in muscle layers. Moreover, co-localization of Nav1.9 and TRPA1 was observed among the area, which is corresponding to the myenteric plexus. In conclusion, our results suggest that at least in newborn rat myenteric plexus of the large intestine functional dependence may exist between Nav1.9 channel and TRPA1. No COI.

2P-081

Characterization of ghrelin-sensitive neurons in the lumbosacral defecation center that are associated with facilitation of colorectal motility in rats.

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We have previously demonstrated that ghrelin applied to the L6-S1 spinal cord level enhances colorectal motility. In situ hybridization showed that neurons in the spinal cord express ghrelin receptor. In this study, we characterized the ghrelin-sensitive neurons in the lumbosacral defecation center, with particular focus on their similarity with those in the hypothalamus. Rats were anesthetized with alpha-chloralose, and the distal colon and anus were cannulated to measure the intracolorectal pressure and propelled intraluminal liquid volume. The intrathecal injection of tetrodotoxin abolished the prokinetic effect of ghrelin. NPY, which is a major neuropeptide released from ghrelin-snsitive neuron in the hypothalamus, did not enhance colorectal motility. Consistently, NPY-Y1 receptor antagonist failed to inhibit the action of ghrelin. Although leptin exerts opposite effects to ghrelin on the activity of hypothalamic neurons, leptin had no effect. We then examined effects of AMP-activated protein kinase (AMPK) activation in the lumbosacral defecation center, since AMPK is known to be related to effect of ghrelin on food intake. Intrathecal administration of an activator of AMPK, AICAR, failed to enhance the colorectal motility. These results suggest that characteristics of the ghrelin-sensitive neurons in the lumbosacral defecation center are different in those of the hypothalamus. No COI.

2P-082

Serotonin enhances colorectum motility through an activation of lumbosacral defecation center in rats.

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Serotonin (5-HT) is one of the transmitters regulating gut motility in the enteric nerve systems. In the present study, we examined effects of stimulation of 5-HT receptors in the lumbosacral defecation center on colorectal motility in rats.Rats were anesthetized with α-chloralose and ketamine, and colorectal intraluminal pressure and propelled intraluminal liquid volume were recorded in vivo.An intrathecal administration of serotonin to the L6-S1 region of the spinal cord elicited periodic increases in colorectal intraluminal pressure that were associated with increases in fluid output through the anal cannula. Similar enhancement of colorectal motility was induced by intrathecal administration of 5-HT2 agonist, α-methylserotonin and 5-HT3 agonist, 1-(3-chlorophenyl)biguanide. In contrast, 5-HT1 or 5-HT4 agonist had no effects. Application of either 5-HT2 or 5-HT3 antagonist partially blocked the prokinetic effect of serotonin, whereas the serotonin effect was totally abolished when these antagonists were applied simultaneously.It is concluded that serotonin promotes propulsive contractions of the colorectum through an activation of 5-HT2 and 5-HT3 receptor in the lumbosacral defecation center. No COI.

2P-083

Firing properties of medullary expiratory neurons during defecation

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Defecation is normally assisted by contraction of diaphragm and abdominal muscles. Experimental evidence suggests that expiratory (E) neurons of caudal NRA closely interrelated with the activities of neurons controlling respiration and automomic functions, because E neurons extend their axons in the lumbar and sacral spinal cord. The purpose of this study is to examine the possible mechanisms interacting with the respiratory neurons and the defecation. The experiments were performed on adult cats anesthetized with urethane-alpha chloralose. The phrenic nerve, L1 and L2 abdominal nerves were prepared for recording. The animals were paralyzed and kept on artificial ventilation. Glass micropipettes were used for extracellular recordings of respiratory neurons. Spinal projection of the respiratory neuron was tested by monopolar stimulation of the lumbar and sacral spinal cord. Defecation was induced by injection of warm Ringer solution into the soft rubber balloon which was inserted in the rectum. E neurons showed the respiratory rhythm in the expiratory phases during defecation. But firing frequencies of E neurons having their descending spinal axons to the lower thoracic and lumbar segments increased during defecation, but those of E neurons to the sacral segments decreased. These results suggest that E neurons to the lower thoracic and lumbar segments may exert excitatory synaptic inputs to the abdominal motoneurons and E neurons to the sacral segments synaptic inputs to the pudendal motoneurons via sacral local circuits during defecation. No COI.

2P-084

A method for recording of afferent and efferent mesenteric sympathetic nerve activity in rats

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We previously demonstrated that an experimental model for simultaneous measurements of mesenteric sympathetic nerve activity (SNA), arteriolar diameter, and blood flow in small intestinal arterioles of Sprague-Dawley rats, to evaluate a quantitative relationship between SNA and arteriolar vasomotion for blood pressure control. The aim of this study was to develop a method for recording of afferent and efferent mesenteric SNA. Using intraperitoneal urethane (1.2 g/kg), the small intestine was exteriorized through a midline abdominal incision and placed on a dish. The mesenteric nerve was exposed between the mesenteric artery and vein under a dissecting microscope. Bipolar silver electrodes were put under the nerve to record. Mesenteric SNA was recorded with a power lab system. Mesenteric SNA was recorded before and after cutting the proximal side of the mesenteric nerve for afferent recordings or cutting the distal side of the nerve for efferent recordings. Mesenteric SNA decreased after cutting the proximal or distal side of the nerve. Efferent mesenteric SNA after cutting the distal side fluctuated than afferent mesenteric SNA after cutting the proximal side. These results suggest that the mesenteric nerve includes both afferent and efferent fibers, indicating that this method may be useful for recording of afferent and efferent SNA. No COI.

2P-085

Endogenously and exogenously newborn enteric neurons in the deep tissue of mouse small intestine underwent transection and anastomosis

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Nonlinear optical microscopy, in particular two photon-excited fluorescence microscopy (2PM), can provide deeper optical penetration (several hundred μm) in ex vivo and in vivo preparations. We have used this approach and obtained clear three-dimensional imaging of newborn enteric neurons that were endogenously generated after gut transection and anastomosis in Thy1-promoter YFP mouse. Neurogenesis has been promoted by oral application of the 5-HT_4-receptor agonist, mosapride citrate (MOS). Most neurons were located within 100 μm of the surface. To clarify the cell source of neurogenesis, mesenchymal stem cells (MSC) from bone marrows and neural stem cells (NSC) derived from the hippocampus and subventricular zone were challenged as candidates. MSC and NSC were cultured with MOS for 4–11 days. Outgrowth of protrusion was facilitated in neurospheres formed by NSC ex vivo. The NSC transplantation from the tail vein was performed after treatment with PKH26. One-two weeks later during when MOS was applied, under a stereomicroscopy nerve cell-like red fluorescence was visible, but uncertain. 2PM imaging made it possible to observe exogenously newborn enteric neurons derived from transplanted NSC showing red fluorescence among endogenously newborn enteric neurons (showing green fluorescence) in the deep tissue of mouse small intestine underwent transection and anastomosis. No COI.

2P-086

Habituation of sudomotor and vasoconstrictor components in bursts of skin sympathetic nerve activity

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A burst of skin sympathetic nerve activity (SSNA) evoked by a variety of arousal stimuli usually contains both sudomotor and vasoconstrictor spikes. The purpose of this study was to explore differences in habituation process between sudomotor and vasoconstrictor components of SSNA bursts during repeated arousal stimulation. SSNA signals were recorded by microneurography from the tibial nerve in eight healthy subjects. Simultaneously, skin sympathetic response (SSR) and skin blood flow response (SKBR) were monitored on an innervated area of foot sole. Superficial electrical stimulation of the median nerve was used to induce arousal response. By referring to occurrence of SSR and SBFR, each SSNA burst was divided into two segments: a sudomotor segment and a vasoconstrictor segment. Time average of SSNA within each segment was calculated as sudomotor SSNA amplitude and vasoconstrictor SSNA amplitude. On average, the sudomotor SSNA amplitude showed significant reduction throughout 30 stimuli. The vasoconstrictor SSMA amplitude significantly decreased during the first 10 stimuli, but did not change during the subsequent 20 stimuli. Sympathetic sudomotor and vasoconstrictor drive may be differently subjected to neural habituation process. No COI.

2P-087

Analysis for pathologic condition of facial hemihyperhidrosis

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Facial hemihyperhidrosis may be often compensatory due to the contralateral anhidrosis, but little is known about the pathophysiological mechanism. We explained in detail of 11 of 14 patients with this symptom about I: Unilateral hyperhidrosis: Cervical disk herniation, II: Segmental hemihyperhidrosis: II-a: Harlequin syndrome, and II-b: Probable cause of cervical disk herniation in this 90th meeting. We express remaining 3 cases of symptomatic segmental hemihyperhidrosis this time. We analyzed these patients by defining the total body sweat distribution patterns using Minor's starch-iodine test, total skin temperature distribution using infrared thermography, and local skin blood flow using laser Doppler flow meter during heating, and imaging to confirm the lesions. Results: Case 1: The excessive extension of the left neck: A 63-year-old woman with hyperhidrosis in the right posterior region of neck, and the left axilla since 40 years old and left Horner syndrome. Case 2: Lung cancer in right upper lobe invading the dorsal rib: A 44-year-old man with hemihyperhidrosis of the right hemitrunk and the right back pain. Case 3: Ross syndrome: A 38-year-old woman with anhidrosis at the right hemiface and the left hemitrunk, left Adie's Tonic Pupil, and areflexia, detected regional cardiac sympathetic denervation by MIBG imaging. For elucidation of the pathologic condition for introductions of precise treatment, the examination of sweating distribution for localized diagnoses is essential for facial hemihyperhidrosis. No COI.

2P-088

Renal afferent fibers regulate body fluid balance through controlling arginine vasopressin release.

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Although it is well known that the renal nerve includes autonomic and sensory fibers, property and functions of renal sensory fibers are still unclear. We hypothesized that the renal afferent nerves may contribute body fluid homeostasis through controlling renal excretory functions. To examine this, renal afferent nerve activity (RANA), plasma arginine vasopressin (AVP) concentration, and urine volume were measured, while intrarenal receptors were selectively stimulated using double infusion (DI) technique. That is, hyperosmotic NaCl solution was infused into the renal artery, while hypoosmotic solution was infused into the inferior vena cava, thus total loaded solution was isoosmotic but the right kidney was stimulated by hyperosmotic solution. DI induced increases in RANA and plasma AVP concentration, and a decreased urine volume. These responses were completely abolished by renal denervation. These data indicate that the renal afferent fibers might sense changes in ionic concentration or osmolarity, and alter renal excretory functions, probably through controlling AVP release. No COI.

2P-089

Renal afferent nerve activity and blood pressure response to intrarenal arterial infusion of ionic solutions in conscious rats.

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We hypothesized that the kidney senses ionic concentration in plasma and send this message to the central nervous system via the renal afferent nerves. To examine this, renal afferent nerve activity (RANA) was measured in conscious rats, while a small intrarenal arterial infusion of hyperosmotic or isosmotic ionic ssolutions. To exclude the sympathetic nerve component from the renal nerve record, hexamethonium was administered in advance of RANA recording. Intrarenal arterial infusion was performed via the chronically implanted catheter into the right suprarenal artery, which allowed us to make a selective stimulation to the right kidney without causing obstruction of renal blood flow. The recording electrodes were chronically implanted on the right renal nerve. Infusion of high concentration solution of NaCl or KCl (100 μL/min for 1 min) evoked an instant transient increase in RANA and a rise in arterial pressure, while 0.9% NaCl or 20% glucose solution did not induce these responses. Administration of losartan attenuated the hyperosmotic NaCl infusion-induced pressor response but did not affect RANA. Our results suggest that kidney senses the elevation of plasma Na^+, Cl^-, or K^+ concentrations to activate the afferent nerve, but the concomitantly occurred pressor response are mainly mediated via renin-angiotensin-aldosterone system. No COI.

2P-090

Arterial blood pressure response to the metabotropic excitatory amino acid receptor agonist L-AP4 injected into the caudal ventrolateral medulla of the rat

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Neurons in the caudal ventrolateral medulla (CVLM) play a pivotal role in the central control of the cardiovascular system and are sensitive to the sulfur-containing amino acid L-cysteine to produce a depressor response that is abolished by both antagonists for ionotropic excitatory amino acids (iEAA) NMDA and non-NMDA receptors (Takemoto 2013). Receptor binding assays using synaptic membrane (Pullan et al. 1987) suggest strong affinity of L-cysteine for L-2-amino-4-phosphonobutanoate (L-AP4) sensitive receptors. The response to L-cysteine could be via L-AP4 related metabotropic EAA group III receptors. However, there is no information about arterial blood pressure (ABP) response to L-AP4 in the CVLM. The present study therefore examined ABP responses to microinjection of L-AP4 in the CVLM of urethane-anesthetized rats, identified with a depressor response to L-glutamate (34 nl, 10 mM). Microinjections of L-AP4 in the CVLM dose-dependently decreased ABP. A single prior injection of an antagonist MK801 (68 nl, 20 mM) alone for NMDA receptors, or another antagonist CNQX (68 nl, 2 mM) alone for the non-NMDA receptors abolished the depressor response to L-AP4 (34 nl, 5 mM). The results indicate that L-AP4 produced the depressor response via both NMDA and non-NMDA receptors possibly in a serial fashion in the CVLM of the rat, different from a parallel mode of both receptors in the depressor response to L-cysteine. No COI.

2P-091

Effect of postural changes and vestibular lesions on responses of arterial blood pressure to neck flection in rabbits

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It has been shown in our previous study that 45-degree head-down postural rotation (HDR) induces a transient drop of arterial blood pressure (ABP) in urethane-anesthetized rabbits. We hypothesize that vestibular organs play a role in the drop of ABP through the suppression of sympathetic nerve outflows because peak activation of aortic depressor nerve, an afferent for baroreceptor reflex, occurred later than the peak suppression of sympathetic nerve. To test this hypothesis, we examined responses of ABP and sympathetic nerve activity to neck flection (NF) in the prone and lateral position in anesthetized rabbits with bilateral vagotomy. NF in the prone position induced a transient decrease of ABP which was greater than that in the lateral position. The decrease was associated with suppression of renal sympathetic nerve. NF did not affect activity of aortic depressor nerve. In vestibular-lesioned (VL) animals, NF induced a small drop of ABP, which was smaller than that in the control rabbits. These results suggest that not only vestibular organs but also neck afferents could induce the transient drop of ABP through the suppression of sympathetic nerve. No COI.

2P-092

Reduction of plasma estrogen level affects circadian rhythms of heart rates and cardiac sympathetic nerve in female rats

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Purpose We aimed to clarify the effect of estradiol (E_2) on heart rates (HR) and arterial pressure (AP), and mechanisms. ***Methods*** Female Wistar rats (n=9, age of 7–9 wk) were bilaterally ovariectomized, and placed a radio transmitter for HR and AP measurements in the abdominal cavity. Two silicon tubes containing E_2 were s.c. placed in one group (n=4, E_2 (+)), and empty tubes for the other (n=5, E_2(-)). The tubes were removed 10 days after the surgery (Day 0). On Day 7 or 21, rats were killed. The left ventricle of heart was taken, and protein contents of β_1 and β_2-adrenoreceptors (AR) was determined by Western blotting. ***Results*** On Day 0, HR was greater ($P<0.05$) in the E_2(-) than that in the E_2(+) group (388±15 and 337±13 beats/min (bpm) in the light phase; and 450±12 and 390±12 bpm in the dark phase, respectively). On Day 14, HR in the E_2(+) group increased (377±15 bpm in the light phase; and 431±14 bpm in the dark phase). On Day 21, HR became lower ($P<0.05$) in the E_2(-) group (330±17 bpm in the light phase; and 388±20 bpm in the dark phase). Mean AP was not different between the two groups on each day. Both β_1-AR and plasma noradrenaline were greater ($P<0.05$) in the E_2(-) than E_2(+) group, on Day 0. On Day 7, both β_1-AR and plasma noradrenaline decreased, however on Day 21, plasma noradrenaline became higher again. ***Conclusion*** A reduction of plasma estrogen level may affect HR, which is resulted from greater sympathetic activity and β_1-AR expression in the heart. No COI.

2P-093

Aging and plasma total homo-cysteine enhance the response of sympathetic nerve function to cold loading

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Purpose: Cold loading is a risk factor of cardio-cerebrovascular diseases (CVD), although it does not influence healthy people. We studied the modification factor of a cold pressor test.
Method: We evaluated the sympathetic-nerve function in response to cold loading or control testing by keeping a plastic cup filled with ice cold water (surface temperature; 5.5°C) or a cup with corrugated paper sleeve (15°C), respectively. We measured heart rate variability, plasma catecholamine concentration, and total homo-cysteine (t-HC): a well-known risk factor of CVD.
Result: (1) The elder group (n=10, 49±5 years) showed higher LF/HF ratio than the younger group (n=11, 22±2 years) in control (5.9±2.5 vs. 3.2±2.5, p=0.02) and cold loading study, (8.4±3.4 vs. 3.2±2.5, p=0.04).(2) When the subjects were divided into the high t-HC group (n=3, 21.2±11.5nmol/mL) (normal range<13.5 nmol/mL) and normal t-HC group (n=7, 10.0±2.1 nmol/mL), LF/HF ratio and plasma epinephrine concentration in response to cold loading were higher in the high t-HC group than in the normal t-HC group (LF/HF: 10.1±4.8 vs. 7.9±3.1, p=0.41; plasma epinephrine: 863±449 vs. 436±143 pg/m, p=0.042, respectively).
Conclusion: The response of the sympathetic nerve function to cold loading was more sensitive in the elder and high t-HC groups, suggesting that their endothelial function is impaired and raises a risk of CVD. No COI.

2P-094

GLP-1 inhibits reflex swallowing via the dorsal medulla in the rat.

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Our previous study revealed that appetite-enhancing peptide orexin-A inhibited reflex swallowing via orexin-1 receptors situated in the dorsal vagal complex (DVC). Glucagon-like peptide-1 (GLP-1), which is an incretin hormone secreted from the intestine, suppresses food intake. It is not much clarified how appetite-suppressing peptides acts on reflex swallowing. In the present study, we examined the effect of GLP-1 on reflex swallowing using anaesthetized rats to clarify how appetite-suppressing peptides acts on reflex swallowing. Swallowing was induced by the electrical stimulation (20 Hz, 20 sec) of the central cut end of the superior laryngeal nerve and was identified by the electromyogram lead penetrated the mylohyoide muscle through bipolar electrodes. GLP-1 was injected into one of the medial DVC or the lateral DVC. The microinjection of GLP-1 but not vehicle into the medial DVC significantly decreased swallowing frequency. The microinjection of GLP-1 into the lateral DVC did not change swallowing frequency. Preinjection of GLP-1 receptor antagonist into the medial DVC disrupted the inhibitory response induced by the microinjection of GLP-1. These results suggest that GLP-1 inhibits reflex swallowing via GLP-1 receptor situated in the medial DVC. No COI.

2P-095

The effect of playing a stringed-instrument ensemble on autonomic nerve function

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Purpose: It is well known that music has good influence on health. However, the mechanism of how music affects physiological function is not understood clearly. Therefore, we studied the effect of playing stringed-instrument ensemble on autonomic nerve function.
Method: Subjects are nine undergraduate (UG) (20.8±1.0years) and seven graduate students (GS) (25.5±1.9 years) at a music college. Their speciality is stringed-instrument: violins (n=4), violas (4), cellos (3), and contrabass (1). While they played the string quartet of Mozart (Eine kleine Nachtmusik and Divertimento), their ECG and spirogram were recorded by using LS-300 (Fukuda,Japan). We calculated heart rate viability (HF and LF/HF) by frequency analysis of R-R interval of ECG.
Result: The heart rate (HR) and respiratory rate (RR) at rest were not different between UG and GS (HR: 75±10 vs. 74±4/min) and (RR: 17±2 vs. 15±4/min). The GS's HR, RR, and HF showed the periodicity fluctuation at the time of play (average HR; 86±6/min, RR; 26±3/min, HF; 149±154mS^2) and rest (73±4, 14±2, 683±392). The periodicity fluctuation of HR and RR was not observed in the US.
Conclusion: The present study demonstrated that respiratory rhythm under playing stringed-instruments synchronized HR in the advanced players, but not in undergraduate students. Since respiration can be adjusted in voluntary, it was suggested that circulation rhythm might be able to adjust in semi-voluntary by RR. No COI.

2P-096

Relationship between RR interval variability with galvanic vestibular stimulation and changes in arterial pressure upon head-up tilt

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R-R interval variability (RRIV) during supine position without and with galvanic vestibular stimulation (GVS), changes in mean arterial pressure (MAP) at the onset of 60° head-up tilt (HUT) without GVS, and their relationship were analyzed in 25 healthy young subjects. MAP increased or decreased less than 5 mmHg upon HUT in 12 subjects (UP), but MAP decreased more than 5 mmHg in 13 subjects (DOWN). Applying sinusoidal GVS of 2 mA at the random frequency between 0.2 and 10 Hz did not change mean R-R intervals and MAP. However, high frequency component (HF) of RRIV increased in both UP and DOWN groups. The increase in DOWN group was larger than that in UP group. Ratio of low frequency component to HF (L/H) increased in UP group with GVS, but did not reach significant level in DOWN group. The changes in HF with GVS were significantly correlated with changes in MAP at the onset of HUT, i.e., the subjects who had larger increase in HF with GVS showed larger decrease in MAP. Thus, GVS or input for the vestibular system during HUT possibly activates vagal nerves, and the dominance of excitation in sympathetic or vagal during vestibular stimulation is important for controlling MAP at the onset of HUT. No COI.

2P-097

Monitoring autonomic nerve activity using time-frequency analysis: A study of time shift width

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Previous studies suggest that autonomic nerve activity can be evaluated by power spectrum analysis of heart rate variability. In these studies, the analysis was performed for a set of data only during certain defined conditions. Therefore, this study aimed to investigate whether power spectrum analysis for data sets with a short time shift width can be used to evaluate autonomic nerve activity indexes, under any given condition. After a 2-min resting period, 10 healthy male volunteers performed a mental arithmetic task (MAT; addition or multiplication in 64 squares), followed by another 2-min resting period. Electrocardiograph (ECG) monitoring, at 1 kHz, was performed throughout the experiment; R-R interval (RRI) data were calculated from the ECG data. Thereafter, 60-s RRI data sets were extracted from the start to the end of the experiment. The starting point of the data sets was set at 10-s intervals, regardless of the participants' status. Alternatively, the starting point was set at 60-s intervals during both resting periods and during the MAT. Time-frequency analyses were performed for the RRI data sets, from which the powers of the low frequency (LF, 0.04–0.15 Hz) and high frequency (HF, 0.15–0.4 Hz) components were calculated. More detailed changes in powers of the LF/HF and HF components could be observed in data sets with a shorter time shift width. Therefore, it is possible that power spectrum analysis can allow for real-time monitoring of the dynamics of autonomic nerve activity regardless of the operating conditions. No COI.

2P-098

Blocking effect of peripheral adrenoceptors on the defensive cardiovascular reaction in the frog

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ohmura, Ayano; Horiuchi, Jouji(*Department of Biomedical Engineering, Toyo University, Saitama, Japan*)

In mammals, stressors evoke a typical cardiovascular response. This cardiovascular response to the stressors is mediated by the sympathetic nervous system. The sympathetic response to the stress is mainly mediated via the hypothalamic areas, especially in the dorsomedial nucleus. However, the defensive hypothalamic area in the frog is not well-evolved compare to that in mammals. The frog has the midbrain where is also recognized as a defense area and is a more primitive part of the brain. In the present study, we examined whether the frog (*Rana catesbeiana*) shows the cardiovascular defensive reaction exposed to mechanical and environmental stressors (pinprick, pinching and 10% NaCl solution) and, if it has, the cardiovascular response to the stress is neurogenic or not. Blood pressure (BP) and heart rate (HR) were measured through a branch of abdominal artery in conscious frogs. Two types of adrenoceptor antagonists were intravenously injected, (an α_1 receptor antagonist, prazosin (0.3mg/kg) and a β_1 antagonist, atenorol (10mg/kg)). The all stressors caused decent pressor and tachycardic responses in the animals. Bolus injections of both prazosin and atenolol significantly inhibited the pressor and tachycardic responses evoked by the stressors. These results suggest that the mechanical and environmental stressors caused typical defensive cardiovascular response similar to mammals and the cardiovascular responses to the stressors are mediated via the sympathetic adrenoceptors in the heart and the blood vessels. No COI.

2P-099

Abnormal bradycardia response to fear in rats with heart failure

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The present study examined autonomic responses to fear in conscious rats with heart failure (HF) after myocardial infarction and central neural mechanisms for the responses. Rats exposed to 5-min white noise sound (90 dB) displayed freezing behavior (an index of fear), renal sympathoexcitation, and bradycardia. The bradycardia was parasympathetically mediated since atropine injected intravenously prevented the response in normal rats (N5). In HF rats (N7, fractional shortening<35%), the bradycardia response to fear was significantly ($P<0.05$) larger than that in normal rats (N9). Of note, in HF rats, arrhythmia appeared in association with the reduction in heart rate seen during fear. Moreover, in normal rats (N7), microinjection into the lateral/ventrolateral periaqueductal gray (l/vlPAG) of muscimol, a GABAA receptor agonist, significantly suppressed the bradycardia response to fear. Lastly, in HF rats (N6), microinjection into the l/vlPAG of Tempol, a superoxide dismutase mimetic, significantly suppressed the bradycardia response to fear. Taken together, these results suggest that fear stimulates the l/vlPAG and parasympathetically causes bradycardia in rats, and that brain oxidative stress in HF leads to dysfunction of the l/vlPAG, thereby exaggerating parasympathetic effects on heart rate and elevating risks for cardiovascular events during fear. No COI.

2P-100

Stimulation of skin osmoreceptor evokes a cardiovascular defensive reaction in the frog

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Takahashi, Tomoyuki; Horiuchi, Jouji(*Department of Biomedical Engineering, Toyo University, Saitama, Japan*)

It has been suggested that the frog skin has a various functions like a kidney (osmoreceptor), a tongue (a chemical sensor of taste buds) or a lung. External environmental condition, especially osmolality can be crucial for the frog, so that almost of all frogs live in a fresh water environment. Thus, change in the external osmolality must be stressful situation in the frog and the osmotic condition may evoke a defensive reaction, which is the cause of stress response. In the present study, we examined the cardiovascular defensive reaction exposed to environmental stressors (NaCl and sucrose solutions) in the frog (*Rana catesbeiana*). Blood pressure (BP) and heart rate (HR) were measured through a branch of abdominal artery in conscious frogs. A frog saline (0.65% NaCl solution) with 2cm^2 absorbent cotton on the back did not change of BP and HR. However, 5, 10 and 15% of NaCl solutions with the absorbent cotton elicited dose-dependent increases in BP and HR. These increases were blocked after the treatment of local anesthetic agent (2% lidocaine) on the back skin. In contrast, isotonic sucrose solutions (50, 108, 163%) compared with NaCl solutions (5, 10, 15%, respectively) did not evoke any changes in BP and HR. These results suggest that the frog skin has a sensor for change in osmolality caused by electrolyte and that the change in osmolality evokes a cardiovascular defensive reaction in the frog. No COI.

Poster Presentations
Motor Function (1)

2P-101

Changed response to microstimulation of the sensorimotor cortex in developmental white matter injury model rat.

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Developing white matter injury (DWMI) caused by hypoxia-ischemia (HI) is associated with permanent neurodevelopmental disabilities such as paralysis and cognitive dysfunction in preterm infants. We made DWMI model rat made by right common carotid artery occlusion followed by 6% oxygen for 1h in P3 Wistar rat. Our model rat showed that the arrested progression to mature oligodendrocyte (OL) with minor neuronal damage. To investigate motor deficits after HI, we performed the behavioral tests; motor deficit score (MDS), rotarod, grip test, horizontal ladder test, and gait analysis. MDS and retention time of the rotarod were reduced in the DWMI rats compared with sham group. However, no significant difference was observed in grip test, horizontal ladder test, and gait analysis. We then investigated the response to stimulus by intracortical microstimulation (ICMS). Under anesthesia, when bipolar pulses (0.2 ms, 0–200 μA, 333 Hz) were given into the layer V sensorimotor cortex using a tungsten electrode, we can detect the evoked twitches in contralateral lower leg joints, which can make the brain map of responses. The response map results indicated that hip representative area was larger in sham group rather than that of PWMI group. Moreover, the altered areas in PWMI model are contributed to evoke the twitches of the other joints. The data suggests that motor coordination rather than primary motor function is impaired in our model, and that sensorimotor cortex is reorganized in neonatal HI. No COI.

2P-102

Arrested progression to mature oligodendrocytes with minor neuronal damage in developing white matter injury model rat

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Developing white matter injury (DWMI) caused by hypoxia-ischemia (HI) is associated with permanent neurodevelopmental disabilities in preterm infants. In addition to selective loss of oligodendrocyte progenitor cells (OPC), arrested progression to mature oligodendrocytes (OL) and disturbance of myelination are reported as a cause of pathological change in DWMI. To analysis neuropathological changes in DWMI model rat, made by right common carotid artery occlusion followed by 6% hypoxia for 1 hour, we investigated the pattern of cellular degeneration, counted the number of OL lineage cells and checked neuronal injury after the damage. Active caspase-3 positive cells were detected in both the white matter (WM) and the sensory-motor cortex within 24 hours after HI. Fluoro-Jade B positive cells were distributed in the deep cortex layer. However, active-caspase3/NeuN double-positive apoptotic neurons were not detected in the cortex. Detection of OL lineage markers revealed that NG2 positive cells increased in the ipsilateral WM (137.2 ± 6.0 % of contralateral WM, n=4) at 2 day after HI and PDGFRα positive cells increased (108.9 ± 2.74 %, n=4) at 7 day after HI. However, mature OL of APC-positive decreased by 84 ± 2.45 % (n=4) at 6 month later. Data suggest that arrested OL lineage progression occur in neonatal HI injury with accompanied by minor neuronal damage. No COI.

2P-103

Projection pattern of the corticospinal tract, its formation and cervico-lumbar variance

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The corticospinal (CS) tract is essential for voluntary movement. Despite numerous studies, what we know about CS tract organization and development remains limited. To reveal total cortical areas innervating a single spinal segment of C7 which controls the forelimb movements, we injected a retrograde tracer into C7 so that they spread to the unilateral gray matter as widely as possible, but not to the ventralmost dorsal column (CS tract). We found that in both infants and adult mice, neurons distributed over an unexpectedly broad area of the rostral two-thirds of the cerebral cortex converge to this segment. Moreover, the cortical areas innervating C7 include areas controlling the hindlimbs (L4). However, with aging from the infant to the adult cell densities greatly declined, mainly due to axon branch elimination. Whole cell recordings from spinal cord cells with optogenetic selective stimulation of CS axons and a cotransfection-expression system that enabled labeling of axons and presynaptic structures showed that the exuberant CS axons make synaptic connections with spinal cells in infants. This suggests neuronal circuits involved in CS tract to C7 are largely reorganized during development. In contrast, the cortical areas innervating L4 were limited to the conventional hindlimb area, and the cell distribution and density did not change during development. No COI.

2P-104

Development and characterization of a monkey model of internal capsular stroke

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We have investigated the mechanisms of functional recovery using a monkey model of cortical lesion, in which a focal lesion is induced in the primary motor cortex (M1). In the present study, as the first step to understand functional recovery mechanisms in a clinically more relevant model, we made a focal lesion in the internal capsule, an area susceptible to stroke. An anatomical MRI scan was performed on Japanese monkeys to identify the part of the internal capsule in which descending motor tracts from the hand digit area of M1 pass though. Endthelin-1, a vasoconstrictor peptide, was then injected into the identified part of the inner capsule (1.5 μL/μg; 15 tracks, 120 μl). The lesion was evaluated using a T2-weighted anatomical MRI scan after injection; the areas of increased T2 signal were observed around the injected area from 3 days to 1 week, then they gradually disappeared within 1 month after lesion. Motor deficit occurred in the contralesional upper limb, as was shown by a decrease in the success rate of a small-object retrieval task, in which monkeys retrieve a small food morsel from a narrow tube. Recovery of gross movements such as reach and power grip occurred during the first week after lesion, while little recovery of dexterous movements including precision grip was observed even at 1 month after lesion. The result is in contrast to that observed in our M1-lesion model, in which drastic recovery of dexterous movements was observed during the first month, suggesting that the compensatory mechanism after internal capsule stroke is different from that after cortical lesion. No COI.

2P-105

Neuronal activity in supplementary motor area of an unrestrained Japanese monkey walking on a treadmill

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To further understand cortical mechanisms for controlling bipedal (Bp) gait in humans, we recorded neuronal activity in supplementary motor area (SMA) of an unrestrained monkey walking either quadrupedally or bipedally on a treadmill. EMG activity was also recorded from trunk and limb muscles. We found that, during quadrupedal (Qp) locomotion, many neurons in trunk/hind-limb regions of SMA modulated their discharge tonically and/or phasically along with the step cycle, while some did not. When the monkey converted its locomotor pattern from Qp to Bp, majority of these (task-related) neurons substantially increased and maintained their discharge frequency tonically with or without enhanced phasic component. Among the rest of recorded (non task-related) neurons, some displayed burst activity closely related to turning movements of head toward the left or right, which was superimposed on the on-going locomotor movements. Meanwhile, activity of trunk and hind-limb muscles during Qp locomotion displayed discrete bursts without tonic activity in each step. During Bp locomotion, the EMG burst was drastically enhanced in all these muscles. Particularly, the trunk muscles displayed substantial tonic activity in addition to the enhanced burst. These results suggest that, during locomotion, monkey SMA contributes to the control of maintaining upright posture so as to accommodate movements of head, trunk and limbs, and may provide insight into the basis of frontal gait disorders in humans. No COI.

2P-106

Intermittent Grasping a High Repulsion Cushion Grip Increases Skin Temperature and Muscle Activity of Hand, Arm and Face as well as Blood Flow in the Prefrontal Cortex

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Cerebral stroke often induces contracture of hand, rigidity, expressionless and speech disturbance. Continuous improper (flexion dominant) afferent stimuli from contracted hand disturb the normal sensorimotor neural network in the spinal cord and brain, and would disturb an appropriate environment for motor output. A high repulsion cushion grip designed by us has a strong repulsive power, thus the stronger it was grasped, the stronger repulsive power was induced. The continuous grasping the grip promoted a prompt recovery from hand contracture after stroke. In this report we investigated the effect of hand movement (intermittent grasp and release of the grip) on temperature and muscle activity of hand, arm and face as well as on cerebral blood flow. Skin temperature of hand, arm, neck and face measured by thermograph increased within minutes. Muscle activities in flexor and extensor of arm, masseter as well as perioral muscle increased. The cerebral blood flow especially in prefrontal cortex was increased. Data indicated that intermittent hand movement increased the activities in not only hand and arm but also those of neck and face as well as the brain. These results would be the base of the effect of repulsion grip for prompt recovery from aftereffects of stroke. No COI.

2P-107

Rewiring of subcortical projections from ventral premotor cortex after primary motor cortex lesion in macaque monkeys

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Neuromotor systems have a capacity to recover motor function following the local damage. We previously reported that the ipsilesional ventral premotor cortex (ip-PMv) was activated during functional recovery of hand movements in the primary motor cortex (M1) lesioned animals. In the present study, we studied the subcortical connections of ip-PMv in the M1-lesioned monkeys after motor recovery in comparison with those in the intact animals, by using biotinylated dextran amine (BDA). A remarkable difference between the lesioned and intact monkeys was observed in the deep cerebellar nuclei, especially in the ipsilesional fastigial nucleus (ip-FN): the BDA-labeled terminals were observed in all of the three M1-lesioned animals, but not in either of two intact animals. The labeled terminals were mainly located in the caudal and medium part of ip-FN, known to contain fastigiospinal neurons. These results suggest that the newly-formed projection from ip-PMv to ip-FN is involved in functional compensation after M1 lesion. No COI.

2P-108

The property of Ia excitation and recurrent inhibition of abdominal motoneurons in the cat

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Abdominal muscles have divergent functions such as respiration, postural control, vomiting and innervated from T6-T13 and from L1-L3 in cats. The present experiments were undertaken to investigate how reflex actions exerted on the motoneurons work and whether recurrent inhibitory pathways are present in cat abdominal motoneurons.Intracellular recordings were made from external oblique (EO), internal oblique (IO), transversus abdominis (TA) and rectus abdominis (RA) motoneurons in L1-L2 on cats anesthetized with sodium pentobarbital. 1)Dorsal roots were intact and muscle nerves electrically stimulated to activate Ia afferents. Ia-EPSPs elicited were found in almost all motoneurones following the stimulation of the homogeneous nerve. They are responsible for the monosynaptic EPSP of motoneurones of their muscles, although these values are longer than those values of the hind limb and the intercostals muscles. 2)Dorsal roots were sectioned and muscle nerves were electrically stimulated to activate motor axons. Recurrent IPSPs elicited were found in some EO motoneurons following the stimulation of EO nerves with an intensity subthreshold activation for axon of the impaled motoneuron. The present results provide evidence of Ia excitation and recurrent inhibition of abdominal motor nucleus. These neuronal circuits might be related to the control of abdominal muscles during various motor activities. No COI.

2P-109

Neuronal activity in the motor thalamus of dopamine-intact and Parkinsonian rats

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Movement difficulties of Parkinson's disease are often associated with the emergence of abnormal beta (13–30 Hz) oscillations in cortex and basal ganglia. The motor thalamus, which mediates the influences of basal ganglia on cortical information processing, is parcellated into two major zones according to their subcortical inputs; the basal ganglia-recipient zone (BZ) and cerebellar-recipient zone (CZ) are bombarded by inhibitory GABAergic inputs from basal ganglia and excitatory glutamatergic inputs from cerebellar nuclei, respectively. To address the key issue of how the activities of neurons in the motor thalamus are disturbed in Parkinsonism, we recorded the spontaneous firing of identified BZ and CZ neurons under anesthesia in dopamine-intact rats as well as Parkinsonian rats with unilateral 6-hydroxydopamine (6-OHDA) lesions. In the Parkinsonian rats, we found no evidence of pathologically-reduced firing rates in either zone of motor thalamus, contrary to classic models. However, after dopamine loss, the tight coupling of firing of BZ neurons to cortical slow (~1 Hz) oscillations was phase-shifted by ~100 °. During cortical activation, many BZ neurons, but not CZ neurons, exhibited pronounced beta oscillations, which were not seen in dopamine-intact rats. We conclude that the thalamocortical substrates of Parkinsonian movement difficulties are more closely related to abnormal firing patterns, as exemplified by excessive beta oscillations, than altered firing rates. No COI.

2P-110

NR2B antagonist ifenprodil improves abnormal forelimb movement induced by D1 agonist SKF38393 in hemi-parkinsonian rat

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For the last decade, the antiparkinsonian action of NR2B selective NMDA receptor antagonist has been studied. However, the ameliorative effect of NR2B antagonist on motor symptoms in animal model of parkinson's disease (PD) or patients with PD is still controversial. Recently we have found that single application of NR2B antagonist ifenprodil demonstrates no anti-parkinsonian effect in hemi-parkinsonian rats (hemi-PD), while co-application of ifenprodil and L-DOPA improves the L-DOPA-induced abnormal forelimb movement in hemi-PD. In this study, to test whether the ameliorative effect of NR2B antagonist on the L-DOPA-induced motor abnormality in hemi-PD is resulted from cooperative action of NR2B antagonist with dopamine D1 receptor agonist to motor system, we investigate the effect of co-application of NR2B antagonist ifenprodil and D1 agonist SKF38393 on the abnormal forelimb movement induced by SKF38393 in hemi-PD by using behavioral test. We performed the cylinder test to estimate forelimb use as motor activity after single administration of SKF38393 or co-administration of ifenprodil and SKF38393. As a result, the co-administration of ifenprodil and SKF38393 completely reversed the dyskinesia-like motor abnormality induced by SKF38393. From this result, we suggest that ifenprodil improves the L-DOPA-induced abnormal forelimb movement in hemi-PD through its cooperative action with D1 agonist to motor system. No COI.

2P-111

Stabilometry test in stroke patients during optokinetic stimulation.

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The postural stability of stroke patients with hemiplegia was studied by using a stabilometric platform with optokinetic stimulation. Four patients who could maintain a sitting posture without assistance were recruited. The patients were asked to sit quietly on a force platform fixed on a stool in a dim room. For optokinetic stimulation, a pattern of random dots, projected onto a screen placed 100 cm in front of the subjects, was moved continuously in horizontal, vertical, or torsional directions with a velocity of 20 deg/s. As a control condition, the same pattern without any movement was presented. In each condition, sway path (SP) and the center of pressure (CoP) position along the antero-posterior (x) and medio-lateral (y) axes were examined. No significant differences were found in the SP values in any stimulus direction when compared with control data. However, the CoP position along the x-axis tended to shift to the paralytic side during horizontal or torsional optokinetic stimulation with paralytic direction, while no clear shift was observed during stimulation with nonparalytic direction. In contrast, the CoP position along the y-axis did not show clear change with any of the directions. These results suggest that the CoP shift induced by optokinetic stimulations can be used as a model for the recovery of deviations in the CoP observed in patients with hemiparesis. No COI.

2P-112

Characteristics of Dynamic Postural Adjustments during floor inclination using Posturography Technique in Rats

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In order to understand characteristics of dynamic postural control of animals, it is important to precisely measure changes of their center of pressure (COP) in response to postural disturbances. In this study, we aimed to examine the properties of dynamic postural adjustment in rats. We measured the COP during floor inclination in the right-left directions at angle from 0 to 30 degrees and at angle velocities of 1.8, 5, 10, 15 deg/sec using a novel posturography technique that we developed. We further measured EMG activities of extensor muscles of fore- and hind-limbs in some animals. The results that we obtained were as follows: 1) during floor inclination at angle from 0 to 30 degrees in right-left directions, two successive dynamic COP changes were commonly observed across animals, 2) regardless of inclination velocities, the inclination angles that dynamic COP changes occurred were at around 8 and 13 degrees, 3) during floor inclination, animals showed tonic EMG activities of extensor muscles, and further exhibited phasic EMG activities corresponding to these dynamic COP changes. From these results, dynamic COP changes together with phasic EMG activities are thought to correspond to compensatory postural adjustments that take place in response to postural disturbances. No COI.

2P-113

Thalamo-cortical inputs to the motor cortex during a self-initiated motor task

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Thalamo-cortical (TC) pathways drive information processing in the cerebral cortex. In the primary motor cortex (M1), TC inputs into layer 1 (L1) are thought to carry basal ganglia information. However, it remains unknown how the activity of L1 TC projections in M1 is related to the execution of a voluntary movement. To address this issue, we conducted two-photon imaging and optogenetic stimulation in M1 while mice performed a self-initiated lever-pull task (Hira et al., 2013). We injected adeno-associated virus that encoded GCaMP6 into the thalamus, and then, performed two-photon calcium imaging of GCaMP6-expressing TC axons in M1 during the task performance. The activity of L1 TC axons was higher during the lever-pull period than during other periods. Next, we perturbed the activity of Channelrhodopsin-2-expressing L1 TC axons during the task performance by blue-light illumination on the cortical surface of M1. We found that the task performance got worse during the photostimulation period. We also found that L1 TC axons innervated the apical dendritic tufts of L5 corticospinal neurons. The results suggest that L1 TC axons send a critical signal directly to L5 corticospinal neurons to execute the lever pull. We are currently examining the temporal correlation between L1 TC axonal activity and the dendritic activity of corticospinal neurons during the lever-pull task. No COI.

2P-114

Purkinje cell activity in the cat cerebellar uvula during optokinetic stimulation

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We have been studying the response pattern of Purkinje cells (P-cells) in the cerebellar nodulus and uvula during sinusoidal head rotation in vertical plane in awake cats. The present study examined the complex spike (CS) and simple spike (SS) during optokinetic stimuli of the P-cells in the uvula. For visual-pattern rotation, a random dot pattern was presented on the monitor that was placed in front of the animal. The random-dot pattern was rotated sinusoidally in horizontal, vertical, or torsional plane under control by a computer. The stimulus parameter of at a frequency of 0.25 Hz with 4 deg amplitude was used for each stimulus plane. In about forty percent of tested cells, clear firing response was observed in CS activities during visual-pattern rotation in either vertical or torsional plane. No clear CS response was found during stimulation in the horizontal plane. On-direction (stimulus direction that increases firing rate) of CS firing was upward for vertical-rotation-responding cells and extorsional direction for torsion-rotation-responding cells. For SS activities, clear response was not observed for all stimulus direction in all tested cells. The results suggest that there is a directional specificity for the CS visual response in the cerebellar region. No COI.

2P-115

Reward-timing dependent bidirectional modulation of spontaneous activity during single-cell operant conditioning with two-photon calcium imaging

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By delivery of a reward when the firing rate of a neuron increases, animals can learn the association between the activity of the neuron and the reward, resulting in specific increase of the activity (single-cell operant conditioning, SCOC). This ability is fundamental to the brain machine interface (BMI) but the underling mechanisms remain unclear. Here we developed SCOC with two-photon calcium imaging in the mouse motor cortex. Head-fixed mice that were transfected with an adeno-associated virus carrying the GCaMP7 gene in motor cortex were trained for 1–2 weeks to perform a self-initiated lever-pull task. After training, we conducted two-photon calcium imaging and mean fluorescence in the target cell was continuously measured. Each time a calcium transient in the cell occurred, a water drop was delivered with a lag time of <300 ms. The activity of target cell specifically increased within 15 minutes. The non-target cells which were accidentally activated before and after the reward increased and decreased the activities, respectively. This reward-timing dependent bidirectional modulation of the activity was reproduced by photostimulation of cells with ChR2. These results indicate that cortical neurons individually respond to the temporal difference between their activities and rewards, which may be fundamental process in BMI learning. No COI.

2P-116

An analysis of the activity of the arm muscles during the service motion in badminton- A longitudinal study during the period between the first and second years of junior high school

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[Objective] Every badminton game starts with a serve, and a player's service skills are considered to influence rallies in a game.With the aim of acquiring knowledge of the development of stroke techniques, the present study, involving players who started playing badminton in the first year of junior high school, examined improvements in their serves for one year. [Methods] The subjects were seven badminton players who started to play when they were in the first year of junior high school - a longitudinal study during the period between the first and second years of junior high school. The subjects were asked to perform short back-hand serves, and the activity of the arm muscles was assessed. [Results] The mean iEMG levels of the musculus extensor carpi ulnaris and musculus flexor carpi radialis were significantly lower when the students were in the second than first year. The mean iEMG level of the deltoid was significantly higher when they were in the second than first year. [Conclusion] These results suggest that players who have continuously practiced tend to use the deltoid instead of forearm muscles during the serve motion. Changes in muscle activity are considered to be related to: a decrease in the time between the decision to hit the shuttlecock and moment the racket contacts it; and improvements in the service accuracy. No COI.

Poster Presentations
Reproduction

2P-117

Arm preference of ophiuroids during locomotion and its implication for the internal control scheme of arm usage

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Ophiuroids are pentaradially symmetrical echinoderms that locomote on the seafloor by the coordinated rhythmic movements of their multijoint arms. How such coordinated movements are achieved is a focus of interest from the viewpoint of neurobiology as well as robotics, because ophiuroids lack the central nervous system that exerts the integrative control over the five arms. To explore the underlying mechanism of the arm coordination, we studied their arm usage during locomotion. Previous studies reported two types of locomotion of ophiuroids, rowing and reverse rowing. In the former, one arm acts as the leading arm, being pointed forward along the axis of locomotion while in the latter, an arm is pointed backward. We found the followings. 1) During rowing, a particular arm preferentially acted as the leading arm. 2) When the preferred leading arm was anesthetized, it was replaced by one of the other arms. 3) Both intact and anesthetized individuals preferentially performed rowing, and finally 4) reverse rowing occurred infrequently even when multiple arms were anesthetized such that it would have allowed more efficient usage of the intact arms. These findings indicate that the ophiuroids' nervous system prioritizes the selection of the leading arm over others for locomotion. An autonomous decentralized control model was developed that accounts for the observed arm usage during locomotion. No COI.

2P-118

Regulation of sperm hyperactivation by serotonin

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On mammalian spermatozoa, capacitation is the essential process to fertile to the egg. Capacitated spermatozoa exhibit an acrosome reaction in head and hyperactivation in flagellum. Recently, it has been investigated that some hormones regulated sperm hyperactivation in a dose dependent manner. In the present study, I examined whether serotonin, which releases from cumulus-oocyte complex, regulated hyperactivation using hamster spermatozoa. Serotonin significantly enhanced sperm hyperactivation in a dose dependent manner although it did not affect sperm motility. Serotonin regulated sperm hyperactivation through two types of serotonin receptor. High concentration (nM - μM) of serotonin enhanced sperm hyperactivation through $5HT_4$ receptor. On the other hand, low concentration (fM - pM) of serotonin enhanced sperm hyperactivation through $5HT_2$ receptor. In general, sperm hyperactivation is regulated through cAMP signals and Ca^{2+} signals. Because $5HT_2$ receptor and $5HT_4$ receptor are associated with Ca^{2+} signals and cAMP signals respectively, it was examined whether serotonin regulates sperm hyperactivation stimulating Ca^{2+} signals and cAMP signals via each receptor. From results, serotonin regulated sperm hyperactivation through IP_3 receptor and PKA when it stimulated spermatozoa via $5HT_2$ receptor. When serotonin stimulated spermatozoa via $5HT_4$ receptor, it regulated sperm hyperactivation through PKA only. In conclusion, serotonin regulates sperm hyperactivation in a dose dependent manner through Ca^{2+} signals and cAMP signals via $5HT_2$ receptor and $5HT_4$ receptor. No COI.

2P-119

Regulation of hyperactivated motility by fluid osmolality in hamster spermatozoa

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Mammalian spermatozoa cannot fertilize with ovum without capacitation. Generally, capacitated spermatozoa exhibit hyperactivation, a specialized flagellar movement with increased bend amplitude to penetrate the zona pellucida. In mammals, osmolalities of the fluids from male internal genitalia (seminal vesicle, prostate, and epididymis) are higher (<420 mOsm) than that of the fluids from female reproductive tract (around 290 mOsm). This means that mammalian spermatozoa experience osmotic shock in the female reproductive tract. However, the impact of osmotic shock on flagellar motility has not been elucidated. In the present study, we investigated the effect of physiological osmotic change on mammalian sperm motility using hamster. The osmolalities of fluids from male internal genitalia and vagina in hamster were higher (around 390 mOsm) than that of blood plasma (around 300 mOsm). When spermatozoa were incubated in various osmotic conditions (220–390 mOsm), osmolality near semen and vaginal fluid suppressed sperm motility activation, and sperm motility was activated as the extracellular osmolality decreased. Moreover, the appearance of hyperactivated motility was delayed at the osmolality near semen and vaginal fluid, and was accelerated as the extracellular osmolality decreased to that of oviductal fluid. Our data suggest that mammalian sperm motility is regulated by fluid osmolality to be hyperactivated at oviduct, where sperm fertilize with ovum. No COI.

2P-120

Identification of the interactive regions between dicalcin and gp41; development of compounds that control the fertilization rate in frogs.

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Fertilization is a well-coordinated and sequential process, beginning with taxon-specific sperm-binding to egg-coating envelope. We recently discovered that *Xenopus* dicalcin, present in the egg-coating envelope, remarkably suppresses fertilization ***in vitro*** through binding to gp41, a glycoprotein of the egg envelope. We have previously reported that two amino-acid regions of dicalcin were candidates of the native binding region on dicalcin for gp41, and the corresponding peptides decreased the fertilization rate in a dose-dependent manner. In the present study, we, oppositely, attempted to determine the binding region on gp41 for dicalcin. To roughly estimate the region, we generated poly-histidine-tagged gp41 and two ZP domains of gp41: ZP-N and ZP-C, and examined their binding to dicalcin. The results showed that dicalcin bound to recombinant gp41 and ZP-C, but not to ZP-N, suggesting that ZP-C domain is the responsible domain for the binding of gp41 to dicalcin. We next prepared a set of deletion mutants of ZP-C domain, and found the potential dicalcin-binding region of gp41, among which we obtained a synthesized peptide that increased the fertilization rate, indicating that this amino acid region is responsible for the native binding of gp41 to dicalcin. Thus, our novel results elucidated the interactive regions between dicalcin and gp41, and help to understand molecular mechanisms of the action of dicalcin, and also lead to development of bioactive peptides that are capable of 'increasing' the fertilization rate as well as 'decreasing' the one. No COI.

2P-121

New intrauterine probe for measuring uterine electrical impedance of mice using four-electrode method

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Biological electrical impedance (EI) measured using a four-electrode method (FEM) has been widely applied to assess body constituents. The FEM involves two pairs of electrodes, an outer pair (current electrodes, CE) and an inner pair (voltage electrodes, VE). EI is calculated by measuring the voltage between VE produced by AC currents passing between CE. The uterine endometrium (UE) changes its conditions in each estrus phase. The aim of this study was to clarify the characteristics of UE EI using mice. Eight adult female Slc:ddy female mice were used in this study. We checked the estrus cycle of mice by the cytological evaluation of vaginal smears. The intrauterine probe (IUP) was composed of two electrodes and a core of tungsten wire of 0.3 mm in diameter. We rolled platinum wire on the core wire as CE and VE at four sites within 10.0 mm from the tip. We measured electrical impedance using an impedance checker between 2.5 and 350 kHz, and calculated characteristic parameters such as the impedance at 0 Hz (R0) and infinity Hz (Ri). Under inspired anesthesia, we exposed the uterus and inserted the IUP into the vagina and advanced its tip into the uterine cavity, and then measured UEI. R0 and Ri were larger in metestrus than in proestrus and diestrus. The UEI parameters measured by FEM might be novel parameters for evaluating UE conditions. No COI.

2P-122

Changes of G-protein-coupled Receptor (GPR) 30 mRNA Levels in the Rat Uterus during Pregnancy, Labor and the Estrous Cycle

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Estrogen induces physiological and morphological changes associated with a large number of gene expressions in the uterus. The G-protein coupled receptor 30 (GPR30) has been reported to be a membrane-associated estrogen receptor (ER). In this study, to investigate the regulation of GPR30 in the uterus, changes of GPR30 mRNA levels in the uterus were examined in rats during pregnancy, labor and the estrous cycle and in estrogen-treated ovariectomized (OVX) rats. No significant change in GPR30 mRNA levels was detected during pregnancy and labor. During the estrous cycle, GPR30 mRNA levels in the morning and afternoon on proestrus were significantly lower compared with those on diestrus, metestrus and estrus. This decline in GPR30 mRNA levels on proestrus was abolished by the administration of ER antagonists, ICI182780 and raloxifene, on diestrus, but not by tamoxifen. After a single injection of 17β-estradiol (E2, 12.5 μg/ rat), GPR30 mRNA levels decreased within 2 h, but showed no significant change 24 h after treatment. The injection of PPT, an ERα agonist, or DPN, an ERβ agonist, decreased GPR30 mRNA levels 3 h after treatment. These results suggest that GPR30 mRNA levels in the rat uterus change during the estrous cycle and that these changes are induced by estrogen, probably via ERα and ERβ. No COI.

2P-123

Essential amino acids deficient diet suppress estrous cyclicity in rat

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Reproductive functions are influenced by circumstances, especially their nutritional status. We investigated whether deficiencies of essential amino acids (EAAs) could be a signal of systemic undernutrition to suppress reproductive functions. Adult female Wistar-Imamichi rats were daily fed a calorically identical diet which lacks one of EAAs, threonine (Thr-def), tryptophan (Trp-def), lysine (Lys-def) or valine (Val-def), and monitored their estrous cyclicity by vaginal smears. All of the EAA deficient treatments decreased food intake, body weight, plasma glucose and triglyceride (TG) compared to a control diet. It also stopped the estrous cycle in different timing among EAAs; the days when continuous diestrus started were 5.8±1.1 in Val-def, 9.7±0.7 in Trp-def, 12.0±0.8 in Thr-def and 18.7±4.5 in Lys-def. Then, rats were subjected to a pair-feeding (PF) experiment in which control diet was restricted to either 100, 66 or 33% of the spontaneous Thr-def intake. Both of the PF-100 which consumed same calorie as the Thr-def, and the PF-66 of which body weight was equally decreased as the Thr-def, did not show a delayed estrous cyclicity. Only the PF-33 group showed a cessation of the estrous cycle in the same timing (12.8±0.2 d) as the Thr-def. Plasma glucose and TG were similar to the Thr-def in the PF-33, but higher in other groups. These results indicated that a deficiency of EAA suppresses reproductive functions in particular manner among EAAs. No COI.

2P-124

Functional decline of alpha4beta2 nicotinic receptors in the aged rat brain

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Our previous study demonstrated that long-term stimulation of nicotinic receptors enhances cortical acetylcholine (ACh) release in adult rats but not in aged rats. In the present study, we examined which subtype of nicotinic receptors contribute to this enhancement of cortical ACh release in adult rats. Adult rats (4–9 months) received sustained subcutaneous infusion of either a selective alpha4beta2 nicotinic receptor agonist (ABT-418, 10 μg/kg/h) or a selective alpha7 nicotinic receptor agonist (GTS-21, 20–100 μg/kg/h) for 14 days. Under urethane anesthesia, we determined ACh release in the parietal cortex induced by electrical stimulation of the nucleus basalis of Meynert (NBM). The stimulus intensity-dependent ACh release in the cortex was greater in rats treated with alpha4beta2 agonist than in saline-treated control rats. On the other hand, the magnitudes of cortical ACh release in rats treated with alpha7 agonist were similar to those in control rats. We conclude that alpha4beta2 nicotinic receptors mediate enhancement of cortical ACh release induced by NBM activation after long-term stimulation of nicotinic receptors in adult rats. These findings, together with our previous studies, suggest the reduction of the alpha4beta2 type nicotinic receptor function in aged rats. No COI.

Poster Presentations
Development, Growth, Aging

2P-125

Hypoplasia of the vascular smooth muscle cell in the ATBF1 KO mouse

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ATBF1 (AT-motif binding factor 1), a 404-kDa transcription factor that contains 4 homeodomains and 23 zinc finger motifs, involved in transcriptional regulations and differentiation on many types of cells including neurons. To gain insights into the physiological functions of ATBF1, we generated ATBF1 deficient mice by gene targeting. ATBF1 knockout (KO) mice are died on the day of birth because of respiratory failure caused by the impairment of air space formation of lung. Body weight was decreased in E13 and E17 ATBF1 KO mouse compared to wild type mouse, and ATBF1 KO mouse appears a dysplasia in some organs. ATBF1 was expressed in vascular smooth muscle cells but not in endothelial cells. Although pulmonary parenchyma such as alveolus epithelial cells was normal, a pulmonary artery diameter was smaller than that of WT. And also we found that number of smooth muscle cell was decreased in coronary artery of ATBF1 KO mouse, and that loss of ATBF1 lead to the down-regulation of PDGFR-a, a key regulatory factor of vascular formation, and induced vascular smooth muscle cell defects. Together, these data indicate that ATBF1 may an important role in the development of vascular smooth muscle. No COI.

2P-126

Erythropoietin facilitates the differentiation of mouse embryonic stem cells into insulin-producing cells

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To date, there are few ways of the fundamental cure for diabetes. Cell replacement therapy has become possible by utilizing artificially generated pancreatic β-cells from embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). However, both the efficiency of the method and potency of differentiated cells are insufficient. It is important to develop the more effective method of generating a pancreatic β-cells for the regenerative medicine. In the present study, we screened several endogenous factors involved in organ development and found that the treatment with erythropoietin (Epo) facilitated the differentiation of mouse ESCs into insulin-producing cells. Moreover, Epo increased *Pdx1* gene expression at the stage of pancreatic progenitors. Next, we examined which differentiation stage Epo acted on. Epo treatment during early stage of the differentiation significantly increased *Sox17* gene expression, as a marker of definitive endoderm, and decreased the expression of *Oct4*, a marker of pluripotency. These results suggest that the treatment with Epo at an early stage is useful for the efficient generation of insulin-producing cells. Epo is a cytokine promoting the production of red blood cells via JAK-STAT signaling pathway. The mechanism of *Sox17* induction by Epo treatment is under investigation. No COI.

2P-127

Cell Functional Differences in Relation to the Distribution of Cytokeratins 13, 14 and 18 during Morphogenesis of the Tongue

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On the morphogenesis of the rat tongue, we could recognize the specific appearance and distribution of cytokeratin 13 (K13), 14 (K14) and 18 (K18). K14-specific immunoreactivity was first detected on the lingual epithelium of fetuses on embryonic day 17 (E17), at which time the lingual epithelium was composed of a few layers of cuboidal cells, and K13-specific immunoreactivity on E19. The number of layers of cuboidal cells in the lingual epithelium increased from E17 to E19. At postnatal stages, K13-specific immunoreactivity became to be evident in the intermediate cells of the interpapillary cell columns and K14-specific immunoreactivity did in the basal and suprabasal cells of the papillary and interpapillary cell columns according to the development of filiform papillae. K13-specific immunoreactivity was evident in cells of the differentiating intermediate cells, while K14-specific immunoreactivity was detected in proliferating cells of the basal and suprabasal layers. K18-specific immunoreactivity was detectable in the single layer of periderm cells that covered the lingual epithelium of fetuses on E13 and E15. Immunoreactivity was distributed throughout the cytoplasm in periderm cells. On E17, K18-specific immunoreactivity was very distinct in the periderm cells, which had become swollen and elliptical. Periderm cells are estimated to have peculiar functions in this process. No COI.

2P-128

The expression of PTEN in the development of mouse cochlear lateral wall

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Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a tumor suppressor gene that regulates various cell processes including proliferation, growth, synaptogenesis, neural and glioma stem/progenitor cell renewal. In addition, PTEN can regulate sensory cell proliferation and differentiation of hair bundles in the mammalian cochlea. In this study we use immunofluorescence, western blot and RT-PCR to reveal the expression of PTEN in the developing cochlear lateral wall, which is crucial for regulating K+ homeostasis. Relatively high levels of PTEN are initially expressed in the marginal cells (MCs) of the lateral wall at embryonic day (E) 17.5 when they start to differentiate. Similarly high levels are subsequently expressed in differentiating root cells (RCs) at postnatal day (P) 3 and then in spiral ligament fibrocytes (SLFs) at postnatal day (P) 10. In the mature cochlea, PTEN expression is low or undetectable in MCs and SLFs but it remains high in RCs and their processes. The expression pattern for PTEN in the developing lateral wall suggests that it plays a critical role in the differentiation of the cellular pathways that regulate K+ homeostasis in the cochlea. No COI.

Poster Presentations
Digestion, Absorption

2P-129

Effects of moxibustion on restraint stress-induced delayed gastric emptying in conscious rats

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Moxibustion (MOX) has been used to treat gastric symptoms. Animal studies have already shown that acupuncture at the ST-36, which located on tibialis anterior muscle, have improved stress-induced delayed gastric emptying (GE). However, the mechanism of the beneficial effects of MOX remains unknown. The purpose of this study was to investigate whether the MOX at ST-36 improves restraint stress-induced alteration in the GE of conscious rats. Rats were given of solid food after 24-h fasting. Immediately after the ingestion, rats were loaded to restraint stress. Ninety minutes after the feeding, rats were euthanized and gastric content was removed to calculate GE. Indirect MOX was performed above the bilateral ST-36 throughout the stress loading. To investigate whether vagal nerve was involved in mediating the stress-induced alterations of GE, atropine was administered (i.p) just before the start of restraint stress, and bilateral truncal vagotomy was performed in 14 days before GE measurement. GE in the 90 min study period was significantly delayed by restraint stress. This delayed GE was significantly accelerated by MOX at ST-36, which had been disappeared by atropine administration and vagotomy. Restraint stress-induced delayed GE is improved by MOX at ST-36. Enhanced vagal nerve activity are involved in mediating the stimulatory effects of MOX on restraint stress-induced delayed GE. No COI.

2P-130

The different pattern of gastrointestinal motility and blood flow following oral glucose and water ingestion

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Oral glucose ingestion increases splanchnic blood flow (SBF), but water does not. We hypothesized that the increased SBF by glucose ingestion may be elicited by the exaggerated elevation of gastrointestinal motility. To investigate this hypothesis, we measured the gastrointestinal motilities of stomach (by electrogastrography; EGG) and small intestine (by electroenterography; EEnG), and the blood flow (BF) of superior mesenteric artery (SMA) as an index of SBF before and every 15 min for one hour after ingesting 350-ml of either 8% glucose (Glu) or water (Wa) in 6 male subjects. The gastrointestinal motility was evaluated the power derived from the spectral analysis to EGG and EEnG recordings before and after ingestion. BF of SMA was measured by pulsed-Doppler ultrasonography. In addition, gastric emptying (GE) rate was evaluated by echo-ultrasonographic images. Compared to the baseline, the SBF was significantly increased not by Wa, but by Glu ingestion. Whereas EGG power was increased after ingestion of both solutions, EEnG power was significantly increased merely by Glu ingestion. GE rate was significantly delayed in Glu to Wa ingestion. These results suggest that glucose ingestion elicits a different pattern of gastrointestinal motility from water ingestion. Especially, the exaggerated EEnG response may be a factor which leads to increasing SBF. No COI.

2P-131

Characteristics of autonomic nervous response in irritable bowel syndrome

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Autonomic dysregulation induced by higher sympathetic nervous activity and lower parasympathetic nervous activity is considered to be the main characteristic in male patients, and visceral hypersensitivity is considered to be the main characteristic in female patients with Irritable bowel syndrome (IBS). However, autonomic characteristic in the female patients is still controversial. The purpose of this study was to clarify the characteristics of autonomic response to physical (cold pressor test) and psychophysiological (stroop test) stimuli in female patients with IBS, along with the mood change associated with the stimuli. Mood states were estimated by a profile of mood states (POMS), and autonomic nervous function was evaluated by a spectral analysis of heart rate variability (HRV), baroreflex sensitivity and blood pressure (BP). Repeated measures analysis of variance (ANOVA) revealed significant interaction of group (IBS and control) x time course after the stimuli in HF amplitude, that is, delayed recovery of HF amplitude after the physical stimulus, and delayed and diminished recovery after the psychophysiological stimulus (stroop test) in female patients with IBS. Repeated measures analysis of variance (ANOVA) also revealed significant main effect of group (IBS and control) in baroreflex sensitivity, that is, the baroreflex sensitivity is generally lower in female patients with IBS, though the time courses in both groups were similar. No COI.

2P-132

Anion secretion induced by short-chain fatty acids in the human terminal ileum

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Short-chain fatty acids (SCFAs), predominantly acetate, propionate and butyrate, are fermented products existing more than 100 mM in all in the large intestinal lumen of mammals including humans. We and some research groups have previously reported that SCFAs not only are absorbed as nutrient, but also stimulate intestinal mucosa inducing a variety of physiological responses, e.g. transepithelial ion transport in the rodents. However so far, there has been no report of the SCFA-induced ion transport in human intestine. This reason seemed to be because SCFAs did not evoke ion transport in the human large intestine clearly. However in the present study, we found that the SCFAs induced an ion transport in the human surgical specimens of terminal ileum by using mucosa-submucosal preparations mounted on the Ussing chambers. In the rat and guinea pig colon, it has been reported that propionate evokes anion secretion, but acetate does not. However in the human terminal ileum, the luminal addition of acetate also concentration-dependently evoked an anion secretion the same as propionate. Moreover, the luminal acetate-induced response was attenuated dependent on the concentration of propionate pretreated, and vice versa. These results suggest that the reflux of SCFAs from cecum to terminal ileum passing through the ileocecal valve may induce a fluid secretion in the human terminal ileum. No COI.

2P-133

Desacetyl bisacodyl induced ion transport in rat colon mucosa

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Bisacodyl, 4,4%-(2-pyridylmethylene)-bis-(phenyl acetate), is a stimulant laxative used for the treatment of constipation. Bisacodyl shows to inhibit intestinal water absorption and to induce net water secretion in rats. Desacetyl bisacodyl is the active metabolite of bisacodyl. Previous reports suggest that the laxative action of bisacodyl is initiated through a direct interaction of luminal side of the colon. However, the mechanism of action of bisacodyl has not yet been elucidated. Aim of the present study is to investigate the mechanism of the desacetyl bisacodyl induced secretion. Methods: Mucosa-submucosal tissue preparation of rat colon and rectum were made of rat tissues. Short-circuit current (I_{sc}) and tissue conductance (G_t) were measured as indices of transepithelial electrogenic ion transport and permeability by Ussing chamber.Results: Mucosal addition of desacetyl biscodyl (10^{-6}–10^{-6} M) concentration-dependently induced a transient decrease and subsequent increase in II_{sc} in rat distal colon and rectum. Neural blockade by tetrodotoxin (10^{-6}M) did not affect, but inhibition of prostaglandin by the pretreatment of piroxicam $^{-5}$ almost completely reduced the desacetyl bisacodyl-induced II_{sc} increase. These results suggest that the secretory effect of desacetyl bisacodyl is possibly interaction in apical side of distal colon and rectum. COI properly declared.

2P-134

Effect of electrical acupuncture on colonic transit measured by a new method using the radiopaque marker in conscious rats.

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Acupuncture has been used for treating functional gastrointestinal (GI) disorders, including irritable bowel syndrome, constipation and diarrhea. However, the precise mechanism of acupuncture on GI function remains unclear. We examined the influence of acute restraint stress (RS) and electrical acupuncture (EA) on colonic transit (CT) of conscious rats by the method using the radiopaque marker. Male Sprague-Dawley rats were used. Under pentobarbital sodium anesthesia, an in-dwelling silastic cannula was inserted into the caecum and positioned to enter the proximal colon. Five days after the surgery, rats were exposed to RS for 90 min. The rats treated with EA (10 Hz, 3 V, 0.5 msec) at ST-36, which located on tibialis anterior muscle, for 20 min before stress loading. Twenty metal radiopaque markers, whose diameter is 1.5mm, were administrated into the proximal colon with saline. It was visible throughout the GI tract via soft X-ray every 30 min until 120 min. CT was significantly increased by RS. Furthermore, nearly all-radiopaque markers passed out of the colon until 60 min during RS. In contrast, CT was delayed by EA regardless of stress loading, and the entire radiopaque markers remained at 120 min in the colon. These results raise the possibility that EA at ST-36 may inhibit stress induced-accelerated CT. EA may improve on GI disorders, like irritable bowel syndrome. No COI.

2P-135

Colonic transit measured by a new method using the radiopaque marker in conscious rats.

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Colonic transit (CT), which measured by bead expulsion time, the number of fecal pellet output or calculating the geometric center, is not proper for evaluating chronologically. On the other hand, through there are individual differences in effects of acupuncture and responses of drugs, it is important that CT repeatedly measures on individual. Therefore, we developed a new method using the radiopaque marker for time-course analysis of CT of rats. Male Sprague-Dawley rats were used. Under pentobarbital sodium anesthesia, an in-dwelling silastic cannula was inserted into the caecum and positioned to enter the proximal colon. Five days after the surgery, 20 metal radiopaque markers, whose diameter is 1.5mm, were administrated into the proximal colon with saline (1.0 ml). It was visible throughout the GI tract via soft X-ray every 30 min until 120 min at 2 consecutive days. CT was calculated by maximum migration length of the radiopaque marker. On the 1st day, maximum migration length was 32.6±15.6 mm at 60 min and 62.4±20.4 mm at 120 min. The next day, it was 24.8±20.4 mm and 51.2±31.4 mm, respectively. There were not significantly differences of maximum migration length between 1st and 2nd day. These results showed that the new method using the radiopaque marker was stable and reliably. This method can measure CT by chronologically and may be useful to elucidate the mechanism of colonic disorders, like IBS. No COI.

2P-136

Curcumin reduces colonic fermentation after the ingestion of milk

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Curcumin is one of the main constituents of turmeric. We previously reported that dietary tumeric activated bowel motility and carbohydrate colonic fermentation. Ninety percents of Japanese adult are milk intolerance. The ingestion of milk causes marked increase in breath hydrogen (H2) by the colonic fermentation. Thus, we wondered how curcumin affect the colonic fermentation after the milk ingestion. Subjects fasted for 12 h and ingested milk with or without curcumin. Breath H2 concentrations were analyzed every 15 min for 6 h by gas chromatography with a semiconductor detector. Ingestion of milk without curcumin showed a delayed and sustained increase of breath H2 in subjects with milk intolerance for up to 9 hours, whereas ingestion of milk with curcumin significantly decreased the rise in breath H2 in a dose-dependent manner. We conclude that curcumin decreases the colonic fermentation caused by the ingestion of milk. No COI.

2P-137

Anti-fibrotic effects of Daikenchuto (TU-100) associated with intestinal myofibroblast TRPA1 channel

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Daikenchuto (TU-100), a traditional oriental herbal medicine used for post-operative ileus and constipation. TU-100, composed of *Zingiberis Rhizoma*, *Ginseng Radix* and *Zanthoxyli Fructus*, generally accelerates gastrointestinal motility, and increases intestinal blood flow and gastrointestinal hormone secretion. However, the mechanism of TU-100's actions remains unclear.

Intestinal myofibroblasts play an important role in fibrotic stenosis. Previously, we reported pathophisiological involvement of transient receptor potential (TRP) channels in colonic myofibroblasts. Micro-array assay with an intestinal myofibroblast cell line (InMyoFib) detected TRPA1 at highest expression among TRP family. The TRPA1 agonist AITC and an active ingredient of TU-100 [6]-shogaol respectively induced Ca^{2+} influx in InMyoFib, which was antagonized by co-treatment with a selective TRPA1 channel blocker HC-030031. Importantly, 24-hour incubation with TU-100 100μg/ml enhanced the mRNA and protein expressions of TRPA1 in InMyoFibs.

We next explored a potential role of TU-100 on fibrosis signal transduction and collagen synthesis associated with TGF-β1. TU-100 increased Type I Collagen expression and the phosphorylation of Smad2 and p38-MAPK at the downstream of TGF receptors.

These results suggest that TU-100-mediated myofibroblastic TRPA1 expression and Ca^{2+} influx could be an important process suppressing intestinal fibrogenesis, the mechanism for which may account for the pharmacological actions of TU-100. No COI.

Poster Presentations
Endocrinology

2P-138

Regional difference in blood flow response to cold pressor test

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To investigate whether vascular responses to increase in blood pressure differ among various blood vessels, we recorded relative changes of mean arterial pressure (MAP) and blood flow values in the middle cerebral artery (MCA), retinal arterial (RA), and choroidal vessels (CV) during cold pressor test (CPT) in 24 healthy males (24.1 ± 0.2 yrs). The CPT was induced by placing hand in cold water (5~10°C) for 2 min. MAP significantly increased from the baseline by 18 ± 2% ($P<0.05$). Blood flow values in MCA, RA, and CV significantly increased by 4 ± 2%, 9 ± 2%, and 16 ± 3%, respectively ($P<0.05$). Conductance index (CI), which was calculated as each blood flow value divided by MAP, in MCA and RA significantly decreased by 10 ± 3% and 6 ± 2%, respectively ($P<0.05$). There was a significant correlation in relative changes of blood flow values between RA and CV ($r=0.55$), while no correlation was obtained among other vessels. There were no correlations in relative changes of CI among any blood vessels. It was suggested that vascular responses to increase in blood pressure during CPT differ among vessels. No COI.

2P-139

Morphological and histological properties of MCH neurons in ES cell-derived hypothalamic culture

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Mouse embryonic stem cell-derived hypothalamic culture (ES-hypo) is reported to generate peptidergic neurons producing vasopressin, neuropeptide Y, or agouti-related protein (Wataya et al., 2008, PNAS). However, it remains unclear whether ES-hypo is identical with the native hypothalamus where a variety of peptidergic neurons are working in harmony. The present study focused on the development of melanin-concentrating hormone (MCH) neurons in ES-hypo. MCH is a hypothalamic neuropeptide controlling feeding behavior and energy handling. We found that MCH-positive neurons increased in number and maturity in ES-hypo during 3rd-5th weeks of induction, which is similar to developmental neurogenesis. These MCH neurons showed histological characters common to native ones *in vivo*: co-expression of MCH with GABA or cocaine- and amphetamine-regulated transcript (CART), and reciprocal synaptic connections with orexin-positive or tyrosine hydroxylase-positive (*i.e.*, dopaminergic) neurons. Moreover, the MCH neurons exhibited various morphologies corresponding to those found in the developing stages. In summary, we have identified MCH neurons co-exist with other peptidergic neurons in ES-hypo, and also they share many characters with native MCH neurons. It is suggested that ES-hypo provides a new experimental system to investigate the development and neurophysiology of the hypothalamic MCH system. No COI.

2P-140

Adiponectin regulates proopiomelanocortin and neruopeptide Y neurons in the hypothalamic arcuate nucleus

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Aim: Adiponectin facilitates insulin sensitivity and regulates energy expenditure in the peripheral organs, It has also been suggested that serum adiponectin secreted from adipocytes enters and exerts effects in the brain. Adiponectin receptors are expressed in several regions of the brain including the hypothalamic paraventricular nucleus and arcuate nucleus (ARC), the centers regulating feeding and energy expenditure. The aim of this study was to determine the effect of adiponectin on the activity of proopiomelanocortin (POMC) neurons and neuropeptide Y (NPY) neurons in the ARC. Methods: Slices containing ARC were produced from the hypothalamus of POMC-GFP or NPY-GFP mice. The effects of adiponectin on the membrane potential and firing in POMC neurons and NPY neurons in slices were recorded under current clamp mode. Food intake was measured from mice which received intracerebroventricular injection of 150 ng adiponectin just before dark period.Results: Bath application of adiponectin (100 ng/ml) depolarized and increased firing in POMC neurons whereas it decreased firing in ARC NPY neuron. Food intake was suppressed by intracerebroventricular adiponectin injection.Conclusion: Adiponectin activates POMC neurons and inhibits NPY neurons in ARC. These effects may be relayed to suppression of food intake by adiponectin. No COI.

2P-141

The expression of the oxytocin-monomeric red fluorescent protein 1 fusion gene in the hypothalamus and spinal cord of adjuvant-induced arthritic rats

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Oxytocin (OXT) is a well-known neurohypophysial hormone that is synthesized in the paraventricular (PVN) and the supraoptic nuclei (SON). Several lines of evidence have suggested that OXT plays an important role in pain modulation and analgesia. However, little is known about the neuronal spinal networks responsible for OXT effects. The present study examined the effects of chronic inflammatory/nociceptive stress on the expression of the OXT-monomeric red fluorescent protein 1 (mRFP1) fusion gene in the hypothalamus and spinal cord, using the adjuvant arthritis (AA) rat model. To induce AA, OXT-mRFP1 transgenic rats were intracutaneously injected heat-killed Mycobacterium butyricum (1 mg/rat) in paraffin liquid at the base of their tails. We observed mRFP1 fluorescence in the PVN, the SON, and the dorsal horn in the spainal cord when AA was established. The expression of the mRFP1, and the OXT genes in the hypothalamus were also measured by *in situ* hybridization histochemistry. OXT and mRFP1 mRNA levels in the PVN and the SON were significantly increased on day 22 in AA rats. The mRFP1 fluorescences in the SON, the PVN, and the dorsal horn were apparently increased on days 15 and 22 in AA rats compared with controls. These results suggest that OXT may play an role in pain modulation and analgesia in AA rats. No COI.

2P-142

Roles of secretin-oxytocin system in the control of social recognition

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It has been known that oxytocin receptors are implicated in autism. Furthermore, mice lacking the oxytocin or oxytocin receptor gene show autistic phenotypes, such as socio-behavioral deficits including social recognition deficits. On the other hand, secretin, a gastrointestinal hormone, has been reported to be a possible treatment for autism. Mice lacking the secretin or secretin receptor gene also show socio-behavioral deficits. Secretin administration facilitates oxytocin release. However, relationship between oxytocin and secretin concerning social behavior remains to be determined.
We have demonstrated that secretin activates oxytocin neurons of the supraoptic nucleus and facilitates oxytocin release. In the present study, we investigated whether secretin facilitates social recognition via activation of oxytocin receptor. An intracerebroventricular injection of secretin increased the capability of social recognition in rats, and the facilitative effect of secretin was blocked by an oxytocin receptor antagonist. Furthermore, local application of secretin into the supraoptic nucleus facilitated social recognition and its action was blocked by an oxytocin receptor antagonist injected into the medial amygdala. All these results suggest that secretin activates oxytocin neurons in the supraoptic nucleus, potentiates dendritic oxytocin release, and facilitates social recognition via the oxytocin/oxytocin receptor system. No COI.

2P-143

Paired-pulse facilitation in parallel fiber-Purkinje synapses were decreased in congenital hypothyroid mice.

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Thyroid hormone (TH) plays a critical role on development of brain. Deficiency of TH during development induces the organizational changes of the cerebellum, causing cerebellar ataxia. However, underling mechanisms of such abnormal development are poorly understood. To clarify the effect of TH on the development of neuronal circuit, we used the dual oxidase maturation factor (DUOXA) mutation mice, which show congenital hypothyroidism. As previously reported, proliferation and migration of granule cells were delayed after P15 in homozygous mice (Duoxa-/-) using Cresyl violet staining. However, organizational changes of the granule cell in Duixa-/- mice disappeared by P25. The branching of dendrite and the size of soma in the Purkinje cell were not significantly different between Duixa-/- and control mice. Although the structure of the cerebellum was not different between Duixa-/- and wild type at P25, cerebellar ataxia was detected in Duixa-/- mice. To examine the mechanism, we next performed the neurophysiological study. We found that paired-pulse facilitation, which is commonly detected in parallel fiber-Purkinje cell synapses, were decreased in Duixa-/- mice especially at P15 without the obvious change of expression levels of protein, which regulate neurotransmitter release. Taken together, we conclude that the anatomical catch-up growth of cerebellum cannot normalize its function because of the function of neuronal circuit itself was continuously affected by hypothyroidism. No COI.

2P-144

Comparison of the effect of current therapeutic agents for diabetes in Cdkal1-deficient mice

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Genetic variations in the Cdk5 regulator associated protein 1-like 1 (CDKAL1) gene have been associated with type 2 diabetes (T2D). Cdkal1 catalyzes 2-methylthio modification at position 37 adenosine in cytosolic tRNALys(UUU). The 2-methylthio modification is critical for the accurate decoding of lysine codon. Deficiency of 2-methylthio modification in pancreatic beta-cell-specific cdkal1-deficient (KO) mice show aberrant proinsulin translation, and resulted in impaired insulin secretion and glucose intolerance. Given the similarity of symptoms between Cdkal1 KO mice and T2D patients carrying risk CDKAL1 variation, we utilized Cdkal1 KO mice as T2D model to investigate the long-term effects of anti-diabetic drugs. We treated Cdkal1 KO mcie with glibenclamide, glucagon like peptide 1 (GLP1) agonists including exendin-4 and liraglutide, and sitagliptin, an inhibitor of GLP-1 protease (DDP-4) up to 8 weeks. Glucose metabolism and gene expression were then investigated. Long-term application of glibenclamide impaired the glucose tolerance and insulin secretion in KO mice. In contrast, GLP-1 agonists and sitagliptin significantly improved the glucose tolerance and insulin secretion. Examination of gene expression showed that both liraglutide and sitagliptin, but not glibenclamide, decreased the expression of unfolded protein response-related genes as well as L-Myc, a dedifferentiation-related gene. Our results suggest that the GLP-1 related drugs will be beneficial for T2D patients carrying risk Cdkal1 variation.

2P-145

Suppression of AMPK activity in skeletal muscle improves metabolic abnormalities of streptozotocin-induced diabetes mellitus in mice.

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AMPK (AMP-activated protein kinase) is activated by decreased intracellular energy level. Here we found that strepotozotocin (STZ)-induced diabetes activates AMPK and its signaling pathways in skeletal muscle in mice. Surprisingly, inhibition of AMPK activity in skeletal muscle in STZ-induced diabetes by preferentially expressing dominant-negative AMPK (DN-AMPK) significantly improved STZ-induced hyperglycemia, and high level of plasma free fatty acids and ketone bodies, although plasma insulin level was low. Moreover, STZ-treated DN-AMPK mice improved atrophy of white adipose tissue (WAT) and skeletal muscle, body weight loss and increased survival rate. In parallel with the WAT mass, STZ-treated DN-AMPK recovered and increased plasma level of leptin and adiponectin, respectively.
IL-6 (Interleukin-6) and a novel myokine, irisin, stimulate lipolysis and fat utilization in WAT. STZ-induced diabetes increased both IL-6 and irisn levels in skeletal muscle and plasma, and those are returned to the control levels in DN-AMPK mice. Infusion of leptin or neutral antibody for IL-6 by osmotic minipump improved the metabolic changes in STZ-induced diabetes, similar to those in STZ-treated DN-AMPK mice. Furthermore, chronic infusion of AMPK inhibitor, compound C, also improved the metabolic abnormities in STZ-induced diabetes. These results thus unveil a key role for muscle AMPK in metabolic abnormities in STZ-induced diabetes, and important role of organ networks in the metabolic regulation. No COI.

2P-146

Alteration of thyroid hormone signaling by treadmill training with different intensity in male rat skeletal muscle.

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Thyroid hormone (TH) controls metabolic activity in a wide range of tissue. Aerobic exercise training facilitates oxidative phosphorylation and glycolysis of skeletal muscle. Thus, we studied if TH signaling pathway is activated by a several intensity of training. Male adult rats received 30 min/day treadmill training with different exercise intensity for 12 days. By lactate levels, rats were divided into stationary control (SC, 0 m/min), aerobic (A, 15 m/min, for 30 min) and anaerobic (AN, 25 m/min, 30 min) training groups. We have previously reported that TSH level was suppressed in both A and AN groups during the exercise. TRβ1 mRNA and protein levels were increased in A group, but not in AN group. In the present study, we examined whether the sensitivity to triiodothyronine (T3) was altered in several TH-target genes including MyoD, and etc. Twenty-four hours after the last training, T3 was injected intravenously. Six hours later, rats were sacrificed and the soleus muscle was dissected out to extract total RNA. Then, semiquantitative RT-PCR was carried out. Particularly, induction of Na^+K^+-ATPase β1 expression by T3 was significantly augmented in A group, but not in AN group. Chromatin immunoprecipitation assay in L6 myoblast-derived clonal cells showed that TRβ1 bound to the nucleotide sequence that is similar to typical TH response element in promoter region of Na^+K^+-ATPase β1 gene. These results indicate that aerobic training alters TH signaling at least in part by increasing TRβ1 expression. No COI.

2P-147

Distribution of TRPC channels expression and their role in catecholamine secretion of adrenal medullary cells.

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Adrenal medullary (AM) cells secrete catecholamines in response to ACh releasing from the splanchnic nerve terminal. This ACh-mediated signal is thought to be received mainly by nicotinic ACh receptors, and the role of muscarinic Ach receptors in the neurotransmission is controversial although muscarinic receptors have biochemically and/or functionally been detected in several species including guinea-pig, rat and human AM cells. We have previously demonstrated that an inhibition of TASK1 channel was responsible for muscarinic activation in rat AM cells. Whereas an activation of nonselective cation channels as well as an inhibition of TASK1 channels was involved in guinea-pic AM cells. The electrophysiological and pharmacological studies suggest TRPC channels are a candidate. In fact, immunoblotting and immunocytochemistry revealed expression of TRPCs1, 4, 5, and 7 in guinea-pig AM cells; TRPCs1 and 4 were mainly localized in the cytoplasm in the resting cells whereas TRPC5 was in and/or near the plasma membrane. In this study, we examined whether or not the localization of TRPCs in guinea-pig AM cells is changed upon the muscarinic stimulation and TRPC1 channels are coupled with TRPC4 or TRPC5 channels. We also utilized PC12 cells to investigate detailed mechanisms of the trafficking of TRPC channels in the muscarinic stimulation. No COI.

2P-148

Ghrelin attenuates incretin effect of GLP-1 in rats

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Ghrelin, an acylated 28-amino acid peptide, reportedly restricts insulin release in islet β-cells and thereby regulates glucose homeostasis. We have reported that ghrelin attenuates cAMP signaling, thereby inhibiting glucose-induced increases in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and insulin release in islet β-cells. This study aimed to clarify whether ghrelin counteracts the effects of glucagon-like peptide-1 (GLP-1), a physiological incretin hormone, on insulin release, cAMP and $[Ca^{2+}]_i$ signaling in islet β-cells. In rat isolated islets under static incubation, glucose (8.3 mM)-induced insulin release was potentiated by GLP-1, and this potentiation was suppressed by ghrelin. The glucose (8.3 mM)-induced cAMP production in islets was enhanced by GLP-1, and this enhancement was blocked by ghrelin. In the presence of 8.3 mM glucose, GLP-1 evoked $[Ca^{2+}]_i$ increases in single β-cells and they were significantly attenuated by ghrelin. The results indicate that ghrelin attenuates glucose-dependent insulinotropic actions of GLP-1 by suppressing cAMP-mediated $[Ca^{2+}]_i$ signaling in islet β-cells. We next performed in vivo studies in rats. In oral glucose tolerance tests in which GLP-1 is released and exerts its incretin effect, intraperitoneal administration of ghrelin markedly attenuated the increase in plasma insulin and enhanced the increase in blood glucose. These findings indicate that ghrelin attenuates incretin effect of GLP-1 and that interaction between ghrelin and GLP-1 plays an important role in physiological regulation of glucose-induced insulin release in islet β-cells. No COI.

2P-149

Effects of Ghrelin on tuberomammillary nucleus neurons

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Ghrelin, known as stimulator of release of growth hormone (GH) and food intake, is produced by stomach, other peripheral organs and some brain regions and acts on GH secretagogue receptors (GHS-Rs) in the some peripheral organs and brain regions. Recent study also suggests that ghrelin participates in regulation of sleep-wakefulness. Tuberomammillary nucleus (TMN), which involves histaminergic neurons that contribute to maintain arousal state, also expresses GHS-Rs. However, direct action of ghrelin on TMN neurons is remained unclear. Thus, we examined electrophysiological effects of ghrelin on TMN neurons using rat brain slice preparations and whole-cell patch clamp recording technique. Application of ghrelin depolarized TMN neurons in both absence and presence of tetrodotoxin, and the depolarization was inhibited by antagonist for GHS-Rs. The ghrelin-induced depolarization was accompanied by increase of membrane resistance, and decreased under high-K^+ and/or Ca^{2+} free extracellular solution. These results suggest that ghrelin depolarizes TMN neurons postsynaptically via GHS-Rs with a dual ionic mechanism including a decrease in K^+ conductance and an increase of Ca^{2+} conductance, and may participate in the regulation of sleep-wakefulness via the excitatory effect on TMN neurons. No COI.

2P-150

Mifepristone upregulated adiponectin secretion during 3T3-L1 adipogenesis.

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Mifepristone, a putative steroid receptor antagonist, clinically serves as an anti-cancer agent, eliciting both cytostatic and cytotoxic effects on malignant cells. However, the metabolic effects of long-term treatment with mifepristone have remained unclear. Here we show that mifepristone also promotes the ability of cultured mouse 3T3-L1 cells undergo adipogenesis elicited induced by co-treatment with Dexamethasone (Dex), 1-methyl 3-isobutylxanthine (IBMX), and Insulin (Ins). Specifically, we observed increases in cells treated with mifepristone-Dex-IBMX-Ins when compared to those with Dex-IBMX-Ins alone in 1) the protein and gene expression levels of adipocyte marker aP2, the adipocyte fatty acid binding protein, 2) the lipid accumulation (Bodipy 493/503 staining), and 3) the adiponectin secretion levels into culture medium. Intriguingly, mifepristone-induced gene expression of aP2 and adiponectin was markedly attenuated by neutralizing antibody to adiponectin (ANOC9104). Because adiponectin, an antidiabetic hormone, is able to enhance adipogenesis in an autocrine/paracrine fashion, we propose that mifepristone may stimulate differentiation of preadipocytes into adipocytes possibly by modulating the expression and secretion levels of adiponectin. These results therefore identify a previously unknown pharmacological action of mifepristone on fat cell metabolism. No COI.

Poster Presentations
Cell Physiology, Molecular Physiology (1)

2P-151

Re-evaluation of regulatory volume decrease of several cell lines by flow cytometric measurements.

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The mechanism of regulatory volume decrease (RVD) after hypotonic stress has been widely investigated. The independent activations of volume regulatory chloride channel and potassium channel are considered to induce the KCl efflux from the cell together with water , causing cell volume decrease. Although this concept is widely accepted, the speed of regulatory volume decrease differs by cell types. In addition, initial swelling after the hypotonic stress differs mong cell species. To re-evaluate the mechanism of RVD, we measured initial cell swelling after hypotonic stress and volume change in HeLa cells, HEK293 cells, and PC12 cells. We observed that the RVD processes of HEK293 cells and PC12 cells are slower compered with HeLa cells. Initial cell swelling of PC12 cells are slower than HeLa cells and HEK293 cells. These initial swelling process may represents the expression level of aquaporins of plasma membrane which increase the water permeability of cell membrane. Further studies would be necessary to prove this possibility. No COI.

2P-152

Cytotoxic anions damaged HeLa cells via volume regulatory chloride channel

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Special anions damages the cells with unknown mechanism. We call those anions cytotoxic anions, including VO_4^{3-}, CrO_4^{2-}, SeO_3^{2-}, WO_4^{2-}, etc. We examined the cytotoxic effects of these anions. SeO_3^{2-} was found to have most cytotoxic effects to HeLa cells with micro-molar concentration. The chloride channel inhibitors, DIDS or NPPB, blocked the effect of cytotoxic anions. When such anions were applied just after hypotonic challenge, the cytotoxic effects were markedly increased. These results suggest that cytotoxic anions damaged HeLa cells via volume regulatory chloride channels. The mechanism of cell damage by these anions after entrance into the cells should be further examined. No COI.

2P-153

AVP neurons possess the cell volume regulation mechanism under hyperosmotic conditions

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It is known that many types of mammalian cells can regulate their cell volume after transient osmotic cell shrinkage under sustained hyperosmotic conditions by the mechanism called the regulatory volume increase (RVI). In contrast, it was reported that brain osmosensory magnocellular neurons isolated from rat supraoptic nucleus (SON) cannot exhibit RVI during hyperosmotic perturbation. In agreement with this report, in the present study, the RVI event was not observed in arginine-vasopressin (AVP) neurons selectively isolated from the SON of AVP-enhanced GFP transgenic rats. In the presence of flufenamic acid (FFA), however, a significant RVI was observed in AVP neurons under sustained hyperosmotic stress. The RVI response observed with FFA was completely inhibited by a stilbene-derivative inhibitor of Cl^-/HCO_3^- anion exchanger (AE), DIDS, or an inhibitor of Na^+/H^+ exchanger (NHE), amiloride. Thus, it is concluded that brain osmosensory AVP neurons possess the RVI ability which is masked by some FFA-sensitive mechanism under normal conditions. No COI.

2P-154

Structural and functional analysis of TRPC6 for the interaction with PKG Iβ

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cGMP/cGMP-dependent protein kinase (PKG) signaling pathway plays an important role in vasorelaxation. In this pathway, PKG plays a key role of lowering the intracellular Ca^{2+} level and/or Ca^{2+} sensitivity of contractile machinery by protein phosphorylation. However, the downstream of PKG is still poorly elucidated. Recently, we reported that a transient receptor potential (TRP) non-voltage-gated cation channel, TRPC6, is a novel PKG substrate and negatively regulated via phoshorylation on its threonine 69 (Takahashi *et al. J Physiol*, 2008). Furthermore, using yeast two-hybrid system to identify responsible binding domains in both TRPC6 and PKG type I proteins, we revealed that two PKG subtypes, both PKG Iα and Iβ, interact with TRPC6 N-terminal domain (the 87th annual meeting of the Physiological Society of Japan, 2010), and that only PKG Iβ showed the cGMP-dependent interaction with TRPC6 N-terminal domain and acidic amino acids, located in the leucine zipper region, are responsible for that interaction (the 89th and 90th annual meeting of the Physiological Society of Japan, 2012 and 2013). In this study, to determine which amino acids are responsible for the interaction between the PKG Iβ and TRPC6, series of mutations are introduced to TRPC6 N-terminal domain, and protein interaction between PKG and TRPC6 was examined by yeast two-hybrid assay. One mutant (K115E) showed the loss of interaction between PKG Iβ and TRPC6. This result indicates that basic amino acid located in the ankyrin-like repeat domain of TRPC6 N-terminal region is responsible for interaction with PKG Iβ. No COI.

2P-155

diDCP-LA-PE stimulates GLUT4 translocation to the cell surface

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The present study investigated the effects of the newly synthesized phospholipid derivatives diDCP-LA-phosphatidylethanolamine (PE), -phosphatidylserine (PS), -phosphatidylcholine (PC), and -phosphatidylinositol (PI), which contain the linoleic acid derivative DCP-LA at the α and β position, on GLUT4 trafficking.diDCP-LA-PE, diDCP-LA-PS, diDCP-LA-PC, and diDCP-LA-PI activated most of the investigated PKC isozymes, but diDCP-LA-PE alone significantly activated PKCλ/ι and -ζ. diDCP-LA-PE, diDCP-LA-PS, and diDCP-LA-PI exhibited a strong inhibition on PTP1B activity, while diDCP-LA-PC enhanced the activity. diDCP-LA-PE stimulated GLUT4 translocation to the cell surface in differentiated 3T3L1-GLUT4myc adipocytes, with the highest potential among the phospholipid derivatives, and the effect was prevented by an inhibitor of PI3K, Akt1/2, or PKC or each knocking-down. The results of the present study show that diDCP-LA-PE stimulates GLUT4 translocation to the cell surface still in the absence of insulin by upregulating insulin signaling along an insulin receptor/insulin substrate 1 (IRS-1)/PI3K/Akt(1/2) axis as a result of suppressing tyrosine dephosphorylation of insulin receptor and IRS-1 in association with PTP1B inhibition and by activating PKCλ/ι and -ζ. No COI.

2P-156

Characteristics of exogenous expression of ROMK K channels with EGFP fused to the different terminus in polarized and non-polarized membranes in cultured M-1 cells

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ROMK K channels in the apical membrane of renal collecting duct plays a key role in potassium secretion to the urine. In this study, ROMK1 K channel gene (*KCNJ1*) with EGFP fused to N- or C-terminus was exogenously transfected to cultured M-1 cells. We used two types of culture conditions. One was single cells, and the other was confluent M-1 cells on membrane inset with polarized apical membrane. Although there was no appreciable difference in fluorescence intensity and the localization between the cells with ROMK1 fused to N-terminal EGFP and to C-terminal EGFP in both single and confluent cells, frequency of channel current observation was significantly different in single cells. Namely, channel current is observed in 71.4 % of the cells transfected ROMK1 with EGFP fused to N-terminus, and in 12.2 % of those fused to C-terminus. However, in the confluent cells, channel current was highly observed in the apical membrane of the cells with ROMK1 fused to either terminus (69.2 % and 66.7 % of EGFP fused to N- and C-terminus, respectively). Since the fusion of EGFP to the specific terminus may disrupt the role of the terminal function, it is suggested that C-terminus of ROMK1 is important for expression of channel activity in single cells, whereas expression of channel activity in polarized apical membrane of M-1 cells required no specific terminal functions of ROMK1. No COI.

2P-157

Calcium holes: a novel PMCA-mediated mechanism for calcium signaling

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Calcium ion is a versatile signaling molecule that regulates various cellular functions. Accordingly, calcium mediates multiple signaling processes concurrently in a single cell, owing to an unveiled cellular machinery that encapsulates many different calcium signaling processes. Here, I propose the concept of "calcium holes" that provide an encapsulated nano-environment for calcium signaling. A calcium hole is a Ca^{2+}-deficient nanodomain having a diameter of about 100 nm that develops around a plasma membrane Ca^{2+}-ATPase (PMCA) molecule. Ca^{2+}-extrusion by PMCA maintains the local Ca^{2+}-level in the calcium holes lower than the global level, providing encapsulated intracellular domains that are insulated from each other, and also from the bulk Ca^{2+}-environment. Experimental evidence indicates that cardiac Na/Ca exchanger (NCX) in whole-cell clamped mouse heart cells is regulated by calcium holes. Selective inhibition of PMCA enhanced the NCX current reflecting an elevated local Ca^{2+}-level, without a change in its reversal potential reflecting an unchanged global Ca^{2+}-level. This indicates the intracellular regulatory Ca^{2+}-binding domain (CBD) of the NCX senses the local Ca^{2+}-level in the calcium hole, of which the local Ca^{2+}-level is elevated by the PMCA inhibition. Numerical simulation of local Ca^{2+}-diffusion predicts the diameter of a calcium hole to be about 100 nm. The regulation of other calcium signaling molecules by calcium holes and their relation to special membrane structures such as lipid rafts are also discussed. No COI.

2P-158

GPRC6A receptor is involved in amino acid-induced glucagon-like peptide-1 secretion from GLUTag cells

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Glucagon-like peptide-1 (GLP-1) is a peptide hormone secreted from enteroendocrine L cells in response to dietary nutrients. However, the precise molecular mechanisms by which dietary nutrients regulate GLP-1 secretion from enteroendocrine L cells remains unclear.

In the present study, we used enteroendocrine L cell line GLUTag cells to clarify molecular mechanism of GLP-1 secretion. First we performed RT-PCR analysis and found that GPRC6A, which is a putative L-amino acids-sensing receptor, is expressed in GLUTag cells. Live cell imaging and ELISA analysis revealed that L-ornithine, a potent stimulus for GPRC6A, induces GLP-1 secretion from GLUTag cells in a dose-dependent manner. To elucidate the intracellular signaling responsible for L-ornithine-induced GLP-1 secretion from GLUTag cells, we examined the effect of a GPRC6A receptor antagonist, a phospholipase C inhibitor and an inositol triphosphate receptor antagonist on L-ornithine-induced GLP-1 secretion. Application of those antagonists significantly suppressed L-ornithine-induced GLP-1 secretion from GLUTag cells. Furthermore, depletion of endogenous GPRC6A expression inhibited L-ornithine-induced GLP-1 secretion.

Taken together, these findings suggest that Gq-coupled receptor, GPRC6A receptor functions as an amino acids sensor and regulate L-ornithine-induced GLP-1 secretion from GLUTag cells. No COI.

2P-159

Vesicular nucleotide transporter regulates ATP storage in secretory lysosome in astrocyte

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Astrocytes have diverse roles including protection of neurons, regulation of vesicular system and modulation of synaptic transmission. In addition to responding to transmitters released from neurons, recent studies have suggested that astrocytes themselves synthesize and release gliotransmitters. However, the precise molecular mechanism that regulates ATP secretion from astrocytes and the source of ATP in astrocytes remains unclear. A vesicular nucleotide transporter (VNUT)/ solute carrier family 17, member 9 (SLC17A9) has been thought to regulate accumulation of ATP into vesicles. In the present study, we found that VNUT was expressed in both primary cultured cortical astrocytes and glioma cell line C6 cells, and mainly localized in lysosome revealed by immunohystochemical analysis. Live cell imaging revealed that the fluorescent intensity of VNUT-associated secretory lysosomes did not change after depolarization was induced. This result indicates that the VNUT-associated lysosomes stay in plasma membrane after lysosomal exocytosis. Inhibition of VNUT activity by Evans Blue decreased the amount of ATP accumulation into secretory lysosome. Furthermore, depletion of endogenous VNUT expression by small interference RNA and inhibition of VNUT activity by Evans Blue decreased the amount of ATP release from astrocytes. Taken together, these findings suggest that VNUT plays a key role in ATP accumulation into lysosome and ATP release via secretory lysosome in astrocytes. No COI.

2P-160

Transient vacuole formation induced by V-ATPase inhibitor-a hint of the mechanism of vacuole formation

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We have previously shown that membrane-permeable weak bases cause persistent vacuole formation and that individual vacuoles are formed around a lysosome. Since the vacuole outside the lysosome was weakly acidic and the inner lysosome was strongly acidic, we hypothesized that vacuoles are formed by extrusion of accumulated free H^+ and water from the lysosome. In this study, in order to clarify this hypothesis in part, the vacuole formation was investigated after treatment of Saos-2 cells with the V-ATPase inhibitor bafilomycin A1 (Baf). Time-lapse microscopy revealed that continuous treatment with Baf caused the formation of vacuoles 15 min after the start of treatment, and vacuoles diminished after 1 h. The individual vacuoles were formed around the spherical organelles. These organelles were immunoreactive for anti-lysosome-associated protein-2 antibody. Staining with amine-reactive dye CFDA-SE indicated that vacuoles contained no protein. The pH indicator Lysosensor Yellow/Blue staining showed that organelles inside the vacuoles were strongly acidic and vacuoles were weakly acidic. After vacuoles diminished, both organelles and cytosol were weakly acidic. These results suggest that Baf-induced transient vacuoles are formed around lysosomes and the vacuole formation is caused by extrusion of H^+ and water from the lysosome. Then H^+ in the lysosomes is depleted by long-term inactivation of V-ATPase and H^+ in the vacuoles diffuses to cytosol, leading to the equalized distribution of H^+ among lysosomes, vacuoles and cytosol, which paralleled by disappearance of vacuoles. No COI.

2P-161

Involvement of Ras-PI3K-mediated calcium signaling in the regulation of endocytosis

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We have reported that PI3K binds to activated Ras in the endosomes, which is involved in the regulation of clathrin-independent endocytosis and the incorporation of a range of substances. In this study, we explored the mechanism by which Ras is activated during endocytosis. Using the FRET-based biosensor Cameleon, transient increases in the intracellular Ca^{2+} concentration were observed upon infection with influenza viruses, which are known to enter host cells via endocytosis. Because buffering of both intracellular and extracellular Ca^{2+} abolished the virus-dependent Ras activation, Ca^{2+} is responsible for the Ras activation. We also revealed that the Ca^{2+} elevation triggered RhoA activation at the membrane. On the other hand, expression of dominant negative form of RhoA inhibited such Ca^{2+} oscillations, which hampered subsequent virus entry and infection. Hence, RhoA and Ca^{2+} constitute a positive feedback loop, with RhoA not only being activated by Ca^{2+}, but also inducing subsequent $[Ca^{2+}]i$ elevation during virus infection. Moreover, this signaling circuit was also found to participate in uptake of other substances, including dextran and transferrin. Taken together, the viruses may exploit signaling pathways reserved for physiological substrate uptake, which is mediated by RhoA and calcium, for their effective entry. No COI.

2P-162

Cytosolic chloride ion is a key factor in lysosomal acidification and function of autophagy in human gastric cancer cell

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The purpose of the present study was to clarify roles of cytosolic chloride ion (Cl^-) in regulation of lysosomal acidification (intra-lysosomal pH (pH_{lys})) and autophagy function in human gastric cancer cell line (MKN28). The MKN28 cells cultured under a low Cl^- condition elevated pHlys and reduced the intra-lysosomal Cl^- concentration ($[Cl^-]_{lys}$) via reduction of cytosolic Cl^- concentration ($[Cl^-]_c$), showing abnormal accumulation of LC3II and p62 participating in autophagy function (dysfunction of autophagy) accompanied by inhibition of cell proliferation via G0/G1 arrest without induction of apoptosis. We also studied effects of direct modification of H^+ transport on lysosomal acidification and autophagy. Application of bafilomycin A1 or EIPA elevated pH_{lys} and decreased $[Cl^-]_{lys}$ associated with inhibition of cell proliferation via induction of G0/G1 arrest similar to the culture under a low Cl^- condition, however unlike low Cl^- condition application of the compound, bafilomycin A1 or EIPA, induced apoptosis associated with increases in caspase 3 and 9 without large reduction of $[Cl^-]_c$ compared with low Cl^- condition. These observations suggest that the lowered $[Cl^-]_c$ primarily causes dysfunction of autophagy without apoptosis via dysfunction of lysosome induced by disturbance of intra-lysosomal acidification. No COI.

Poster Presentations
Environmental Physiology

2P-163

Orexin contributes to feeding and drinking behaviour induced by methamphetamine

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Methamphetamine is an addictive drug and known to have anorectic and hyperactive effect. Recently, orexin has been suggested to contribute to drug addiction and rewarding. Orexin is a neuropeptide localized in hypothalamus and is known to play an important role in many physiological systems including feeding behaviour, motivation and maintaining wakefulness. Therefore, we investigated whether orexin contributed to changes in feeding and drinking (as a maker of drug-seeking) behaviour in response to oral administration of methamphetamine. In orexin knockout (KO) mice and their wild type (WT), we provided methamphetamine containing drinking water (0.005%) for 21 days and measured food intake, drinking, body weight and locomotor activity during the treatment. Methamphetamine treatment decreased food intake by 25±3% (n=6, $P<0.05$) during the first three days of treatment in WT mice, but not in KO mice. Methamphetamine increased the amount of drinking by 17±4% (n=6, $P<0.01$) in WT mice, but not in KO mice. Larger amount of drinking indicate an increase in drug-seeking behaviour, suggesting addictive effect of methamphetamine. These data suggest that orexin contributes to methamphetamine-induced anorectic and addiction. No COI.

2P-164

Effects of intracerebroventricular administration of colchicine on oxytocin-mRFP1 expression in transgenic rats

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Colchicine blocks the axonal transport, resulting in peptide accumulation in the cell body. Recently, we generated a transgenic rat that expresses the oxytocin (OXT)-monomeric red fluorescent protein 1 (mRFP1) fusion gene in the hypothalamo-neurohypophysial system (HNS). In the present study, we investigated the effects of intracerebroventricular (icv) administration of colchicine on OXT-mRFP1 expression in these transgenic rats. In vehicle-treated rats (controls) OXT-mRFP1 fluorescence was observed in the HNS, including the magnocellular neurosecretory cells of the supraoptic and the paraventricular nuclei and the nucleus circularis, and posterior pituitary (PP). Icv administration of colchicine caused marked increase of OXT-mRFP1 fluorescence in the hypothalamic MNCs, while decrease in the PP in comparison with control rats. The OXT-mRFP1 fluorescence was not observed in the ectopic area of neither controls nor colchicines-treated rats. The expected changes of OXT-mRFP1 fluorescence in the HNS after icv administration of colchicine indicate that OXT-mRFP1 fusion protein may be transported by axonal flow and secreted from the PP into the systemic circulation. The OXT-mRFP1 transgenic rats are useful animal model to study dynamic changes of OXT in the HNS. No COI.

2P-165

Hypergravity- and microgravity-induced plastic alteration of the vestibular system and its countermeasure.

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The vestibular system is known to be highly plastic; thus, it is possible that if animals or subjects are in different gravitational environment, functions of the vestibular system are altered. To examine this, a series of experiments were performed. Effects of hypergravity: The vestibular-mediated pressor response and the vestibular-related motor skill (measured by rotarod) were impaired, if rats were reared under 3 g condition for 2 weeks. Effects of microgravity: The vestibular-mediated pressor response induced by head-up tilt was examined in two astronauts before and after 4-month stay in International Space Station. It was 16 mmHg before launch, but completely abolished at the 2nd day of landing and 2 weeks after landing, but recovered by 2 months after landing. Countermeasure: Not only the effect of microgravity but also hypergravity on the vestibular system are due to the reduced phasic input to the vestibular organs, since head movement-related vestibular input was reduced to < 20% under 3 g condition. Thus, externally applied vestibular stimulation might improve the vestibular function. To examine this, galvanic vestibular stimulation (GVS; ±50 μA biphasic square wave, 1 s duration) was applied throughout 2-week hypergravity condition. GVS significantly improved the vestibular-mediated pressor response and rotarod skill. These results suggest that, GVS can be used for a countermeasure to improve the vestibular function seen in astronauts. No COI.

2P-166

Candidate mechanism of skin vasodilation caused by contact with high concentration-CO_2-water in the anesthetized rat

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Hot spring water containing high concentration (>1 g/L) carbon dioxide (CO_2-hot spring) has long been applied to the patients of cardiovascular diseases. The skin surface in the CO_2-hot spring is covered with small bubbles and its color turns red within several minutes of bathing even at the water temperature less than 35 °C. The skin reddening is reproducible by artificially made bath-water containing comparable CO_2-concentration to CO_2-hot spring (CO_2-water) and is caused by vasodilation followed by an increase in skin blood flow. Although skin reddening is not obvious, the skin-vasodilation is observed in laboratory animal rats also. In this study, we investigated endogenous substances which might be involved in the mechanism of skin vasodilation caused by soaking in CO_2-water. Experiment was performed using male Wistar rats under urethane anesthesia. Control tap-water immersion for 30 min followed by CO_2-water immersion for 30 min (water temperature of about 35 °C) was repeated 2 times to confirm a reproducibility of the response in each rat. Skin vasodilation during CO_2-water immersion was inhibited by treatment of cyclooxygenase inhibitor indomethacin, but not by NOS-inhibitor L-NAME. Among vasodilatory prostaglandins (PGE_1, PGE_2, PGI_2), PGE_2 level increased by about 30% in the homogenate of the skin removed from rat during CO_2-water immersion. Local PGE_2-production may be involved in the skin vasodilation observed under this experimental condition. No COI.

2P-167

The role of TRPV1 that contribute to the thermal effects of immersion to the carbon dioxide-rich water

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Carbon dioxide-rich water (CO_2) bathing have been used for balneotherapy of patients with hypertension or peripheral occlusive arterial disease. We have previously reported that CO_2 bathing exerts thermal effects than fresh water (FR) bathing. Currently, the modification of the activities of skin warm and cold receptors by CO_2 application is widely accepted. As a result, subjects feel a neutral sensation may be lowered by 2°C during CO_2 bathing. The transient receptor potential vanilloid 1 (TRPV1) is non-selective cation channel that can be activated by heat above 43°C and various chemical agonists are known including such as capsaicin or H+. However, the mechanism of the action of CO_2 on thermoreceptor has only been partially clarified in humans. We examined the role of TRPV1 channel related to an increase in thermal sensation when it was immersed into CO_2 of FR at 33°C after capsaicin application to the skin of the forearm. Thermal sensation of immersion into the CO_2 was higher about 30% than immersion into the FR. These results suggest that the CO_2 might have activated the TRPV1. We also report changes in thermal sensation of immersion into the CO_2 when it inhibits the activity of sodium channels by 5% of lidocaine application to the skin. No COI.

2P-168

Physiological responses to passive heating in younger and elderly

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One older (aged 59 years) and two younger (aged 22 and 24 years) healthy men were exposed to a standard heat stress. These subjects sat in a controlled environment at ambient temperature 35.1±0.3°C and relative humidity 48.9±7.2% to be received the heat stress placing the lower legs and feet in a water bath at 42°C, for 60 min. The eardrum temperature (ET) in both the older and younger men increased during passive heating. But ET at the last 30 min of younger men were lower than that of the older. These stress increased the local sweat rates (LSR, mg/cm2/min) on thigh and chest for both the older and the younger men, but induced no differences between two groups for LSR. Systolic and diastolic blood pressure (mmHg), mean arterial pressure (mmHg), and heart rate (beats/min) changed similarly between the older and younger men during passive heating. Now, we are trying to increase the number of observations, and to measure the inflammatory cytokines. No COI.

2P-169

Effects of lumbar warming on dysmenorrheal pain and uterine blood flow

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More than 80% of menstruating women complain of dysmenorrheal pain, which is mainly caused by pain-producing substances in the uterine endometrium. Warming the lower abdominal or lumbar region is known to relieve dysmenorrheal pain. Especially, a heat and steam-generating sheet (HSG) which warms the attached skin area to around 40 °C for 5–8 hours is effective for dysmenorrheal pain relief. However, the mechanism of this pain relief is still unknown. The aim of this study was to clarify the mechanism by investigating pain relief and uterine blood flow, which may remove pain-producing substances. Seven university students (mean: 21.7 ± 0.3 yrs old) were included in this study. On the second or third day of menstruation, each subject applied an HSG sheet of 54 cm^2 to her lower lumbar region for 60 min. We measured velocities of uterine blood flow using ultrasound Doppler flowmetry every 15 min. We detected maximum (Vmax) and minimum (Vmin) velocities and evaluated the resistance index, RI=(Vmax-Vmin)/Vmax, which is known to reflect peripheral vascular resistance. The subjects also self-rated dysmenorrheal pain using numerical rating scale (NRS), from 0 to 10, at the same time as flowmetry. Five of the subjects experienced pain relief within the 60-min warming. The RI also decreased in all subjects. However, no marked correlations were observed between the RI and NRS. There may be no association between pain relief and a uterine RI decrease. No COI.

2P-170

Effect of hyperthermia on adult rats after neonatal hypoxic-ischemic encephalopathy on learning ability

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A maternal body temperature higher than 38.5°C is known to be one of the most serious risk factors worsening the prognosis in human neonatal hypoxic-ischemic encephalopathy (HIE). However, its effects on learning ability after growth have not been clarified. We investigated the effect of hyperthermia after neonatal HIE on the learning ability of an adult rat model. The left carotid artery of 7-day-old rats was ligated under anesthesia. After 1-h recovery, all rats were exposed to 8% oxygen at an ambient temperature (Ta) of 40°C for 15 min, and then placed in a chamber with Ta of 40°C (n=10; H group). A sham group (n=6; S group) was also established. Eleven to twelve weeks after the operation, the learning ability of the rats was assessed using a step-down passive avoidance test. Before measurement, we decreased the rats' body weight by 15% by fasting. On the first day of measurement, rats were placed on an insulated rubber platform onto a metal-grid floor. Once the rats stepped onto the floor, they experienced discomfort via an electrical shock to the foot. We performed this procedure until the rats no longer stepped down onto the floor. The interval between rats moving from the rubber to metal floor was measured once a day for the next three days. On the last day of measurement, although the S group rats stayed on the rubber floor for 595 sec, the H group rats stepped onto the floor after an interval of 181 sec. This indicates a higher hyperthermia in labor with HIE may result in a restricted learning ability after growth. No COI.

2P-171

Comparison of average changes in brain level blood pressure between SHR and WKY rats during acceleration stress

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Gz stress greatly affects normal blood circulation in the brain and causes loss of consciousness. Increased aortic valve blood pressure imparts a protective effect against Gz stress. Hypertension involves higher aortic valve blood pressure, but blood pressure regulation is different from normotension. We hypothesized that hypertension is not an advantageous factor against sustained Gz and estimated the effect of nifedipine on blood pressure during Gz loading in hypertensive rats. We used male spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats. The SHR were randomly allocated to the nontreatment (nSHR) and treatment (tSHR) groups. The tSHR were administered 0.1% nifedipine diet for 2 weeks. Blood pressure at the level of the brain (APLB) and heart rate (HR) were continuously measured during Gz loading using a centrifuge for small animals. The mean APLB was significantly higher in the nSHR and tSHR than the WKY rats before centrifugation, and the mean APLB was lower in the tSHR than the nSHR. During +5 Gz loading, average changes in APLB was significantly higher in the nSHR than in the tSHR and WKY rats; however, no significant difference was observed between these changes in the tSHR and WKY rats. No significant difference was observed in HR between both groups. These results suggest that APLB decreases easier in the hypertensive rat than in the normotensive rat during Gz loading, and this is improved by care for hypertension. Hypertension is not an advantageous factor against Gz loading. No COI.

2P-172

Changes in cerebral and muscle oxygenation during normoxic and hypoxic exercise.

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The purpose of this study was to examine the influence of acute hypoxia and exercise on oxygenation responses in brain and exercise muscle. Ten male judo athletes performed an incremental treadmill exercise for 6 minutes while breathing room air (normoxia) and hypoxic gas (FIO2=0.112). Near-infrared spectroscopy (NIRS) was used to monitor concentration changes of oxy- and deoxyhemoglobin (d[O2Hb], d[HHb]) in the both right frontal cerebral cortex and left vastus lateralis muscle. Change in total Hb (d[THb]=d[O2Hb]+d[HHb]) and was used as an index of the change in regional blood volume. Both during normoxic and hypoxic exercise, d[O2Hb] and d[THb] in cerebral and muscle, after initial dip, increased steadily afterwards. Increment in d[O2Hb] in both tissues at normoxia was greater than that during hypoxia and a significant difference was detected earlier in muscle tissue than in cerebral tissues. On the other hand, increment in d[THb] in both tissues at hypoxia was greater than that at normoxia and a significant difference was detected earlier in muscle tissue than cerebral tissue. These findings would suggest that dysoxygenation in brain as well as muscle was compensated by relatively preserved blood flow and that brain maloxygenation in brain tissue might be one of the exercise limiting factors during hypoxic condition. No COI.

2P-173

In utero exposure to hydroxylated polychlorinated biphenyl induces hyperactivity in adult male mouse offspring

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Polychlorinated biphenyls (PCBs) are recognized as persistent environmental pollutants that can affect neurobehavioral development. Here, we determined whether *in utero* or lactational exposure to hydroxylated-PCB 106 (OH-PCB 106; 4-hydroxy-2',3,3',4',5'-pentachlorobiphenyl) affects the spontaneous locomotor activity in adult male mouse offspring. For *in utero* exposure, pregnant C57BL/6J mice received corn oil vehicle, 0.05 or 0.5 mg/kg b.w. of OH-PCB 106 via gavage every second day from gestational day 10 to 18. For lactational exposure, dams received corn oil vehicle, 0.05 or 0.5 mg/kg b.w. of OH-PCB 106 via gavage every second day from postnatal day 3 to 13. After male mice offspring reached adulthood, their spontaneous locomotor activity in the home cage and open field were evaluated. Prenatally OH-PCB 106 -exposed mice showed significantly increased spontaneous locomotor activity compared to the control group both in the home cage and open field. Conversely, lactational exposures to OH-PCB 106 had no significant effects on the spontaneous locomotor activity under such conditions. These results suggest that the neurotoxic effects of OH-PCB 106 exposure may be different depending on the period of exposure, and *in utero* OH-PCB 106 exposure can induce hyperactivity in adult male mouse offspring. No COI.

2P-174

Effects of nursing on PER2::LUC rhythm in mouse mammary tissue and endocrine organs

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Mammalian circadian system consists of master and peripheral circadian clocks. The master clock is located in the hypothalamic suprachiasmatic nucleus and the peripheral clocks are found in many organs throughout the body. Current understanding is that the master clock entrains the peripheral clocks. The entraining signals contain neural and humeral signals, body temperature, activity and possibly many others. For nursing females, suckling behavior of pups may act as the time cue for the circadian clocks in endocrine organs related to nursing. In the present study, we examined the effects of nursing on circadian rhythm in the mammary tissue, pituitary gland and ovary by using Period2::Luciferase knock in mouse. Nursing dams were deprived from their pups for 6 hours during the first half of the day from lactating day1 (L1) to L7. The tissue samples were collected from nursing dams on L7, and bioluminescence was recorded from the cultured tissues to analyze PER2::LUC rhythm. In non-deprived control mammary tissue, anterior and posterior pituitaries showed the peak phase of the rhythm at early night, while the ovary had the peak somewhat earlier. In the deprived group the phase of the anterior pituitary was significantly advanced. This result suggests that nursing pattern is a time cue for the circadian clock in the anterior pituitary. No COI.

2P-175

Effects of daily exercise and mental stress on forearm endothelial function in male and female university students

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Mental stress or physical inactivity is a risk factor for atherosclerosis. The underlying mechanisms are not clearly known, but endothelial function could be involved. We examined the effects of daily exercise and mental stress on endothelial function of brachial artery using echo and Doppler ultrasound in male and female university students. Young healthy subjects were subjected to mental stress evoked by the modified STROOP Color-Word Test (CWT) in 10 min. They underwent reactive hyperemia (RH) for flow-mediated dilation (FMD) and maximal increase in blood flow (%) assessments before and at 5 min after the CWT. In addition, 5-min hand-grip exercise (HGEX) was performed for the estimation of FMD response to HGEX-induced increase in shear stress. The basal level of FMD in RH was higher in the exercise group compared with the sedentary group in men, but not in women. The CWT induced a slight vasoconstriction and a reduction of blood flow in brachial artery. However, there was no significant difference among men and women, or among the exercise and sedentary groups. FMD in RH was increased at 5 min after CWT compared with the basal level only in the exercise group of men, but not in another groups. In addition, FMD in HGEX was higher in the exercise group compared with the sedentary group in men. These findings suggest that daily exercise had beneficial effects on forearm endothelial function at the basal condition and recovery phase from mental stress in men. No COI.

2P-176

Central ketone bodies regulate sleep homeostasis in mice

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The ketone bodies are generated from the breakdown of fatty acids, and they are major metabolic fuels for the brain under conditions of low glucose availability. Ketogenesis is known to be modulated by the activity of peroxisome proliferator activated receptor alpha (PPARα). In our previous study, treatment with a PPAR activator has induced a marked increase in plasma acetoacetate (AcAc) and decreased β-hydroxybutyrate (BHB) in mice, accompanied by increased slow-wave activity (SWA) during non-rapid eye movement (NREM) sleep. In the present study, we have investigated the role of ketone bodies in the sleep regulation. Six-hour sleep deprivation increased plasma ketone bodies. Moreover, sleep deprivation increased mRNA expression of ketogenesis such as PPARα and 3-hydroxy-3-methyl- glutarate-CoA synthase 2 (Hmgcs2) in the brain and decreased ketolytic enzymes such as succinyl-CoA: 3-oxoacid CoA transferase (Scot). Additionaly, central injection of AcAc, but not BHB, markedly increased SWA during NREM sleep and suppressed glutamate release. These results suggest that central metabolism of ketone bodies (especially AcAc) play a role in the regulation of sleep homeostasis. No COI.

2P-177

Physiological responses when viewing aquarium fishes and their changing movement activities

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In order to investigate physiological effects of viewing aquarium fishes, we measured EEG and heart rate. Subjects were young healthy 4 men. A kind on goldfish, Ryukin was kept in plastic aquarium (29×30×34 cm), which was placed 1 m in front of a subject in an experimental room. To induce behavior change of fishes, bait was dropped two times 2.5 minute interval. Comparing to EEG during watching a white board, amplitudes of α and β band were suppressed during viewing aquarium fishes. When bait was dropped into the aquarium, goldfishes took the bait and activity of their movement was increased. When the feeding motion was activated, amplitudes of α and β band were suppressed much more at that time. The suppression suggested that neural activity of Fz was reduced by watching fishes and more effectively watching active motion. Slope of power spectral density (PSD) of EEG, which showed averaged neural activities between δ to β band, increased negatively in Fz and O2 of two subjects during viewing aquarium fishes. This meant that viewing aquarium fishes reduced activity of frontal and occipital neural activity. In other two subjects, a reduction in slope of PSD on Fz was also suppressed, suggesting that frontal activity was not stimulated relatively comparing to O2, as a representative of the occipital area, which corresponded to the visual cortex. These data suggested that viewing aquarium fishes rested general neural activity of subjects, namely leading to relaxation in feelings, though neural activity in visual sensory system should be activated because of watching goldfishes. No COI.

2P-178

Chronic exposure of extremely low-frequency magnetic field stimulates aldosterone secretion in H295R cell

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An extremely low-frequency magnetic field (ELF-MF) is generated by power lines and household electrical devices. Many studies have investigated whether ELF-MF has biological effect using cell line, animal model, human subject, and epidemiological study, but the effects remain controversial. We previously reported that chronic ELF-MF exposure is increased plasma corticosterone and aldosterone without enhancement of hypothalamic-pituitary-adrenal axis in mice. Also in vitro, corticosterone and aldosterone secretion was increased significantly by 24 h ELF-MF exposure in mouse adrenal cortex-derived Y-1 cell. These previous studies suggest the possibility that chronic ELF-MF exposure affects the adrenal stroidgenesis also in humans. In the present study, we carried out chronic (6h, 12h, 24h, and 48h) ELF-MF exposure (1.5 mT intensity) to human adrenal cortex-derived H295R cell and estimated the effect for adrenal steroid biosynthesis. As the results, 12 and 24 h ELF-MF exposure significantly stimulates aldosterone secretion in H295R cell. On the other hand, the secretion of cortisol did not affect significantly. The results suggest the possibility that chronic ELF-MF exposure stimulates steroidgenesis in zona glomerulosa, not zona fasciculate of adrenal gland in humans. No COI.

2P-179

Exploration of the mechanism for extremely low-frequency magnetic field-induced adrenal steroids up-regulation in Y-1 cell

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Extremely low-frequency magnetic field (ELF-MF) is generated by power lines and household electrical devices. Many studies have investigated biological effect of ELF-MF by using human subjects and experimental animals. However, the effect remains controversial. We previously reported that chronic ELF-MF exposure was increased plasma corticosterone level and depression-like behavior without enhancement of hypothalamic-pituitary-adrenal axis in mice. *In vitro*, the synthesis of corticosterone was also increased in mouse adrenal cortex-derived Y-1 cell by 24 hours ELF-MF exposure. However, the mechanism is unknown at present. Some reports indicate that ELF-MF affects various cellular processes via reactive oxygen species (ROS) production. In this study, we measured intracellular ROS in ELF-MF treated (1.5mT intensity) Y-1 cell to reveal how the up-regulation of corticosterone synthesis was elicited. As the results, 24 hours ELF-MF treated Y-1 cells showed the decrease of intracellular ROS compared with sham-treated control. Our evidence suggests the possibility that the ROS-scavenging mechanisms are involved in the up-regulation of adrenal steroids synthesis. No COI.

2P-180

Changes of systemic arterial pressure,common carotid arterial flow and heart rate after onset of 90 degrees head-up tilt in anesthetized young rats

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We previously reported the initial changes of the systemic arterial blood pressure (BP), the blood flow in the common carotid artery (BF) and the heart rate (HR) after onset of 90° head-up tilt (HUT) from supine position. To elucidate these initial changes and the role of baroreflex during HUT for 30 min in anesthetized young rats (urethane, 1.0–1.5 g/kg), we measured the BP, BF and HR by analog-digital recording system (MP-36; Biopac, USA). Under anesthesia, we inserted a catheter into a right common carotid artery toward the heart for BP measurement, attached a probe of ultrasound flowmeter (T206; Transonic, USA) to an artery for BF, and placed ECG electrodes on a subcutaneous of a chest for HR. After onset of HUT, BP and BF immediately decreased to 86.4±9.4 mmHg and 2.96±0.45 ml/min at 2.7±0.7 sec, respectively, compared with the values under supine before HUT (102±10.1 mmHg, 3.82±0.65 ml/min; n=6, $p<0.01$). Both of traces in BP and BF turned to the values before HUT, and the average of HR slightly increased. These results indicated that the initial changes in BP and BF produced by the hydrostatic pressure gradient due to HUT were similar to those in the adult rats. From the viewpoint of HR change in each animal, it was unclear that the baroreflex work actively. No COI.

2P-181

Responses of serotonin release in the central nucleus of the amygdala to cutaneous pinching of rats- Contribution of type 1 and 2 corticotropin releasing receptors in the dorsal raphe nucleus

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We have shown that responses of serotonin (5-HT) release in the central nucleus of the amygdala (CeA) to cutaneous pinching were abolished after administration of a non-selective corticotropin releasing factor (CRF) receptor antagonist, α-helical-CRF (9-41), into the dorsal raphe nucleus (dRN) in anesthetized rats. In the present study we aimed to define the contribution of both type1 and type2 CRF receptors in the dRN to the responses, with use of selective type1 and type 2 CRF receptor antagonists. A coaxial microdialysis probe was stereotaxically implanted in the CeA and perfused with modified Ringer's solution at a speed of 1 μl/min in anesthetized rats. Pinching was applied to the back for 10 min. After vehicle injection into the dRN, pinching increased the 5HT release. The responses of 5-HT to pinching were abolished after injection of antisauvagine-30 (ASV-30), a type 2 CRF receptor antagonist, while those were not influenced by injection of antalarmin, a type 1 CRF receptor antagonist. These results suggest that responses of 5HT release in the CeA to cutaneous pinching are mediated via the type 2 CRF receptors in the dRN. No COI.

Poster Presentations
Drug Actions

2P-182

Evaluation of muscarinic actions of the antipsychotic clozapine and its metabolite N-desmethylclozapine by using cultured hippocampal neurons

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Clozapine is a unique antipsychotic drug, and has been used for treatment-resistant schizophrenic patients. Clozapine and its active metabolite, N-desmethylclozapine (NDMC), interact with various types of neurotransmitter receptors. Recent biochemical studies suggested that NDMC exhibits M_1 muscarinic agonist action, which is unique and not shared by any other antipsychotics. However, direct electrophysiological evidence for the muscarinic action of NDMC on intact neurons has been scanty. In rat cultured hippocampal neurons, we previously reported that application of a muscarinic agonist, oxotremorine-M (oxo-M), induced suppression of the basal outward K^+ current at -40 mV in a PLC-dependent manner. In the present study, we examined agonist-antagonist properties of NDMC and clozapine using this muscarinic response. The oxo-M-induced suppression of K^+ current was sensitive to both 0.1 μM pirenzepine (M_1 antagonist) and 3 nM DAU5884 (M_3 antagonist), indicating involvement of M_1/M_3 muscarinic receptors. NDMC suppressed the K^+ current, which was reversed by the muscarinic antagonist atropine. Clozapine was much less effective in suppressing the current. Next, antagonist actions were examined by using oxo-M. The effect of oxo-M was reversed partially by NDMC, and more strongly by clozapine. Coapplication of NDMC and clozapine exhibited weak agonist and strong antagonist actions. These results indicate that NDMC alone can act as muscarinic agonist, but its agonist action is suppressed by the antagonistic action of clozapine when the both coexist. No COI.

2P-183

Mesenchymal stem cells ameliorate central and peripheral neural pathologies in mice with spinocerebellar ataxia type 1

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Spinocerebellar ataxia type 1 (SCA1) is a devastating neurodegenerative disorder caused by the expansion of a polyglutamine tract in the ataxin-1 protein. We found that intrathecal injection of only 3 x103 MSCs significantly mitigated the cerebellar and spinal cord pathologies seen in SCA1-transgenic (SCA1-Tg) mice. Although the Purkinje cells (PCs) of 24-week-old non-treated SCA1-Tg mice displayed a multi-layer arrangement and dendritic atrophy, MSCs-injected SCA1-Tg mice displayed mono-layer PCs and well-developed dendrites. Furthermore, MSCs injection partially restored delayed nerve conduction velocity in spinal motor neurons in SCA1-knock in mice. Finally, behavioral tests demonstrated that intrathecal injection of MSCs normalized deficits in motor coordination in SCA1-Tg mice, which was replicated by an intrathecal and following intravenous injections of MSCs-conditioned medium. Thus, MSCs likely exert their trophic effects in a paracrine manner and future studies determining the trophic factors would be worth a try to develop a treatment for SCA1 patients. No COI.

2P-184

Lansiumamide B and SB-204900 isolated from *Clausena lansium* inhibit histamine and TNF-α release from RBL-2H3 cells

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Aims Mast cells play a central role in allergic and chronic inflammation. Extracts from *Clausena lansium* (Lour.) Skeels (Rutaceae) possess many pharmacological effects including anti-inflammatory, anti-oxidant, anti-cancer, and anti-trichomonal activities. In addition, the leaves and fruit are used in Chinese folk medicine. We have isolated and identified four known cinnamamides from this plant: lansiumamide C, lansamide I, lansiumamide B, and SB-204900. However, the biological activities of these compounds are not yet understood. The purpose of this study is to clarify the pharmacological effects of these compounds on mast cells. **Methods** We measured inflammatory molecules in A23187-stimulated rat basophilic leukemia cells (RBL-2H3) treated with these compounds using HPLC, ELISA, and immunoblotting methods. In addition, some signaling molecules were investigated by immunoblotting. **Results** Lansamide I, lansiumamide B, and SB-204900 significantly decreased histamine release. Furthermore, lansiumamide B- and SB-204900-treated cells also reduced the protein and/or mRNA levels of TNF-α. Although IL-6 protein expression on lansiumamide B- and SB-204900-treated cells were not detected, their compounds tended to reduce its mRNA levels. SB-204900 markedly suppressed the phosphorylation of p38 MAPK. **Conclusion** Our findings suggest that lansiumamide B and SB-204900 attenuate mast cell-induced inflammation. No COI.

2P-185

Triptolide suppresses the adjuvant-arthritis in rats.

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Various extracts of the Chinese herbal medicine Tripterygium wilfordii Hook. f. (TWHF) have been reported to be therapeutically effective for rheumatoid arthritis (RA) in China, but their action mechanism has not been understood. Although triptolide, a diterpenoid triepoxide from TWHF, suppresses the inflammatory cytokine production in vitro, the influence of triptolide on immune system is not fully understood well. The present study, therefore, was designed to examine the effects of triptolide on cytokines production in vitro and in vivo by using adjuvant arthritis rats.The first part of these experiments was designed to examine the effects of triptolide on arthritis rats. Adjuvant arthritis was induced in male Wistar rats by a single subcutaneous injection of 0.1 mL complete Freund's adjuvant into hind paw. Arthritis rats were injected intraperitoneally with various doses of triptolide once a day for 1 week, starting one week after adjuvant injection. The minimum concentration of triptolide, which caused significant suppression of paw swelling, was 0.1 mg/kg. The second part of these experiments was carried out to measure the ability of cytokine production, and transcription factor about the cytokine production by lienal lymphocytes from the arthritis rats which were treated with triptolide. Triptolide caused suppression of IL-1β production in lymphocytes which was activated by Concanavalin A.These data suggest that the therapeutic effects of TWHF in RA are in part due to the novel chondroprotective effects of triptolide via suppression of IL-1β production. No COI.

2P-186

Effect of glucosamine on MMP production of the fibroblast cells derived from the osteoarthritis knee

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Osteoarthritis of the knee (knee OA) is one of the important diseases in the field of orthopedics. Recently it was reported that glucosamine might be effective for knee OA. Extracellular matrix splitting enzyme (MMP) which is produced by inflammatory cell, synovia cell and cartilage cell has the important role for alteration of articular cartilage. This study was examined the effect of glucosamine hydrochloride (GH) on MMP production in the knee OA fibroblasts.The subjects were five cases knee OA patients who visited for total joint replacement surgery at Showa University hospital. In the experiment, the separated fibroblasts from articular synovial tissue collected at the time of surgery were used. The first part of these experiments was designed to obtain the optimal concentration of TNF-α on the fibroblasts. In the second part of these experiments, MMP mRNA expression in the presence of GH was measured using RT-PCR method, and TIMP production, TNF-α-dependent MMP and transcription factor in the presence of GH were measured using ELISA methods.As the results of stimulation with TNF-α (2.5ng/ml) fibroblasts, MMP in the culture supernatant (MMP-2, MMP-3 and MMP-13) levels were significantly increased. GH (1.0mg/ml) inhibited TNF-α dependent MMP production of fibroblast cells, and had no effect on TIMP production. Then GH decreased NF-κB production and MMP mRNA expression in TNF-α dependent.GH showed the production of MMP inhibitory effect on articular cartilage degeneration. It was suggested that GH intake was useful for QOL improvement of the knee OA patients. No COI.

2P-187

Effects of Herbal Medicines in Rats with Chronic Constriction Injury ~ Comparative Study of Yokukansan and Kamishoyosan

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Chronic pain causes stress and psychiatric symptoms, such as anxiety and depression,; therefore, it is necessary to provide mental health care in association with pain management. Kamishoyosan (KSS) is used to treat neuropsychiatric symptoms. Recently, yokukansan (YKS), which contains a formula similar to KSS, has been reported to be effective against pain disorders, such as headaches and chronic pain. In this study, the effects of these medicines on neuropathic pain and stress caused by pain were investigated using Chronic Constriction Injury (CCI) model rats. The CCI model rats were prepared according to the model of Bennett. Two weeks after a surgery, a decrease in the mechanical withdrawal threshold was confirmed in the CCI rats using the von Frey Test; the drugs had been administered for this two-week period. Four weeks post-operatively, the levels of plasma corticosterone (Corti) and chromogranin A (CgA), markers of mental stress, significantly increased in the CCI rats. Moreover, the pain threshold significantly decreased and the activation of spinal astrocytes, which are involved in the expression of chronic pain, was observed. The increase in the levels of Corti and CgA was significantly suppressed by the administration of YKS and KSS. However, the activation of astrocyte was controlled and the decrease in the pain threshold was reduced following the administration of YKS only. We conclude that treatment with YKS effectively reduces both neuropathic pain and the stress caused by pain. No COI.

2P-188

Suppressive effect of Hochuekkito on lung metastasis of B16 melanoma cells in mice

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Hochuekkito (HET) is well known to be one of Kampo (Japanese herbal) medicine consisted of ten component herbs, and used for the supplemental therapy of cancer patients with remarkable success. However, the precise mechanisms by which HET could favorably modify the clinical conditions of cancer patients are not well defined. The present study, therefore, was undertaken to examine the possible therapeutic mechanisms of HET on cancer using experimental mouse model.In the first part of expeiment, HET was well mixed with rodent chow at concentrations of either 0.1%, 1.0% or 3.0%, and administered orally ad libitum, which was started one week before tumor cell injection and continued throughout the experiment. Oral administration of HET at concentration 1.0% and 3.0% into C57BL/6 male mice significantly inhibited tumor metastasis in lungs, which was induced by the intravenous injection of 1×10^5 B16 melanoma cell. The second part of experiment was to measure the cytokine production ability of lienal and lung lymphocytes from the first experiment mice.HET caused increase of INF-γ production in lymphocytes which was activated by lipopolysaccharide (LPS). These results strongly suggest that oral administration of HET caused increase in the production of IFN-γ, which is responsible for the activation of both NK cell and NKT cell, in the lungs and results in inhibition of B16 melanoma cell metastasis in the lungs. No COI.

2P-189

Search of Hyugatouki (Angelica Furcijuga) extract fraction which stimulated melanogenesis

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PurposeAngelica furcijuga (AF) is an endemic species and perennial herb of Japanese parsley department that grows wild in the South Kyushu island in Japan. It is faced with extinction but its usefulness is attracted attention by success of organic grow.We have ever reported that the AF extracts from lobe and stem, stimulated melanogenesis in mouse B16 Melanoma Cell (B16 cell) and mouse hair, and effect of melanogensis in fraction of water and ethyl-acetate layer.In the present study, on the effects of the each extract solvent fraction of the AF's extracts from lobe and stem,melanogenesis were examined using the B16 cell.MethodsThe B16 cell were cultured with the each fraction of AF's extracts, and drug susceptibility test was conducted. And then the melanin content and tyrosinase activity were measured in the B16 cell, the effect to the ability of melanin production was checked. Results and DiscussionWe observed different effects of each fraction on melanogenesis and tyrosinase activity. This study suggests the certain fraction of AF's extracts contains the melanin production promoting substances. No COI.

2P-190

Influence of Palmatine for bone metabolism in OVX mouse

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Receptor activator of nuclear factor- κB ligand (RANKL) or osteoprotegerin (OPG) was found to be a key osteoclast relation molecule that regulates its cognate receptor, RANK, on osteoclast precursor cells. The present study was designed to examine an inhibitory effect of the palmatine, an isoquinoline alkaloid originally isolated from *Coptis chinensis*, on an osteoclast differentiation in vivo by using ovariectomized (OVX) mice.The first part of experiments were designed to examine by histological approach of shank-proximal obtained from OVX mice treated with palmatine. They were divided into four groups of 5 mice each:the sham-operated (Sham), OVX, OVX-palmatine intake groups (1mg/kg, 10mg/kg), randomly.OVX mice were served by sonde forcibly with various doses of palmatine once a day for 12 week, starting two-weeks after OVX-operation. The second part of experiments was examined the cytokine level of serum from OVX mice treated with palmatine. The third part of experiments was examined the influence of Palmatine for the osteoblast-like cell (MC3T3-E1) in vitro.Palmatine caused significant suppression of the osteoclast number on tissue. In the mice treated by palmatine, RANKL decreased, and OPG increased. These results suggest that palmatine attenuates osteoclast differentiation through inhibition of RANKL expression, and activation of OPG expression from the osteoblast. Therefore, palmatine might be a candidate for an anti-resorptive agent of disorders such as osteoporosis. No COI.

2P-191

Antinociceptive Effect of Yokukansan, a Traditional Herbal Medicine, in Rats with Morphine Tolerance

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Morphine is one of the most commonly used drugs to treat severe and chronic pain; however, its chronic use results in antinociceptive tolerance as well as addiction. It has been reported that glial cells may be involved in the development of morphine tolerance. In addition, we previously reported that an herbal medicine, Yokukansan (YKS), controls activated microglia in rats with chronic inflammatory pain. In the present study, the effects of YKS in rats with morphine tolerance were investigated.

Male Wistar rats (7 weeks of age) were injected with morphine hydrochloride (10mg/kg) daily for seven days. The hot plate test (47.5°C) was used to assess the thermal nociceptive threshold. The threshold began to decrease on day 3 following the administration of morphine, and the antinociceptive effect completely disappeared within four days. However, the administration of YKS (manufactured by Tsumura & Co.) significantly inhibited this decrease. Moreover, glial cells (microglia and astrocytes) were observed in the posterior horn of spinal cord immunohistochemically. The glia cells were significantly activated in the morphine-treated rats; however, this activation was reduced by the administration of YKS.

Our findings reveal that YKS has an effect in reducing morphine tolerance by controlling the activations of microglia and astrocytes. No COI.

2P-192

Spectrum of dose-response relationship for radical scavenging activity of edaravone

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Edaravone is a powerful free radical scavenger and has been clinically used for the treatment of cerebral infarction. But data concerning its spectrum of dose-response relationship against multiple free radicals are still sparse as far as the authors surveyed. Thus we evaluated direct scavenging activity of edaravone against multiple free radicals and estimated dose-response curves and EC_{50}'s. Antioxidant activity on peroxidation in brain homogenate was also evaluated by thiobarbituric acid reactive substances (TBARS) assay. Six kinds of free radicals were measured by ESR; O-centered radicals: HO·, $O_2^{\cdot-}$, ascorbate free radicals (AFR), *t*-BuOO·; N-centered radicals: NO, DPPH radicals. Free radicals were quantified by spin-trapping method using DMPO or CYPMPO and peak height of spin adducts relative to internal standard Mn^{2+} were calculated. Edaravone scavenged all free radicals examined; EC_{50}'s are as follows; HO· 0.4 mM, $O_2^{\cdot-}$ 4 mM, *t*-BuOO· 0.3 mM, NO 40 μM, DPPH radicals 5 μM ($p < 0.001$). Edaravone significantly inhibited AFR at 5 mM ($p < 0.01$), but EC_{50} was not significant ($p = 0.098$). Antioxidant activity of edaravone on peroxidation in brain homogenate was significant against HO· and AFR with EC_{50}'s of 60 μM and 100 μ, respectively. It is concluded that edaravone had a broad spectrum of radical scavenging activity. Although its EC_{50} against each radical varied, its antioxidative activity is, at least, partially attributable to its direct free radical scavenging activity against multiple free radicals. No COI.

2P-193

The newly synthesized naftopidil analogue HUHS1015 induces apoptosis in malignant mesothelioma cells by activating caspase-4

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The aim of the present study was to assess the antitumor effect of newly synthesized naftopidil analogues on human malignant mesothelioma cell lines NCI-H28, NCI-H2052, NCI-H2452, and MSTO-211H cells. We newly synthesized 21 naftopidil analogues, and of them 1-[2-(2-methoxyphenylamino)ethylamino]-3-(naphthalene-1-yloxy)propan-2-ol (HUHS1015) most effectively reduced cell viability for all the investigated malignant mesothelioma cell lines in a concentration (1-100 μM)-dependent manner. Treatment with HUHS1015 (100 μM) for 12 h markedly increased TUNEL-positive cells in all the investigated malignant mesothelioma cell lines, and HUHS1015 activated caspase-3 and -4, without activating caspase-8 and -9. The results of the present study, thus, indicate that HUHS1015 induces apoptosis in malignant mesothelioma cells, possibly by activating caspase-4 and the effector caspase-3. This raises the possibility that HUHS1015 could be developed as a promising anticancer drug for treatment of malignant mesothelioma. No COI.

2P-194

Spinal neuronal responses elicited by intradermal pruritogen injection in hairless mice

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Antipruritic effect of drugs has been evaluated by using pruritogen-induced scratching behavior. In order to better understand the detail mechanisms of antipruritic drug, we examined spinal neuronal responses elicited by intradermal injection of serotonin, and the time course was compared with that obtained from scratching behavior. HR-1 hairless mice were deeply anesthetized with urethane. After dorsal surface of the lumbar spinal cord was exposed, extracellular recordings were made from spinal dorsal horn neurons. For behavioral experiments, the number of scratching behavior was counted for 30 min after injection of serotonin. Serotonin injected in the ipsilateral paw skin increased the frequency of action potentials elicited in spinal neurons, and the action was lasted for more than 20 min. The number of scratching behavior was also significantly increased by serotonin skin injection, and the time course was quite similar to that of serotonin-induced spinal responses. These results suggest that spinal long-lasting responses elicited by intradermal serotonin injection may be related to itch. No COI.

2P-195

Reduction of TRPV1-mediated pain sensation by TMEM16A inhibition

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A calcium-permeable channel TRPV1 is important for the pain sensation because TRPV1 is activated by pungent compounds including capsaicin. A calcium-activated chloride channel TMEM16A is co-expressed with TRPV1 in DRG neurons. This suggests that TMEM16A could be activated by calcium influx though TRPV1 activation. TMEM16A activation induces depolarization because a resting potential is lower than the equilibrium potential of chloride in DRG neurons. Therefore, we hypothesized that TMEM16A inhibitor operates to reduce pain. To confirm the TRPV1-TMEM16A functional interaction, we performed whole-cell patch-clamp recordings in HEK293T cells. The capsaicin-induced currents were larger in the cells expressing TRPV1 and TMEM16A than in the cells expressing TRPV1 alone. Moreover, this interaction depended on calcium influx through TRPV1 activation. Next, we investigated the effect of a TMEM16A blocker (A01) in DRG neurons. A01 reduced the capsaicin-induced currents by approximately 50 %. These findings suggest that TRPV1-mediated nociception could be inhibited by TMEM16A blocking. Therefore, we investigated capsaicin-induced pain-related behaviors in mice. Time of licking and biting was shorter with a concomitant administration of A01 with capsaicin than in administration of capsaicin alone. These results suggest that TMEM16A inhibition would be an effective treatment of acute peripheral pain involving TRPV. No COI.

2P-196

Effects of melatonin administration on motor function recovery after internal capsule hemorrhage

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Intracerebral hemorrhage (ICH) causes severe disability. Free radical produced from blood metabolites is involved in the brain damage after ICH. To investigate whether melatonin (ML), a radical scavenger, could reduce brain damage by ICH, rats were injected collagenase (15 Units, 1.4 μl) into the internal capsule with ML (15 mg/kg) that was orally administrated for 7 days. Motor function was assessed by motor deficit score test. Surviving corticospinal neurons were evaluated by retrograde tracer fluorogold (FG) into cervical cord. Immunostaining of 8-hydroxy-2'-deoxyguanisine (8-OHdG) was carried out for DNA damage assessment. Measurements of motor map in ipsilateral motor cortex were done by intracortical microstimulation (ICMS), in which bipolar pulses (0.2 ms, 0–200 μA, 333 Hz) were given in layer V sensorimotor cortex to evoke twitches in contralateral lower leg joints. Behavioral tests revealed that ML significantly ameliorated motor dysfunction. In addition, more FG-labeled neurons were shown in the motor cortex in ML-treated rats. ICMS revealed that ML results in lower threshold for evoking forelimb movement than control. Less 8-OHdG positive cells were detected in ML in peri-hematoma area. Data suggested that oral ML administration to ICH model rats reduces the damage of peri-hematoma area including corticospinal pathway by scavenging radicals, resulting in better functional recovery. No COI.

2P-197

Long-term feeding of rare sugar D-psicose preserved pancreas beta cells and thus prevented diabetes development in T2DM model OLETF rats

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Prevalence of global obesity, mostly due to excess calorie intake and insufficient physical activities leading to one of its complications, type 2 diabetes mellitus (T2DM) has been increasing with alarming health problems. This circumstance demands health screening from early life with the intake of age-adjusted balanced food in obese-tendency subjects. We introduce a zero-calorie food additive, D-psicose, a sweet rare sugar produced in Kagawa University Rare Sugar Research Centre. Long-term feeding of more than 60 weeks of 5% D-psicose in drinking water has been proven to control body fat accumulation and thus prevented excess body weight increase in comparison to non-treated control rats. D-psicose improved insulin resistance through constant maintenance of blood sugar levels with the preservation of insulin producing pancreas beta cells. Subsequently, serum levels of pro-inflammatory and anti-inflammatory adipocytokines were also controlled well by D-psicose drink. These findings demonstrate that D-psicose might be a promising anti-obese and anti-diabetic agent. No COI.

2P-198

Slowing of Mg^{2+} influx by TRPM7 inhibitors in rat ventricular myocytes

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Adult rat ventricular myocytes were isolated enzymatically. Intracellular free Mg^{2+} concentration ($[Mg^{2+}]_i$) was estimated using a fluorescent indicator furaptra (mag-fura-2). Incubation of the cells in a Mg^{2+}-depleting solution (high-K^+, divalent-free) caused a decrease in $[Mg^{2+}]_i$ by 0.4–0.6 mM. The lowered $[Mg^{2+}]_i$ was recovered by perfusion with Ca^{2+}-free Tyrode's solution. The time course of the recovery could be well fitted by a single exponential function, the first derivative of which at time 0 was analyzed as an initial Mg^{2+} influx rate. Administration of TRPM7 inhibitors, 2-Aminoethoxydiphenyl borate (2-APB, 100 μM) and NS8593 (10 μM) suppressed the initial rate of Mg^{2+} influx, respectively, to 43±10% and to 12±8.6%. These compounds also inhibited the rate of Ni^{2+} influx estimated by quenching of furaptra fluorescence during the initial 10 min. The half inhibitory concentrations (IC_{50}) of 2-APB for Mg^{2+}- and Ni^{2+}-influx rates were comparable: 17 μM and 20 μM , respectively. The IC_{50} values of NS8593 (2.0 μM for Mg^{2+} influx and 4.4 μM for Ni^{2+} influx) were also comparable. We measured MIC currents (I_{MIC}) likely via TRPM7 channels activated by removal of intracellular and extracellular divalent cations under the whole-cell patch-clamp configuration. I_{MIC} at -120 mV was inhibited by 2-APB with IC_{50} of 50 μM, and was reduced to 50±12% by 10 μM NS8593. Thus, Mg^{2+} influx was significantly slowed by TRPM7 inhibitors, which support our previous conclusion [J Phy No COI.siol Sci 63:S273, 2013] that TRPM7/MIC channels serve as a major physiological pathway of Mg^{2+} influx in rat ventricular myocytes. No COI.

Poster Presentations
Membrane Transport

2P-199

Activation of the Na^+-H^+ exchanger by Ca^{2+} and cAMP signals during bicarbonate secretion from rat salivary ducts

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To characterize the Na^+-H^+ exchanger (NHE) activation during bicarbonate secretion from rat salivary ducts, we measured intracellular pH changes induced by Ca^{2+} and cAMP signals. Rat parotid glands were removed, minced and incubated with collagenase to separate acini and ducts. The intralobular duct segments were loaded with the pH-sensitive fluorescence dye, BCECF. The intracellular pH changes were measured with the ARGUS-HiSCA system. Application of carbachol (CCh) did not affect the intracellular pH, while forskolin+IBMX (F+I) decreased the pH. CCh restored the pH level that had been reduced by F+I. In the presence of the NHE inhibitor, 5-(N,N-dimethyl) amiloride (DMA), CCh and F+I decreased the intracellular pH compared with their effects in the absence of DMA, indicating that the NHE was activated during bicarbonate secretion induced by the stimulations. The rate of F+I-induced decrease in pH was greater than that of CCh-induced decrease in the presence of DMA, suggesting that the rate of H^+ generation by F+I is higher than that by CCh. When bicarbonate and H^+ were at an equilibrium state under the inhibition of the carbonic anhydrase by methazolamide, addition of CCh increased the pH, while F+I did not. Results obtained suggest that CCh strongly activates the NHE due to the high pH set point. F+I may activate the generation of bicarbonate and H^+ by the carbonic anhydrase rather than H^+ extrusion by the NHE. No COI.

2P-200

Adenosine A_{2B} receptor regulates CFTR in pancreatic duct cells

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Introduction: Pancreatic acini secrete ATP and nucleotide-modifying enzymes that include CD39 and CD73. Adenosine, the end product of ATP, stimulated cystic fibrosis transmembrane conductance regulator (CFTR) in pancreatic duct cells. However, the identity of adenosine receptors has not been extensively investigated. **Objectives:** The present study aimed to identify functional adenosine receptors in pancreatic duct cells. **Methods:** The molecular basis of adenosine receptors was revealed by RT-PCR analysis and immunostaining. We measured equivalent short-circuit current (I_{sc}) in human adenocarcinoma cell line (Capan-1) monolayer. **Results:** Capan-1 cells expressed adenosine A_{2B} receptor (*Adora2b*) as reported previously. Adenosine A_{2B} receptor localized in luminal membrane of pancreatic ducts and Capan-1 monolayer. The luminal addition of adenosine significantly increased I_{sc} in Capan-1 monolayer. The effect was consistent with anion secretion in epithelia. PSB 603, an adenosine A_{2B} receptor antagonist, inhibited this increase. BAY 60-6583, an adenosine A_{2B} receptor agonist, increased I_{sc}, which was inhibited by CFTRinh-172. **Conclusion:** These results indicated that the adenosine A_{2B} receptor regulates CFTR in Capan-1 cells. Furthermore, the adenosine A_{2B} receptor may be involved in anion transport in pancreatic ducts. No COI.

2P-201

Optimization of a mathematical model of HCO_3^- secretion by pancreatic duct cell

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Pancreatic duct cell produces isotonic fluid secretion containing ~140 mM HCO_3^-. We have been constructing a mathematical model of pancreatic duct cell that various ion transporters, channels, and pumps are allocated in the basolateral and apical membranes by using MATLAB/Simulink. In the present study, we have tried to optimize the permeability of several transporters/channels/pumps at one time using an algorithm "fminsearch" which is based on the Nelder-Mead method. The permeability values to be optimized included those of Na^+-K^+ pump, K^+ channel, NBC1 $1Na^+$-$2HCO_3^-$ cotransporter, and AE2 $1Cl^-$-$1HCO_3^-$ exchanger in the basolateral membrane, and of CFTR anion channel (P_{HCO3^-}/P_{Cl^-} was set at 0.4) and SLC26A6 $1Cl^-$-$2HCO_3^-$ exchanger in the apical membrane. The values were optimized to reproduce the published experimental data of interlobular ducts isolated from guinea-pig pancreas. The data included (1) intracellular pH, [Cl^-], and membrane potential in the resting and cAMP-stimulated ducts luminally-perfused with low HCO_3^- (25 mM HCO_3^--125 mM Cl^-) or high HCO_3^- (125 mM HCO_3^-–25 mM Cl^-) solution and (2) the maximal rate of fluid secretion (3.5 nl/min/mm^2 epithelium) and fluid [HCO_3^-] (140 mM) into the closed ducts. The standard errors of experimental data were set as acceptable variation ranges for optimization. The permeability values of transporters were successfully optimized to reproduce HCO_3^- secretion and intracellular parameters within acceptable ranges. No COI.

2P-202

Short-chain fatty acid might increase intracellular Ca^{2+} concentration in rat colonic surface and crypt cells

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Short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate are synthesized from dietary carbohydrate by colonic bacteria fermentation. These SCFAs are considered that they may contribute not only energy source or prevention of cancer but also regulating ion transport by affecting SCFA receptor, GPR43. This receptor couples G-protein type G_q (and G_i), thus when SCFAs bind this receptor, intracellular Ca^{2+}([Ca^{2+}]$_i$) might change. However, these effects only identified in culture cells. Thus, I investigated that whether butyrate can increase [Ca^{2+}]$_i$ in rat colonic surface and crypt cells with Fura-2. As a result that butyrate showed elevation of [Ca^{2+}]$_i$ in colonic surface and crypt cells. This butyrate-induced [Ca^{2+}]$_i$ elevation was not inhibited by atropine, which showed inhibition the effects of acetylcholine on colonic crypt cells but not surface cells. To evaluate the contribution of butyrate absorption via Na^+-coupled monocarboxylate cotransporter 1 (SMCT1) and H^+-coupled monocarboxylate transporter 1 (MCT1), I investigated the effect of ibuprofen, SMCT1 inhibitor, or α-cyano-4-hydroxycinnamic acid (α-CHC), MCT1 inhibitor on butyrate-induced [Ca^{2+}]$_i$ increase. Although ibuprofen didn't show any effects, α-CHC showed comparable inhibition on colonic crypt, but not on surface cells. These results indicated that butyrate might increase [Ca^{2+}]$_i$ without affecting intracellular butyrate which absorbed via SMCT1 or MCT1. No COI.

2P-203

Short-chain fatty acid inhibits cAMP-activated short-circuit current in rat distal colon

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Introduction: Short-chain fatty acids (SCFAs) are produced in colon of omnivore, from dietary fiber fermented by enteric bacteria. The main SCFAs in colon are acetate, butyrate, and propionate and they are thought to be absorbed via colonic epithelia inhibiting colorectal cancer or provided as nutrients for epithelia. SCFA are also thought that it reduces Cl^- secretion and following intestinal fluid flow. This regulation might be induced via SCFA receptor GPR43 on colonic epithelia, however, it has not been clear yet. Thus we focused on GPR43 which couples to G_i protein. **Method:** We tested how SCFA affects cAMP-activated Cl^- secretion, which may be via cystic fibrosis transmembrane conductance regulator (CFTR) with increasing intracellular cAMP condition with colorectal mucosal on Ussing Chamber to measure short-circuit current (I_{SC}). We also tested the effect of 4-chloro-α-(1-methylethyl)-N-2-thiazolylbenzeneacetamide (4-CMTB), an agonist of GPR43. **Result:** Apical addition of SCFA with 10 mM reduced cAMP-activated I_{SC}. After this process, I_{SC} was not change with adding CFTRinh-172, an inhibitor of CFTR on apical side. On the other hand, further addition of 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), a nonspecific Cl^- channel inhibitor, showed additional reduction of I_{SC} and made it turn to initial state. 4-CMTB also reduced I_{SC} in similar way of SCFA. **Conclusion:** These results indicated that SCFAs reduced cAMP-activated Cl^- secretion by declining cAMP via GPR43 and it may regulate intestinal fluid secretion. No COI.

2P-204

LAT1 Is a Critical Transporter of Essential Amino Acids for Immune Reactions in Activated Human T Cells.

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Sufficient nutrient supply is important to support immune reaction in T cells. Since our results demonstrate that a specific inhibitor of L-type amino acid transporter 1 (LAT1) suppresses the activation of T cells, LAT1 is seemed to be crucial for immune function of T cells. Purified human peripheral blood T cells were activated with anti-CD3 and anti-CD28 antibody in the presence or absence of JPH203, a specific inhibitor of LAT1, and LAT1 expression, [^{14}C]-labeled L-leucine uptake or cytokine production were analyzed. The up-regulation of DNA-damage-inducible transcript 3 (DDIT3) in LAT1-treated activated T cells was confirmed by real time PCR. The reporter genes for which expression is promoted by NF-κB or NFAT were co-transfected with a DDIT3 expression vector into Jurkat T cells, and the activity of each transcription factor was determined by a reporter assay. LAT1 expression was dramatically increased by anti-CD3 and anti-CD28 stimulation in human T cells, but this expression was inhibited by NF-κB or AP-1 inhibitor. Leucine uptake and cytokine production in activated T cells were inhibited by JPH203. DDIT3 expression was facilitated by JPH203 treatment in activated T cells. DDIT3 inhibited the function of NF-κB and NFAT. These results suggest that induction of LAT1 expression mediated by NF-κB and AP-1 is indispensable to achieve maximum incorporation of essential amino acids for normal immune reaction in human T cells. No COI.

2P-205

Mechanisms of regulation of clathrin heavy chain CHC22 assembly

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Clathrin heavy chain CHC22 is required for formation of the GLUT4 storage compartment (GSC). CHC22 controls protein sorting from late endosomes to the trans-Golgi network. It is also associated with GLUT-4mobilization in human skeletal muscle. We have compared the biochemical properties of CHC22 to those of CHC17 to define species-restricted characteristics of the membrane traffic pathway that leads to human GSC formation. Cell starvation caused reduction in membrane-associated CHC22 along with its interacting adaptor molecules. Stimulation of AMPK activity by AICAR phenocopied this effect, but treatment of cells with rapamycin, another mTOR antagonist did not. Thus CHC22 membrane dissociation results from AMPK activation, a pathway that increases GLUT4 availability. Notably however, insulin treatment did not affect CHC22 membrane association. CHC22 is missing the binding sequence for the uncoating ATPase Hsc70, which interacts with auxilin to uncoat CHC17, and was not disassembled by this protein complex. These observations suggest that insulin signaling affects GLUT4 release after it is sorted in a CHC22 pathway, but that the CHC22-dependent GLUT4 sorting might be affected by exercise. Thus, we hypothesize that due to its membrane association properties CHC22 contributes to forming a human GSC that is more stable than the murine GSC, and consequently more prone to insulin resistance. No COI.

2P-206

Estimation of activity of electro-neutral ion transporter by establishing mathematical model of Cl^- secretion in epithelial cells based on measurement of transcellular ion transport and membrane ion conductance

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Epithelial cells play key roles in prevention of our body from environmental changes by regulating transepithelial transport of ions and water; e.g., the liquid covering epithelial apical membrane surface produced by Cl-secretion-driven water movement plays an essential role in protection of our body from bacterial and viral infection. Transepithelial (transcellular) Cl^- secretion is composed of two steps; i.e., entry and releasing steps. In this study, we established mathematical models describing transcellular Cl^- secretion based on following three pathways.

(1) the entry step of Cl^- into the intracellular space across the basolateral membrane via electro-neutral ion transporter

(2) the secretion step of Cl^- from the intracellular space across the apical membrane via Cl^- channel

(3) the recycle step of Cl^- across the basolateral membrane via Cl^- channel

In addition to measurements of the transcellular Cl^- secretion by electrophysiological technique (short-circuit current), we measured apical/basolateral Cl^- conductance in Ussing chamber using Cl^- channel blocker (NPPB). The present study enables us to measure the activity of electro-neutral ion transporter, NKCC, by electrophysiological techniques, providing us with deep understanding in the research field of ion transport. No COI.

2P-207

Regulation of the Cl^- extrusion activity in osteoclasts via farnesyl diphosphate synthase

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Farnesyl diphosphate synthase (FDPS), an important enzyme in mevalonic acid metabolism catalyzes the production of geranyl pyrophosphate and farnesyl pyrophosphate, resulting in increase prenylation in small GTP proteins. Nitrogen-containing bisphosphonates (NBPs) have well known to inhibit FDPS, leading to inhibit bone resorption in mature osteoclasts. However, little is known whether FDPS regulate directly the hydrochronic acid transporters in mature osteoclasts. We found that the FDPS were combined with cytoplasmic domain of ClC-7 Cl^- transporters. The aim of present study was to investigate the expression and functional role of FDPS on the extrusion activity of ClC-7 Cl^- transporters in osteoclasts. FDPS were expressed in osteoclast precursors and slightly upregulated by RANKL during osteoclastogenesis. FDPS was colocalized with ClC-7 Cl^- transporters in mouse osteoclasts. Extracellular acidification induced outwardly rectifying Cl^- currents and decreased intracellular Cl^- concentration ($[Cl^-]_i$) associated with in ClC-7 Cl^- transporters in osteoclasts. Zoledronic acid a NBP suppressed acid-induced Cl^- currents and $[Cl^-]_i$ reduction in a dose-dependent manner. In contrast, the inhibitory action of zoledronic acid was rescued by addition of geranylgeranyl acid, a derivative of mevalonic acid metabolism. The results suggest that FDPS may contribute to regulate the bone resorption activity, especially the ClC-7 Cl^- transporter activity in mature osteoclasts because NBPs directly suppressed the activity of Cl^- transporters. No COI.

Poster Presentations
Oral Physiology (2) / Heart, Circulation (3) / Muscle Physiology (2)

2P-208

Involvemnet of thermosensitive TRP channel in rapid wound healing in oral epithelia.

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The oral cavity provides an entrance to the alimentary tract and serves as a protective barrier against a drastic variation of stimuli from food and drink. Because of their location, oral mucosa is susceptible to injury. However, wound in oral mucosa is known to heal faster than skin with little scar formation. Here we showed that transient receptor potential vanilloid 3 (TRPV3), a thermosensitive cation channel activated by warm temperatures (>33°C), is functionally expressed in oral epithelia. We explored the physiological significance of TRPV3, and found delayed closure of wound after tooth extraction in TRPV3-deficient (TRPV3KO) mice. Furthermore, TRPV3 expression was up-regulated during the wound healing process. We also found that TRPV3 activation increased the number of proliferating cells in primary cultured oral epithelial cells from wild-type but it is not found in the cells from TRPV3KO. Interestingly, the number of proliferating cells in oral epithelia was also markedly reduced in TRPV3KO mice.These results suggest TRPV3 in oral epithelia promote the proliferation of oral epithelial cells and contribute rapid wound repair. No COI.

2P-209

Functions and localization of a calcium sensor NCS-1 as a mediator of the nuclear calcium regulation in cardiomyocytes

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In cardiomyocytes (CMs), cytoplasmic calcium (Ca^{2+}) is essential for excitation-contraction coupling, whereas nuclear Ca^{2+} has been implicated in gene expression leading to hypertrophy. Several studies demonstrate that a key molecule for the regulation of nuclear calcium levels ($[Ca^{2+}]_n$) is inositol 1,4,5-trisphosphate receptor (IP_3R), an intracellular Ca^{2+} release channel. However, precise mechanism of controlling $[Ca^{2+}]_n$ in CMs remains unclear. We recently reported that a Ca^{2+} sensor protein, neuronal calcium sensor-1 (NCS-1), interacts with IP_3Rs in the heart and regulates cardiac hypertrophy. To clarify whether NCS-1 together with IP_3Rs are involved in regulation of $[Ca^{2+}]_n$, we examined the effects of receptor stimulation which activates IP_3Rs on nuclear Ca^{2+} transients, subcellular localization and expression patterns of NCS-1 as well as IP_3Rs, and interaction between NCS-1 and IP_3Rs using wild type and *Ncs1* knockout (KO) mouse CMs. We found that 1) receptor stimulation-induced elevation of $[Ca^{2+}]_n$ was lower in KO CMs. 2) NCS-1 was colocalized with IP_3Rs in the perinuclear region. 3) Receptor stimulation increased the expression of NCS-1 in the nucleus where IP_3Rs were mainly expressed as well as in the cytoplasm and membrane fractions, and 4) strengthened the interaction between NCS-1 and IP_3R. These results suggest that NCS-1 contributes to the regulation of $[Ca^{2+}]_n$ through direct modulation of IP_3R activity in the perinuclear region of CMs. No COI.

2P-210

Dysregulation of Cav1.2 Ca2+ Channel by Calpastatin was Involved in Myocardial Hypoxic-Ischemic Injury

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Our previous study revealed that calpastatin domain L (CSL) could reprime activity of Cav1.2 Ca2+ channel from run-down in cell-free patches. Recently, it was reported that CSL can bind to intracellular C-terminal region of Cav1.2 channel and regulate its activity. However, the pathophysiological role of Cav1.2-channel-regulating function of calpastatin was largely unknown. In this study, we investigated the changes in expression and function of Cav1.2 channels associated with calpastatin in both in vitro H9c2 hypoxia model and in vivo myocardial infarction (MI) model of rats. We found that both mRNA and protein levels of Cav1.2 and calpastatin decreased at the late stage of hypoxic-ischemic (H-I) injury. At the same time, influx of Ca2+ induced by KCl was attenuated in H9c2 cells confronted to hypoxia. In addition, colocalization of Cav1.2 and calpastatin on the cytoplasmic membrane was observed in sham-operated rats, but it was only detected in cytosol in rats encountered MI. Knockdown of calpastatin with siRNA in H9c2 cells resulted in both decreased Cav1.2 levels and depressed Cav1.2 channel activity. Taken together, these results indicate that dysregulation of Cav1.2 Ca2+ channel by calpastatin was involved in myocardial H-I injury, which provide insights that proper regulation of Cav1.2 Ca2+ channel by calpastatin was needed in maintaining the normal cardiac function. No COI.

2P-211

Spin-spin relaxation of 1H NMR signals from myofibril suspension of rabbit skeletal muscle with or without ADP

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The dynamic changes of water molecules structure surrounding contractile proteins might play an important role in cross-bridge cycling during contraction. The spin-spin relaxation process of 1H-NMR signals from suspension of myofibrils prepared from rabbit psoas muscle could be well represented by the summation of several exponentials indicating that water molecules in the suspension could be conveniently grouped into several components based on the relaxation time constant (T2). The slowest two components dominated over faster relaxation components at the myofibril concentration range studied. With increase in the concentration of myofibrils, water component that relaxed with T2 around 0.15 s progressively replaced the slowest component of T2 > 0.4 s. An equivolumic point for these two components was found at 12 mg/ml and 20mg/ml myofibril concentration at 20 °C in the absence and presence of MgATP respectively. In the absence of MgATP myofibril affects water molecules within 500 nm from its surface, and releases many water molecules in the presence of ATP. In the presence of ADP myofibrils may release many water molecules. This release might play an important role in cross-bridge cycling during contraction. No COI.

Poster Presentations
Ionic Channel, Receptor (3)

3P-001

Development of light-induced insulin secretion system using channelrhodopsin

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Channelrhodopsin-2 (ChR2) is a membrane protein that has a function to transport cation into the cell by the stimulation of blue light. Cells are depolarized when Na^+ flows via ChR2, and activation of this channel is strictly regulated by light stimulation. In this study, we tried to develop the light-induced insulin secretion system using ChR2.We constructed ChR2-expressing lentivirus vector fused to Gaussia luciferase, which generates luminescence of 475 nm blue light region via catalyzing coelenterazine (CTZ). Therefore, we utilize this luminescence for ChR2 activation. Lentiviruses were transduced into mouse islets, and ChR2 expression levels were analyzed by Western blotting analysis. The channel activity was also measured by voltage-clamp recording. We next quantified insulin secretion, and the cells showed moderate level of secreted insulin by the addition of CTZ. However, this level is less than that by high glucose stimulation. It might be due to low levels of protein expression and membrane trafficking. Further improvement is needed for sufficient and controllable insulin secretion via ChR2.Artificially generated pancreatic β-cells from iPS cells are desired for regenerative medicine of diabetes. However, potency of differentiated cells is shown to be insufficient, as response by glucose stimulation is extremely low. It is possible that our system using ChR2 may be a useful tool for the induction of insulin secretion in these cells. No COI.

3P-002

Reproduction of cyclic GMP-dependent Channel Current under various light intensity by Retinal Rod Phototransduction Model

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The phototransduction system in retinal photoreceptor cells converts light signal to an electrical signal. In this system, visual pigments activated by the incident light stimulate transducin and the activated transducin raise the cyclic GMP (cGMP) decomposition activity of phosphodiesterase. The cGMP-dependent channels in retinal photoreceptor cell membrane tend to be closed by the reduced cGMP concentration. In addition, the cGMP concentration recovers through the calcium-dependent guanylate cyclase activity. In this system, a single visual pigment may activate hundreds of transducin, amplifying the incoming signal. Efficacy of the signal amplification system is reported to be light intensity-dependent. In experiments, positive correlation between light intensity and activated transducin has been observed at a low stimulation range, whereas activated transducin starts to decrease at very high light stimulation. In the physiological condition, succeeding cGMP-dependent channel current shows negative correlation with activated transducin. Since none of the proposed phototransduction model could reproduce the complex characteristics of the signal transduction system of retinal photoreceptor cell, we propose a model which can reproduce the rod current of cGMP-dependent channels under various light intensity. The model includes the light intensity-dependent amplification of incoming signals in rods. The present model could successfully reproduce the experimental data. No COI.

3P-003

Bi-stability of chimeric channelrhodopsins

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[Introduction] Channelrhodopsin1 and 2 (ChR1 and ChR2) are light-gated cation channels, each of which has a seven-pass transmembrane protein with a covalently bound retinal. Light absorption is followed by the photoisomerization of the all-trans retinal to a 13-cis configuration and subsequent conformational changes of the molecule, which allow the channel structure to become permeable to cations. Recently it has been experimentally evidenced that the cation-permeable states is stabilized by the introduction of point mutations in C128 or D156 of ChR2. The counterparts of these amino acid residues, C167 and D195 lie close to all-trans retinal and form retinal-binding pocket under crystallographic study of C1C2/channelrhodopsin-wide receiver (ChR-WR), a chimeric ChR. Here we tested the hypothesis that these residues are also involved in the stabilization of cation-permeable state of C1C2/ChRWR. [Method] The expression plasmids were transiently transfected in ND7/23 cells using Effectene Transfection Reagent (Qiagen). The photocurrent was measured under conventional whole-cell patch clamp.[Results and Discussion] The mutation C167 or D195 position markedly prolonged the OFF phase of the photocurrent of C1C2/ChRWR or ChRFR, another chimeric ChR. The results suggest that the interaction between these positions and 13-cis retinal is conserved against helix exchange between ChR1 and 2. No COI.

3P-004

Activation of peripheral mGluR5 contributes thermal and mechanical hyperalgesia via TRPA1 and TRPV1

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Objective: Peripheral tissue injury causes glutamate release from keratinocytes, resulting in thermal hyperalgesia. We have reported that peripheral glutamate injection induces thermal hyperalgesia. However, it is still not understood the mechanisms underlying hyperalgesia following peripheral glutamate injection. The aim is to clarify the involvement of peripheral TRPA1, TRPV1 and PKCε in glutamate-induced thermal and mechanical hyperalgesia. Methods: We analyzed nocifensive behaviors to heat, cold and mechanical stimulation following antagonists administration 1 week after continuous subcutaneous injection of glutamate into the facial skin. Next, we examined the expression of TRPA1- and mGluR5-immunoreactive (IR) and TRPV1- and mGluR5-IR TG neurons innervated to facial skin. Moreover, we performed a neuronal recording from the TG neurons following glutamate injection. Results: Head-withdrawal threshold to cold, heat and mechanical stimulation 1 week after glutamate injection were significantly decreased compared to vehicle-injected rats, and the decreased head-withdrawal threshold was significantly recovered by antagonists administration.TRPA1-and mGluR5-IR neurons and TRPV1- and mGluR5-IR neurons were observed in the TG. Neuronal activity in TG neurons was significantly increased following glutamate treatment.Conclusions: Present findings suggest that TRPA1 or TRPV1 activation through mGluR5 signaling via PKCε is involved in facial thermal and mechanical hyperalgesia. No COI.

Poster Presentations
Sensory Function (3)

3P-005

Analysis of physiological functions of transient receptor potential vanilloid 2 (TRPV2) in adult primary sensory neurons using a tissue specific conditional knockout mouse.

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TRPV2, is a heat-sensitive ion channel expressed in a subset of the primary sensory neurons (PSN) in adult animals. This channel has been proposed to be a heat transducer (>52°C) in nociceptive neurons, and recently revealed to contribute to stretch-dependent events in developing peripheral neurons and other non-neuronal cells. However, a role in adult animals remains unclear. To understand the functions of TRPV2 in the adult PSN, we have established a conditional knockout (cKO) mouse lacking TRPV2 in PSN, using the Cre/loxP recombination system. Using several types of behavioral assay methods, we found that TPRV2-cKO mice had impaired mechanical nociception, but normal heat nociception. Next, we examined the Ca^{2+} response of acutely dissociated PSN with Fura-2. In the neurons from the normal littermates, about 20% of the cells showed stretch-induced Ca^{2+} increase and 12.9% of the cells were probenecid (a TRPV2 activator)-sensitive. However, in the neurons isolated from TRPV2-cKO mice, the probenecid-sensitive cells and the stretch-sensitive cells, especially with relatively high mechanical threshold, were largely diminished. These results suggest that TRPV2 plays an important role in the high-threshold stretch-dependent excitation of the PSN and mechanical nociception in adult animals. No COI.

3P-006

Skilled motor training increases synaptic delivery of AMPA receptors at layers II/III synapses in primary motor cortex

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Synaptic plasticity via AMPA receptor trafficking at synapse terminals is associated with memory and learning. To investigate the neuronal mechanism of motor learning, we performed a rotor rod test and made acute brain slice to analyze layers II/III neurons in the motor cortex using patch clamp method. Motor skill consistently improved within 2 days of training in all animals (n = 12). In current clamp analysis, motor learning significantly decreased firing threshold and increased firing probability, since the resting membrane potential significantly increased after motor learning (Mann Whitney test, $p < 0.05$). In voltage clamp analysis, motor learning significantly increased the frequency and amplitude of miniature EPSC (mEPSC) but not those of miniature IPSC (mIPSC). In addition, motor learning produced a significant correlation in the amplitude between mEPSC and mIPSC ($\rho = 0.28$, $p < 0.05$). The average AMPA/NMDA ratio at glutamatergic synapses did not change significantly, probably due to an increase of NMDA current. Further, paired-pulse analysis showed that motor learning decreased paired-pulse responses (ANOVA and Fisher's test, $p < 0.05$), suggesting an increase in presynaptic glutamate release. Histological analysis was also performed to examine AMPA receptors in spines. These results suggest that motor learning derived AMPA receptor delivery at excitatory synapse in layers II/III neurons of primary motor cortex. No COI.

3P-007

Taste-related aversive behaviors evoked by microinjection of $GABA_A$ receptors agonist into rat ventral pallidum

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Previously we showed that aversive taste reactivity responses to a conditioned stimulus (CS) in taste aversion learning were decreased by the blockade of $GABA_A$ receptors in the ventral pallidum (VP). In addition, the intraoral infusion of a CS elevated GABA efflux in the VP. These results suggest that VP GABA mediates the expression of aversive responses. In the present study, we investigated whether the stimulation of VP $GABA_A$ receptors induces taste-related aversive responses. Rats received microinjections of the $GABA_A$ receptor agonist muscimol (0, 10, 100 ng/100 nl) into the VP, then their behaviors were videotaped. Low dose (10 ng) of muscimol evoked ingestive behaviors such as rhythmic mouth movements and floor licking. In contrast, higher dose (100 ng) elicited a large number of chin rubbing and forelimb treading, which are known as aversive responses in the taste reactivity response test. To determine the brain regions involved in the aversive responses to higher dose of muscimol, we examined Fos-like immunoreactivity (FLI) as a marker of neural activation in multiple brain sites. The higher dose of muscimol induced greater number of FLI in the lateral habenula, lateral hypothalamus, supramammillary nucleus, and ventral tegmental area. It is likely that the increased activation of VP $GABA_A$ receptors affected these brain regions to cause the alteration in behavioral expression from ingestive to aversive. No COI.

3P-008

Role of presynaptic P2X3 receptor in excitatory inputs onto GABAergic inhibitory interneurons in Substantia Geratinosa of rat spinal cord

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Neurons in the spinal dorsal horn process sensory information and transmit it to several brain regions which are responsible for pain perception. Spinal GABAergic interneurons on the other hand in the superficial dorsal horn have an important role on modulation of sensory information. However, which kind of afferent fibers interact with GABAergic interneurons is poorly understood. Here, we examined the effect of α,β-methylene ATP, a P2X3 agonist, on synaptic responses elicited in GABAergic neurons using whole-cell patch clamp recordings from spinal cord slices of vesicular GABA transporter (VGAT)-venus rats. α,β -methylene ATP increased the frequency and amplitude of spontaneous EPSC in venus-labeled neurons. In the presence of tetrodotoxin, α, β-methylene ATP also increased the frequency but not amplitude of miniature EPSC. These results suggest that activation of spinal presynaptic P2X3 facilitates excitatory synaptic responses in GABAergic interneurons. The P2X3-mediated activation of GABAergic interneurons may have an important role on spinal modulation of itch or pain information. No COI.

3P-009

P2X7 receptor in microglia contributes tongue cancer pain in rats

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[Introduction]Although, $P2X_7$ receptor is implicated in both neuropathic and inflammatory pain, $P2X_7$ receptor mechanism in pathological pain is unclear. Thus, we developed a rat model of tongue cancer pain, and investigated the involvement of $P2X_7$ receptor in the tongue cancer pain. [Materials & Methods]Squamous cell carcinoma (SCC) cells subcutaneously were inoculated into the tongue. The mechanical or heat stimulus intensity evoking neck EMG activity was defined as the head-withdrawal reflex threshold (HWT) under light anesthetization. On days 3, 4 and 14 after SCC cell-inoculation, the microglial activation and $P2X_7$ receptor expression were immunohistochemically examined in trigeminal subnucleus caudalis (Vc). We also examined the HWT in SCC cells-inoculated rats with successive intrathecal selective $P2X_7$ receptor antagonist (A-438079) administration.[Results]The HWT significantly decreased following SCC cell-inoculation into the tongue. However, there were no significant changes in the HWT to heat stimulation in SCC cell-inoculated rats. On days 3, 4 and 14, microglia was activated and $P2X_7$ receptor localized in activated microglia in Vc. Intrathecal A-438079 administration suppressed the decrement of HWT and the activation of microglia in SCC cell-inoculated rats. [Conclusion] These findings suggest that microglial activation in Vc via $P2X_7$ signaling is involved in tongue mechanical allodynia following SCC cells inoculation into the tongue. No COI.

3P-010

Identification of cutaneous low-threshold mechanoreceptors related to inhibition of cardiovascular responses to noxious somatic stimuli

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We have reported that touch inhibited cardiovascular responses to noxious somatic stimuli. Such effects were dependent on the texture of surface of the touching objects. We aimed to identify cutaneous mechanoreceptors that inhibit somatically-induced cardiovascular responses. Single or a few multiple unitary afferent activities were recorded from saphenous nerve in anesthetized rats. Touch was applied to the inner thigh for 10 min using 1) a soft elastomer brush (microcone), which inhibited somatically-induced cardiovascular responses, and 2) a soft elastomer flat disc (flat disc), which did not influence. An increment of firing rate during each type of touch was compared for each unit, and a difference by less than 10% was considered as no difference. A total of 33 slowly adapting units were obtained (Aβ, Aδ and C fibers; 13, 12 and 8 units, respectively). Mechanical threshold of all units was less than 0.4 g. Of Aβ units, firing rate was greater during microcone in 5 units and during flat disc in 5 units, and not different in 3 units. Of Aδ units, firing rate was greater during microcone in 11 units and during flat disc in 1 unit. Of C units, firing rate was greater during microcone in 6 units and during flat disc in 2 units. Mean increments of Aβ, Aδ and C units during microcone and flat disc were 0.55 vs. 0.80 Hz, 0.57 vs. 0.26 Hz, and 0.31 vs. 0.17 Hz, respectively. The present study suggests that excitation of cutaneous low-threshold mechanoreceptive Aδ and C units may responsible for inhibition of somatically-induced cardiovascular responses. No COI.

3P-011

Cholinesterase inhibitor improves contrast sensitivity in freely behaving rats

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Acetylcholine (ACh) is known to modulate neuronal activity in the rodent primary visual cortex (V1). We recently examined effects of a microionophoretically and topically administered ACh in V1 of anesthetized rats, finding that ACh facilitated or suppressed visual responses to varying stimulus contrasts by multiplying the control responses, i.e. response gain control. These ACh effects showed a laminar bias, where the response suppression and facilitation prevailed in layers 2/3 and layer 5, respectively. In facilitated cells, ACh improved the signal-to-noise (S/N) ratio, while in suppressed cells it enhanced the F1/F0 ratio without any concurrent reduction in the S/N ratio. These effects on S/N and F1/F0 ratios were observed in regular-spiking cells, but not in fast-spiking cells. Our findings suggest that ACh promotes the signaling of grating-phase information from supragranular cells to higher-order areas by the suppressive modulation, and enhances feedback signals with a high S/N ratio from infragranular cells to subcortical areas by the facilitatory modulation. To examine whether such fine regulation of visual information processing by ACh contributes to the improvement of visual performance in behaving animals, we trained rats to detect visual stimulus in a two-alternative forced-choice task combined with a staircase method, finding that donepezil, a cholinesterase inhibitor, improved the contrast sensitivity depending on the stimulus conditions. No COI.

3P-012

Extracellular matrix proteoglycan plays a pivotal role in mechanical sensitization by low pH of thin muscle afferent fibers

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Exercise lead to tissue acidosis, which induces acute muscle pain and mechanical hyperalgesia. Corresponding to this, enhanced thin-fiber afferent responses to mechanical stimulation have been recorded in vitro at low pH. Recently we have demonstrated using the whole cell patch-clamp method that the low pH-induced mechanical sensitization in rat cultured dorsal root ganglion neurons were inhibited by chondroitin sulfate and chondroitinase ABC, and proposed a novel mechanism for sensitization that involves extracellular proteoglycan, versican (Kubo et al, J. Physiol. 2012). We therefore examined whether this mechanism works also in tissue level. We recorded single fiber activities in muscle-nerve preparations in vitro obtained from Sprague-Dawley rat. After identifying single Aδ- or C-fiber afferents, mechanical stimulus (from 0 to 196 mN in 10 s) was applied to the receptive field on the muscle. The response magnitude was significantly increased by pH 6.2, and the mechanical threshold was significantly lowered. Injection of 5-μl of 0.3% chondroitin sulfate near to the receptive field significantly reversed the lowered threshold and increased response magnitude (n=26). Injection of pH 7.4 control solution did not have any significant effects (n=15). It was not possible to examine whether sensitized afferents had versican, but the majority of them are considered to have versican based on the previous report that over 70% of thin muscle afferents have it. Our present result supports the novel mechanism for sensitization that involves extracellular proteoglycan, versican. COI properly declared.

3P-013

Effect of forelimb stimulation on the hindlimb stimulation-induced propagating excitation in the rat sensorimotor cortex detected by optical recording system

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We have developed an optical recording system using a voltage-sensitive dye. Using this system, we have reported that the neural excitation induced by a somatic stimulation spreads over the sensorimotor cortex like a propagating wave initiated from the somatotopically corresponding site. In this study, to analyze the interaction between these propagating waves, we examined the influence of forelimb stimulation on the hindlimb stimulation-induced response. The sensorimotor cortex including the hind- and fore-limb regions was exposed and stained with a voltage sensitive dye (RH-414). An electrical forelimb stimulation was given prior to hindlimb stimulation. The inter-stimulus intervals were 0, 20, 50, 100, 200 or 300 msec. The propagating pattern was analyzed on the basis of isochrone maps. When the interval was 0 or 20 msec, propagating waves ran into each other somewhere in between and did not appear to exceed the collision line. When the interval was longer than 50 msec, the propagating wave of the forelimb response should have passed the initiation site of the hindlimb response before it occurred. For the intervals less than 100 msec, the hindlimb response, if any, was overridden by the forelimb response. For longer intervals, i.e., 200 or 300 msec, hindlimb response was observed but the extent of the propagation area decreased. These results suggest that the somatic evoked wave, on the way of propagation, suppresses the occurrence and reduces the propagation of another one. No COI.

3P-014

Activity is required not for initial formation but for later reorganization of neuronal functions in visual cortex

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Visual functions of cortical neurons are established by activity-independent and -dependent mechanisms. However, the precise roles of neuronal activity in the development of two prominent visual functions, orientation and direction selectivity, are still debated. Here we show that in the mouse primary visual cortex (V1), the development of orientation and direction selectivity proceeds in two steps, which differ in their activity dependency. The responsiveness and tuning sharpness of neurons rapidly mature within one day of eye opening, but there is a strong bias in the distributions of preferred orientations and directions. We found that this initial bias reduces more slowly than the maturation of tuning sharpness. To study the roles of neuronal activity in these processes, we genetically suppressed the activity of neurons in V1 during development only. We found that neurons with suppressed activity acquired responsiveness and tuning sharpness normally, but the bias reduction was blocked. Thus, the rapid maturation of responsiveness and tuning sharpness is largely activity-independent, whereas the later process of bias reduction is activity-dependent. This result suggests that the initial neuronal functions are specified by activity-independent mechanisms, such as genetic factors, but the adult functions are reorganized by activity-dependent mechanisms to represent more diverse information. No COI.

3P-015

Characterization and synaptic connections of a new class of neurons in layer II of the cerebral cortex

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It is now established that pyramidal neurons are excitatory neurons of the cerebral cortex. Whether non-pyramidal excitatory neurons exist in cortical layers other than layer IV, however, remains to be elucidated. Here we combined multiple whole-cell recordings and histological methods to investigate the morphological and functional properties of a group of layer II neurons located at the border of layer I and layer II. We refer to these neurons as layer II roof neurons (L2RN). All L2RNs were regular-spiking neurons, having highly homogenous intrinsic membrane properties. Many L2RNs were non-pyramidal in shape. In paired-recordings, L2RNs, as presynaptic neurons, always excited postsynaptic neurons with latencies less than 1.5 msec. L2RNs excited layer I fast-spiking neurons and received inputs from other layer II/III and layer V neurons. Taken together, our results suggest that a portion of L2RNs are non-pyramidal excitatory neurons, integrating inputs from both deep layers and upper layers. No COI.

3P-016

Two-photon imaging of lateral inhibition among neuronal ensemble in the superficial layer of the superior colliculus

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The superior colliculus (SC) is a brainstem center which plays a key role in mediating the signal for sensory-motor translation. The superficial layer of the SC (sSC) is directly innervated by the optic tract and visual space is represented in the retinotopic coordinates. In the visual pathway, firing activity of neurons in response to the stimuli presented in their response field are often inhibited by stimuli presented outside their receptive field. This effect is known as "lateral inhibition". It has been debated whether horizontal inhibitory connections are responsible for the lateral inhibition in the sSC; however, the way of its neural implementation remains elusive, especially at the neuronal population level. Therefore, we applied an ***in vivo*** two-photon Ca^{2+} imaging to sSC in anesthetized mice, and tried to elucidate neuronal population activities and those micro-circuit mechanisms. First, we examined stimulus size tuning properties in the sSC. The larger the stimulus size, the less the neural activity of the neurons which were located near the response center. Second, we confirmed either excitatory or inhibitory effects by two-point stimulus presentation, which depended on the distance between the two stimuli. The smaller separation caused mutually potentiating responses. On the other hand, mutual inhibition was observed when the two stimuli were presented with large separation (>9 deg in the visual space). No COI.

3P-017

Involvement of rostral ventromedial medulla (RVM) cells in cortex stimulus-induced anti-nociceptive effects in normal and chronic constriction injury rats

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Motor cortex stimuli provide anti-nociceptive effects in chronic constriction injury (CCI) rats by spinal cord inhibition. It has also been shown that even in normal rats spinal cord neurons reduce their responses to nociceptive stimulation during motor cortex stimuli, but the precise mechanism of this spinal cord inhibition remains to be unknown. In the present study, we tested the rostral ventromedial medulla (RVM) involvement in this motor cortex stimulus-induced spinal cord inhibition in normal rats and in CCI rats made by unilateral sciatic nerve ligation. Single unit activity of the RVM cell was recorded with tungsten microelectrode under pentobarbital anesthesia. Prior to cortical stimuli, the RVM cells were classified into three groups, ON-, OFF-, and Neutral cells, based on their responses to nociceptive pinch stimulation applied at the hind paw. Cortical stimulus current intensity was ranged 30–110μA. We found that in normal rats RVM cell activity changed during cortex stimuli, and in CCI rats the change of the activity prolonged. These results suggest that anti-nociceptive effects of motor cortex stimuli are derived from the RVM activity. No COI.

3P-018

In vivo analysis of sensory synaptic responses evoked in parasympathetic preganglionic neurons in the rat spinal cord.

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Coordinated movement of the bladder, urethra and external urethral sphincter in the lower urinary tract (LUT) is essential for the bladder filling and voiding. Parasympathetic preganglionic neurons (PGN) in the lumbosacral spinal cord play an important role of in regulating different pelvic organ function including micturition and defecation. Although pharmacological and behavioral approaches have been well utilized to study the spinal control of LUT functions, the cellular mechanism, however, is still unclear. In this study, we developed an *in vivo* extracellular recording and whole-cell patch-clamp recording techniques to investigate how the spinal cord controls the LUT function at the synaptic level. The firing frequency of spinal dorsal horn neurons in the PGN was synchronously changed with the intravesical pressure. The electrophysiological properties of PGN in L6 spinal cord slices with an attached dorsal root were also studied. Monosynaptic EPSCs mediated through Aδ and C fiber were elicited in PGN. And also these neurons were expressed a choline acetyltransferase. The newly developed *in vivo* recording techniques in addition to the lumbosacral slice-patch recording are useful for elucidating the detailed mechanism for spinal control of LUT function. No COI.

3P-019

Effect of CCL-1 on synaptic transmission in the spinal superficial dorsal horn

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Cytokines are well-known to have effects on neurotransmission in the spinal cord. However, the detail mechanism how chemokine (C-C motif) ligand 1 (CCL-1), a well-characterized chemokine secreted by activated T cells, modulates spinal neurotransmission remains unclear. Here we examined action of CCL-1 on synaptic transmission in the spinal superficial dorsal horn by using slice patch-clamp recordings. Pain-related behavioral tests, von Frey filament test, Randall-Selitto test and tail flick test, showed that CCL-1 induced mechanical allodynia and hyperalgesia, but not thermal hyperalgesia after intrathecal injection of CCL-1 recombinant. CCR-8, the receptor for CCL-1, was detected in the superficial dorsal horn neurons. Bath application of CCL-1 (50 ng/ml) enhanced the frequency of spontaneous and miniature excitatory postsynaptic currents elicited in superficial dorsal horn neurons at a holding potential of -70 mV. Our results indicate that CCL-1 increases glutamate release from presynaptic terminal in the superficial dorsal horn. This synaptic facilitation may account for CCL-1-induced mechanical allodynia and hyperalgesia. No COI.

3P-020

Facilitation of spinal inhibitory synaptic transmission by optoactivation of the locus coeruleus in the brain stem

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Locus coeruleus (LC) neurons in the brain stem send noradrenergic projections throughout the neuroaxis and are implicated in the control of many homeostatic functions such as arousal, cardio-respiratory control. In addition, the LC is also a major source of noradrenergic projections to the spinal superficial dorsal horn which play a significant role in pain modulation. Under urethane-anesthesia, in vivo whole-cell current- and voltage-clamp recordings were made from spinal superficial dorsal horn neurons. Bath application of noradrenaline to the surface of the spinal cord elicited an outward current and facilitated inhibitory postsynaptic currents. Optoactivation of the LC neurons expressing ChR2, however, elicited a barrage of IPSCs in spinal superficial dorsal horn neurons without eliciting any postsynaptic slow outward currents. Slice patch-clamp recordings from GABAergic neurons in the spinal cord showed that noradrenaline excites GABAergic neurons mediated through alpha 1 receptors. These results suggest that activation of pontospinal noradrenergic system facilitates spinal GABAergic neurons to inhibit nociceptive information. No COI.

3P-021

Spinal cord imaging during post-ischemic numbness in mice

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We experience numbness after transient ischemia of extremities. However, the mechanisms are unknown. In our previous study, conduction block of peripheral sensory nerves induced abnormal sensitization of the spinal neural circuits. Therefore, it is possible that similar spinal cord mechanisms may be responsible for post-ischemic numbness. To test this hypothesis directly, we investigated spinal cord responses during post-ischemic numbness using flavoprotein fluorescence imaging in anesthetized mice. Vibratory stimuli applied to the hindpaw produced fluorescence responses in the ipsilateral spinal cord. Vibratory stimulation applied to each digit of the hindpaw produced clearly separated and ordered spinal cord responses. The transient ischemia was produced by application of high pressure into a rubber cuff around the hindpaw for 30 min. The ischemia eliminated the spinal responses elicited by vibratory stimuli applied to the hindpaw. However, the responses appeared again, and were potentiated after removal of the ischemia compared with those recorded before the ischemia. It is known that type II metabolic glutamate receptor (mGluR) is responsible for the changes in spinal neural circuits after conduction block of peripheral nerves. Application of LY354740, a mGluR agonist, on to the surface of the spinal cord suppressed the post-ischemic potentiation of spinal responses. These results clearly indicate the presence of spinal mechanisms underlying post-ischemic numbness. No COI.

3P-022

Somatosensory cortical responses after crossing nerve transfer in mice

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We investigated cortical changes after crossing nerve transfer of brachial plexus using flavoprotein fluorescence imaging in mice. The distal cut ends of the left median and ulnar nerves were connected to the central cut ends of the right median and ulnar nerves with a sciatic nerve graft at 8 weeks old. Eight weeks after this operation, S1 responses elicited by vibratory left forepaw stimulation were visualized. In control mice, direct responses (DRs) mediated via thalamic input was observed in the contralateral S1. Weak indirect responses (IRs) were also observed in the ipsilateral S1. In mice with crossing nerve transfer, DRs were observed in the ipsilateral S1, and clear IRs were observed in the contralateral S1. The relative amplitudes of IRs normalized by those of DRs in mice with crossing nerve transfer were significantly larger than those in control mice. It is expected that DRs were initiated by thalamic inputs to layer 4, while IRs were initiated by callosal inputs to layer 2/3. In control mice, layer specific flavoprotein fluorescence responses were investigated using a macroconfocal microscope. The DRs in layer 4 were slightly larger in amplitude and faster in latency compared with those in layer 2/3. For investigating cortical plasticity after crossing nerve transfer, we are going to perform similar analyses on IRs in mice with crossing nerve transfer. No COI.

3P-023

Firing pattern of spinal dorsal horn neurons receiving pruriceptive afferents in the adult rat spinal cord

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Itching is a common symptom in dermatologic diseases. We have previously shown by using an in vivo patch-clamp technique that pruritic synaptic responses are evoked in spinal dorsal horn (SDH) neurons of rats. Topically application of 5-HT to the skin increased the frequency of the large amplitude of spontaneous EPSCs in about 30 % of the neurons recorded. In this study, we examined the firing patterns of the SDH neurons receiving 5-HT-sensitive afferent fibers. In current-clamp mode, current pulses were applied to SDH neurons to elicit action potentials. SDH neurons tested were classified into five types based on their firing patterns. Most (about 70%) of the 5-HT-responsive SDH neurons were the delayed and sustained repetitive firing types, although 5-HT-unresponsive neurons were also found in these two types of SDH neurons. The relationship between the morphological class of SDH neurons and their firing patterns has previously shown that vertical cells exhibit the delayed or sustained firing type. In combination with the previous studies, the present results suggest that pruriceptive 5-HT-sensitive afferent fibers evoke excitatory synaptic responses mainly onto delayed firing and sustained repetitive firing type neurons which are morphologically classified as the vertical cell. No COI.

3P-024

Involvement of lysophosphatidic acid-evoked TRPA1 and TRPV1 activation in peripheral itch sensation of mice

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Lysophospahtidic acid (LPA) is a bioactive phospholipid reported as a main mediator of cholestatic itch, or pruritus, while it is also reported to induce acute pain. Whether LPA induces itch, pain or both in the periphery is unclear. Although how LPA induces pain has been investigated in detail, the cellular and molecular mechanism of LPA-induced itch remains unclear. Transient receptor potential ankyrin 1 (TRPA1) and vanilloid 1 (TRPV1) channels are calcium-permeable non-selective cation channels reported to contribute to the itch transduction in peripheral sensory neurons.We investigated the effects of LPA on the intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in the dorsal root ganglion neurons obtained from wild-type, TRPA1KO, TRPV1KO or TRPA1/TRPV1 double KO mice, and found that LPA induced $[Ca^{2+}]_i$ increases through both TRPV1 and TRPA1 activation. We also observed LPA-induced TRPA1-mediated single channel openings in HEK293T cells expressing mouse TRPA1. Furthermore, we observed LPA-induced scratching behaviors in a cheek-injection model, which were significantly reduced in TRPA1KO, TRPV1KO and TRPA1/TRPV1 double KO mice. Thus, we concluded that both TRPA1 and TRPV1 are involved in the LPA-induced itch sensation in mice. No COI.

3P-025

Esophageal peristalsis is regulated by capsaicin-sensitive intrinsic neural circuit in rats

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The purpose of this study was to clarify the role of a local neural reflex consisting of capsaicin-sensitive primary afferent neurons and intrinsic neurons in esophageal peristalsis. Rats were anesthetized, and esophageal intraluminal pressure and propelled intraluminal liquid volume were recorded. In the experimental system, an intraluminal pressure stimulus evoked periodic changes in intraluminal pressure of the esophagus, which were consistently accompanied by intraluminal liquid propulsion. Bilateral vagotomy abolished changes in intraluminal pressure as well as liquid propulsion. These results indicate that the novel method is appropriate for inducing peristalsis in the esophagus composed of striated muscles. Then, by using the method, we examined functional roles of the local reflex in esophageal peristalsis. The esophagus of capsaicin-treated rats showed a multi-phasic rise in intraluminal pressure, which may due to non-coordinated contractions of esophageal muscles, whereas a mono-phasic response was observed in the intact rat esophagus. In addition, destruction of capsaicin-sensitive neurons increased the propelled liquid volume and lowered the pressure threshold for initiating peristalsis. These results suggest that the local neural reflex consisting of capsaicin-sensitive neurons and intrinsic neurons contributes to coordination of peristalsis and suppresses mechanosensory function of vagal afferents in the esophagus. No COI.

3P-026

Reduction of pain threshold induced by joint immobilization modulates the negative affective component of pain

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Immobilization of joint by cast is commonly used for resting the injured joint. However the reduction of pain threshold for mechanical stimuli is often induced by cast immobilization of the hind limb. In this study using rats, we examined whether the hypersensitivity induced by cast immobilization alters the pain-induced place aversion as an aspect of the affective component of pain. To examine the effects of cast immobilization on pain behaviors in rats, one hind limb was immobilized for 2 weeks with a cast and observation of pain behavior was conducted after cast removal for 3 weeks. A wire mesh cast was wrapped around the one hind limb to keep the ankle joint almost straight. The pain threshold was measured by using calibrated von Frey filament test before and after cast immobilization. The joint immobilization elicited the reduction of pain threshold which continued almost over 10 day period after a cast removal. The negative affective component of pain was assessed in rats with cast treatment by using the conditioned place aversion induced with the intraplantar injection of formalin. The formalin-induced conditioned place aversion was decreased in the rats with a cast treatment. These results suggest that the reduction of pain threshold by joint immobilization fails to form the associate learning between the painful stimulation-induced aversive emotion and the pain-conditioned environment. No COI.

3P-027

Effect of thermal therapy on cutaneous pain threshold and muscular pain threshold in healthy subjects

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There are many patients suffering from chronic musculo-skeletal pain such as the low back pain. In the clinical treatment for muscular pain, thermal therapy such as hot pack (HP) is frequently used as one of the physical therapies. Although some hypotheses are proposed in analgesic mechanism, evidence of effects of HP is poor. Furthermore, usually treatment time is used 20 min, but the effective treatment time is uncertain. Therefore, we examined effects of HP on the mechanical nociceptive thresholds of muscles and skin. Effects of different treatment time were also examined.HP was applied on the right lower leg of healthy subjects (n = 9). Unilateral application of HP for 20 min increased muscular pain thresholds of both sides. These effects lasted for more than 20 min after HP. HP application for 5 min increased muscular pain threshold of the ipsilateral side only for 10 min. Unlike the effects of muscular pain threshold, HP application for 20 min did not affect cutaneous pain threshold in both sides.That HP application to one side for 20 min increased muscular pain threshold on both sides, but that there was no effects on cutaneous pain threshold suggest that the descending pain inhibitory system may act more strongly to muscular nociception than cutaneous nociception in humans. HP for 5 min is not enough to develop full activation of the descending pain inhibitory system. No COI.

3P-028

Involvement of MeCP2 in heat sensitivity and inflammatory pain in tongue

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[Introduction]
Regulation of gene expression can be achieved through DNA CpG methylation which induces chromatin remodeling and gene silencing through a transcriptional repressor complex comprising methyl-CpG-binding protein 2 (MeCP2). Though it is well known that noxious sensitivity decreased in Rett syndrome patients who have mutations in *MeCP2* gene, the mechanism remains unclear. In the present study, we determined if *MeCP2* was associated with altered heat sensitivity of the tongue following complete Freund's adjuvant (CFA) injection into the tongue.
[Methods]
CFA solution was injected subcutaneously into the tongue in wild type and *mecp2* knockout female mice. Heat stimulation (35–55°C, 1°C/sec, cut off: 60°C) was also applied to the tongue by using a contact heat probe under light anesthetization. The threshold temperature for evoking EMG activity by heat stimulation to the tongue was defined as the heat head-withdrawal reflex threshold (HHWT).
[Results]
The HHWT in *mecp2* knockout mice is higher than that in wild type. The HHWT significantly decreased following CFA injection into the tongue in wild type mice. However, there were no significant changes in the HHWT following CFA injection into the tongue in mecp2 knockout mice.
[Conclusion]
The present findings provide the first evidence that MeCP2 is involved in heat sensitivity and heat hyperalgesia following tongue inflammation. No COI.

3P-029

The effects of music listening on the pain threshold

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Pain plays a crucial role in transmitting hazard signals to the body, but it causes discomfort. Various studies have shown that pain perception is reduced when we are concentrating on something such as sports. In this study, we examined the effects of music listening (popular music or ballads) as well as several oral functions (biting, chewing and tasting) on pain perception. Forty-five subjects were given stimulation by CO_2 laser on their ankles with each condition. Subjects assessed the intension of pain level using visual analog scale (VAS) by themselves. The thresholds of pain on oral areas (tongue, buccal mucosa, and jaw gingiva) were investigated by pain vision PS-2100 while the subjects were listening to popular or classical music, or ballads. Furthermore, the blood oxygenation level-dependent BOLD signals in the cingulate cortex were analyzed using functional magnetic resonance imaging (fMRI), when ten subjects were given electrical stimulation of 80μA on their ankles while listening to music. In the VAS levels, we found significant pain reduction when the subjects were listening to music (Shaffe's test, $p<0.001$), whereas no significant pain reduction was seen with the oral functions. The thresholds of pain on oral areas were significantly higher when the subjects were listening to ballads or classical music than those without music (Wilcoxon test, $p<0.05$). In the fMRI study, listening to music attenuated BOLD signals in the cingulate cortex in 5 subjects. These findings suggest that listening to music is capable of reducing pain perception. No COI.

3P-030

Repeated forced swim stress enhances CFA-evoked thermal hyperalgesia and affects the expressions of pCREB and c-Fos in the insular cortex

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Exposure to stressors causes substantial effects on the perception and response to pain. In several animal models, chronic stress produces lasting hyperalgesia. The insular (IC) and anterior cingulate cortices (ACC) are the regions exhibiting most reliable pain-related activity. And the IC and ACC play an important role in pain modulation via descending pain modulatory system. In the present study we examined the expression of pCREB and c-Fos in the IC and ACC after forced swim stress and CFA injection to clarify changes in the cerebral cortices that affect the activity of descending pain modulatory system in the rats with stress-induced hyperalgesia. Forced swim stress (day 1, 10min; days 2–3, 20min) induced an increase in the expression of pCREB and c-Fos in the anterior IC (AIC) and ACC. CFA injection into the hindpaw after the forced swim stress shows significantly enhanced thermal hyperalgesia and induced a decrease in the expression of c-Fos in in the AIC and the posterior IC (PIC). Quantitative image analysis showed that the numbers of c-Fos-IR neuron in the left AIC and PIC were significantly lower in the forced swim stress + CFA group (L AIC, 95.9±6.8; L PIC, 181.9±23.1) than those in the naive group (L AIC, 151.1±19.3, $p< 0.05$; L PIC, 274.2±37.3, $p< 0.05$). These findings suggest a neuroplastic change in the IC after forced swim stress, which may be involved in the enhancement of CFA-induced thermal hyperalgesia through dysfunction of descending pain modulatory system. No COI.

3P-031

Lowered Threshold for Self-Motion Perception to Galvanic Vestibular Stimulation (GVS) in Patients with Weather-Related Pain

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Several clinical studies have demonstrated a consistent relationship between changes in barometric pressure and pain intensity in patients with chronic pain. We have demonstrated that the barometric pressure sensor located in the inner ear (vestibule) contributes to the mechanism of weather-related pain. However, it has not been known whether the vestibule of patients with weather-related pain is more sensitive than that of healthy subjects. The present study, therefore, aimed to investigate the threshold for self-motion perception to GVS in patients with weather-related pain.

Sixteen healthy subjects (age 22–77 yrs, mean 38 yrs), fourteen patients with weather-related pain (age 31–73 yrs, mean 52 yrs), and eight patients with weather-independent pain (age 25–73 yrs, mean 53 yrs) were included in this study. Sinusoidal GVS was delivered binaurally via surface electrodes applied over the mastoids at stimulus frequencies 0.5 Hz. The subject becomes slightly dizzy (self-motion perception) with increasing current, and the perception threshold was measured. Average threshold of subjects with weather-related pain (1.0 ± 0.2 mA, mean ± SE) was significantly lower than that of weather-independent pain group (2.2 ± 0.3 mA) and healthy control group (3.1 ± 0.4 mA). These results showed that patients with weather related-pain are sensitive to vestibular inputs. No COI.

3P-032

Thermal preference test in rat orofacial formalin model

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The affective/emotional component of pain helps animals to change their behavioral programs to avoid potentially aversive and harmful situations in the future. Such function of pain depends on central plasticity and makes it a strong driver of negative emotion that forms basis for pain suffering in chronic pain patients. However, in contrast to the human subjects that report the state of pain "also" in a verbal form, objective evaluation of pain affect in animals has been difficult, compared to that of nocifensive behaviors, such as the paw withdraw and reflexogenic escape, widely used until today. Recently, evaluation of place aversion or preference using the nociceptive or analgesic experiences as unconditioned stimuli for Pavlovian learning is used to address this issue, assuming that the degree of learning represents the degree of pain suffering. To develop a method for more objective evaluation of spontaneous pain in animals, we employed two-temperature preference paradigm. Rats were allowed to move freely between two equivalent plates of different temperatures that were near-aversive range for some periods within 30 min of observation. We optimized the programmed changes in plate temperatures so that the rats spontaneously move back and forth between the two plates in a highly reproducible manner and evaluated the effects of chronification process of inflammatory pain (6 to 24 hours after orofacial formalin injection) on the time and frequency spent in colder plate. The results suggest that the chronified inflammatory pain affects the temperature-dependent decision-making process. No COI.

3P-033

Efficacy of electro-acupuncture for paclitaxel-induced peripheral neuropathy

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Paclitaxel is a microtubule-binding compound that is widely used as a chemotherapeutic in the treatment of common cancers, including breast and ovarian cancer. However paclitaxel has neurotoxic effect, and paclitaxel exposure frequently causes peripheral neuropathy at a side effect.The SD rats were randomly divided into three groups; the Paclitaxel group (PTX), the Paclitaxel and acupuncture group (PTX-A), and the control group. All rats were injected intraperitoneally (i.p.) on four alternate days (days 1, 3, 5, and 7) with vehicle (saline) or 2.0 mg/kg paclitaxel. Electro-acupuncture which caused slight muscle twitch was applied to the ZuSanli acupoint (S36) in the limbs on every other day (right side, 1Hz, 20 min., 3–5V). Behavioral assays were carried out by the heat-hyperalgesia test and mechano-allodynia test of the hind paws (sciatic nerve territory). All rats were sacrificed on day 28, and the lumbosacral vertebral column, and the segment of the sciatic nerve from mid-thigh were collected for light microscopy examination. PTX group produced significant mechanical allodynia in both the hind paws. However the PTX-A group did not show the decrease of the mechanical threshold. Additionally, the difference of heat-hyperalgesia and axonal β-tubulin was not shown between the PTX group and the PTX-A group.In conclusion, our study suggests that a acupuncture stimulation relieved to paclitaxel-induced painful peripheral neuropathy. It was suggested that satellite cells in the DRG might play a role in the development of paclitaxel-induced painful peripheral neuropathy. No COI.

3P-034

Neural correlates of a target position represented in a background coordinate: an f-MRI study

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Our brain represents a target position in terms of our body parts, like in retinal or craniotopic coordinates. However, recent studies have shown that a target position can be represented in terms of a frame in the background. In this study, we aimed to dissociate neural correlates of the background coordinates from the retinal and craniotopic coordinates by using an f-MRI adaptation technique. A target was presented at different locations on a screen, in combination with a rectangular frame that was also presented at different locations, while participants looked at a fixation cross. When a target was presented at the same location in the background coordinate, significant decreases of f-MRI signals (due to adaptation) were observed in the bilateral precuneus, the right dorsal premotor cortex, the right transverse occipital sulcus, and the right middle temporal cortex. On the other hand, no region showed adaptation, when a target was repeatedly presented in terms of the retinal or craniotopic coordinates. These results clearly show that neural correlates of the background coordinate is distinct from those of the retinal and craniotopic coordinates, and reside in several areas including the precuneus. No COI.

3P-035

Possible plasticity of the Stevens' power law

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Objective
The subjective magnitude of sensation grows as a power function of the stimulus intensity, known as the Stevens' power law (1957). The power law was studied for visual and auditory sensations as a practicum for nursing students.
Methods
Subjects were instructed to assign numbers proportional to the apparent magnitude of light (brightness) and sound (loudness) and the apparent frequency of sound (tone) stimuli presented (n=50; Match). The subjects were instructed to adjust brightness, sound and tone until it seemed to match the number given (n=45; invMatch). Inverse matching was studied after "training session" using stimulators with exponents >2 (n=7; invMatch/Tr).
Results
In Match, exponents for brightness, loudness and tone were estimated to be 0.25, 0.31 and 0.69, respectively ($p<0.001$). In invMatch, exponents were 0.91, 0.71 and 0.82, respectively ($p<0.001$). Exponents in invMatch/Tr were 1.18, 1.14 and 0.89 ($p<0.001$).The exponent for loudness was significantly larger in invMatch/Tr than in invMatch ($p=0.013$).
Discussion
The three modalities obeyed the Stevens' power law in Match. They also followed the power law in invMatch, but it is interesting that exponents significantly differed after training. These results may indicate possible plasticity of the power function. No COI.

3P-036

Effect of dopamine on excitatory synaptic transmission in the rat spinal parasympathetic preganglionic nuclei

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Dopamine is known to modulate synaptic transmission over wide areas of the CNS. Dopaminergic neurons innervate their axons also into the lumbosacral spinal cord, and are thought to control activity of visceral organs. In this study, we examined how dopamine acts on excitatory synaptic responses evoked in parasympathetic preganglionic nuclei neurons in the adult rat lumbosacral spinal cord. Whole-cell current-clamp and voltage-clamp recordings were made from parasympathetic preganglionic nuclei neurons in spinal cord slices, and dopamine was bath-applied for 1–2min. Parasympathetic preganglionic nuclei neurons had a resting membrane potential more negative than -60 mV. Under voltage-clamp conditions at a holding potential of -70mV, parasympathetic preganglionic nuclei neurons exhibited spontaneous excitatory postsynaptic currents. Dopamine dose-dependently decreased the frequency of spontaneous excitatory postsynaptic currents in most of the parasympathetic preganglionic nuclei neurons tested. The present results suggest that inhibitory action of dopamine on spinal excitatory transmission may have an important role on parasympathetic control of visceral organs by dopamine. No COI.

3P-037

Effects of Vasopressin on reciprocal synaptic transmission between mitral and granule cells in the mouse accessory olfactory bulb

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Central vasopressin facilitates social recognition and modulates numerous complex social behaviors in mammals. Recent analysis of transgenic rats engineered with an enhanced green fluorescent protein reporter for vasopressin synthesis identified new population of vasopressin neurons in the accessory olfactory bulb (AOB). The AOB has been demonstrated to be a critical site for mating-induced mate recognition (olfactory memory) in female mice. A behavioral study revealed that inactivation of the vasopressin 1b receptor gene impaired the olfactory block to pregnancy. The effect of vasopressin, however, on the synaptic transmission between dendrites in the AOB of female mice is largely unknown.
To address this issue, we examined the effect of vasopressin on the reciprocal transmission between mitral and granule cells by stimulating a mitral cell and recording the evoked IPSCs from the same cell with the patch-clamp technique in whole-cell configuration. AOB slices were prepared from 23- to 36-day-old Balb/c female mice. To evoke IPSCs, a depolarizing voltage step from -70 to 0 mV was applied to a mitral cell.
In Mg^{2+}-free solution, vasopressin significantly blocked the IPSCs. The suppressive effect of vassopressin on the IPSCs was diminished by an antagonist for vasopressin receptors (AVRs), [β-mercapto-β,β-cyclopentamethylenepropionyl-O-me-Tyr, Arg] vassopressin. The present results suggest that AVRs are involved in reciprocal transmission between mitral cells and granule cells in the mouse AOB. No COI.

3P-038

A role for vasopressin in synaptic plasticity in the accessory olfactory bulb of male mice

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The accessory olfactory bulb (AOB) has been demonstrated to be a critical site for mating-induced mate recognition in female mice. However, a role for the AOB of male mice in social recognition is largely unknown. To investigate the functional implications of the male AOB, we examined the effect of vasopressin on synaptic plasticity of glutamatergic transmission from mitral to granule cells in AOB slices from male mice. The strength of synaptic transmission from mitral to granule cells can be analyzed by lateral olfactory tract (LOT)-evoked field potentials in parasagittal slices. We measured the maximal initial slope of field EPSPs of granule cells to monitor the strength of glutamatergic transmission from mitral to granule cells. When sub-threshold LOT stimulation that only produced short-term potentiation was paired with vasopressin, robust LTP was induced. Next, we examined the effect of vasopressin on the reciprocal transmission between mitral and granule cells by using whole-cell patch-clamp techniques. Vasopressin suppressed reciprocal synaptic currents triggered by endogenous glutamate release from mitral cells in the AOB. This study suggests that vasopressin facilitates LTP induction at the mitral-to-granule cell synapse in the male AOB by reducing dendrodendritic inhibition. No COI.

3P-039

Functional MRI during odorant induced olfactory stimulation revealed brain activation with 7 Tesla MRI

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Piriform cortex forms the main part of the primary olfactory cortex, yet its precise functional role within the brain remains unclear. Previously, functional magnetic resonance imaging (fMRI) has been used to assess odor-induced piriform cortex activation in humans, however the results have been inconsistent. Two main factors which are attributed to this inconsistency are: 1) BOLD-signal changes evoked by olfactory stimuli are comparatively small, and 2) brain regions involved in olfaction tend to be susceptible to spatial distortion and signal loss within the image. In order to increase BOLD-signal strength, we hypothesized that the use of ultra high field (7 Tesla) MRI may be beneficial. In this study, we optimized an echo-planar imaging pulse sequence for fMRI using a 7T MRI (GE) system to minimize susceptibility artefacts, and to investigate odorant induced olfactory cortex BOLD signal activation. Odorant stimulation (isovaleric acid, peppermint and coffee odor) induced activations in the piriform cortex, amygdala, orbitofrontal cortex and hippocampus. We observed differences in the spatial extent of BOLD signal activation for each odorant, with some spatial overlap within the piriform cortex. These results suggest that the piriform cortex play an important role for odor discrimination, and that the odor quality may be mapped in the piriform cortex. No COI.

3P-040

Inhibitory action of 6-methylthiohexyl isothiocyanate on nasal inflammatory responses induced by toluene2, 4-diisocyanate in rats

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The influence of 6-methylthiohexyl isothiocyanate (6-MTITC) extracted from Wasabia japonica on allergic rhinitis induced by toluene2, 4-diisocyanate (TDI) were examined in F344 rats. F344 male rats (4-weeks of age) were sensitized by intranasal instillation of 10% TDI once a day for 5 days. These rats were exposed to various concentrations of 6-MTITC for 4 hours/day for 5 consecutive days, which was started 10 days after final TDI instillation. Nasal symptoms, sneezes and nasal rubs for 10 minutes, induced by 10% TDI nasal challenge were evaluated 24 hours after 6-MTITC exposure. 6-MTITC at 0.1% but not 0.05% and 0.01% could attenuate the appearance of nasal symptoms and decreased the number of both sneezes and nasal rubs. We then examined the concentrations of substance P (SP), calcitonin gene-related peptide (CGRP) and nerve growth factor (NGF) in nasal lavage fluids obtained from TDI-sensitized and 6-MTITC-inhaled F344 rats 60 minutes after TDI challenge. Exposure of TDI-sensitized rats to 0.1% 6-MTITC could suppress the increase in SP, CGRP and NGF levels in nasal lavage fluids, which were increase by TDI challenge. These results clearly showed that exposure to 6-MTITC could attenuate the development of allergic nasal symptoms through the suppression of the production of neuropeptides, such as SP, CGRP and NGF. It is also suggest that aromatherapy with 6-MTITC will be a good candidate for the treatment of allergic rhinitis. No COI.

3P-041

Involvement of actin polymerization in long-term potentiation at synapses in the mouse accessory olfactory bulb

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Microcircuits in the mouse accessory olfactory bulb (AOB) include the prominent reciprocal dendrodendritic synapse between mitral cell projection neurons and granule cell interneurons. Long-term potentiation (LTP) at the AOB synapses is expected to underlie pheromonal memories in female mice. The formation of LTP is thought to involve the reinforcement of synaptic connections that depends on protein synthesis, and the changes in the structure of synaptic connections that result from actin cytoskeleton reorganization. In the previous meetings, we reported that LTP at the AOB synapses requires synthesis of proteins, one of which would be atypical protein kinase C. Here we examined whether LTP at the AOB synapses also requires actin polymerization. Using AOB slices, we measured field EPSP derived from granule cells to examine the effects of some reagents that modify actin polymerization on the LTP. Bath application of an actin polymerization inhibitor, cytochalacin D, blocked the LTP induced by the tetanic stimulation (100 Hz × 4), but had no effects on the short-term potentiation. Under bath application of an actin polymerization activator, jasplakinolide, LTP is induced by the tetanic stimulation (100 Hz × 2) that elicits only short-term potentiation. The results are consistent with the hypothesis that actin polymerization is required for the formation of LTP at the AOB synapse. No COI.

3P-042

Hard-diet and soft-diet feeding change neurogenesis in the subventricular zone and olfactory functions

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The subventricular zone (SVZ) generates an immense number of neurons, which migrate to the olfactory bulb (OB), even during adulthood. Recent studies have shown that a reduction of mastication impairs both neurogenesis in the hippocampus and brain functions. Ingestion of hard diet induced higher number of expression of Fos-ir cells at the principal sensory trigeminal nucleus (Pr5) of female mice than those of soft diet or no diet did, suggesting that soft-diet feeding mimic impaired mastication. After 1 month, the density of Bromodeoxyuridine-immunoreactive cells, newly generated cells, in the SVZ was lower in the soft-diet-fed mice than in the hard-diet-fed mice. Avoidance behaviors to butyric acid were also reduced by the soft-diet feeding. We then explored the effects of the hard-diet feeding on olfactory functions and neurogenesis in the SVZ of mice impaired by soft-diet feeding. At 3 months of hard-diet feeding, avoidance behavior to butyric acid and responses to odors were recovered, as was neurogenesis in the SVZ. The present results suggest that feeding with a hard diet improves neurogenesis in the SVZ, which in turn enhances olfactory function at the OB. No COI.

3P-043

Orexin neurons are involved in antinociceptive effect of olfactory input

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Some odor molecules were reported to have antinociceptive effects. However the antinociceptive effect mediated by the olfactory system and the central nervous system has not yet examined. In this study, we demonstrated that odor exposure of an odor molecule (odorant X) induced antinociceptive effect and the effect was mediated by olfactory system. Furthermore, we demonstrated the involvement of hypothalamic orexin neurons, one of the key neurons involved in pain processing, in the antinociceptive effect of olfactory input. We performed hot-plate tests and formalin tests for assessment of nociceptive response and examined the effect of odorant X exposure. When the odorant X was exposed to wild type mice, the latency to nociceptive responses in hot-plate tests significantly increased and the nociceptive responses times in formalin tests significantly decreased. These data indicated that the exposure of odorant X induced antinociceptive effect. Additionally, we performed the formalin tests with olfactory bulbectomy mice. The antinociceptive effect by odor exposure was disappeared, indicating that the antinociceptive effect was mediated by olfactory input evoked by the odorant X vapor. Next, in formalin test with the mice whose orexin neurons were genetically ablated, the antinociceptive effect by odor exposure was disappeared. In conclusion, we found that hypothalamic orexin neurons were involved in the antinociceptive effect of olfactory input evoked by the odorant X vapor. No COI.

3P-044

The effective period of anti-inflammatory treatment in the traumatic olfactory dysfunction

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Previous studies have reported that recovery in the olfactory system depends on the severity of injury and that treatment with anti-inflammatory drug as steroid is effective in improving recovery during an acute phase of head injury. It is unknown when we should start steroid for improving olfactory dysfunction. We investigated the effective period of anti-inflammatory treatment using mice performed olfactory nerve transection (NTx). Subcutaneous injection of dexamethazone sodium phosphate (DXM) for consecutive 5 days was started 7, 14, 28 and 42 days after the NTx. Histological assessment of nerve recovery in the olfactory bulb was made at 5, 14 and 42 days after injury using X-gal staining and immunohistochemical staining of glial fibrillary acidic protein (GFAP) and CD68. Animals that DXM was injected 7 days after the NTx had less injury-associated tissue with fewer astrocytes and macrophages and better nerve recovery compared to control mice. However, those that had 14 days or longer intervals between the NTx and DXM injection did not show significant difference. An anti-inflammatory treatment using steroids for olfactory dysfunction by head trauma may be effective until 7 days but ineffective 14 days or longer after head injury. These findings suggest that different therapeutic strategy from inhibition of inflammation may be needed for traumatic olfactory dysfunction in a chronic phase. No COI.

3P-045

Aversive olfactory learning is prevented by intrabulbar infusion of tunicamycin

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The endoplasmic reticulum (ER) is an organelle in which secretory and transmembrane proteins are folded or processed, and is susceptible to various irritants, such as tunicamycin, that provoke the accumulation of unfolded protein response and induce ER stress in the lumen, which is linked to neuronal death in various neurodegenerative diseases, including Parkinson's disease, amyotrophic lateral sclerosis, Alzheimer's disease, and many others.Young rats can learn their mother's odor and approach her without vision. They do this in part by learning their mother's odor as a conditioned stimulus that is paired with an unconditioned somatosensory stimulus given by maternal care. To establish aversive olfactory learning, an artificial odor can be paired with foot-shock during training.Tunicamycin (0.2, 0.25, 0.5 and 1.0 mM) infused into the bilateral olfactory bulbs during odor-shock training on postnatal day 11 dose-dependently impaired aversive olfactory learning tested on the next day without affecting memory retention one hour after the training. These results suggest that tunicamycin-induced ER stress causes the selective impairment of the long-term memory process of aversive olfactory learning in young rats. No COI.

3P-046

Evaluation of olfactory function in the elderly using a card-kit of odor identification test for Japanese

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We have already examined olfactory function of more than 500 healthy volunteers with Open Essence. It is a card-kit of odor identification test for Japanese which consists of 12 odorants with 6 alternatives. The number of correct answers is defined as the score on this test. Elderly people are known to decrease olfactory function gradually. Our results also revealed that women over 70 years old and men over 65 years old show significantly lower scores on Open Essence compared with young people. Then we analyzed the data in detail from this study in order to reveal the properties of olfactory function of elderly people. All participants were diagnosed as "non-dementia." We obtained the results as follows: [1] Elderly women show gradual olfactory impairment as aging. Especially over 85 years old Open Essence scores decline significantly compared with younger people. [2] Over 70 years old men show low scores on Open Essence. Even if they get old scores no longer change significantly. [3] Although in elderly women the number of "Detectable but not recognizable" answers is increasing as aging, the number of "No smell detected" answers does not change. It suggests that odor detection at the peripheral olfactory organ is maintained in senior women. [4] In women self-evaluation of olfactory function show good correlation with scores on Open Essence. It seems that women are capable of estimating their olfactory function correctly. No COI.

3P-047

The odor of Osmanthus fragrans modulates feeding behavior

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Odors have been shown to exert an influence on various physiological and behavioral activities. Here we show that the neural transmission by Osmanthus fragrans (OSM) decreased the mRNA expression of orexigenic neuropeptides, such as agouti-related protein, neuropeptide Y, melanin-concentrating hormone and prepro-orexin, while increased anorexigenic neuropeptides, such as cocaine- and amphetamine-regulated transcript and proopiomelanocortin in rats. The latency to eating and the feeding time for a fixed amount of pellets were extended in the OSM rats. EMG recordings from both the digastric and masseter muscles showed two distinct patterns of bursts corresponding to the gnawing and chewing phases. During the OSM odor exposure, the magnitude of the bursts became smaller in gnawing phase in both masseter and digastric muscles, the burst duration became longer in both phases in masseter muscle. Consequently, the burst frequency in both phases in masseter muscle was decreased, consistent with sluggish masticatory movements. This study suggests that the OSM odor decreases food intake accompanied by changing eating pattern, which contrasts markedly with the facilitatory feeding pattern in rat intracerebroventricularly-injected with orexins. No COI.

3P-048

Influence of aging on visual field for color vision

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Background Decay of sight with aging has been studied; decline in light sensitivity with age (Spry et al., 2001), decrease in retinal nerve fiber layer thickness (Da Pozzo et al., 2006) and the number of optic nerve fibers (Frisen et al., 1991). But knowledge about the effects of aging on visual field (VF) is sparse. It is well known that VF varies among color, but there is no study on effects of aging on VF for color vision as far as the authors know. The authors investigated influence of aging on VF for color vision in elderly. Methods Thirteen healthy elderly (≥70 yrs) and nine healthy adults (<70 yrs) participated as unpaid volunteer subjects. VFs were measured by a perimeter with ϕ8 mm targets (100–300 lux). VFs for detection (VFdet) and discrimination (VFdis) of five colors (white, red, green, blue, yellow) were compared. Results No significant difference was observed in VFdis for each color between the elderly and the control groups. There were significant differences in VFdis among five colors in subjects under 70 yrs ($p < 0.01$), VFs being blue > white > yellow > red > green. But there were smaller differences in VFdis observed among colors in the elderly over 70 yrs. Significantly narrower VFdet was observed in the elderly than in the control.Conclusions The present study showed VFdet might be narrower in the elderly than the control, indicating decay in peripheral vision by aging. VFdis in the elderly did not differ from those in the control group, which might indicate no decay in color vision process in the elderly. No COI.

3P-049

The integration of gustatory and olfactory information in the agranular insular cortex

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Gustatory information processing could be modulated by olfaction. Not only gustatory but also olfactory are processed in the insular cortex (IC). However, little information is available how these sensory inputs are converged in the IC. The present study, aimed to examine the spatiotemporal dynamics of excitatory propagation induced by simultaneous stimulation (5 train of pulses at 50 Hz) of (1) the parvicellular part of ventroposteromedial thalamic nucleus (VPMpc) and olfactory bulb (OB), or (2) the lateral olfactory tract (LOT) and chorda tympani nerve (CT). We performed in vivo optical imaging with a voltage sensitive dye.

The VPMpc stimulation evoked excitatory propagation in the dysgranular IC (DI). On the other hand, the ventral OB stimulation evoked excitatory propagation that spread in the piriform cortex (PC) and the agranular region (AI) , which locate adjacent to the DI.The excitatory regions by stimulating the OB/VPMpc were segregated except for the AI. The excitatory signals in the AI showed an additive effect of OB/VPMpc stimulation .Our data raise the possibility that a part of integration of gustatory and odor sense were carried out in AI. The similer additive excitation in the AI was observed by the simultaneous stimulation of the LOT/CT.

These results suggest that the AI may play a role in integrating the gustatory and olfactory information. No COI.

3P-050

Gustatory innervation in taste bud is stable for the rapid reconnection to the ever-renewing taste receptor cells

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Taste perception provides flavors in food intake and eventually affects our moods. Its impairment brings about the profound deterioration on quality of life. Taste receptor cells (TRCs) in lingual taste buds respond to various chemicals and comprise three major subtypes; type I- (mildly salt-sensitive), type II- (umami-, bitter- or sweet-sensitive) and type III- (sour-sensitive) cells. Remarkably, TRCs continuously renew and thus the consistency in taste perception should require the stable maintenance of neural connections once established. The loss of input usually atrophies the network structures, but what happens if ever renewing TRCs are ablated for a long period? Here we eliminated the pre-synaptic type III cells in the mouse taste buds by conditional chemo-genetic cell ablation technique called TRECK (diphtheria Toxin Receptor-mediated Cell Knockout). TRECK resulted in the sole deterioration of sour sensitivity in behavioral test and nerve recording as expected. When the TRECK was suspended, the recovery of the sour sensitivity occurred rather promptly within 2 days on average. Histological investigation revealed that the gustatory nerve terminals persisted normally inside the taste bud even during the TRECK ablation. Thus, these results suggest that the gustatory innervation in the taste buds should be maintained stably and independently from the pre-synaptic type III cells. No COI.

Poster Presentations
CNS Function

3P-051

Zinc-induced currents in the rod cells of frog taste disc

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TRPM5 is activated by the rise of intracellular calcium concentration and plays an important role in taste transduction mechanism in mammals. Frogs have suffered a loss of the gene. However, the rod cells in frog taste disc display the appearance of an inward current in response to the rise of intracellular calcium concentration. In the present study, we investigated the effect of intracellular zinc on the membrane properties of the rod cells. When 100 μM zinc was dialyzed with cesium in whole-cell configuration, the rod cells displayed the increase of inward current (-19.4 ± 5.6 pA/pF at -50 mV, n=6) and the membrane potential depolarized from -36 ± 5 mV to -2 ± 2 mV (n=6). The EC_{50} of zinc for eliciting the inward current was 24 μM, while the EC_{50} of calcium was 2.5 μM. Gadolinium (30 μM) inhibited the inward currents almost completely. Intracellular alkalization (pH 8.27) with TAPS also induced large inward currents of -53 ± 16 pA/pF at -50 mV (n=6) in the rod cells. It has been reported that TRPA1 channels could be activated by intracellular zinc, calcium and alkalization. The results suggest that TRPA1 channels express in the frog rod cells. No COI.

3P-052

Human auditory steady state responses to fine structure periodicity

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Auditory steady state responses (ASSR) are oscillatory brain responses that are phase-locked to the temporal envelope of periodic sounds such as click trains or amplitude modulated (AM) white noises/tones. The envelopes of these sounds are created by their well-defined on and off gating patterns and most prominent oscillatory responses are obtained for 40 Hz periodicity. We previously observed that in case of 40 Hz AM white noises, when we used the same white noise segment for the on-periods of the signal, the evoked 40 Hz ASSR amplitudes were significantly increased. Based on this finding we investigated whether it would be possible to obtain ASSR in response to a continuous periodic sound that has a flat temporal envelope. We have created special short noise segments that have the lowest possible amplitude variations in time domain and concatenated these segments to prepare periodic sounds with 40- and 80 Hz repetition rates. Using these novel sounds we recorded auditory evoked cortical responses in 15 healthy adults using magnetoencephalography and performed the following analyses: 1) dipole analysis of the magnetic field data to define the auditory cortex as region of interest, 2) spatial filtering of the sensor data to obtain single-trial source waveforms, and 3) time-frequency analysis of single-trials. Our data showed that compared to non-periodic control noise sounds, 40- and 80 Hz periodic noises evoked significant oscillatory activity in the auditory cortex. Our findings suggest that in addition to the periodic envelope, repetitive fine structure also plays a role in ASSR generation. No COI.

3P-053

Cortical top-down input evokes dendritic spike in somatosensory L5 neurons

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Our previous study using current source density analysis with a linear probe in primary somatosensory cortex (S1) during direct cortical microstimulation (DCMS) of secondary motor cortex (M2) suggested that M2 top-down input arrives in cortical layers (L) 6 and 2/3, rather than in L5. However, this was inconsistent with multi-unit data that showed the highest firing activity in L5. One explanation for this inconsistency could be that M2 synaptic input to L2/3 caused local Ca^{2+} spikes in L5 apical dendrites that led to their increased firing. To substantiate whether M2 top-down input evokes dendritic Ca^{2+} activity in L5 neurons, we performed 2-photon imaging and measured dendritic Ca^{2+} activity in the presence and absence of CNQX during DCMS of M2 in anesthetized mice. Cortical application of CNQX significantly reduced the averaged response to top-down input in the whole field of view. In individual dendrites, CNQX had much larger effects on dendrites with a larger initial dendritic response. Dendritic Ca^{2+} activity caused by backpropagating action potentials (BPAPs), but not by dendritic spiking, should not be affected by glutamatergic blockers. Moreover, dendritic spiking is known to induce larger fluorescence changes in distal dendrites compared to BPAPs. These data are therefore consistent with the small dendritic Ca^{2+} responses we observed in S1 arising from BPAPs, and the large responses from local, dendritic Ca^{2+} spikes evoked specifically by the observed top-down programmed coincident inputs from M2. No COI.

3P-054

Cortical Top-Down Input Regulates Conscious Perception

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Our previous study demonstrated that a secondary motor cortex (M2) projection regulates dendritic spiking and somatic firing in somatosensory cortex (S1) layer 5 pyramidal neurons. To test whether this projection influences conscious perception, we used an optogenetic approach to specifically inhibit the top-down M2 to S1 projection during a somatosensory-associated task in behaving mice. We injected AAV-GFP or ArchT into M2, and illuminated M2 projection fibers in S1. In the spontaneous place preference test, which uses tactile cues, mice expressing GFP alone or ArchT without LED-illumination also exhibited a strong texture preference. However, upon inactivation of the M2 to S1 projection via LED-illumination, ArchT mice lost the texture preference. We also applied optogenetic inactivation to mice highly trained in a tactile discrimination task. Optogenetic inactivation of the M2-S1 projection significantly degraded accurate discrimination in this task. Moreover, we conducted several general behavioral tasks, including an open field test, gait analysis, etc., but no significant differences were observed between illumination conditions. Together, these results suggest that M2 top-down inputs modulate S1 activity, and specifically alter sensory perception in behaving animals. No COI.

3P-055

Identification of indirect top-down pathways in the sensory-motor circuit through the thalamus

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Top-down input from a higher cortical area toward a sensory area is essential for sensory perception and behavioral execution. Recent physiological studies have shown that mouse sensory-motor circuits consist of long-range recurrent horizontal projections between primary somatosensory cortex (S1) and secondary motor cortex (M2), the so-called direct pathway. However, other studies have shown that each cortical region forms thalamic connections, called indirect pathways. Little is known about the physiological functions of these indirect pathways during sensory perception. Here, we focused on the S1-M2 circuit through the thalamus and investigated its functions during M2 top-down input to S1. In order to anatomically find the indirect pathway, we labeled M2 fibers by injecting adeno-associated virus-green fluorescent protein into M2 as an anterograde tracer and labeled thalamic somata by injecting cholera toxin subunit-B into S1 as a retrograde tracer. We found that labeled M2 axons and neurons projecting to S1 coexisted in the ventral lateral nucleus (VL), which is part of the motor thalamus. This anatomical result suggested that VL relays M2 top-down signals to S1. To test this hypothesis, we extracellularly recorded neural firing activity from VL, S1, and M2 during hind paw stimulation that caused M2 feedback input to S1. We will present these anatomical and physiological results and discuss the functions of the indirect pathway in sensory perception. No COI.

3P-056

Non-noxious skin stimulation activates the nucleus basalis of Meynert and promotes NGF secretion in the parietal cortex via nicotinic ACh receptors in anesthetized rats.

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We have previously demonstrated that stimulation of the basal forebrain nucleus (the nucleus basalis of Meynert; NBM) increases extracellular nerve growth factor (NGF) secretion in the parietal cortex by the activation of nicotinic ACh receptors (nAChRs) [Neurosci Res 63: 122, 2009]. Here we examined whether non-noxious skin stimulation activates NBM, resulting in the promotion of NGF secretion in the cortex. We used anesthetized and artificially ventilated rats. Innocuous skin stimulation was delivered to the left hindlimb with a soft-hair brush. Firstly, extracellular NGF in the right parietal cortex was collected and measured by microdialysis methods and an ELISA. Brushing stimulation, repeated at a frequency of 1 Hz for 100 min, produced an increase in extracellular NGF level in the parietal cortex. The response of NGF was not observed in rats pretreated with a nicotinic cholinergic blocking agent mecamylamine (20 mg/kg, i.v.). Secondly, by means of functional MRI, we examined the blood oxygen level dependent (BOLD) signal evoked by brushing (at 3 Hz for 1 min to the left hindlimb) in the NBM. BOLD signal in the right NBM was higher during brushing compared with baseline. These results indicate that non-noxious mechanical skin stimulation can promote NGF secretion in the parietal cortex by activating nAChRs, possibly through excitation of cholinergic nerves from the NBM. No COI.

3P-057

Single neuronal activity in the rhesus monkey orbitofrontal cortex related to reward value processing during the decision-making schedule task

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When we make a choice from the alternatives, we consider their values and workloads. To understand the neuronal mechanism of such a decision-making process, we developed a decision-making schedule task and recorded single unit activity from monkey orbitofrontal cortex (OFC) which has been reported to be one of the important brain areas for the reward-guided behavior.The monkey was trained to perform a reward schedule task which consists of 1, 2 or 4 trials of visual discrimination to earn 1, 2 or 4 drops of liquid reward. After learning this task, the decision-making schedule task in which two kinds of choice target (CT) were sequentially presented was introduced. The CT brightness and length indicated reward amount and required number of trials, respectively. Then, these two CTs were simultaneously reappeared (choice phase). The monkey was required to choose one of them, and then the chosen reward schedule started. We recorded from 137 neurons in the OFC and analyzed the neuronal activity during the second CT period. Some neuronal activities were correlated with differential value of the two CTs (7.3%). Other 7.3% of neurons showed larger/smaller responses when the two CT values were close. These results suggest that OFC neurons play an important role in the decision-making by reward value information processing. No COI.

3P-058

Neuronal activity in the monkey frontal cortex during a novel shape-manipulation task

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Humans manipulate tools or machines by using a sequence of steps or actions to achieve certain goals. The monkey lateral prefrontal cortex (lPFC) and premotor area (PM) are thought to play important roles in planning the steps to attain a behavioral goal. To investigate the involvement of these areas in planning goal-directed actions, we introduced a novel shape-manipulation task that required step-by-step movements with a manipulanda. The goal of the task was to fit the test shape to the sample shape by manipulating angle and size by rotating, expanding, and contracting the test shape. After training monkeys to perform this task, we recorded neuronal activity in the lPFC and PM using linear-array multi-contact electrodes while the animals were performing the task, and examined how the frontal neuron activity reflected behavioral planning under different task conditions.This research is supported by the MEXT of Japan (#24120703), FRIS of Tohoku Univ. and CREST. No COI.

3P-059

Neural response predicting social stimuli in monkey striatum

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Striatal neurons which receive strong projection of midbrain dopamine neurons have been reported to show predictive response of rewards. The striatum, especially in the ventral part, also receives neural projection from the brain areas that process social information such as the amygdala. Thus, it is possible that social information also affects the activity of the striatal neurons. In this study, to elucidate the involvement of the striatum in social information processing, we recorded the activity of the monkey ventral striatal neurons while an arbitrary geometric pattern was associated with one of the social images belonging to several categories such as the opposite sex or a face showing negative emotion. The precedent geometric patterns and the following social images were separated by a brief delay period. 49 of 77 neurons recorded from the ventral striatum of a macaque monkey showed significant responses in relation to at least one of the task events of geometric pattern presentation, delay period and social image presentation. To estimate how well individual neurons discriminate social stimulus categories, we computed the temporal change of ROC value in 49 responsive neurons by comparing neural activity between socially preferable and unpreferable stimuli. Results showed that ROC value increased while the geometric patterns that predicted the socially preferable stimuli were presented. These results indicate that the monkey ventral striatal neurons are involved in prediction of preferable social information. No COI.

3P-060

Real-time change in the firing rate of hippocampal CA1 neurons before, during, and after the exposure to a specific episode

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Peoples remember the episodes of their first love or sexual relationship. To monitor the process of episodic memory, we recorded neuronal activity of hippocampal CA1 neurons in freely moving young male rats before, during, and after the first encounter with young female rats. Spontaneous behavior and sounds were simultaneously recorded using a digital-video recording system. Prior to the experiment, we implanted multi-unit recording electrodes into the pyramidal cell layer of CA1 in young males, unisexually housed after weaning. On the day of experiment, we started to record the spontaneous firing of CA1 neurons in habituated home cage, but the firing rate was low. Ten min after the recording, young female rat was put into the home cage for 10 min. Male rats showed an interest in female rats, and frequency of the firing was increased simultaneously. A few minutes after the female rat was taken out, spontaneous high frequency firing (~100 Hz) was suddenly observed for seconds. Minutes after the high-frequency firing, the rats began to show ripple-like firing (~50 msec) frequently. This observation shows a real-time change in the firing rate of hippocampal CA1 neurons before, during, and after a specific episode. No COI.

3P-061

The endogenous opioids related with antinociceptive effects induced by electrical stimulation of the amygdala

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Previous studies demonstrated that brief electrical stimulation of the amygdala depressed neural discharge in the cingulate areas when noxious stimulation was applied to peripheral tissues. To clarify whether endogenous opioids are involved in antinociception induced by electrical stimulation of the amygdala, we investigated immunohistologically the number of c-Fos expression and the distribution and the quantitative changes of endogenous opioids (β-endorphin, enkephalin, dynorphin A) secreted in the brain when electrical stimulation was applied to the amygdala with or without noxious stimulation to the peripheral tissue. Male wistar rats were used for this study. As the noxious stimulation, formalin solution (0.1ml) was injected into the right hind paw. Electrical stimulation (2μA, 100Hz, 15sec) was applied to the central nucleus of the right amygdala. C-Fos expression was increased in the ipsilateral anterior cingulate cortex (ACC), which suggested that electrical stimulation into the amygdala activated ACC neurons. However, only a few endogenous opioids were observed in the ACC. On the other hand, the amount of dynorphin A in the periaqueductal gray (PAG) was increased by electrical stimulation of the amygdala. The results suggest that the increase of dynorphin A in the PAG induced by electrical stimulation of the amygdala activates the descending antinociceptive system, and depresses the nociceptive response in the ACC indirectly. No COI.

3P-062

Higher expression of cocaine and amphetamine regulated transcript (CART) peptide in the amygdala rather than the medial prefrontal cortex in ADHD model rat

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Spontaneously hypertensive rat (SHR) are the most used animal model of attention deficit hyperactivity disorder. Previously we found that environmental enrichment (EE) for 5 weeks from postnatal day 25 (P25) to P60 decreased hyperactivity and anxiety-like behavior in SHR and that mRNA of cocaine and amphetamine regulated transcript (CART), known to influence locomotor activity, anxiety and stress, increased in medial prefrontal cortex (mPFC) and amygdala (Amy). To confirm the protein level and localization of CART in mPFC and Amy, Western blot and immunohistochemistry were carried out. Western blot was performed in 16% tricine-gel using preproCART polyclonal antibody. The antibody (1:800, Proteintech) recognized three bands (14, 7.7, 5.3 kDa) in the arcuate nucleus as the positive control. Antibody did not detect any bands in mPFC but several bands in Amy, indicating that the amount of CART peptides in mPFC is under the detection level. CART localization was investigated using the antibody (1: 10,000, Phoenix Pharmaceuticals Inc). A few CART positive cells was detected in the mPFC only the rats grown in EE. Strong immunoreactivity for CART was observed in the Amy, especially central nucleus of Amy (CeA). Data suggest that the amount of CART peptide is larger in Amy compared to mPFC, probably relating to anxiety-like behavior in SHR. No COI.

3P-063

The functional role of the prefrontal cortex and basolateral nucleus of amygdala in value comparison between reward and punishment

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Comparison between reward value and punishment value are essential to decide whether to execute the action or not, if outcomes of the action are both reward and punishment. As a mechanism of comparison, we hypothesized that prefrontal cortex (PFC) and basolateral nucleus of amygdala (BLA) play a role in value evaluation since both areas regulate reward seeking and punishment avoidance behaviors. In the present study, we investigated whether the ventral-medial PFC (v-mPFC), v-lateral PFC (v-lPFC) and BLA could have functional role in the value-comparison task, in which the food-restricted rats received one pellet and electric foot shock simultaneously after lever-pressing during tone presentation. In this task, the current intensity of foot shock became stronger each time the rats pressed the lever 10 times, and the rats allowed to chose whether to press the lever or not. We evaluated maximum current intensity (MCI) in which lever press probability was more than 25 %. Inactivation of the v-mPFC or BLA by local treatment with GABAA and GABAB agonists elevated MCI, whereas v-lPFC inactivation was lowered MCI. On the other hand, inactivation of these three regions did not affect the probability of lever press for only reward. These results suggest that the v-mPFC and BLA may contribute to overestimate punishment, while v-lPFC may contribute to underestimate punishment in value-comparison task. In conclusion, the v-mPFC, v-lPFC and BLA provide a functional role in value evaluation under the conflicting situation. No COI.

3P-064

Synaptic potentiation in the nociceptive amygdala in orofacial inflammatory pain model of rats

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A large majority of the spinal cord nociceptive projection neurons send axons to the lateral parabrachial nucleus (LPB) and then to the capsular part of the central amygdala (CeC), a subnuclus dubbed as "nociceptive amygdala". This non-thalamocortical pathway is central in the nociception-emotion link and undergoes robust plasticity in various pain models (Ikeda et al., 2007; Nakao et al., 2012). Interestingly, in sub-acute inflammatory pain models such as arthritis (Ji et al. 2009), the potentiation always occurs in the right CeC regardless of the side of inflammation in contrast to the chronic nerve injury models, in which the potentiation occurs in the CeC contralateral to the injury. Anatomically, the LPB receives inputs also from the spinal trigeminal nucleus, which sends bilateral projections to the CeC with ipsilateral predominance, unlike the dorsal horn neurons (Li et al., 2006). To identify the LPB-CeC plasticity in the trigeminal pain model, we injected 5% formalin into the left upper lip of adult Wistar rats, which showed robust nocifensive behaviors for 1 hour after injection, and recorded LPB-CeC transmission 6 hours later and found that the LPB-CeC transmission was potentiated only in the right CeC. This study is the first to describe the synaptic potentiation in the trigemino-parabrachio-amygdaloid pathway and suggests that the laterality of LPB-CeC synaptic transmission is determined not by the side of nociception but rather by the type of pain models. Supprted by Kakenhi. No COI.

3P-065

Synaptic plasticity and electrophysiological properties of layers II/III neurons in primary motor cortex at the early stages of motor learning

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Rotor rod test is widely used for the assessment of motor coordination and learning. Rodents can reach a plateau score after 2 consecutive days of the training. After the training, we found an increase of both AMPA and NMDA current in the motor cortex, followed by plastic changes involving structural reorganization in spine architecture (Yang et al., 2009). However, the effects of motor training on electrophysiological properties of layers II/III neurons are still known. On the 1st day of training, motor skill was significantly improved within 10 trials in all animals (n = 8). Then, we prepared acute brain slices of primary motor cortex to analyze the electrophysiological properties and the synaptic plasticity using whole cell patch clamp methods. In voltage clamp analysis, the trained rats showed significantly higher AMPA/NMDA ratio than untrained control rats, but the increase was not observed in rats 2 days after the training (Fisher's test, $P < 0.05$). Further, motor training did not affect paired-pulse responses, suggesting that AMPA receptor-mediated postsynaptic plasticity rather than presynaptic glutamate release is involved in the motor learning. These results suggest a possible neural mechanism at early stage of motor learning. No COI.

3P-066

The enhancement of the reward prediction error signal in the midbrain dopamine neuron by the cost paid for the reward

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"One of the greatest joys in life is an ice cold beer after a hard day of work." As this phrase indicates, the value of a reward can be modulated by the effort or the cost put into its achievement. However, relatively few studies have shown the neural basis of the effect caused by the paid cost on the value of the reward. When calculating the values of rewards, the activity of dopamine neurons, which represent the reward prediction error, is used to update the value. Here, we examined whether the activity of the dopamine neurons in response to reward predictive cues was increased by the cost preceding to these cues.

Two macaque monkeys performed a saccade task. After fixation on a fixation point, the subjects were required to make a saccade to a condition cue and then a target appeared. In the high or low cost condition, long or short fixation to the target was required, respectively. After fixation on the target, the subjects made a saccade to the reward cue. While the subjects performed the saccade task, the activities of dopamine neurons were recorded from the SNc in the midbrain. The neuronal response to the low-cost cue was larger than that to the high-cost cue. In contrast to the responses to the condition cues, the responses to the reward cue after the high-cost condition were larger than those to the reward cue after the low-cost condition. We suggest that information about the cost is transmitted to the dopamine neurons and the paid cost increases the reward prediction error signal in the dopamine neurons. No COI.

3P-067

Memory-guided navigation behavior of mice in a virtual linear track

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Although virtual reality (VR) has increasingly been used to investigate navigation-related neural activity in head-fixed mice, it is still unclear whether the mice use their spatial memory when they navigate within a VR environment. To address this issue, we have established a new VR spatial memory task. In this task, mice begin to run from one end of a virtual linear track to seek for rewards dispensed at a target zone in the middle of the track. The training protocol consists of the following two phases. (1) Non-delayed task: mice are rewarded immediately whenever they enter and pass through the target zone. (2) Delayed task: mice have to navigate to the same target visually and stay there for 1-2 sec to receive rewards. In the non-delayed phase of the training, mice demonstrated steady increase in distance traveled, running speed and the number of rewards. In the delayed phase, however, the number of rewards dropped but gradually recovered as they learned to stop and stay in the target in most trials. We trained transgenic mice that express the G-CaMP7 fluorescent calcium sensor proteins in hippocampal CA1 pyramidal neurons similarly and imaged the neuronal activities during the behavior by *in vivo* two-photon microscopy. Our preliminary analysis suggests that a population of neurons with virtual place-specific activity can be optically identified. This VR task thus provides an experimental paradigm to study plasticity of hippocampal memory circuits with imaging and electrophysiology. No COI.

3P-068

Short-term changes in behavioral efficiency and brain activity associated with response inhibtion

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Behavioral performance is improved through learning, and this improvement may entail developmental neural changes even within one experimental session. However, previous studies of response inhibition have overlooked such short-term changes in performance, which may have helped in investigating neural mechanisms of response inhibition. In the present fMRI study, we measured brain activity changes within a short range (~ 1 hour) of fMRI runs, and tracked signal increase/decrease in broad sets of brain regions. Two sets of brain regions were identified that showed increase or decrease of brain activity following performance improvement: the inferior frontal cortex (IFC), the pre-supplementary motor area (preSMA), the caudate nucleus and the cerebellum showed increase of brain activity, whereas the medial prefrontal cortex and the anterior cingulate cortex showed the brain activity decrease. Given the pivotal roles of the IFC and the preSMA in response inhibition, these results suggest that the caudate and the cerebellum also play an important role in response inhibition in coordination with these cerebral regions. No COI.

3P-069

Therapy of stress-induced depressive-like state of mouse by tail vein injection of adipose stem cell-conditioned medium

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Adipose stem cells (ASC) have the ability to differentiate into multiple cell lineages and used for clinical application against various diseases. Recently, the therapeutic effects of stem cells are considered not by the transplanted cells, but by secreted growth factors. In the present study, we examined the therapeutic effects of ASC-conditioned medium (ASC-CM) on stress-induced depression-like state of mouse. The depressive-like state of male C57BK/6J mouse was produced by application of repeated restraint stress (3 hours for 3 days). When treated mice were examined by tail suspension test or forced swim test, they became immobile state more rapidly than non-treated mice. The depression-like state in mice continued more than two months. We prepared ASC from fat tissue of female C57BK/6J mouse, and cultured in DMEM with 10% FBS. ASC-CM was obtained by culturing adipose stem cells in PBS containing 1mM $CaCl_2$ for 24 hours. The medium was collected and filtered. We injected ASC-CM (0.4 ml) into tail vein of stressed mice, and measured their response on tail suspension test or forced swimming test. The immobility period was reduced gradually and recovered to the level of control mice by 3 weeks. We found that the conditioned medium of muscle stem cells (satellite cells) also had therapeutic effect. However, no change was observed when buffer was injected into stressed mice. This result shows that stem-cell conditioned medium is effective for therapy of brain disease even by vein injection. No COI.

3P-070

Identification and characterization of a top-down circuit in the mouse somatosensory system

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Despite the fundamental role of top-down control in behavior, the structure and operation of these neural circuits are poorly understood. Here, we report the identification and characterization of a top-down circuit in the mouse somatosensory system. Using wide-field cortical voltage-sensitive dye (cVSD) imaging, we detected that primary somatosensory (S1) and secondary motor (M2) cortex were activated in succession during hindpaw stimulation. In the awake state, hindpaw stimulation evoked a significantly larger cVSD response in M2, but not in S1 and other areas, relative to the anesthetized state. Application of TTX to S1 significantly decreased cVSD activity in M2, while application to M2 significantly decreased a late component of cVSD activity in S1 during hindpaw stimulation. We next examined the detailed anatomical connectivity between S1 and M2. To anterogradely label axons, a viral tracer (AAV-CAG-GFP) was injected into each area, while the retrograde tracer cholera toxin B subunit conjugated to Alexa 555 was used to label the somata of projection neurons. The axonal innervation pattern of fibers from M2 to S1 showed targeting to layer1 (L1) and deep cortical layers while avoiding the middle layers, suggestive of top-down connectivity. Reverse tracing experiments also revealed a predominance of projecting neurons from L2/3, L5a and L6 of S1 to M2, in a bottom-up connectivity pattern. These physiological and anatomical results indicate a reververating circuit between S1 and M2. No COI.

3P-071

Cortical top-down programmed input to primary sensory area in mice

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We have found that primary somatosensory cortex (S1) and secondary motor cortex (M2) form a reverberating circuit via long-range reciprocal connections, indicating that M2 can execute top-down control of sensory perception. However, the structure and information flow of this top-down input to the S1 column is poorly understood. Here, we report the identification and characterization of a top-down control circuit in the mouse forebrain somatosensory system. During hindpaw stimulation, S1 and M2 were activated in succession. M2 then provided feedback input to S1, initiating dendritic spiking and increased firing of layer 5 neurons. The dendritic spike and the layer 5-selective output were also observed by direct cortical microstimulation of M2, and blocked by TTX application to M2. By performing current source density analysis, we found that M2 synaptic inputs selectively contacted S1 bottom and top layers, consistent with anatomical tracing studies. This finding indicates that M2 top-down inputs induce firing in the S1 column in a programmed temporal sequence of layer-specific activity. We have described this coordinated input pattern to the lower and upper layers of S1 as "programmed coincident input" (PCI), because it relies on the ordered recruitment of activity from lower to higher layers, coordinated with direct input to the distal dendrites timed to most effectively produce dendritic spikes. No COI.

3P-072

Gene expression of the transcription factors in the monkey cerebral cortex during postnatal development

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Transcription factors recognize specific DNA sequences and regulate gene expressions. In the central nervous system, they are involved in the control of multiple biological phenomena including cellular differentiation, regional differentiation, and maintenance of regional speciation. Therefore, we analyzed gene expression patterns of transcription factors in the cerebral cortex of macaque monkeys, which has well differentiated cortical areas, at 3 stages in postnatal development (postnatal day 70, 1 year and at adult). Developmental change in the gene expression was compared between sensorimotor areas (M1 and S1) and the association areas (PFC, TE, and PE) by using DNA microarray system (Agilent, 4×44K). Among transcription factor genes whose expression was developmentally regulated, expression patterns were categorized into 10 patterns from logically possible 14 patterns. Expression level of some transcription factor genes was in accordance with the extent of myelination either positively or negatively. Expression level of some other transcription factor genes was developmentally up-regulated and always higher in the association areas. These area-specific and development-specific expression of specific transcription factor may be related to area-specific structure and function. No COI.

3P-073

Human Brain Areas Activated by Attention-related Stimuli in Relation to Genetic Variations

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Brain activities are "intermediate" phenotypes between genes and behavior. To assess the intermediate phenotype in humans, brain imaging by fMRI is most suited. We employed ANT (attention-networks test) that has been applied to fMRI brain imaging (Fan J. et al., 2005). The task in ANT is to request participants to report the direction of an arrow projected on a screen. Three attention-related processes can be resolved, i.e., 1)alerting by presenting a cue, 2)orienting by spatial cues, and 3)conflict resolving by simultaneously presenting flanker arrows heading towards the other end. Unlike the predictions made so far, most of the activated areas were detected in duplicate in two or all of the three processes ($p<0.05$, FWE corrected). These activated areas were left higher-order visual and inferior parietal cortices (occipitoparietal projection), left higher-order visual and adjacent temporal association cortices (occipitotemporal projection) and right SMA. Exception was that right occipitotemporal projection area and right anterior cingulate cortex were activated only in the conflict resolving. We are investigating variations (SNPs) in candidate genes to find the genetic polymorphism in the activation pattern detected. In this way we may be able to investigate the relationship between genes and behavior, and the attention-related neural substrate and function in human brain. No COI.

3P-074

Neural correlates of personality traits: A resting-state fMRI study

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Neural correlates of personality traits still remain controversial although a number of brain regions have been suggested. A further understanding of its neural basis would also provide better insight into personality disorders. Therefore, the aim of this study was to examine the correlation of personality estimates (obtained from Cloninger's Temperament and Character Inventory, TCI) with global functional connectivity as measured by fMRI ("regional global connectivity," rGC). We used a 3-Tesla MRI (Philips) to obtain structural and resting-state functional images from healthy male subjects (N=89, 18–24 years old). After preprocessing of BOLD signals through SPM8 and MATLAB, cross-correlation coefficients of each voxel (6×6×6 mm) with all other voxels were calculated and averaged to determine rGC in each voxel. Significant positive correlations between most of TCI scores (Novelty Seeking, Reward Dependence, Persistence, Self-Directedness and Self-Transcendence) and rGC were revealed ($p<0.05$, corrected for multi-comparison with Monte Carlo simulation) at distinct gray matter regions (including middle/inferior frontal gyri, orbitofrontal cortex and cerebellar hemisphere), while significant negative correlations were revealed for Cooperativeness score at precuneus. The results suggest that these regions, each with distinct function, are included in the brain network related to personality formation. No COI.

3P-075

Neonatal dopamine depletion results in a decrease of anxiety-related behaviors in the adulthood

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Dopamine neurons originating in the midbrain is implicated in several important functions, including motor control, attention and emotion. The effects of dopamine depletion on the neural functions probably depend on the lesioned developmental period. As an example, dopamine depletion in the adulthood causes an akinetic motor activity, and that in the neonate causes a motor hyperactivity. Although some reports have shown an increase of anxiety-related response caused by dopamine depletion in the adulthood, effects of neonatal dopamine depletion on anxiety-related response in the adulthood are still unclear. To clarify characteristics of behavioral response to anxiogenic stimulation in the adulthood of rat with neonatal dopamine depletion, we performed the behavioral analyses with an open field (OF) test, a light/dark box (L/DB) test and an elevated plus maze (EPM) test using the rats that received intra-ventricular injection of 6-hydroxydopamine 5 days after birth. In the adulthood, the rats with neonatal dopamine depletion showed a significant increase in distance traveled and time spent in center area in the OF test, and a significant increase in the number of open arm entries and head dips in the EPM test. There is no significant effect of neonatal dopamine depletion on the anxiety-related behavior in the L/DB test. These results indicate a lack of anxiety-related behavior in part, and suggest that dopamine system has a crucial role in the development of information processing system for behavioral response to anxiogenic stimulation. No COI.

3P-076

The development of attentional prioritization of own face during adolescence.

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Adolescence is the developmental stage characterized by the abrupt change of physical and psychological attributes triggered partly by the gonadal hormone secretion. Human development researchers argue that a sudden surge of the interest in own body-image is observed during this stage. However, at this point, such claim is based largely on the anecdotal or observational evidences, and few experimental studies have verified this contention. The primary aim of the present study is to objectively quantify the interest in own body image, and track the developmental course of it during adolescence. To this end, we measured the visuo-spatial attention directed towards own face, familiar friend's face and unfamiliar stranger's face in early- and middle-adolescent participants using eye-tracker.The results have shown that the participants in the early adolescence spend longer duration on fixating to friend's than own face. Those in the middle adolescence have exhibited the reverse pattern of attentional prioritization. That is, they spend longer on looking at own face than on the friend's face. There was no discernible difference in the fixation duration on the stranger's face between early- and middle-adolescent participants. These results indicate that the pattern of attentional prioritization qualitatively changes during the adolescence, and that this change is characterized mainly by the increased attentiveness towards own face consistently with the existing view that there is a surge of self-consciousness during adolescence. No COI.

3P-077

Age-related human brain changes detected by MRI T1W/T2W ratio

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MRI T1w/T2w ratio signal intensity (calculated by T1 weighted image signal intensity divided by that of T2 weighted image) cancels the receiver coil bias and increases the contrast related to myelin content. We examined the age-related T1w/T2w signal change to evaluate the usefulness of the T1w/T2w ratio image for studying the normal human brain functions and various brain diseases. Normalized T1w/T2w ratio images were created for 33 healthy subjects (23–60 years old). At each voxel, correlation between the signal intensity and the age were calculated. Significant negative correlations were found ($p<0.05$, corrected for multi-comparison with Monte Carlo simulation) at corpus callosum, pyramidal tract, corona radiata, and left optic radiation in white matter and at insular, basal ganglia, cingulate gyrus, parieto-occipital cortex in the gray matter, which corresponds to the age-related myelin reduction. Significant positive correlations were found at right inferior frontal, left insular white matter, and right external capsule and the signal intensity peak was around the age of 45, which corresponds to the late development of the myelination in these areas. Age-related signal intensity change in T1w/T2w ratio image indicates the possibility of quantitative analysis of human normal brain and pathology of various brain diseases including developmental disorders and degenerative nervous diseases. No COI.

3P-078

Neuronal responses to pure-tone and complex sound stimuli in the putamen, globus pallidus and amygdala of awake cats

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The basal ganglia and amygdala have been known to be highly involved in goal-directed behaviors and emotional functions. To date, little is known about how the sensory signals are processed in these nonauditory neural loci. Here, we investigated the acoustic response properties of neurons in putamen (PU), globus pallidus (GP), lateral amygdala (LA) and central amygdala (CA) of the awake cats. We found that 1) PU neurons well responded to the pure-tone stimuli. They had shorter response latencies, lower intensity thresholds and larger response magnitudes. The responsiveness to pure-tones gradually decreased in the order of PU, GP, LA and CA. 2) The neural responsiveness to complex sounds (vocalizations of con-species and other species and nonliving environmental sounds) also deteriorated in the order of PU, GP, LA and CA, while the selectivity for the complex sounds increased (response to a small fraction of the stimuli). 3) The proportion of neurons preferred to complex sounds rather than pure-tones was larger in LA and CA than in PU and GP. The ratio between the response magnitudes of complex sounds and pure-tones was higher in CA. Our results suggest that PU and GP might receive more direct auditory inputs, which can provide precise sensory references meeting the requirements to make a rapid motor response. On the contrary, LA and CA might process more indirect auditory signals to fulfill the emotional and/or cognitive functions. No COI.

3P-079

An experimental model of an emotional response that induced by auditory stimulation, which are linked to the experience-stimulation coupling

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Perception of environmental sensory stimuli through sounds induces several kinds of emotions, despite the underlying biological mechanism is still unclear. To clarify the mechanism, we have attempted to establish useful experimental models. In an animal model study, we exposed mice to these sounds within a sound proof box. Serotonin concentration in the frontal forebrain was shown to be higher in the sound-exposed mice than in mice kept in silence. Auditory environmental surroundings can influence emotional response through serotonergic neuron system. However, hearing a sound often elicits an emotion that is induced by not instinct but experience. In light of this sound effect, we tried to examine whether or not the hearing experience actually evokes emotional response using an experimental mouse model, in which mice were exposed to environmental sound stimulation coupled to different housing conditions. We exposed mice to an artificial sound stimulation under each of the pleasant and the unpleasant housing conditions. After mice were spent in each of the two conditions for several days, we analyzed some physiological parameters of mice while exposing to a sequence of sound stimulations. Among the parameters, hart beat rate was significantly decreased by presenting a sound coupled to the pleasant housing condition. This study provides an model to understand neuronal mechanisms of emotion induced by auditory perception. No COI.

3P-080

Enhanced sociality by umami intake during the period of development is mediated by vagus nerve afferent in ADHD model rat

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Spontaneously hypertensive rat (SHR) are the most used animal model of attention deficit hyperactivity disorder that is characterized by hyperactivity, impulsivity and inattention. We found that oral intake of monosodium L- glutamate (MSG), a taste substance for umami, for 5 weeks from postnatal day 25 (P25) to P60 altered social behavior in SHR that was grown in single isolated condition. How oral MSG intake during the period of development induced enhanced sociality in adulthood is unknown. To investigate the effect of brain-gut communication via vagus nerve on the MSG effect, SHR in isolation environment received vagotomy at P25 followed by MSG (0.6% solution) intake for 5 weeks, and then behavioral tests (open field test: OFT, social test: ST) were carried out. In OFT, no difference was observed in the vagotomy-group as compared to sham-operated MSG-treated group. However, in ST, sniffing time and riding number in the vagotomy-group increased back to same level of non-MSG treated group. Data suggest that MSG effect on social behavior during the period of development was mediated by the afferent of vagus nerve from the gastrointestinal tract receptor. No COI.

3P-081

Neural activities to frequency-modulated sounds in the frequency bands of the primary auditory cortex of guinea pigs observed by optical recording

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Neural activities to frequency-modulated (FM) sounds with different FM sweep rates in the primary auditory field (AI) of the left and right auditory cortices of the guinea pig were investigated using optical imaging with a voltage-sensitive dye (RH795). Eight guinea pigs were anesthetized with ketamine (80mg/kg) and xylazine (40mg/kg). Activity patterns to the FM sounds (upward and downward linearly swept frequency: 0.5–16.5 kHz in 16–400ms duration or FM sweep rate 0.04–1 kHz/ms) at 75 dB SPL and the tones (0.5, 16 kHz) were recorded from the AI. The peaks of the activity to FM sounds and tones were measured in the dorsal and ventral regions of the 0.5, 1, 2, 4, 8, 16-kHz frequency bands (FB). The peaks to the 0.5-kHz tone in the 0.5-kHz band did not change much in amplitude with respect to the duration (with small maxima at 40 and 640 ms) and those in the other FBs showed the similar duration function with smaller amplitudes. The peaks to the FM sounds in the 8- and 16-kHz bands showed the duration function with the maximal amplitude at 16 ms and decreased amplitudes beyond 64 ms. This tendency in the duration function was salient in the dorsal but not the ventral region of these bands and neither in the other lower FBs. We discuss the functional difference of the frequency processing in AI of guinea pigs. No COI.

3P-082

Temporal information processing of visual and auditory cues in monkey prefrontal cortex during a duration discrimination task

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We examined neuronal activity in monkey PFC during a duration discrimination task with visual (Vis, a green square) and auditory (Aud, 2000Hz tone) cues. In the task, two cues (first cue, C1 and second cue, C2) were presented consecutively for different duration ranging from 0.2 to 1.8 sec. Each cue was followed by 1 sec delay period. Subjects were required to choose the longer presented cue after the second delay (D2) period. There are four kinds of cue modality combinations; Vis-Vis, Vis-Aud, Aud-Vis and Aud-Aud. Duration of two cues, order of cue duration (long-short, LS or short-long, SL), and cue modality combination were pseudo-randomized. Out of 860 PFC neurons examined, 64 and 139 neurons responded to the C1 and C2, respectively. More than 80% of C1 response neurons were modality-specific, responded to either the visual or auditory C1, while 14% of the neurons were bimodal. More than 70% of C2 response neurons were also modality-specific, while 25% of the neurons were bimodal. Out of 192 D2 response neurons, 57 neurons exhibited greater D2 activity in the LS trials than the SL trials, while 85 neurons showed greater activity in the SL trials than the LS trials. More than half of these D2 response neurons responded similarly after the visual and auditory C2 and did so among the four cue modality combinations. These results suggest that the PFC integrates interval information on different modes of sensory stimuli and contributes to temporal discrimination between these stimuli after the two cue presentations. No COI.

3P-083

Sound-shape association memory tested using a M-maze in mice

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We can recall a particular shape from a sound stimulus intimately associated with the shape. To investigate this sound-shape association memory in mice, we developed a M-maze equipped with a screen and a speaker. First, a combination of sound A and shape A, or sound B and shape B was presented to water-deprived mice. After that, shape A and shape B were presented at the two inlets of the M-maze branches. If the mice choose the branch with the same shape presented before, they could obtain a small amount of water. This trial was repeated 20 times per each day. Mice learned to select the correct shapes after about 20 sessions. We further tested whether the mice could select the shapes based on the sound cues only, and we confirmed that the trained mice selected the correct shapes successfully, indicating that mice have sound-shape association memory. After the training, we anesthetized the mice, and cortical responses to the sound stimuli with or without presentation of the shapes were recorded in the auditory cortex using flavoprotein fluorescence imaging. The responses to the sound-shape stimuli were larger than those elicited by the sounds only. The areas ventral to the auditory cortex were also weakly activated by the sound-shape stimuli. Such positive interaction between the sounds and shapes was not observed in control mice. These results suggest the auditory cortex and the ventral areas may play an important role in sound-shape association memory. No COI.

3P-084

Visual responses for one's own hand during hand manipulation movement of hand manipulation-related and mirror neurons.

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During motor behavior, our brain represents "body schema", that is spatiotemporal dynamic organization of the body, based on interactions between motor output signal and multisensory feedback. The hand manipulation-related or mirror neurons in inferior parietal area AIP/PFG of the macaque monkey that are heavily involved in visual and motor integration, may correlate one's own body schema. The aim of this study was to investigate whether these neurons encode visual feedback during self-generated hand action. We examined activity of single neurons from AIP/PFG of two monkeys during execution of grasping action viewing video monitor that presented their online movements, and during fixating video clips of their own grasping action filmed from the monkey's point of view and then experimenter's grasping action. We recorded 54 hand manipulation-related neurons (including 33 mirror neurons) that responded to video clips of one's own and/or experimenter's hand movement. Of these, 25 neurons (including 13 mirror neurons) also responded to the video clips of one's own hand movement without the image of the target object. These results suggest that some of hand manipulation-related or mirror neurons in the AIP/PFG visually responded to one's own hand kinematics, representing the body scheme rather than representing the goal of action. No COI.

3P-085

Relationship between crossed hand illusion and autism spectrum quotient score

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Neurotypicial individuals experience a subjective reversal of temporal order judgments when the two hands are stimulated while they are crossed (Yamamoto & Kitazawa, 2001) and it may be caused by a conflict between an egocentric and an allocentric stance (Shore et al., 2002). Recently, we found that the crossed hand illusion was significantly smaller in autistic than in neurotypical children (Wada et al., 2013). In this study, we investigated a relationship between the crossed hand illusion and Autism Spectrum Quotient (AQ) scores.Young boys who join a self-help group for mild developmental disorders and also attend a regular school class participated in the study ($n = 12$, 16.3 ± 0.4 y.o.). They were required to judge temporal order of two tactile stimuli that were delivered to their both ring fingers with their arms crossed. After the order-judgment probabilities that the right hand was stimulated earlier were fitted by the double flip model (Yamamoto & Kitazawa, 2001), we found that both right-to-left and left-to-right flip probabilities were negatively correlated with AQ score ($R = -0.77$, $P < 0.01$; $R = -0.70$, $R < 0.05$) during the arm crossed condition. Present results suggest that the young boys who have higher AQ scores generally showed smaller amounts of the crossed hand illusion, which was consistent with our previous study that used autistic children (Wada et al., 2013). No COI.

3P-086

Prenatal low-dose bisphenol A enhances behavioral alterations induced by predator odor

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Bisphenol A (BPA) is one of the 'environmental endocrine disrupters' (EEDs). Past our study showed that pre- and postnatal administrations of low-level BPA induced depression-like behavior in rats. We focused on the stress vulnerability which was considerably related to the depression. In order to investigate the relationship between the depressive-response and the stress vulnerability, we designed one behavioral experiment using the predator odor as a stressor. We made cross-form plastic chamber, in which, predator odor (fox odor) was located at two opposite corners. Rats were located at the center position, and evaluated the locomotor activity and the avoidance response. In this study, pregnant Wistar rats were exposed to low-dose BPA during 7 days just before birth. The male and female offspring were evaluated at the age of 10 weeks. Both control and BPA groups showed the reduced locomotor activity under the predator odor, especially BPA group was remarkable. The odor-avoidance response was significant only in BPA rats. BPA exposure rats were obviously sensitive to the predator odor. It suggested that prenatal BPA exposure rats had a background regarding the vulnerability to stress such as the predator odor. That may have some relationships to the enhancement of depression-like behavior in the forced swimming test. No COI.

3P-087

Recordings of neuronal activity in the pig brain related to the snout function

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The pig's snout is not only the main organ related to the sense of smell, but also is chief tactile organ as well as chief effector, spade as the human hand. In this study, we examined neuronal activity in the pig brain, related to the snout function. In the freely-moving condition, local field potentials (LFP) were measured by use of the wireless technique. Large negative waves in the LFP were recorded in the hippocampus, prior to pushing a switch by the snout to get a reward during operant learning tasks. Under general anesthesia, evoked potentials were recorded in the hippocampus as well as in the posterior part of the rostral region by contralateral electrical stimulation on the surface of the upper part in the snout. The hippocampus in the pig may be a receptive field to the afferent input from the snout and may, in part, be involved in motor control for the snout as well as learning and memory. Further investigations need to reveal neuronal mechanisms generating the large negative waves in the pig hippocampus associated with pushing the switch during the operant conditioning tasks. No COI.

Poster Presentations
Nutrition, Metabolism, Thermoregulation

3P-088

Evening-based feeding habit impaires insulin sensitivity via central AgRP

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Disturbance of feeding rhythm has been understood as a risk factor for development of insulin resistance, however, it is not elucidated the detailed mechanism. In this study, 3 groups of C57BL/6J mice were given lab chows freely during dark phase (ZT12–24, Control group), first 4-hour in dark phase (ZT12–16; Morning group), or last 4-hour in dark phase (ZT20–24, Evening group) for 8 weeks. Mice in Evening group showed impaired whole body insulin sensitivity by insulin tolerance test despite mice in the group ingested the smaller food intake than that of Control group, while mice in Morning group showed normal insulin sensitivity. We observed higher triglyceride (TG) content, increased gene expression of fatty acid synthase (FAS) and impaired insulin signals in skeletal muscle in Evening group compared to other group. These effects were not observed in liver. On the other hand, mRNA expression of agouti-related protein (AgRP) was increased in hypothalamus in Evening group. We observed that acute and/or chronic ICV-injection of AgRP increased FAS expression and TG content in skeletal muscle. Moreover, inhibition of central AgRP expression by antisense oligo improved insulin resistance in Evening group. These results indicate that feeding rhythm like as ingestion only in the evening impairs insulin sensitivity because of TG accumulation in skeletal muscle mediated by hypothalamic AgRP. No COI.

3P-089

Hindbrain responsiveness to anorexinergic hormones is reduced in animals showing binge-like overconsumption

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Previous studies suggest that the feedback inhibition of food intake by postprandial visceral stimulation is blunted in animal models showing binge-like overconsumption. However, it is unclear whether the response to gut anorexinergic peptides is reduced in these animals. In the present study, we focused on neural responses in the nucleus of tractus solitarius (NTS) and parabrachial nucleus (PBN) to an intraperitoneal injection of peptide YY (PYY), one of gut peptide hormones, in C57BL/6J mice using c-fos immunoreactivity as a neural activation marker. Mice were given limited access to sucrose for 4 hour/day with (trained group) or without (control group) food restriction. Trained mice showed binge-like sugar overconsumption. In control animals, many c-fos-positive cells were seen in the NTS and PBN in response to the PYY injection, especially in the area receiving gastrointestinal but not taste-related information. In contrast, in trained mice, the number of c-fos-positive cells activated by the peripheral PYY injection was smaller in both nuclei compared with control mice. The present results suggest that binge-like sugar overconsumption attenuates hindbrain responsiveness to gut anorexinergic peptide. No COI.

3P-090

Effect of the number of chewing on diet-induced thermogenesis and postprandial appetite

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The present study was to examine the effect of the number of chewing on diet-induced thermogenesis (DIT) and subjective appetite after eating. After baseline measurements in the overnight fasting state, ten healthy normal-weight subjects chewed 300-kcal solid food as long and many times as they could before swallowing in the S trial, while they swallowed the same but fractured food as fast as they could in the R trial. DIT was calculated from oxygen uptake and body mass. Subjective score of appetite was assessed by visual analog scale (no appetite: 0 - appetite: 100) every 15 min. DIT and appetite score were recorded until 90 min after eating. Duration and the number of chewing were significantly greater in the S trial than in the R trial (497±45 vs. 103±11 s, 702±108 vs. 137±15 times, respectively, $p<0.05$). DIT was significantly greater in the S trial than in the R trial (11±2 vs. 1±1 kcal / 90 min, $p<0.05$). Appetite score decreased significantly from resting baseline level; this was shown in immediately after meal and continued until 30 min in the S trial, while, solely at 75 min after meal in the R trial. Appetite score was significantly lower in the S trial than the R trial until 90 min after meal (44±7 vs. 61±5, immediately after meal; 50±6 vs. 67±5, 90 min; respectively, $p<0.05$). These results suggest that fewer number of chewing a food inhibits the increase in DIT and the decrease in appetite, implying an association between eating rapidly and overweight. No COI.

3P-091

Effect of systemic ghrelin administration on tail-hiding behavior in a cold exposure in male rats

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INTRODUCTION Rats place their tails underneath their body trunks in the cold (tail-hiding behavior). In a cold (15°C), body temperature (T_b) in 42-h fasted rats decreased when a tail-hiding behavior was prevented experimentally (Uchida et al., 2012). Thus, tail-hiding behavior is a thermoregulatory behavior in fasted rats. The aim of the present study was to elucidate whether ghrelin that is an increasing hormone by fasting is involved in tail-hiding behavior in rats during a cold exposure. **METHODS** Male Wistar rats were divided into 'fed', '42-h fasting' and ghrelin groups. The rats received an i.p. saline or 30 μg ghrelin injection, then exposed to 27°C or 15°C for 2-h with continuous T_b and tail-hiding behavior measurements. **RESULTS** At 15°C, T_b decreased only in the fasted group. The duration of tail-hiding behavior increased in the fasted and ghrelin groups than the fed group at 15°C. The onset of tail-hiding behavior advanced in the ghrelin group than the fed and fasted groups at 15°C. **CONCLUSION** These results suggested that ghrelin might modulate a tail-hiding behavior during a cold exposure in rats. No COI.

3P-092

Skin warm perception is enhanced by regular endurance training

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Increase in skin and esophageal temperature (T_{sk} and T_{es}) induces autonomic and behavioral thermoregulatory responses with the perception of both temperatures. It has been reported that regular endurance training enhances autonomic thermoregulatory responses and may modify whole body thermal perception. We assessed whether skin thermal perception was also modified with regular endurance training.Methods: Ten trained (< 3 days of training/week) and seven untrained healthy young men underwent measurements of noticeable increase/decrease (±0.1℃/sec) of skin temperature (warm/cold threshold) at chest by using a thermode (6.25 cm^2) and of subjective whole body thermal sensation (visual analogue scale) in normothermia (NT, T_{es}; 36.6±0.2℃) and hyperthermia (HT, T_{es}; 37.3±0.1℃, lower legs immersion in 42℃ water). T_{es} and T_{sk} were measured continuously.Results: In both groups, cold threshold was decreased and subjective whole body thermal sensation were increased with T_{es} and T_{sk} in HT compared with NT (all, $P<0.05$), while warm threshold remained unchanged. In NT and HT, warm threshold was lower (more sensitive) in trained than untrained men ($P<0.05$), although cold threshold and subjective whole body thermal sensation were similar in both groups. Conclusions: Skin warm perception but not skin cold perception or whole body thermal sensation is more sensitive in trained than untrained men. No COI.

3P-093

The effect of the head or neck cooling on body core temperature and thermal pleasantness in humans.

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Aim Some animals have mechanisms that selectively cool the brain during hyperthermia. However, it remains controversial if humans have the mechanism. In the present study, we investigated the effect of the head or neck cooling on tympanic temperature as an indicator of brain temperature. ***Method*** Eight healthy male subjects performed four local cooling trials (facial fanning, cooling of the forehead or neck, and the control). Subjects sat on a chair, putting on a water-perfusion suit to maintain skin temperature at 33 ℃ (normothermic condition) or 38 ℃ (hyperthermic condition). Tympanic, esophageal, and skin temperatures were continuously monitored. After 10-min baseline period, local cooling was conducted for 15 min. Temperature sensation and thermal pleasantness were reported by the subjects. ***Results*** During the head or neck cooling, while facial skin temperature was significantly decreased, there was no significant difference between tympanic and esophageal temperatures in normothemic condition. Only in hyperthermic condition, a significant difference between tympanic and esophageal temperatures was observed. After facial fanning, tympanic temperature was lower than esophageal temperature. ***Discussion*** These results may suggest that facial fanning cools the brain in hyperthermic condition. However, local cooling of the forehead or neck may not effect on the selective brain cooling. No COI.

3P-094

Changes in behavioral thermoregulation in β-amyloid-infused rats

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We investigated the behavioral thermoregulation of β-amyloid (Aβ)-infused rats by measuring their selected ambient temperatures (T_s). Male Wistar rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and implanted in the intraperitoneal cavity with a temperature transmitter. A solvent of 35% acetonitrile and 0.1% trifluoroacetic acid was used as the vehicle for Aβ peptide (4.9-5.5 nmol). The osmotic pump contained 234±13.9 μl of Aβ solution was subcutaneously inserted into the back and was cannulated into the left cerebral ventricle. Moreover, 0.5 μg of $AlCl_3$ was injected into the right cerebral ventricle. Vehicle-infused rats were used as control rats (CN). After 2 weeks, rats were placed in a thermal gradient and their intra-abdominal temperature (T_{ab}) and T_s were measured for 3 days. After the behavioral test, rats were subjected to heat tolerance test, i.e. they were exposed to the ambient temperature of 36℃ for 3 h. Then, rats were anesthetized and brain was removed for immunohistochemical analysis. Although there were clear day-night variations of T_s and T_{ab} in CN, the levels of T_s and T_{ab} in the Aβ-infused rats were altered. However, magnitudes of rises in T_{ab} of CN and Aβ-infused rats during heat tolerance did not change. Immunohistochemical analysis showed that the expression level of c-Fos protein in the hypothalamus, a center of autonomic thermoregulation, did not differ between CN and Aβ-infused rats. These results suggest that Aβ-infusion in the lateral ventricle changes behavioral thermoregulation maybe without affecting autonomic thermoregulation in rats. No COI.

3P-095

Heat acclimation affects circulating levels of PGE2 COX-2 and orexin

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We examined serum levels of prostaglandin E2 (PGE2), cyclooxygenase (COX)-2 and orexin before and after heat acclimation (HA) to test the hypothesis that decreased basal body temperature due to HA correlate with circulating levels of these key thermoregulatory molecules. Nine healthy human male volunteers were recruited (age, 21.9 +/- 2.7 years). The subjects were exposed to half-body immersion in hot water (42 +/- 0.5oC) at the same time of day (2–5 pm) on alternate days for 60 days. The HA protocol included 10 bouts of 30 min immersion. All experiments were performed in an automated climate chamber (temperature, 26.0 +/- 0.5oC; relative humidity, 60 +/- 3.0%; air velocity,1 m/sec). Tympanic and skin temperatures were measured, and mean body temperature was calculated. The difference in body weight was used to estimate total sweat loss. Serum levels of PGE2, COX-2 and orexin were analyzed before and after HA. Body temperature decreased significantly after HA, whereas sweat volume increased significantly. Serum PGE2, COX-2 and orexin concentrations decreased significantly compared to those at pre-acclimation. Our data suggest that decreased basal body temperature after HA is associated with decreases in thermoregulatory molecules, such as PGE2, COX-2 and orexin.Keywords prostaglandin E2, cyclooxygenase-2, orexin, heat acclimation, body temperature. No COI.

3P-096

Increase in circulating levels of free fatty acids during passive heat loading

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The purpose of this study was to determine whether heat acclimation (HA) is closely related to sweating function and circulating levels of free fatty acids (FFA) during passive heat loading (PHL, lower body immersion in hot water, 42 +/- 0.5 C, 30 min) in human. The subjects were 17 male university students (age, 22.14 +/- 3.27 yr; height, 173.52 +/- 4.10 cm; weight, 70.40 +/- 5.64 kg; body fat, 19.84 +/- 3.28%; muscle mass, 22.49 +/- 4.19 kg). To mimic heat acclimation, repeated lower body immersion in hot water (42 +/- 0.5 C, 30 min/day) was performed 6 times every other day for 2 weeks. The results showed that the sweat function improved, and waist circumference significant decreased after HA. The level of FFA was significantly altered after HA in Post-PHL and there were significant correlations between body temperature and FFA, both pre- and post- HA treatment in Post-PHL (r2=0.595, r2=0.716, respectively). The threshold of body temperature for lipolysis was decreased by HA. In conclusion, HA improved lipolysis and decreased waist circumference during PHL. Keywords: Heat acclimation, Free fatty acids, Sweat function, Body temperature, Lipolysis. No COI.

3P-097

Activation of G protein-coupled receptor 40, one of free fatty acid receptors, induces the enzymes of polyunsaturated fatty acids synthesis and facilitates differentiation of cultured neural stem cells

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Polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid (DHA) and arachidonic acid, are essential for the growth and functional development of the brain. Moreover, decreasing PUFA levels and decreasing mRNA levels of enzymes for PUFA synthesis were observed in postmortem human brain tissues of Alzheimer's disease, depressive disorder, and schizophrenia. Therefore, enhancement of PUFA synthesis in the brain is an important target to prevent and treat these diseases. The present study examined effects of activation of free fatty acid-activated G protein-coupled receptor (GPR) 40, which is one of free fatty acid receptors, on the enzymes of PUFAs synthesis in the cultured rat fetal neural stem cells (NSCs). GPR40 activation by GPR40 agonist GW9508 increased the levels of Tuj-1 (a neuronal marker), GFAP (an astrocyte marker) mRNA expression as well as the percentage of Tuj-1- and GFAP-positive cells; suggesting that GPR40 activation enhanced neuronal and glial differentiation. GW9508 increased the mRNA levels of stearoyl-CoA desaturase, delta-5 and delta-6 desaturases, and fatty acid elongase-5 via sterol regulatory element-binding protein (SREBP) 1c transcriptional activation. SREBP1c is known as a main regulator of PUFA synthesis. These results suggest that GPR40 is related to SREBP 1c-mediated activation of PUFA synthesis and enhances differentiation of NSCs. No COI.

3P-098

Orexin involves lipid metabolism in liver

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Orexin is a hypothalamic neuropeptide that regulates motivated behaviors, including feeding and awake-sleep cycle, and, is also involved in energy homeostasis. Orexin knockout (OxKO) mice significantly gained more weight than wild-type (WT) mice, although food intake was not significantly different between both genotypes, suggesting that OxKO mice might have lower rates of metabolism compared with WT mice. However, it is not clear whether orexin affects metabolic rates of mice or not. Thus we compared metabolism, focused on lipid metabolism in the liver, between OxKO and littermate WT mice. Livers in the OxKO mice were significantly larger than those of WT mice and were yellowish in appearance. Oil red O staining revealed higher lipid accumulation in the livers of the OxKO mice compared with WT mice. The total cholesterol in peripheral blood was higher than WT mice (WT: 32.8±15.1 mg/dL, n=12; OxKO: 156.5±29.2 mg/dL, n=12, $p<0.05$), although no significant differences in free fatty acid and serum triglyceride were observed between OxKO and WT mice. Moreover, mRNA levels of the hepatic lipogenic genes, PPAR-γ, CD36, and fatty acid synthase in OxKO mice were higher than that in WT mice. These results suggest that orexin is likely to contribute to regulation of lipid metabolism in the liver. No COI.

3P-099

The effect of meal duration and timing of high fat diet on energy metabolic rhythm of mice

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It is known that the overeating at dinner and/or at night snack time, or breakfast skipping lead to become obesity in humans. Therefore, meal timing and meal frequency may affect body weight and body fat gain, and will be associated with obesity. In our previous experiment, we gave high fat diet (HFD) or normal diet (ND) to mice at active period or at inactive period in 2 meals per day schedule, and amount of food was regulated by feeding duration time (2, 4, and 8hrs per half day). As a result, the body weight and body fat were higher in 8hrs group than those in 4hrs group. Although 2hrs group kept small food intake than 4hrs group, the body weight and body fat were higher in 2hrs group than 4hrs group. Moreover, we measured peripheral clock phase in liver using In Vivo Imaging System (IVIS). Mice fed with HFD at inactive period showed the advance of liver clock as compared with those fed with HFD at active period.In current experiment, we asked whether above feeding conditions may affect the energy metabolic rhythm. Metabolic rhythm was measured by metabolic measuring system after we bred mice with each protocol for 3 or 4 weeks. As the result, mice fed with HFD at inactive period consumed less energy from fat in comparison to those fed with HFD at active period. This result suggested that feeding of HFD at inactive period could not consume much energy from fat. No COI.

3P-100

Seasonal differences in thermoregulation, blood pressure and melatonin secretion in obese subjects

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Obesity has been increasing in the world during the past several decades. Obese people have an increased incidence for developing cardiovascular, renal, and hormonal diseases and sleep disorders. We have recently studied the differences of thermoregulation, melatonin secretion during sleep and 24 hours blood pressure in obese subjects in Japan in two seasons, summer vs. winter. Five obese (BMI, 32±5 kg/m^2) and 5 non-obese (BMI, 23±3 kg/m^2) men participated in this experiment at latitude 35°10′ N and longitude 136°57.9′ E. The average environmental temperature was 29±1°C in summer and 3±1°C in winter. Tympanic temperature and sweat rate were measured during leg water immersion at 42°C for 30 min. Blood pressure was measured over the course of 24 hours in summer and winter. Saliva samples for melatonin were collected at 11 pm, 2 am and 6 am. The relationship between tympanic temperature and sweat rate was significantly different between obese and non-obese subjects in both seasons, there being a lowered sweat rate for any core temperature in obese subjects. Melatonin concentrations during sleep in obese subjects were significantly lower than those in non-obese subjects in the winter. Systolic and diastolic blood pressures in obese men were significantly higher in winter than in summer. The thermoregulatory responses, melatonin secretion during sleep and blood pressure control were attenuated in winter season in obese. No COI.

3P-101

Search of the suitable protein content and kinds of amino acid which entrains mouse peripheral clocks

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It is well known that insulin secretion may be important step for the entrainment of peripheral clock by restricted feeding (RF) (Hirao et al., 2009; Thara et al., 2011), and we found that faster digestible carbohydrate caused big phase-shift of peripheral clock. (Itokawa et al., 2013). However we have not yet found the ratio of protein in food and also roles of amino acids on peripheral clock entrainment. In this research, we prepared 100% casein only (100C), 50% casein + 50% β-cornstarch (50C), and 100% β-cornstarch only (0C) groups, and in some experiments we prepared changing ratio of casein and β-cornstarch (3C, 6C, 20C, 40C, 86C) in AIN-93M formula diet. Mice were fed with each food at day time for two days. We measured phase-advance of liver, kidney and, submandibular gland (Sub Gla) clock using in vivo imaging system (IVIS). In liver and kidney, values of phase-advance were not dependent on protein content but on total calorie. On the other hand, in Sub Gla phase-advance was positively dependent on content of carbohydrate, suggesting that Sub Gla is responsive to carbohydrate, and liver and kidney are well responsive to protein. In parallel to above experiment, we screened whether oral administration of each amino acid (5mM / kg) in 20 different amino acids caused phase-shift of peripheral clocks using IVIS. We found cysteine and histidine entrain peripheral clock, suggesting that casein substituted by cysteine and/or histitidine is interesting. No COI.

3P-102

The relevance between estrogen decrease and cold constitution

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Menopausal women frequently suffer from uncomfortable cold constitution, which is at least in part caused by peripheral circulatory imperfection resulted from vasoconstriction due to the decline of estrogen secretion. In this study, we examined the blood flow (shoulder, waist and femur) and the skin temperature (shoulder, femur and planta) before and after cold-load (1°C, 1hr) ovariectomized rats (OVX) as a menopause-model rat, and compared the findings with those obtained from sham-operated rats (Sham) and non-treated rats (Control) . In addition, we also studied the effects of β-estradiol administration on the blood flow and the skin temperature in these rat groups. Ovariectomy produced a significant decrease in the blood flow of shoulder, waist and femur before the cold-load, but not the skin temperature. The recovery from the cold-load was delayed in the skin temperature of femur in OVX. The administration of β-estradiol led to a significant increase in the blood flow and tended to fasten the recovery of the skin temperature after the cold-load. These findings suggest that the estrogen reduction due to ovariectomy may deteriorate the vascular function, resulting in the decrease of the blood flow. The fact that the decrease of the blood flow did not affect the skin temperature, but affect the recovery process of the skin temperature following the cold-load suggests that the decrease of estrogen in menopausal women may cause the onset of cold constitution. No COI.

3P-103

The noradrenergic vasoconstrictor response in leg skin in young women complaining of unusual coldness

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In Japan, we often encounter women complaining of physical coldness, especially in the acral portion of the lower extremities, even under normal temperature conditions. In this study, we examined the sympathetic cutaneous vasoconstrictor response in the lower extremities in ten young women complaining of unusual coldness (C group) and nine young women not suffering from coldness (N group). In protocol 1, the room temperature was decreased from 29.5°C to 23.5°C for increasing noradrenergic sympathetic activity in the skin. In protocol 2, cutaneous vasoconstrictor responses to iontophoretic application of norepinephrine (NE) were examined at 29.5°C. In both protocols, laser-Doppler flow (LDF) was monitored from the calf and dorsal foot. Cutaneous vascular conductance (CVC) was calculated as the ratio of LDF to blood pressure. In protocol 1, the slopes of relationship between mean skin temperature and CVC in the calf during mild-cold exposure did not differ in both groups, while the slope in dorsal foot was steeper ($P<0.05$) in the C group than in the N group. In protocol 2, the CVC in the calf similarly decreased in both groups during NE iontophoresis, but the reduction of CVC in the dorsal foot was greater ($P<0.05$) in the C group. Thus, the C group showed a greater cutaneous vasoconstrictor reflex response in the peripheral region in the lower extremities to cold. It was suggested that the augmented sensitivity of the cutaneous vasoconstrictor response in the C group was due to the altered noradrenergic sensitivity of the legs. No COI.

3P-104

Hypoxia-induced hypothermia mediated by the GABAergic transmission in the rostral ventromedial medulla in anesthetized rats

Osaka, Toshimasa (*National Institute of Health and Nutrition*)

Hypoxia evokes a regulated decrease in the body core temperature (T_c) in a variety of animals. This response is beneficial for animals because a lower T_c increases the affinity of hemoglobin for oxygen and reduces the oxygen demand of the tissue. The neuronal mechanisms of this response include activation of the carotid chemoreceptors, release of noradrenaline and nitric oxide in the rostral ventromedial preoptic area, and glutamatergic activation in the lateral preoptic area (LPO). Here, I examined the hypothesis that glutamate-sensitive neurons in the LPO activate GABAergic transmission that mediates hypoxia-induced hypothermia in the rostral ventromedial medulla (RVMM) in urethane-chloralose-anesthetized, neuromuscularly blocked, artificially ventilated rats. Unilateral microinjection of GABA (15 nmol/50 nl) in the RVMM elicited a prompt increase in tail skin temperature and decreases in T_c, oxygen consumption rate, and heart rate. The GABA-sensitive site was localized in the RVMM, including the rostral raphe pallidus and adjacent parapyramidal area. Pretreatment with the $GABA_A$ receptor blocker bicuculline methiodide (10 pmol/100 nl), but not vehicle saline, bilaterally microinjected into the RVMM greatly attenuated the hypothermic responses evoked by hypoxic (10%O_2 and 90%N_2 for 5 min) stimulation or by bilateral microinjections of glutamate (5 nmol/100 nl) into the LPO. The results suggest that hypoxia-induced hypothermia was mediated, at least in part, by activation of $GABA_A$ receptors in the RVMM. No COI.

3P-105

Topographical study of activated microglia and c-Fos immunoreactive neurons following acute restraint stress

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Recent studies have shown that exposures of animals to stress, either acute or chronic, induce robust microglial activation in the brain. The stress-induced microglial activation has been well documented in regions which are susceptible to clinical neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, and depression. In the present study, we investigated the spatial distribution of c-Fos and activated microglia in various brain regions by employing sham-operated, adrenalectomized, and adrenalectomized and oral corticosterone treated rats, following 2 h period of restraint stress. The present study showed that: 1) acute 2 h restraint stress significantly increased c-Fos immunoreactive neuron number in various regions, such as the thalamus, hypothalamus, periaqueductal gray (PAG), substantia nigra (SN), but, not in the hippocampus. The acute stress induced more c-Fos immunoreactive neurons in these brain regions in adrenalectomized rats than in sham-operated rats; 2) the microglia activation occurred in accordance with c-Fos immunoreactive neurons most of these brain regions except for the hippocampus. The microglial activation was significantly enhanced by the adrenalectomy; 3) oral administration of corticosterone consistently suppressed both c-Fos immunoreactive neurons and microglial activation in these regions. Thus, the present study demonstrates that neuron-microglia may have close interactions each other during the time of acute stress and that glucocorticoids may provide inhibitory signals to both neuronal and microglial activity. No COI.

Poster Presentations
Pathophysiology

3P-106

Neuroprotective effect of water-soluble vitamin E derivatives as radical scavengers/antioxidants

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We evaluated neuroprotective effect of newly developed water-soluble vitamin E derivatives, ETSGS (glutathione + vitamin E) and EPCK1 (vitamin C + vitamin E) against ischemia-reperfusion injury (IRI) by phosphorous NMR spectroscopy (^{31}P-NMR) and by electron spin resonance (ESR) spectrometry.

Brain slices were incubated in a NMR sample tube with standard artificial cerebrospinal fluid with ETSGS or EPCK1 (0, 10, 100 μM) at 27.5°C. Brain slices were exposed to IRI by halting the perfusion for 1 hr, followed by the reperfusion for 3 hrs. High-energy phosphates in brain slices, phosphocreatine (PCr) and ATP, and intracellular pH were serially measured. Direct free radical scavenging activity was evaluated by ESR using spin trapping method.

Both ETSGS and EPCK1 demonstrated neuroprotective effect against IRI at 100 μM, but not at 10 μM; PCr had significantly better recovery than control ($p < 0.05$). In ESR study, those derivatives significantly scavenged multiple free radicals examined (HO·, O_2^{-}·, ascorbate free radicals, *t*-BuOO·, NO, DPPH radicals) with EC_{50}'s from 10^{-4} to 10^{-2}M. EC_{50} for each radical (scavenging profile) varied between ETSGS and EPCK1.

It is concluded that vitamin E derivatives might be neuroprotective against IRI through their antioxidative activity, which is at least partially attributable to their direct scavenging activity against multiple free radicals. No COI.

3P-107

A new therapeutic approach for glioblastoma using tumor selective cell-penetrating peptide

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Cell-penetrating peptides (CPPs) has been expected as a new biomedical tool from the perspective of its high efficiency and minimal invasiveness. However, since low specificity to target cells, clinical application of the CPPs for cancer remains challenging. In this study, we attempted identification of novel cancer-specific CPPs for targeting glioblastoma multiforme (GBM), which is often refractory and resistance to treatment. Here, we conducted screening of CPPs which having affinity to human GBM cell line U87MG, from mRNA display random peptides library. Based on amino-acid sequence of the candidate CPPs obtained from the screening, we synthesized fluorescent peptides and examined transduction efficiency for various cell lines derived from histologically different types. In addition, some modifications of amino-acids of the CPP resulted in enhancement of cell-penetrating activity. Furthermore, GBM-selective CPP fusion p16Ink4a functional peptide induced cellular apoptosis, which demonstrate that this CPP could deliver p16 peptide into U87MG cells, and consequently had an antitumor effect against GBM. These results indicate that the novel CPP identified in this study permeate with highly affinity into GBM cells and suggest its potential for imaging and therapeutic by selective delivery of any cargo molecules to tumor tissue. Further studies are required to evaluate the tissue specificity and the distribution of the CPPs in GBM model mouse. No COI.

3P-108

Tumor-associated macrophages in experimental gliomas in the rat brain

Gotoh, Katsuhiro; Kusakawa, Akari; Horiuchi, Mika; Sugimoto, Kana; Takahashi, Hisaaki; Yano, Hajime; Tanaka, Junya (*Dept. of Molecular and Cellular Physiology, Graduate School of Medicine, Ehime Univ., Ehime, Japan*)

We analyzed the functions of tumor-associated macrophages (TAMs) in experimental gliomas in the rat brain. The gliomas were induced by transplanting C6 glioma cells into the neonatal rat brains within 24 h after birth. Immunohistochemical staining on the cryostat sections of the brain with glioma tumor revealed highly dense accumulation of macrophages that were descendants of bone marrow cells but not of resident microglial cells as revealed by bone-marrow transplantation experiments. TREM2, a protein responsible for recognition of apoptotic cells, were expressed by TAMs located in the periphery of the tumor mass. TAMs in and around necrotic tumor mass strongly expressed CD68, a marker for phagocytes. Progranulin, a cell growth factor was abundantly and diffusely expressed by almost all TAMs. Isolated TAMs expressed proinflammatory factors such as inetreleukin-1beta and chemokines CCL2, CCL3, and CCL4 at quite high levels, whereas the expression of the factors were much suppressed in the tumor mass, even when the expression levels were normalized with the level of Iba1 (a marker for macrophages). These results indicate that proinflammatory nature of TAMs in the tumor mass were highly suppressed, resulting in the protection of tumor cells from the destructive infiltration of inflammatory cells. No COI.

3P-109

Oct-3/4 promotes tumor angiogenesis in glioblastoma through VEGF production

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Accumulating evidence shows that the expression level of Oct-3/4, a self-renewal regulator in stem cells, is positively correlated with the progression of various solid tumors. However, little is known regarding the influence of Oct-3/4 in the tumor angiogenesis of glioblastomas. In the present study, we subcutaneously transplanted Oct-3/4-overexpressing human glioma U251 cells (U251/EGFP-Oct) into the right thighs of nude mice to evaluate the roles of Oct-3/4 in the glioblastoma angiogenesis. Both tumor size and the number of large vessels growing in the tumor were markedly increased. In an in vitro model of angiogenesis, conditioned media from U251/EGFP-Oct cells significantly accelerated capillary-like tube formation compared with conditioned media from U251/EGFP cells that were devoid of Oct-3/4 overexpression. In comparison with U251/EGFP cells, U251/EGFP-Oct cells had markedly elevated expression of vascular endothelial growth factor-mRNA under the control of HIF1alpha. In U251/EGFP-Oct cells, enhanced protein expression and nuclear translocation of HIF1α were observed in normoxic conditions. Our results demonstrate that Oct-3/4-expressing glioma cells have the ability to adapt to low-oxygen environments within tumor masses by promoting tumor angiogenesis, and thus Oct-3/4 may be a promising target for treatment of malignant gliomas. No COI.

3P-110

Contributions of TGFbeta1 on insidious glioma cell invasions in the experimental glioma and exploration of the source of the TGFbeta1

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Insidious invasions of glioma cells from primary tumor site toward surrounding normal brain parenchyma cause untreatable pathological conditions at the recurrence after surgical resection. Although suppression of this invasion has long been desired as a promising therapeutic target, it has yet to be developed.We have established the insidious invasion model in KSN nude mice by xenografting C6 rat glioma cells into the brain, and observed the invasion accompanied by CD105/Endoglin (+) but CD309/VEGFR2 (-) blood vessels, implying dominant contribution of TGFbeta1 rather than VEGF in insidious glioma invasions. Moreover, the invasion was prevented by inhibition of Sodium ion/proton exchanger 1 (NHE1) using an NHE1 inhibitor. Now we are on the way to determine whether C6 cells themselves are the source of TGFbeta1 in the glioma brain accompanying insidious invasions. On the one hand, NHE1 has been reported to acts on the conversion of latent form of TGFbeta1 secreted and deposited on extracellular matrix (ECM) toward active form, via regulation of ECM construction. Inhibition of NHE1 by an inhibitor did not affect the mRNA levels of TGFbeta1. We are also on the way to determine the consequence of NHE1 in TGFbeta1 conversion in insidious glioma invasions, and would like to discuss about the possibility of NHE1 as therapeutic target for the invasions. No COI.

3P-111

Exploration of the mechanism of TGFbeta1 production from glioma associated macrophage-like cells in experimental glioma

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Insidious invasions of glioma cells from primary tumor site toward surrounding normal brain parenchyma cause untreatable pathological conditions at the recurrence after surgical resection. Primary glioma mass contains significant number of macrophage-like cells as well as glioma cells. The macrophage-like cells are different from resident microglia in many aspects, for example the higher expression of sodium ion/proton exchanger 1 (NHE1), while their roles in glioma cell invasions are yet to be elucidated.We have established the insidious invasion model in KSN nude mice by xenografting C6 rat glioma cells into the brain, and observed the invasion accompanied by CD105/Endoglin (+) but CD309/VEGFR2 (-) blood vessels, implying dominant contribution of TGFbeta1 rather than VEGF in insidious glioma invasions. Moreover, the invasion was prevented by inhibition of NHE1 using an NHE1 inhibitor.Now we are on the way to determine whether glioma associated macrophage-like cells are the source of TGFbeta1 in the glioma brain accompanying insidious invasions. On the one hand, NHE1 has been reported to acts on the conversion of latent form of TGFbeta1 secreted and deposited on extracellular matrix (ECM) toward active form, via regulation of ECM construction. We would like to discuss as to the consequence of NHE1 in TGFbeta1 conversion in insidious glioma invasions, and the possibility of NHE1 as therapeutic target for the invasions. No COI.

3P-112

Effects of interactions of microglia/macrophages and C6 glioma cells in co-culture on the proinflammatory functions of microglia

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Microglia/macrophages massively accumulate in and around a tumor mass of an experimental rat glioma model, in which C6 glioma cells are transplanted into the neonatal rat brain parenchyma. Many studies have shown that tumor-associated macrophages (TAMs) enhance the tumor growth and invasion. Although a large number of activated microglia were located as they were surrounding the tumor mass, it has not been well elucidated what kinds of effects the microglia elicit on the gliomas. In this study, we compared the changes in nature of microglia/TAMs when cocultured with C6 glioma cells. Quantitative real-time RT-PCR experiments revealed that cocultured macrophages downregulate expression of mRNA encoding proinflammatory cytokines such as interleukin-1beta, tumor necrosis factor alpha, and interferon-alpha, whereas microglial cells in the coculture did not show any significant functional changes. Immunohistochemical staining revealed that TAMs but not microglia cells in coculture often expressed phagocyte markers CD68 and TREM2. Furthermore, there is no apparent inflammatory reactions around the tumor mass, in spite of the marked accumulation of activate microglial cells. These results suggest that microglial cells and TMAs play distinct roles in the growth of glioma. No COI.

3P-113

Oct-3/4 promotes chemoresistance of glioblastoma cells through the expression of ABC transporters

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Glioblastoma is the most malignant type of primary brain tumor that has been shown to contain a small population of cancer stem cells (CSCs). CSCs share many of the properties of normal stem cells, such as self-renewal, differentiation into multilineage cells, and drug resistance. Although chemotherapy kills most of cells in tumors to chemotherapeutic agents, CSCs may be left behind, which then cause local tumor recurrence. Recent studies have demonstrated increased Oct-3/4 expression, a self-renewal regulator in stem cells, in glioblastomas. However, little is known regarding the influence of Oct-3/4 in the chemoresistance property of glioblastoma. In this study, we established Oct-3/4-overexpressing glioblastoma cells (U251/Oct-3/4 cells) to assess the chemoresistance property. To determine the chemosensitivity in U251/Oct-3/4 cells, we exposed these cells to various concentrations of chemotherapeutic reagents including temozolomide, carboplatin, and etoposide (VP16) for 48 hr, and performed cell viability assay determined by LDH release. Compared with control cells, U251/Oct-3/4 cells exhibited greater resistance to these agents. This ability was canceled in these cells by suppressing the expression of Oct-3/4 using tet-off system. Furthermore, U251/Oct-3/4 cells express higher levels of multidrug resistance-related gene, ABCG2. These results suggested that future treatments should target Oct-3/4 positive CSCs in tumors to improve the survival of brain tumor patients. No COI.

3P-114

Response of glial cells to IL18 produced in the ischemic rat brains

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We have found that interleukin-18 (IL-18) and transforming growth factor-beta1 (TGFb) are the most abundantly produced cytokines in core lesions of the ischemic rat brains whose middle cerebral artery transiently occluded for 90 min. It is plausible that these abundant cytokines infiltrate into the adjacent peri-infarct tissue and act on glial cells. It has been shown that TGFb induces expression of NG2 chondroitin sulfate proteoglycan (NG2) by primary cultured microglial cells also to stimulate their migration activities. In fact, NG2+ resident microglial cells are located in the peri-infarct tissue and they appeared engaged in elimination of degenerated neurons. In this study, we particularly addressed the actions of IL-18 on glial cells in the peri-infarct tissue. To examine the response of glial cells in vitro, we used mixed glial cultures started from the neonatal rat forebrains. The culture contained astrocytes, microglial cells and NG2 glial cells (or oligodendrocyte progenitor cells) but not neurons. When mixed glial cultures were incubated with IL-18, mRNA expression that encode type I interferons, nestin, NG2 and hepatocyte growth factor (HGF) was increased in a dose-dependent manner. These results suggest a possibility that IL-18 may be at least partly involved in activation of glial cells and/or glial scar formation in the ischemic brains. No COI.

3P-115

Treadmill exercise as rehabilitation for stroke model; its effects through increased serum corticosterone level

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Although rehabilitation may be the most effective therapy for stroke, mechanisms underlying the curative effects are not fully elucidated. We addressed the short-term effects of light treadmill exercise on ischemic brain edema and neurological dysfunction. Wistar rats were subjected to transient (90min) right middle cerebral artery occlusion (MCAO) that produced large stroke lesion. The area of the lesion was measured with magnetic resonance imaging (MRI) on the next day or 1 day-post reperfusion (1dpr), and only rats with substantially large ischemic lesion were grouped into exercise and non-exercise ones. Treadmill was horizontally set and its speed was at 4–6m/sec and the rats ran only for 10 min/day at 2, 3, and 4 dpr. On the 5dpr, the brain lesions were again examined with MRI. The severity of brain edema was evaluated by dividing the volume of the right hemisphere by that of the left one. Consequently, the light exercise was significantly reduced brain edema and ameliorated the motor function that was evaluated one month after MCAO. The ameliorating effect of the exercise was abolished when anti-glucocorticoid agent mifepristone or anti-mineralocorticoid agent spironolactone. Orally administered low dose of corticosterone suppressed the brain edema in the non-exercise group rats. These results show that the forced light exercise controlled brain edema by enhancing secretion of adrenal corticosterone. No COI.

3P-116

The light treadmill exercise after the cerebral infarction controls cellular edema: the involvement of aquaporin 4 and sodium/hydrogen exchanger 1

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We have recently found that light treadmill exercise ameliorates brain edema using a rat stroke model, in which the right middle cerebral artery of a male Wistar rat was transiently occluded for 90 min. The amelioration of brain edema may be related to the increased level of blood corticosterone. We investigated expression of mRNA encoding aquaporin 4 (AQP4) and sodium/hydrogen exchanger 1 (NHE1) in the three parts of cerebral cortex that were contralateral, peri-infarct and ischemic core tissues. AQP4-mRNA was much increased in the peri-infarct tissue and NHE1-mRNA in the peri and core tissues. Light treadmill exercise suppressed these mRNA expressions. Forced treadmill exercise has been shown to increase blood corticosterone level. In vitro study suing rat primary mixed glial culture showed that corticosterone at 10nM but not 100nM suppressed the expression of AQP4- and NHE1-mRNA. Anti-glucocorticoid or anti-mineralocorticoid agents partly prevented the corticosterone-induced enhanced expression of the mRNA expression. These results suggest that treadmill exercise suppress the influx of sodium ion and the subsequent water into the cytoplasm of glial cells by suppressing expression of AQP4 and NHE1, leading to amelioration of edema of the stroke brain. We are currently conducting in vitro experiments to determine whether NHE1 is actually involved in cellular edema using C6 glioma cells. No COI.

3P-117

Role of glutamate transporters in functional compensation at the intact hemisphere contralateral to a stroke

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After ischemic brain stroke, the corresponding area contralateral to the lesion may partly compensates for the loss of function. However, the underlying processes in the contralateral hemisphere after stroke have not yet been fully elucidated. Recent studies have shown that astrocytes may play critical roles in synaptic reorganization and functional compensation after a stroke. Thus, we aim to clarify the contribution of astrocytes using a mouse stroke model. In vivo Ca2+ imaging showed a significantly large number of astrocytes in the contralateral SSC responding to ipsilateral limb stimulation at the first week after infarction. Simultaneously, extracellular glutamine level increased, indicating the involvement of astrocytes in the conversion of glutamate to glutamine. Such process may be important for functional recovery. This hypothesis was supported further by the observation that application of TFB-TBOA, a glial glutamate transporter blocker, disturbed the functional recovery. These findings indicate the involvement of astrocytes in functional remodeling/recovery in the area contralateral to the lesion. Our study has provided new insights into the mechanisms underlying synaptic remodeling after cerebral infarction, especially, critical role of the glutamate transporters on functional recovery after stroke. This time, we present the recent results for activating the astrocyte and glutamate transporters using several drugs. No COI.

3P-118

Pre-conditioning exercise upregulated superoxide dismutase activity and prevented sensori-motor dysfunction after brain ischemia in rats

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We investigated pre-conditioning exercise can enhance antioxidant activity and ameliorate motor dysfunction following transient middle cerebral artery occlusion (MCAO). Male Wistar rats (5 weeks old) were assigned to 4 groups: Sham (n = 6), Ex + Sham (n = 6), MCAO (n = 8), and Ex + MCAO (n = 12). Ex + Sham and Ex + MCAO were forced to run on a motorized treadmill at a speed of 15 m/min for 30 min every day for 3 weeks. MCAO and Ex + MCAO were done by a 90 min left-MCAO using an intraluminal filament after 3 weeks. 24 hours after the surgery, animals were assessed for neurological deficits (ND), ladder test, limb placing test (LP) and beam walking test (BW). Then, superoxide dismutase (SOD) activity of all groups (Ex + Sham: n = 6, Sham: n = 6, MCAO: n = 7, Ex + MCAO: n = 7) was quantified to evaluate antioxidant enzyme activity. Left somato-sensory cortex was removed and assayed SOD activities using SOD assay kit- WST (Dojindo, Kumamoto, Japan). ND, ladder test, and LP were improved by 3 weeks exercise, but BW was not significantly improved. SOD activity of Ex + MCAO was higher than MCAO. These data suggest pre-conditioning exercise attenuates motor dysfunction followed MCAO. These results might be closely associated with reduction of oxidative brain damage via the protective effect of SOD activity. No COI.

3P-119

Role of K -Cl cotransporter(KCC2) for neural reorganization after cerebrovascular disease

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KCC2 has been known to play an important role in neural circuit formation through the regulation of intracellular chloride concentration. Moreover, down-regulation of KCC2 has been observed in immature and injured neurons, which results in an excitatory response from GABA transmission (Moorhouse, A.J. and Nabekura, J; ISBN,2011). My research is focused on understanding how changes in KCC2 expression are associated with the recovery phase after stroke. This is especially important since the physiological significance of KCC2 expression levels on behaviour is not known yet. To elucidate the possible role of KCC2, we generated Tet-off transgenic mice whose KCC2 gene expression is under the control of the calcium calmodulin dependent protein kinase 2 and tetracycline inducible system. When comparing these transgenic mice to control mice in a model of cerebral ischemia we found differences in cerebral infarct size in the acute phase and motor paralysis in the recovery period. In this session I will discuss the role of KCC2 reduction after cerebral infarction. In the future, I would like to study the possible role of KCC2 in neuronal plasticity. No COI.

3P-120

Suppression of draining lymph node metastasis of oral squamous cell carcinoma by inhibition of the factors secreted from primary tumor site at pre-metastatic phase

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Progression of oral squamous cell carcinoma largely depends on the metastasis on to draining lymph node. Since the metastasis toward draining lymph node precedes systemic metastasis in many cases, suppression of this metastasis is expected as a promising strategy for prevention of this cancer progression. Lymph node metastasis has long been understood as a result of filter function in lymph nodes for lymph fluid, and the molecular events concerns with this metastasis attracted less attention than pre-metastatic events in other organs such as lung.We have found pre-metastatic tissue reorganization in draining lymph nodes assumed as pre-metastatic niche formation, by establishing metastasis model by xenografting highly metastatic human squamous cell carcinoma cell line SASL1m, toward KSN nude mice tongue. Moreover, we observed secretion of the factors including TGFbeta1 from pre-metastatic primary tumor site induces CD31-positive blood vessel-like structure in draining lymph node.Now we are on the way to assess the effects of inhibition of secreted factors from SASL1m primary tumor on to lymph node metastasis, and would like to discuss as to the possibility of anti-metastatic therapy No COI.

3P-121

In vitro effects of nicorandil on lower esophageal sphincter tone in rats

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The lower esophageal sphincter (LES) is a specialized region of the esophageal circular smooth muscle that allows passages of a swallowed bolus to the stomach. Functional disorder of an esophagus such as achalasia displays a diminished peristalsis in lower esophagus. It has been reported that nitric oxide plays a major role in LES relaxation. However, the detailed mechanism involved in the regulation of LES activity is still elusive. Nicorandil possesses dual properties of a nitrate and K^+ATP channel agonist. We hypothesized that nicorandil reduced the LES contraction by NO and/or K^+ channel dependent mechanism(s). The aim of this study was to investigate the effects of nicorandil on LES tone in the rat isolated LES preparation contracted with carbachol or high concentration of external K^+ (50 mM). LES tissues of rats were placed in a standard organ bath and activities were recorded using the software Chart Pro v 4.0. After contraction with carbachol, nicorandil was added directly to the tissue bath in cumulatively increasing concentrations, resulting in a significant relaxation of the LES in a concentration-dependent manner. On the other hand, nicorandil failed to cause a relaxation when the LES tissues were contracted with high K^+ solution. RT-PCR revealed that Kir6.1, Kir6.2, SUR1 and SUR2B subunit, which compose K^+ATP channel, were expressed in rat lower esophagus. These findings suggest that nicorandil has a suppressor effect on LES contractions associated with K^+ATP channel function. No COI.

3P-122

Oxidative stress in rats with monocrotaline-induced pulmonary hypertension

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Monocrotaline (MCT) causes pulmonary hypertension (PH) and/or acute hepatic injury in a dose-dependent manner. For PH model, numerous investigators have studied mainly on the cardiopulmonary changes in rats with a single standard dose of MCT (60mg/kg). We recently reported that MCT-PH in rats accompanied marked CO production in the liver, suggesting MCT-induced hepatic injury were associated with ROS. However, pathophysiological roles of oxidative stress in the MCT-PH rats have not been established. The present study examined alterations of oxidative stress in MCT rats. Based on the standard methods for PH studies, MCT-PH and control rats (male SPF-SD) were made. Three weeks later, animals under anesthetics and mechanical ventilation were subjected to the measurements of plasma BAP and d-ROM, and of urine 8-OHdG/creatinine ratio. In MCT rats, urine 8-OHdG/creatinine ratios were increased and plasma BAP/d-ROM ratios (latent anti-oxidant potential) decreased. These results indicated that oxidative stress were involved in rats with MCT-induced PH. We will also present the localization of oxidative stress by the immuno-histochemical studies and discuss the association with the CO production in the liver. No COI.

3P-123

Anti-inflammatory effects of dexamethasone on microglia/macrophages: inhibition on JAK/STAT pathway

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Activated microglia may be one potential cause of neuronal death in various neurological disorders. This is at least partly due to their production of pro-inflammatory mediators. Glucocorticoids have been shown to suppress such harmful reactions of microglial cells on neurons very effectively. Although the anti-inflammatory effects of glucocorticoids have been believed to exert through their binding to glucocorticoid receptor (GR), some literatures show GR-independent effects of glucocorticoids. We have recently found that a synthetic glucocorticoid dexamethasone (Dex) suppresses phosphorylation of signal transducer and activator of transcription1 (STAT1) in rat primary microglia and peritoneal macrophages that were incubated with rat recombinant interferon gamma for 15 or 30 min. It may be presumable that Dex elicits the inhibitory effect on JAK/STAT pathway through a way independent of GR. We have prepared microglial cells/macrophages that are devoid of GR in order to investigate GR-independent effects of Dex using siRNA. Our preliminary results show that Dex can suppress phosphorylation of STATs in the absence of GR in the siRNA-treated microglial cells. Dex completely suppressed LPS-induced NO production by macrophages, and the suppression was still partly observed even in the siRNA-treated cells. The present study might reveal the effects of glucocorticoid that are independent of GR. No COI.

3P-124

Activated microglia in the substantia nigra pars reticulata of rats with 6-OHDA-induced Parkinsonism

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Injection of 6-hydroxy dopamine (6-OHDA) into the substantia nigra causes Parkinson's disease symptoms such as bradykinesia and rigidity. 6-OHDA injection also caused activation of glial cells as well as dopaminergic neuronal degeneration in the substantia nigra pars compacta (SNpc). In this study, we have conducted immunohistochemical analyses on glial cell reactions with special emphasis on microglial cells. Microglial cell activation was observed not only in the SNpc but also the SN pars reticulata (SNpr), where no neuronal death was observed. Microglial cells in the SNpr were characterized with the expression of CD68, a marker for phagocytes, suggesting that the cells may be engaged in phagocytosis. Immunoblotting study using anti-synaptophysin antibody showed the decrease of synapses in the SN. Furthermore, quantitative real-time RT-PCR demonstrated the decrease in the level of mRNAs encoding glutamate receptors, NR2D (one typ2 of NMDA receptors), mGluR1 and mGluR4. Taken the observation that microglial cells sometimes eliminate synapses through phagocytosis. CD68+ activated microglial cells in the SNpr may be involved in suppression of glutamate-dependent neurotransmission, while suppressing the over-activation of GABArgic neurons and preventing the manifestation of the neurologic symptoms. No COI.

3P-125

An outdated hypnotic bromvalerylurea ameliorates murine model of dermatitis

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Bromvalerylurea (BU) is an outdated hypnotic/sedative with a molecular weight 223. Recently, we have found that BU suppressed nitric oxide (NO) production by LPS-stimulated microglia/macrophages. The inhibitory effect may be mediated through inhibition of phosphorylation of STAT1. In this study, BU was used to treat murine models of atopic dermatitis and contact dermatitis; the former was induced with an ointment conatinig dead mite bodies and the latter with 2,4,6-trinitro-1-chlorobenzene (TNCB). BU was dissolved in vaseline at 1 % w/w and spread over the back skin (atopic model) or the ear skin (TNCB model). BU ointment ameliorated erosion and bleeding in the atopic model and decreased the thickness of the ears of the TNCB model within 5 h after the first treatment. Immunoblotting study has revealed that BU ointment suppressed the phosphorylation of JAK1 and STATs 1, 5 and 6 in the skin samples of TNCB models that was prepared 1 h after the spread of the ointment. Furthermore, BU ointment increased SOCS expression in the skin. These data show that BU with a small molecular size rapidly infiltrates into the skin and ameliorates dermatitis by suppressing phosphorylation of STATs. No COI.

3P-126

An outdated hypnotic bromvalerylurea as a novel agent for sepsis: the inhibitory action on JAK/STAT pathway

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Bromvalerylurea (BU) is an outdated hypnotic/sedative and currently is rarely used in clinics. Recently we found that BU suppressed nitric oxide (NO) production by LPS-stimulated microglia. In this study, BU was used to treat a rat model of sepsis induced by cecum-ligation and puncture (CLP). Peritonitis progressed rapidly with marked swelling of the ileum 24 h after CLP. Measurement of serum interleukin (IL)-6 protein levels in septic rats by ELISA was used as an index of severity of sepsis, and was elevated 20 fold. The increase of serum creatinine levels and deterioration of arterial blood gas data in septic rats suggested they had developed multiple organ failure as a consequence of sepsis. The mortality rate of sepsis rats was 80% 1 week after CLP. Progress of these fatal symptoms was markedly inhibited by subcutaneous injection of BU twice/day, resulting in the decrease mortality by 40 % at 1 week. BU suppressed the phosphorylation of STAT1 when peritoneal macrophages (PMs) were incubated with interferon-gamma (IFNg). When PMs were knocked-down of their JAK1 or STAT1 expression by the use of respective siRNA, the effects of BU were abolished. These data indicate that BU prevented the progress of systemic inflammation through the inhibition of IFNg-induced JAK1/STAT1 activation that may lead to the systemic inflammation. No COI.

3P-127

Celastrol inhibits interleukin-17A-stimulated rheumatoid fibroblast-like synoviocyte migration and invasion

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Interleukin-17A (IL-17A)-induced migration and invasion of fibroblast-like synoviocytes (FLSs) is critical for the pathogenesis of rheumatoid arthritis (RA). More than 30% of RA patients are resistant to available therapies, despite the introduction of novel biologic agents. Therefore, it is necessary to develop new anti-arthritic agents. Recent studies have demonstrated that celastrol has anti-arthritic activity in an adjuvant-induced arthritis (AIA) model. However, the effect and molecular mechanisms of celastrol on the migration and invasion of RA-FLSs are not yet understood. Results showed that treatment of RA-FLSs with celastrol suppressed the IL-17A-induced migration and invasion abilities of the cells. In addition, celastrol inhibited IL-17A-induced matrix metalloproteinase (MMP)-9 mRNA and protein expression, and the proteolytic activity of MMP-9 in RA-FLSs. Furthermore, our results revealed that celastrol inhibited the transcriptional activity of MMP-9 by suppression of the binding activity of nuclear factor-κB (NF-κB) in the MMP-9 promoter, and inhibited IκBα phosphorylation and nuclear translocation of NF-κB. In conclusion, celastrol can inhibit IL-17A-induced migration and invasion by suppressing NF-κB-mediated MMP-9 expression in RA-FLSs. These results provide a strong rationale for further testing and validation of celastrol as an adjunct with conventional drugs for the treatment of RA in humans. No COI.

3P-128

Roles of a transcription factor interferon regulatory factor 8 (IRF8) in proinflammatory reactions of macrophages and microglia

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Interferon regulatory factor 8 (IRF8) is belonging to IRF family proteins that mediate signals originating form type I interferon. IRF8 has been implicated in the differentiation process of myeloid lineage cells into monocytes and macrophages. However, the roles of IRF8 in the functions of macrophages and related cells have not well documented, compared to other IRF proteins. Recently, it has ben shown that IRF8 plays a critical role in the transmission of pain in the spinal cord by controlling microglial functions. In this study, we are addressing the roles of IRF8 in proinflammatory reactions of macrophages and microglial cells. Macrophages were isolated from the lungs (alveolar macrophages; AMs) and the peritonea (peritoneal macrophages; PMs) of Wistar rats. Microglial cells were obtained from rat primary mixed glial cultures. Total RNA was prepared from AMs, PMs and microglia and reverse transcribed to cDNA. mRNA encoding IRFs were quantitatively analyzed using quantitative real-time RT-PCR. Although the three types of cells expressed IRF8-mRNA significantly, the level was less than those of IRF1, 3 and 7 mRNA. When IRF8-mRNA was knocked down by the use of siRNA and IRF8 protein was actually reduced, LPS-induced NO production was reduced in PMs and microglia. By contrast, AMs increased NO production. These results suggest that IRF8 play distinct roles in different cell types. We are currently investigating how IRF8 is involved in the proinflammatory reactions. No COI.

3P-129

Iba-1 positive macrophage is activated in the anterior pituitary in the chronic inflammatory pain model rat

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Non-hormonal cells, such as a macrophage and a folliculostellate cell, exist in the anterior pituitary lobe (AP), however, the function of these cells remains largely unclear. In this study, we examined non-hormonal cell responses to chronic pain using two chronic pain models: CFA induced chronic inflammatory pain model and a chronic neuropathic pain model (Seltzer model). We determined the Non-hormonal cells proportion and morphology using anti Iba-1 antibody (marker of pan macrophages) and anti S-100 antibody (marker of folliclostellate cell). We revealed that Iba-1 positive macrophage occupied 3% of proportion of the total AP cells in the adult intact male rat. In the chronic inflammatory condition, Iba-1 positive cell proportion was increased and the cells morphologically changed: more rounded soma with enhanced lamellipodia. These changes were not observed in the chronic neuropathic pain model rat. We also determined the S100 positive folliculostellate cell as well, but cell proportion and morphology were no difference among that of a control group and two chronic pain models. Furthermore, we revealed that IL-1β positive cell was increased in the AP in the chronic inflammatory pain rat, and most of IL-1β immunoreactive cell (89%) merged Iba-1 immunoreactive cell. These results indicate that the chronic inflammatory pain had activated Iba-1 positive macrophage in the anterior pituitary, which synthesized IL-1β modulating pain sensation. No COI.

3P-130

Oct-3/4 induces CpG demethylation in MGMT promoter to acquire temozolomide resistance in glioblastoma cells

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Temozolomide is the most effective cytotoxic alkylating agents used for malignant gliomas. A cellular DNA-repair enzyme, MGMT, reverses alkylation at the O6 position of guanine, thereby the expression level of MGMT is closely related to the sensitivity of brain tumors to Temozolomide. MGMT expression is controlled by a methylation/demethylation of the CpG islands in the promoter region of MGMT gene. Oct-3/4, a self-renewal regulator in stem cells, has been known to express on various kinds of solid tumors including glioblastoma, and has been involve in tumor progression, malignancy in glioblastomas. Therefore, in the present study, we investigated whether Oct-3/4 involves in the sensitivity of temozolomide through the expression of MGMT. Compared with control cells, Oct-3/4 overexpression resulted in decreased susceptibility to temozolomide, assessed by LDH assay. In Oct-3/4-expressing cells, expression of MGMT mRNA was upregulated by qPCR analysis. The methylation status of 27 CpG sites within the MGMT promoter was analyzed by genomic sequencing of bisulfite-modified DNA. Oct-3/4-expressing cells showed enhanced demethylation of CpG islands. These results suggest that Oct-3/4 promotes to acquire temozolomide-resistant phenotype of glioblastoma cells by upregulation of MGMT expression through the epigenetic change of MGMT promoter region. We also investigate the effect of Sox-2, an essential gene for stemness, against the epigenetic regulation of MGMT gene. No COI.

3P-131

Role of plasminogen activator inhibitor-1 in the development of insulin resistance and osteoporosis in obese female mice

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We previously demonstrated that plasminogen activator inhibitor-1 (PAI-1), an inhibitor of fibrinolysis, is involved in type 1 diabetic bone loss in female mice. PAI-1 is well known as an adipogenic factor induced by obesity. We therefore examined the effects of PAI-1 deficiency on bone, glucose and lipid metabolism in high fat and high sucrose diet (HF/HSD)-induced obese female mice. Female wild-type (WT) and PAI-1-deficient (KO) mice were fed with HF/HSD or normal diet for 20 weeks from 10 weeks of age. HF/HSD increased the levels of plasma PAI-1 in WT mice. PAI-1 deficiency improved glucose intolerance, insulin resistance and hyperlipidemia induced by obesity. Bone mineral density (BMD) at trabecular bone as well as the levels of osteogenic genes in tibia were decreased by HF/HSD in WT mice, and those changes by HF/HSD were not affected by PAI-1 deficiency. HF/HSD increased the levels of plasma tumor necrosis factor-alpha (TNF-α) both in WT and PAI-1 KO mice, and the levels of plasma TNF-α were negatively correlated with trabecular BMD in tibia of female mice. In conclusion, we demonstrated that PAI-1 deficiency does not affect the trabecular bone loss induced by obesity despite the amelioration of insulin resistance and hyperlipidemia in female mice. Thus, the changes of BMD and bone metabolism by obesity might be partly through TNF-α and independent of PAI-1, glucose and lipid metabolism. No COI.

3P-132

Voluntary Exercise Suppresses Development of Diabetes Mellitus and Improves Exercise Performance in OLETF Rats

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The aim of this study was to examine whether voluntary wheel-running (WR) suppress development of diabetes mellitus (DM) and improve exercise performance (EP) in a type 2 DM model rat OLETF. Five-week-old OLETF rats were housed either in cages equipped with wheels (OLETF-WR) or in standard cages (OLETF-SED) for more than 1 year. Eleven-month-old rats underwent an oral glucose tolerance test (OGTT) and an examination of HbA1c. Fourteen-month-old rats underwent 10-day voluntary WR, treadmill performance, hanging and grip-strength tests. There was a negative correlation between total WR distance and body weight in OLETF-WR. In OLETF-SED, blood glucose (BG) increased after the age of 33 weeks, while BG in OLETF-WR and LETO remained unchanged throughout the experimental period. In OGTT, BG of OLETF-WR and LETO with normal HbA1c levels, returned to normal level 2 hours after glucose administration, whereas BG of OLETF-SED with higher HbA1c levels, remained high. LETO and OLETF-WR showed the highest and moderate EP in treadmill performance, hanging and grip-strength tests, respectively, while OLETF-SED diabetic rats the lowest EP. Thus, long-term voluntary WR could suppress DM development and improve EP in OLETF rats. No COI.

3P-133

Secondary Structure and Thermal Stability of FcαRI β Chain Polymorphism (L172I, L174V, E228G)

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The β chain of the high affinity IgE Fc receptor (FcεRI) acts during an allergic reaction as a signal amplifier in mast cells. Genetic polymorphisms that result in three amino acid changes in FcεRI β chain have been identified as candidates that associate with allergic reaction. We have investigated the structure of mouse FcεRI β chain wild type (aa 143-235) and polymorphisms (L172I, L174V and E228G). The far-UV CD spectra of the β-wild type (WT) and polymorphisms are indicative of an α-helical protein and polymorphisms do not have any loss or collapse of α-helical content. We investigated the transition curve for thermal denaturation of β-WT and β-polymorphisms, and calculated the Gibbs free energy (ΔG). ΔG values of β-L172I and β-L174V are almost the same as that of β-WT, however, ΔG value of β-E228G is lower that that of β-WT. These data suggest that β-E228G affects the thermal stability of FcεRI β chain. β-E228G may play some sort of roles in allergic reactions via FcεRI β chain in mast cells. No COI.

Poster Presentations
Oral Physiology (3)

3P-134

Gustatory neuron profiles in rat reticular thalamic nucleus

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Department of Pharmacology, Nihon University School of DentistryThe parvicellular portion of the ventroposteromedial nucleus of thalamus (VPMpc) relays gustatory information from the parabrachial nucleus to the gustatory insular cortex (GC). In addition, the VPMpc projects to the reticular thalamic nucleus (RTN), which also receives excitatory inputs from layer VI neurons in the GC. The principal neurons in the RTN are GABAergic, and they project to the VPMpc, and therefore, the VPMpc, RTN, and GC constitute a feedback circuit to process gustatory information. However, the neural responses of the RTN neurons to gustatory stimulation have not been well understood. The present study aimed to examine (1) which region of the RTN connects to the GC, and (2) the neural profiles of RTN neurons in response to gustatory stimuli. An anatomical experiment using a retrograde tracer, FluoroGold, showed that the rostroventral part of the RTN (RTNrv) received projection from the GC. A part of neurons in the RTNrv responded to gustatory stimulation to the oral cavity: ~15% and ~8% of neurons increased and decreased their firing frequency, respectively. We could not find the relationship between the spontaneous firing frequency and the change of frequency by gustatory stimuli. These results suggest that neurons in the rostroventral part of the RTN neurons receive gustatory information, and likely to modulate neural activities in the VPMpc. No COI.

Poster Presentations
Physical Fitness, Sports Medicine

3P-135

Changes of intensity and palatability by mixing taste solutions

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There are small studies to evaluate intensity and palatability for the mixed taste solutions in human. In this study, we investigated how human subjects evaluated them by using the Labeled Magnitude Scale (Green et al., 1993) and the Labeled Hedonic Scale (Lim et al., 2009).A total of 30 males evaluated intensity and palatability for some taste solutions. Following solutions were used as taste stimuli; 0.3–10mM saccharin Na(Sacc), 0.03–1.0M NaCl(Na), 0.1–0.3mM QHCl(Q), the mixed taste solutions of 5mM Sacc and one of 0.03–1.0M Na, and the mixed taste solutions of 5mM Sacc and one of 0.1–0.3mM Q. As results; there was significant main effect for salt or bitter intensity between pure Na or Q, and mixed solutions with Sacc. When Sacc was mixed with Na or Q, palatabilities for mixed solutions were higher than those for pure solutions. These results suggest that mixing of different type of taste stimulus changes intensity and palatability for original taste stimulus. No COI.

3P-136

High concentration CO_2-water bath may reduce the muscle hardness and soreness after resistance exercise

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The sedative effect on sympathetic nerve function of CO_2-water bath may imply a possibility of the facilitation of muscle fatigue recovery. We investigated whether the immersion of extremities including agonist muscles into artificially made CO_2-water (CO_2 > 1000 ppm) influences recovery of muscle hardness and soreness in fatigue after resistance exercise in twelve male subjects (Ss). On the first day of experiment, Ss performed 100 times calf raise exercise and immersed lower legs into tap-water or CO_2-water (35 ℃, for 10 min) after the exercise. Hardness of the gastrocnemius was determined by indentation method at pre-exercise, immediately after exercise, and after 10 min recovery. The muscle soreness was assessed using a visual analogue scale. The leg bath was performed every 24h for 5 consecutive days after the exercise day, and the muscle parameters were measured before and after the leg bath each day. A same subject was tested with another bath-water after a series of measurement. Maximum muscle hardness lasted for 3 days after exercise irrespective of the kind of bath-water, then decreased gradually to base line. In CO_2-water immersion compared with tap-water immersion, muscle hardness decreased quicker. Muscle soreness in tap-water peaked at the second day, and then reduced gradually. In CO_2-water case, muscle soreness disappeared at the third day. The present results suggest that bathing into artificial high-concentration CO_2-water may contribute to a recovery from the muscle fatigue. No COI.

3P-137

LDL-C / HDL-C ratio may be a factor of cardiac sudden death during exercise

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[Introduction] Increasing in the Plasminogen activator inhibitor - 1 (PAI-1) level may be a factor of coronary artery disease. Coronary artery disease is one of the factors of cardiac sudden death during exercise. The aim of this study was to evaluate whether a difference in the LDL-C / HDL-C ratio affects the PAI-1 level during acute strenuous exercise.
[Subjects and Methods] Thirteen healthy trained men aged 19 to 23 years participated in this study: 7 of these were categorized in the LDL-C / HDL-C ratio < 2.0 group (L/H < 2.0 group), and 6 in the LDL-C / HDL-C ratio > 2.0 group (L/H > 2.0 group). Venous blood samples were collected from the subjects before and after they performed the Cooper 12-min test (running as far as possible within 12 min). LDL-C concentrations (mg / dL), HDL-C concentrations (mg / dL), PAI-1 level (ng / mL) were measured by using blood samples.
[Results] LDL-C / HDL-C ratio was significantly higher in L/H > 2.0 group (2.3 ± 0.07) than in the L/H < 2.0 group (1.2 ± 0.07; $P < 0.05$). PAI-1 level before (30.4 ± 1.9 ng / mL) and after (25.8 ± 3.4 ng/mL) exercise did not change significantly in the L/H < 2.0 group, whereas it increased significantly in the L/H > 2.0 group (41.6 ± 10.0 ng/mL to 61.8 ± 11.7 ng/mL; $P < 0.05$).
[Conclusions] PAI-1 level increased after strenuous exercise in high LDL-C / HDL-C ratio group. Consequently, the present study suggested that LDL-C / HDL-C ratio may be a factor of cardiac sudden death during exercise. No COI.

3P-138

Exercise training improves peripheral vascular function in type I diabetic mice as measured by X-ray microangiography

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We investigated whether exercise training improves hindlimb blood flow and endothelial function in streptozocin induced type I diabetic mice.Mice were divided into the sedentary and exercise training groups 2 weeks post streptozocin injection (200 mg/kg). The exercise training was performed with treadmill level running for 60 min/day, 5 days/week, for 4 weeks. Thereafter, 1) in vivo hindlimb vascular imaging using X-ray microangiography, or 2) femoral blood flow measurement using transonic flow probe were performed.The mice were anesthetized with urethane - α-chloralose, then tracheostomized and cannulated into carotid artery and jugular vein for blood pressure monitoring and acetylcholine (ACh) administration, respectively. An additional catheter was inserted into the femoral artery for injection of iodinated contrast agent, or the flow probe was placed around the femoral artery.The mice hindlimb arteries (inner diameter: 50 to 200 μm) were clearly visualized by X-ray imaging, furthermore dynamic changes in vessel diameter in response to ACh were observed. The exercise training improved the ACh-induced vasodilation in diabetic mice, and the effect was mostly found in the smaller arteries (<100 μm). However, ACh-induced increases in femoral blood flow were not significantly different between diabetic and exercise groups. These data suggested exercise training has potential to ameliorate the endothelial vasodilator dysfunction of the peripheral skeletal muscle arteries in diabetes. No COI.

3P-139

Effectiveness of autonomic control of the circulation is enhanced with increased plasma volume after intense exercise

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We assessed whether increased plasma volume (PV) enhances the effectiveness of autonomic control of the circulation after intense exercise. **METHODS**: Seven healthy men (~23 yrs) underwent two trials those of which differ in the timing of post-exercise intake of macronutrients. In both trials, subjects kept seated rest for baseline (BL), then performed a 72-min intense exercise (8 sets of 4 min at 80% VO_{2max} + 5 min at 20% VO_{2max}) and kept seated rest during 0-5th and 22–23rd hour of recovery. They took protein and carbohydrate (3.2 kcal and 0.18 g protein/kg) just after exercise (0H) or 2 hours later (2H). Percent change in PV from BL (δ%PV) was determined and beat-to-beat blood pressure, R-R interval, heart rate, and stroke volume (SV) were measured during 5-min spontaneous and fixed rate (0.2 Hz) respiration at BL and every hour during recovery phases. Dynamic arterial-cardiac baroreflex sensitivity (BRS) was calculated by sequence analysis. **RESULTS**: δ%PV in 0H was higher than 2H at 5th and 23rd hour of recovery ($P < 0.05$). There was no difference in BRS between trials, while SV and effective arterial-cardiac baroreflex gain estimated as BRS x SV were higher in 0H than 2H at 5th and 23rd hour of recovery ($P < 0.05$). **CONCLUSIONS**: Increased PV enhances the effectiveness of autonomic control of the circulation with an increased SV after intense exercise. No COI.

3P-140

Effects of the gum chewing on cardiac autonomic balances and anxiety levels during the origami competition in the young people.

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We aimed to explore effects of the gum chewing on cardiac autonomic balances with using HRV during the origami competition (origami C) in 13 male and 5 female students. Subjects were paired to balance to each other in origami skills. Each pair repeated the origami C on two different days. One of each pair was the first gum-chewer and the other was the second. Numbers of the first and the second gum-chewers were balanced between both. Subjects were explained about the experimental flow.During the 10 min resting period, a pair sat on chairs directed in parallel with each other and saw their own scenery photo on the room wall. The gum chewer chewed during the latter half of this time and threw out it at the end of this period . Then, they corrected the sitting direction to face each other and played origami C for 5min. A questionnaire of the STAI (A-state) was answered after the explanation, and after the experiment, too. Gum chewing did not improve the performance of the origami and winners were the same in 7 of 9 pairs. The parasympathetic activity assessed by HF was significantly reduced in winners with gum chewing ($p<0.05$, n=9), but not in winners without gum ($p=0.23$, n=8). The gum chewing has significantly reduced anxiety scores of the A-state, in both loser and winner. Present results may mean that gum chewing could lead the mind to concentrate on the competition, particularly in the advantageous case. No COI.

3P-141

Combined effects of hyperthermia and mental fatigue on endurance exercise capacity in the heat

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The aim of the present study was to examine the effects of hyperthermia and mental fatigue on endurance exercise capacity in the heat. Eight male volunteers completed four cycling trials at 80% maximum oxygen uptake until volitional exhaustion in a climatic chamber (30°C, 50%RH). Volunteers cycled after: 90 min seated rest (CON), 90 min of a demanding cognitive task to induce mental fatigue (MF), 30 min immersion in 40°C water to induce hyperthermia following 60 min seated rest (HT), or 30 min immersion in 40°C water with a demanding cognitive task following 60 min of a demanding cognitive task to induce both mental fatigue and hyperthermia (MF+HT). Rectal temperature at the start of cycling was 36.7 ± 0.5 (± SD), 36.8 ± 0.2, 38.1 ± 0.4, and 38.0 ± 0.1°C in CON, MF, HT, and MF+HT, respectively. Exercise time to exhaustion was significantly less in MF+HT than in CON and MF (CON 18 ± 7 min; MF 17 ± 7 min; MF+HT 9 ± 3 min, $P < 0.05$). At the point of volitional exhaustion, rectal temperature, mean skin temperature, heart rate and cutaneous vascular conductance were not different between trials. In conclusion, a combination of hyperthermia and mental fatigue elicits significant reductions in endurance exercise capacity in the heat. No COI.

3P-142

Ubiquitin ligase Nedd4 expression and atrophy in unloaded rat plantaris muscle

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Purpose: It is well documented that two muscle specific ubiquitin ligases, atrogen-1/MAFbx and MuRF1, are involved prominently in the skeletal muscle atrophy. Recent studies have suggested that other ubiquitin ligase, Nedd4 (neural precursor cell expressed developmentally down-regulated protein 4) plays a more encompassing role in denervation-induced muscle atrophy. Therefore, we investigated the involvement of Nedd4 in unloading-induced muscle atrophy with specific focus set on age dependency. Methods: F344 female rats (4-, 10-, and 20-month) were divided into three groups; caged control group, hindlimb-unloaded group, and hindlimb-unloaded and intermittently reloaded group in each age. Rats of reloaded group were exercised on resistance exercise device with a load of 30% of body mass for 3 weeks (30 min/day, 6 days/week). Plantaris muscles were analyzed. Result: Muscle mass and maximum force in caged control group increased from 4-month to 10-month and decreased from 10-month to 20-month. The hindlimb-unloading decreased muscle mass and maximum force at any age, but most prominently in 20-month rats. Significant increase in the expression of Nedd4 was found only in 20-month rats. Intermittent reloading during hindlimb-unloading period inhibited muscle atrophy independent of age. However, the effect of reloading on the Nedd4 expression was not significantly observed even in 20-month rats. Conclusion: The ubiquitin ligase Nedd4 may participate in severe muscle atrophy at least at the old age, although it may not intimately linked with atrophy process. No COI.

3P-143

Abdominal obesity augments the reduction in cough peak flow by supination in middle-aged and elderly women

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The aim of the present study was to examine whether abdominal obesity and the different posture, sitting or supination, affected on the cough capacity in middle-aged and elderly women. Method: Twenty-four middle-aged and elderly women consisted of 10 with (obese group) and 14 without (non-obese group) abdominal obesity were included in this study. In all subjects, we evaluated the changes in vital capacity (VC) and cough peak flow (CPF) in two different positions, sitting and supination. Results:CPF as well as VC were significantly lower in supination than in sitting in both groups (280.3±67.0 vs. 313.1±70.2 L/min in obese group, 298.1±82.1 vs. 316.8±77.4 L/min in non-obese group, $p<0.05$). Although no difference was found in VC or CPF between the groups at each position, the changes in both VC and CPF by postural change were greater in obese group than in non-obese group (5.2±4.4% vs.3.7±5.3% in VC, 10.7±3.9% vs. 7.0±5.6% in CPF, $p<0.05$). In obese group, the changes in CPF was positively correlated with waist circumference ($r=0.63$, $p<0.05$). Conclusion:Our results suggested that, in the subjects with abdominal obesity, cough capacity was affected more markedly by taking supination, because their diaphragm resistance was further increased in supination due to stuffed abdominal cavity with excessive fat contents. No COI.

3P-144

Post-transcriptional suppression of lipopolysaccharide-stimulated inflammatory responses by macrophages in middle-aged mice

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The intensities of macrophage inflammatory responses to bacterial components gradually decrease with age. Given that a reduced rate of protein synthesis is a common age-related biochemical change, which is partially mediated by increased phosphorylation of eukaryotic initiation factor-2α (eIF-2α), we investigated the mechanism responsible for the deterioration of macrophage inflammatory responses, focusing specifically on the age-related biochemical changes in middle-aged mice. Peritoneal macrophages isolated from 2-month-old (young) and 12-month-old (middle-aged) male BALB/c mice were stimulated with lipopolysaccharide (LPS). Although LPS-stimulated secretion of tumor necrosis factor-α (TNF-α) by the macrophages from middle-aged mice was significantly lower than that from young mice, LPS caused marked increases in levels of TNF-α mRNA in macrophages from middle-aged as well as young mice. Moreover, LPS evoked similar levels of phosphorylation of c-Jun N-terminal kinase (JNK) and nuclear factor-κB (NF-κB) in young and middle-aged mice. In contrast, the basal level of phosphorylated eIF-2α in macrophages from middle-aged mice was higher than that in macrophages from young mice. Salubrinal, an inhibitor of the phosphatase activity that dephosphorylates eIF-2α, suppressed the LPS-stimulated inflammatory responses in a murine macrophage cell line RAW264.7. These results suggest that post-transcriptional suppression of macrophage inflammatory responses during middle age requires phosphorylation of eIF-2α. No COI.

3P-145

Evaluation of functional reach in aged people by the correlation with the reach and height of young adult people

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The functional reach test is used to measure balance abilities in the clinical field. The functional reach (FR) was correlated with the height in young adult people (Morishita et al., 89th Annual Meeting of PSJ, 2011). In this study, we evaluated FR in aged people by the regression line with the height in young adult people. FR distance was measured in 58 healthy aged participants (58.7±19.1 years old). Rate of FR was calculated by the comparison with estimated FR by the further regression analysis between FR and height in young adult people. The rate of FR was significantly reduced with age ($r=-0.31$, $p < 0.02$). But raw FR distances showed weak correlation with age ($r=-0.23$, $p =0.08$). In the four participants with a fall showed smaller ratio such as 39±17%. Therefore the rate of FR estimated by our regression line may be useful to evaluate balance abilities in aged people. No COI.

3P-146

The naftopidil analogue HUHS1015 induces apoptosis in gastric cancer cells

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The present study investigated the antitumor effect of the newly synthesized naftopidil analogue HUHS1015 (1-[2-(2-methoxyphenylamino)ethylamino]-3- (naphthalene-1-yloxy)propan-2-ol) on human gastric cancer cells. HUHS1015 reduced cell viability in a treatment time (24-48 h)- and concentration (1-100 μM)-dependent manner, the viability reaching almost 0% of control at 100 μM both in MKN-28 and MKN-45 human gastric cancer cells. Cisplatin, an anticancer drug widely used, also reduced viability of MKN-28 and MKN-45 cells in a concentration (1–100 μM)-dependent manner, but to a lesser extent than that for HUHS1015. HUHS1015 significantly increased the number of TUNEL-positive cells as compared with that for cisplatin. Moreover, HUHS1015 apparently increased the population of PI-positive and annexin V-positive cells, which corresponds to late apoptosis/secondary necrosis. Taken together, these results indicate that HUHS1015 exhibits a more beneficial antitumor effect on human gastric cancers than cisplatin. No COI.

Poster Presentations
Cell Physiology, Molecular Physiology (2)

3P-147

Regulatory mechanisms of the G1 to S phase cell cycle progression via changes in the intracellular concentration of Cl^- in MKN28 human gastric cancer cells

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We previously clarified that the reduction of intracellular chloride concentration ($[Cl^-]_i$) inhibits the cell proliferation of gastric cancer MKN28 cells by diminishing the transition rate from G1 to S cell cycle phase. If there are oscillatory changes in the activity of Cl^- transporters and/or Cl^- channels during the cell cycle progression, oscillatory changes of the $[Cl^-]_i$ would also occur. However, mechanisms involved in the modulation of cell cycle progression by Cl^- transporters and/or Cl^- channels are still poorly understood. To clarify the underlying mechanisms, we measured the mRNA and protein expression of Cl^- transporters (NKCC1 and KCC1) and Cl^- channels (CLC-2 and CLC-3). We also analyzed the $[Cl^-]_i$ of the cells in each cell cycle stage (G1, S and G2/M) released from synchronization by using a double thymidine block method. The $[Cl^-]_i$ was reduced in the M phase followed by the elevation of $[Cl^-]_i$ in the G1 and S phase. The protein expression of NKCC1 was low in the M and early G1 phases. However, protein expressions of KCC1, CLC-2 and CLC-3 were unchanged during cell cycle progression. We also tried to clarify plasma membrane expressions of NKCC1, KCC1, CLC-2, and CLC-3. These results strongly suggest that cell cycle-dependent expression of NKCC leading to oscillatory changes of $[Cl^-]_i$ plays important roles in the G1 to S phase cell cycle progression in MKN28 cells. No COI.

3P-148

Intracellular localization of fluorescent glucose derivatives taken up into mammalian tumor cells

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D-glucose is a fundamental energy as well as carbon source for cells. However, its dynamism in cellular uptake is yet to be thoroughly understood at the microscopic level in many organisms. Fluorescent derivatives of D-glucose such as 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-D-glucose (2-NBDG) has been effectively used as a tool to investigate such uptake processes especially in mammals, although their intracellular fate is still unclear. In the present study, we examined intracellular localization of 2-NBDG in mammalian tumor cells by using a confocal microscopy in combination with various markers for organelles. These data may be useful as a basis for understanding phosphorylation, degradation, and extinction of the fluorescent probe. No COI.

3P-149

Regulation of Helix Repeat protein function by S100 proteins -Regulatory mechanism of tumor cell growth by beta-catenin-

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S100 proteins are small calcium binding proteins with "Helix-loop-Helix" structure. In human, more than 25 different S100 proteins have been reported and they showed different targets and physiological functions. Helix Repeat proteins contain repeated helix structures and include HEAT, Armadillo, TPR (Tetratricopeptide repeat), and Ankyrin repeat families. We have previously reported that S100 proteins regulate the activity of protein phosphatase 5 that contains TPR repeat. Here, we report that S100 proteins regulate the function of beta-catenin which contains the Armadillo repeat. In the normal cells, the amount of beta-catenin is kept low by the destruction complex in the cytoplasm. However, the genetic mutations dysregulate the function of destruction complex and increased level of beta-catenin protein enhances the transcription of cell growth related genes resulted in the unlimited tumor cell growth. We found that S100A2 and S100A6 bound to beta-catenin and inhibited the interaction between beta-catenin and BCL9. S100A6 decreased the transcriptional activity of beta-catenin-TCF complex and inhibited the tumor cell growth. Our findings suggest that S100 proteins could regulate the beta-catenin function by inhibiting the transcriptional activity of beta-catenin-TCF complex through BCL9. No COI.

3P-150

Regulation analysis of thioredoxin interacting protein (TXNIP) for the development of new cancer therapies

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Thioredoxin interacting protein (TXNIP) is an anti-tumor protein down-regulated in cancer cells. Molecular analysis concerning the regulation of TXNIP amount could lead to develop novel cancer therapies. We have reported that a monosaccharide D-allose significantly up-regulates the TXNIP expression, resulting in the inhibition of cell cycle progression in various cancer cell lines. Here we elucidated the mechanisms regulating TXNIP amount in hepatocellular carcinoma cell line HuH-7.

We analyzed signaling mechanisms of TXNIP up-regulation caused by D-allose. D-allose transiently activated p44/p42 MAPK pathway. Inhibition of p44/p42 MAPK phosphorylation by PD98059 reduced expression of TXNIP. D-allose also activated p38MAPK, and inhibition of p38MAPK by LY2228820 reduced the expression level of TXNIP. These results suggest that both p44/p42 MAPK pathway and p38MAPK pathway participate in the TXNIP up-regulation caused by D-allose.

We also examined mechanisms of TXNIP decrease caused by serum stimulation. Upon the addition of fetal calf serum, TXNIP rapidly decreases. A proteasome inhibitor lactacystin inhibited this decrease. This result suggests that TXNIP is degraded through the ubiquitin-proteasome pathway upon the serum stimulation. Further molecular analysis and in vivo administration of D-allose would lead us to have better understanding of the new cancer therapies using D-allose. No COI.

3P-151

PPARα modulation of Ca^{2+}-regulated exocytosis in guinea pig antral mucous cells: activation of NOS1 via PI3K/Akt pathway.

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In antral mucous cells, the Ca^{2+}-regulated exocytosis is activated by acetylcholine (1 μM) consisted of an initial transient increase (initial phase) followed by a second slower decline (late phase). Our previous report demonstrated that an autocrine mechanism via PPARα stimulates NOS1 and enhances the initial phase of Ca^{2+}-regulated exocytosis mediated via NO/cGMP accumulation. Inhibition of this autocrine mechanism by inhibitors of PKG, NOS1 or PPARα abolished the enhancement of initial phase and induced a transient increase in the late phase. However, at present, we do not know how PPARα activates NOS1 in antral mucous cells. On the other hand, PPARs activates phosphatidylinositol 3 kinase (PI3K)/Akt pathway in vascular endothelial cells. We examined the effects of an inhibitor of PI3K (50 nM Wortmannin) and an inhibitor of Akt (100 nM Akt 2/2 kinase inhibitor) on the Ca^{2+}-regulated exocytosis in antral mucous cells. Both inhibitors abolished the enhancement of the initial phase stimulated by a PPARα agonist (50 nM GW7647) but transiently increased the late phase. These observations suggest that PPARα stimulates NOS1 mediated via PI3K/Akt pathway leading to an enhancement of mucin release in antral mucous cells. No COI.

3P-152

Ca^{2+}-regulated exocytosis enhanced by AA/PPARα autocrine mechanism in guinea pig antral mucous cells

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In antral mucous cells, acetylcholine (ACh, 1 μM) activates Ca^{2+}-regulated exocytosis, consisting of an initial peak that decline rapidly (initial phase) followed by a second slower decline (late phase) lasting during ACh stimulation. We have reported that the initial phase of Ca^{2+}-regulated exocytosis is maintained by an autocrine mechanism via AA/PPARα in antral mucous cells. However, it still remains uncertain how PPARα enhances Ca^{2+}-regulated exocytosis in antral mucous cells. The aim of this study is to clarify the mechanism following the PPARα activation in antral mucous cells.GW7647 (a PPARα agonist) enhanced the frequency of the initial phase stimulated by ACh. GW6471 (a PPARα blocker) abolished the enhancement of the initial phase induced by GW7647, but produced a delayed and transient increase in the frequency of the late phase. Moreover, a PKG inhibitor or a NOS1 inhibitor abolished the enhancement of the initial phase and induced a delayed and transient increase in the frequency of the late phase, similarly to GW6471. In antral mucosae, ACh or GW7647 increased NO production and cGMP contents. Analysis of Western blotting and immunohistochemistry demonstrated that NOS1 exists in antral mucous cells.Thus, the AA/PPARα autocrine mechanism stimulates NO production via NOS1 and cGMP accumulation, which enhances the initial phase of Ca^{2+}-regulated exocytosis in antral mucous cells. No COI.

3P-153

Involvement of MARCKS phosphorylation by PKCδ in exocytotic amylase secretion in rat pancreatic acinar cells

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In pancreatic acinar cells, stimulation of cholecystokinin (CCK) induces exocytotic amylase secretion. In the amylase secretion, activation of protein kinase C (PKC) is thought to be an essential step. However, regulation of amylase secretion via the activation of PKC is unclear. Myristoylated alanine-rich C kinase substrate (MARCKS) is known as a major cellular substrate for PKC. MARCKS has been implicated in various cellular functions such as motility, phagocytosis, mitogenesis, and membrane trafficking. The MARCKS phosphorylation by PKCδ has been reported in human pancreatic carcinoid tumor cells. Here, we investigated the involvement of MARCKS phosphorylation by PKCδ in rat pancreatic amylase secretion. MARCKS, phosphorylated-MARCKS and PKCδ in the acinar cells were detected by Western blotting. Translocation of MARCKS in the acini was observed by immunohistochemistry. Amylase activity in the medium and the cell lysate was measured by Bernfeld's method. MARCKS protein was detected in rat pancreatic acinar cells. CCK (100 pM) induced MARCKS phosphorylation. CCK had no effect on the total amount of MARCKS. CCK induced PKCδ activation. A PKCδ inhibitor, rottlerin, inhibited the CCK-induced MARCKS phosphorylation and amylase secretion. MARCKS-related peptide as the MARCKS inhibitor inhibited the CCK-induced amylase secretion. These findings suggest that the MARCKS phosphorylation by PKCδ is involved in the pancreatic amylase secretion. No COI.

3P-154

CAFFEINE INHIBITS FLUID SECRETION IN INTERLOBULAR DUCTS ISOLATED FROM GUINEA-PIG PANCREAS

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High concentrations of ethanol and bile acids induce sustained elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in pancreatic acinar and duct cells,which is thought to be related to the pathogenesis of acute pancreatitis. Previous studies reported that caffeine inhibited ethanol- or bile acid-induced $[Ca^{2+}]_i$ elevation in pancreatic acinar cells. In the present study we examined the effects of caffeine on fluid secretion and $[Ca^{2+}]_i$ in duct cells. Interlobular ducts were isolated by microdissection. During 3hour culture both ends of the ducts sealed spontaneously thus isolating the luminal space from the bath. The sealed ducts were superfused with the standard HCO_3^--buffered solution and the rate of fluid secretion was calculated from the increment in the luminal volume and expressed as secretory rate per unit area of epithelium (nl min^{-1} mm^{-2}). $[Ca^{2+}]_i$ was measured by microfluorometry in ducts loaded with fura2. Caffeine (2 mM) reversibly inhibited secretin (1 nM)-stimulated fluid secretion from 1.27 ± 0.04 to 0.59 ± 0.06, respectively. Caffeine (2 mM) reversibly inhibited acetylcholine (10 μM)-stimulated fluid secretion from 0.71 ± 0.07 to 0.28 ± 0.07 ($p < 0.05$), respectively. Caffeine (2 mM) reduced the level of $[Ca^{2+}]_i$ in secretin-stimulated ducts. Caffeine (2 mM) reversibly inhibited acetylcholine-induced elevation of $[Ca^{2+}]_i$. In summary,caffeine inhibited secretin- and acetylcholine-stimulated fluid secretion in pancreatic duct cells,which was associated with decrease of $[Ca^{2+}]_i$. No COI.

3P-155

Elastic fiber formation of ductus arteriosus in Brown-Norway rat has a unique character trait

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Patent ductus arteriosus (PDA) is a common congenital cardiovascular disease in newborn. Its pathogenesis is strongly concerned in structural remodeling disorders of DA constitution like internal elastic laminae (IEL), extracellular matrix, and smooth muscle cells. Brown-Norway (BN) inbred rat has with a high incidence of PDA compared to other laboratory rat strains. BN rat develops several elastin-related arterial impairments such as ruptures of the IEL in abdominal aorta and aortic elastin deficit in adult. Therefore BN rat has systemic elastin-related arterial impairments that can cause PDA. However, molecular mechanisms in the DA of BN rat have not been sufficiently cleared. We then aimed to identify the mechanisms. First, we found that the DA in four neonates of six BN neonates still remained open although that in all F344 neonates closed within one hour after birth. Next immunostaining revealed that intimal cushion formation was poor, and migration of smooth muscle cells from the media into subendothelial space was obscure in the DA of the BN neonates. The IEL was thicker and the elastic lamellae in the media were scarcer than those of the DA in the F344 neonates, suggesting that the irregular elastic fiber formation was a common feature of the DA in the BN rat strain. In conclusion, the irregularity of elastic fiber formation is a unique vascular remodeling that may contribute to the cause of PDA in BN rat strain. No COI.

3P-156

Vidarabine, a selective cardiac adenylyl cyclase inhibitor, prevents ventricular arrhythmias in Calsequestrin 2 knockout mice

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The beta-adrenergic receptor/adenylyl cyclase (AC) signaling is critical for catecholamine-induced physiological responses of cardiomyocytes. Previously, we found that Vidarabine, an anti-herpesvirus agent, is one of the selective inhibitors of cardiac AC subtype. Cardiac isoform of calsequestrin, calsequestrin 2 (Casq2), plays an important role in Ca^{2+} handling. Casq2 gene-knockout (Casq2KO) mouse has been known as a model of catecholaminergic ventricular arrhythmias. First, we assessed the antiarrhythmic effect of Vidarabine in Casq2KO mice. Intraperitoneal (ip) injection of isoproterenol (ISO, 1.5 mg/kg) induced premature ventricular contractions (PVCs) in Casq2KO mice. Vidarabine reduced the number of PVC occurred during 20 minutes after ISO challenge (56% reduction at 60 mg/kg ip, $P<0.01$). In addition, norepinephrine-induced phosphorylation of Ryanodine receptor 2 (RyR2), which is related to the leaky RyR2, was blunted by Vidarabine in ventricles of wild type (WT) mice (18% reduction, $P<0.05$). Consistently, in ventricular myocytes isolated from WT mice, Vidarabine attenuated ISO-induced Ca^{2+} leak and spontaneous Ca^{2+} release from SR, which has been considered as an potential arrhythmogenic trigger (27% reduction, $P<0.05$ and 58% reduction, $P<0.01$). Our results suggest that Vidarabine suppress the development of catecholamine-induced ventricular arrhythmias via prevention of abnormal Ca^{2+} release from SR. No COI.

3P-157

Mitochondria-endoplasmic/sarcoplasmic reticulum Ca crosstalk and cellular function

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Mitochondrial Ca plays important roles in regulating mitochondrial as well as cellular functions such as energy metabolism and apoptosis. The dynamic change of cytoplasmic Ca also depends on mitochondrial function, which in turn regulates cellular function. We recently reported that NCLX (slc24a6) functions as a mitochondrial Na-Ca exchanger in B lymphocytes and cardiomyocytes. In both types of cells, NCLX provides Ca from mitochondria to endoplasmic/sarcoplasmic reticulum (ER/SR), thereby modulating the cellular Ca response to antigen receptor stimulation in B lymphocytes and the automaticity in cardiac cell line HL-1 (Kim et al., *J Physiol*, 2012; Takeuchi et al., *Sci Rep*, 2013). We proposed that Ca extruded from mitochondria via NCLX accumulates in a narrow mitochondria-ER/SR subspace, from which ER/SR Ca pump (SERCA) efficiently takes up Ca.

In the present study, we further examined this hypothesis by performing simulation analyses using comprehensive cell models of B lymphocytes and cardiac pacemaker sinoatrial (SA) node cells, by newly incorporating mitochondria-ER/SR subspace Ca dynamics. It was shown that the larger the fraction of mitochondria facing mitochondria-ER/SR subspace, the larger the ER/SR Ca content becomes. Contribution of mitochondria-ER/SR subspace on the cellular functions will be discussed in detail. No COI.

3P-158

Involvement of K^+ channel activity in the cytotoxicity of TNF-α in cultured human proximal tubule cells

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TNF-α is known to cause cell injury in various organs, including the kidney. Since there are some reports suggesting that changes in K^+ channel activity played roles in the renal tubular cell injury induced by endotoxemia or ischemia, it is possible that the cytotoxic effects of TNF-α would partly be mediated by its action on K^+ channels in renal tubular epithelia. However, little information is available, regarding the relationship between the effects of TNF-α on K^+ channel activity and cell viability. In this study, we investigated the effects of TNF-α on K^+ channel activity and cell viability in cultured human proximal tubule cells (RPTECs), using the patch-clamp technique and fluorescent imaging. In cell-attached patches, TNF-α (20 ng/ml) acutely stimulated the activity of an inwardly rectifying K^+ channel, which was abolished by a soluble TNF receptor analog, etanercept (10 μg/ml). Furthermore, a MAPK inhibitor, U0126 (20 μM), blocked the stimulatory effect of TNF-α. The cytotoxicity of TNF-α was examined by using two fluorescent dyes, calcein and propidium iodide (PI). When the cells were treated with TNF-α for 24 h, PI-stained dead cells increased, which was partly blocked by the concomitant presence of U0126 or a K^+ channel inhibitor, tertiapin (1 μM). These results suggested that TNF-α stimulated the activity of an inwardly rectifying K^+ channel in RPTECs through its biding to the specific receptor and activation of MAPK, and that the stimulation of K^+ channel would partly be involved in the cytotoxicity of this cytokine. No COI.

3P-159

Enhancement of ciliary beating by Carbocystein (CCys) in distal airway ciliary cells of mice.

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The effects of CCys (a mucolytic) on the bend angle (CBA) and the frequency (CBA) of ciliary beating were examined in airway ciliary cells isolated from mouse lungs. Our previous study revealed that an increase in intracellular pH (pH_i) increases CBF and CBA, and a decrease in intracellular Cl^- concentration ($[Cl^-]_i$) increases CBA. CCys (100 μM) increased CBA by 30 %, while it increased CBF by 5 % in the presence of HCO_3^-. The present study demonstrated that CCys decreases $[Cl^-]_i$ via activation of CFTR resulting in an increase in CBA. On the other hand, we inhibited the Na^+/HCO_3^- cotransport (NBC), the Cl^-/HCO_3^- exchange (AE) or both using an Na^+-free solution, a Cl^--free solution or DIDS. The results suggest that CCys activates NBC and AE leading to an increase in HCO_3^- (an increase in pH_i). This may cause a small increase in CBF (5 %). In conclusion, CCys increases CBA by 30 % via a decrease in $[Cl^-]_i$ and CBF by 5% via a small increase in pH_i. No COI.

3P-160

Modulation of ciliary beat frequency by PDE1 during procaterol stimulation in distal airway ciliary cells of mice.

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We examined the effects of procaterol (Proc, an β-agonist) on ciliary bend angle (CBA) and ciliary beat frequency (CBF) in airway ciliary cells. Proc initially stimulates an increase in CBA and then CBF via cAMP accumulation. Thus, upon stimulating ciliary cells with Proc, a CBF increase was delayed. However, IBMX increased both CBA and CBF in a similar time course. On the other hand, a decrease in $[Ca^{2+}]_i$ stimulated an increase CBF, whereas an increase in $[Ca^{2+}]_i$ did not. Moreover, in the experiments of W-7 (a calmodulin inhibitor), the recoveries of ciliary beating after removing W-7 were markedly delayed in PKI-treated cells, compared with non-treated cells. These observations suggest that the concentration of cAMP in the microdomain regulating CBF (outer dynein arm, ODA) is controlled by a Ca^{2+}-dependent phosphodiesterase (PDE1). A selective PDE1 inhibitor (8MmIBMX) increased CBF, not CBA, and then a further stimulation with Proc similarly increased both CBA and CBF. An immunohistochemical examination using electron microscopy demonstrated that PDE1 exists in the microdomain near the ODAs in the cilia. In conclusion, PDE1 modulates CBF by regulating ODAs in airway ciliary cells. No COI.

3P-161

2-photon FLIM imaging of SNARE assembly in endocrine and neuron cells.

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We have investigated the SNARE configurations in the plasma membranes of beta cells in the pancreatic islets and presynaptic terminals of cortical neurons, using two-photon fluorescence lifetime imaging (FLIM) of Forster resonance energy transfer (FRET). We constructed the FRET probes of SNAREs, VAMP2 and syntaxin1 and SNAP25, by labeling with Turquoise (donor) or Venus (acceptor). In pancreatic beta cells, most SNAREs were unassembled in the plasma membranes, except for a small fraction (6%) of SNAP25 forming a binary complex with syntaxin. In contrast, a significant fraction of SNAP25 and syntaxin formed the ternary trans-SNARE complexes in the boutons, and was reversibly converted into cis-form upon stimulation. Furthermore, the active zone had a propensity to assemble trans-SNARE complex even though either SNAP25 or syntaxin were inactivated by botulinum toxins, and that phorbol-ester facilitated formation of the trans-SNARE complexes. We also found the oligomerization of SNAP25 at synaptic terminals in the domain-swapped configuration. Thus, the SNARE configurations are diverse and regulated to allow the ultrafast exocytosis in the active zone, while slow exocytosis in the islet beta cells. No COI.

3P-162

Measurement the history of the neural activity with AIME-MRI

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The activation-induced manganese-enhanced MRI (AIME-MRI) is one of the candidate methods for measuring the neuronal activity in vivo. This method has the potential to measure the history of the neuronal activity, since the Mn^{2+} can enter the active neuron through voltage-dependent Ca^{2+} channels. However, it has not been clear that the relation between the neuronal activity and the accumulation of Mn^{2+} in the cell. Therefore, we evaluate the ability of AIME-MRI for measurement of the neuronal activity.At first, we confirmed that the longitudinal relaxation time of H^+ (T_1) measured by means of MRI apparatus was related to the $[Mn^{2+}]$. The inverse of T_1 ($1/T_1$) was proportional to $[Mn^{2+}]$, thus $[Mn^{2+}]$ can be measured by MRI quantitatively. Next, we investigated the relation between the neuronal activity and the accumulation of Mn^{2+} in the striatal GABAergic neurons. The amount of the Ca^{2+} influx was correlated to the neuronal activity evoked by tetanic stimulations with various frequencies. In the same slice preparation, the amounts of the fluorescence quench induced by the tetanic stimulations under the condition of 50 μM $MnCl_2$ administration were recorded. As a result, the amount of the quench was proportional to the amount of the Ca^{2+} influx. These results suggested that a cell, which has higher activity, accumulated larger amount of Mn^{2+} in the neuron. Thus, our results supported that AIME-MRI can measure the history of the neuronal activities in the whole brain noninvasively in vivo.In addition we have attempted this method for neural disease mouse model. No COI.

3P-163

Effects of thyroid hormone on microglial function

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L-tri-iodothyronine (3, 3', 5-triiodothyronine; T_3) is an active form of thyroid hormone (TH) essential for development and function of the central nervous system (CNS). Clearance of debris and apoptotic cells by migratory response and phagocytosis of microglia is a critical step for restoration of damaged neural network. Here we report effects of T_3 on microglial function studied with time-lapse videomicroscopy, 48-well microchemotaxis Boyden chamber and immunocytochemistry staining using primary cultured murine microglia. Exposure to T_3 increased microglial motility, chemotaxis and membrane ruffling. The T_3-induced microglial migration was inhibited by antagonists of phosphoinositide 3-kinase (PI3K), mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK), Na^+/K^+-ATPase, $G_{i/o}$-protein, and by inhibitors of $GABA_A$ and $GABA_B$ receptors. Inhibition of TH transporters and receptors (TRs) suppressed T_3-induced microglial migration and morphological remodelling. Moreover, control and T_3-induced migration of microglia isolated from wild-type mice was greater than that of TRα-knock-out mice. *In vivo* stab wound model showed that attraction of microglia to the site of lesion was also affected by T_3. These results demonstrate that T_3 regulates multiple functional responses of microglia. No COI.

3P-164

High Extracellular Ca^{2+} Enhances the Adipocyte Differentiation of Bone Marrow Stromal Cells Through Suppression of ERK Activity

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Mesenchymal stem cells found in bone marrow stromal cells (BMSCs) are the common progenitors for both adipocyte and osteoblast. We have recently reported that treatment with insulin and dexamethasone, high $[Ca^{2+}]_o$ enhanced adipocyte but not osteoblast accumulation in BMSCs, suggesting that increases in $[Ca^{2+}]_o$ caused by bone resorption might accelerate adipocyte accumulation in aging and diabetic patients. In this study, we used primary mouse BMSCs to investigate the mechanism by which high $[Ca^{2+}]_o$ enhance adipocyte accumulation. 10.8 mM of $[Ca^{2+}]_o$ partially suppressed the phosphorylation of ERK activated by PMA (phospho-ERK positive cells; 50% vs. 65% comparing to 1.8 mM of $[Ca^{2+}]_o$ group). We next determined whether inhibition of ERK by inhibitors for MEK (U0126 and PD0325901) alter the expression levels of adipocyte markers. Treatment with insulin and dexamethasone for BMSCs significantly increased the mRNA levels of C/EBPα (1.8 times) and PPARγ (1.3 times), which are important adipogenic transcription factors. 5 μM of U0126 enhanced the mRNA expression of C/EBPα (2.5 times) and PPARγ (2.3 times). Similar effects were observed in 20 nM of PD0325901-treated group. These data suggest that high extracellular Ca^{2+} enhances the adipocyte differentiation of BMSCs through suppression of ERK activity. Increased $[Ca^{2+}]_o$-suppression of ERK signaling may be a new target for therapy for anemia and fractures caused by accelerated marrow adipocyte accumulation in aging and diabetic patients. No COI.

3P-165

High Extracellular Ca^{2+} Enhances the Adipocyte accumulation of Bone Marrow Stromal Cells Through a decrease in cAMP

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[Background] Mesenchymal stem cells found in bone marrow stromal cells (BMSCs) are the common progenitors for both adipocyte and osteoblast. We have recently suggested that increases in $[Ca^{2+}]_o$ caused by bone resorption might accelerate adipocyte accumulation in atherogenic condition. In this study, we investigated the mechanism by which high $[Ca^{2+}]_o$ enhance adipocyte accumulation. **[Methods and Results]** We used primary mouse BMSCs and evaluated the level of adipocyte accumulation by measuring Oil Red O staining. High concentration (10.8 mM) of $[Ca^{2+}]_o$ enhanced the accumulation of adipocytes in BMSCs by about two times under the treatment of both insulin and dexamethasone (atherogenic condition), whereas high $[Ca^{2+}]_o$ alone did not induce adipocyte accumulation. Using the ELISA method, we showed that high $[Ca^{2+}]_o$ decreased the concentration of cAMP by about 20% in BMSCs. Increasing the intracellular concentration of cAMP (1 μM forskolin and 1 μM db-cAMP) hampered the enhancement in adipocyte accumulation by about 40–50% under high $[Ca^{2+}]_o$ in BMSCs. On the other hand, this hampered effect was not observed in BMSCs cultured in basal concentration (1.8 mM) of $[Ca^{2+}]_o$. Using real-time RT-PCR, we showed forskolin and db-cAMP suppressed the expression of important adipogenic transcription factors (PPARγ and C/EBPα) under high $[Ca^{2+}]_o$ in BMSCs. **[Conclusions]** These data suggest that increases in $[Ca^{2+}]_o$ might accelerate adipocyte accumulation through a decrease in cAMP in atherogenic condition. No COI.

3P-166

RANKL-induced osteoclast differentiation on RAW264.7 cells is suppressed by the direct effect of IL-17A

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Bone is continuously remodeled by bone formation and resorption, and the cooperative bone metabolism is tightly regulated to maintain homeostasis. But, bone resorption mechanism has not been fully elucidated. IL-17A is a proinflammatory cytokine that is mainly secreted by activated T cells. IL-17A plays an important role in many autoimmune and inflammatory diseases. IL-17A sensitizes osteoclast precursors to the key osteoclast factor RANKL by increasing RANK expression on osteoclast precursors. However, the direct effects of IL-17A on the differentiation of osteoclast precursors into osteoclasts have not been clarified. Therefore, we studied the role of IL-17A in the direct differentiation and activation of osteoclast precursors. We tested the effect of IL-17A on the proliferation and the differentiation of RAW264.7 cells and also on the expression of IL-17 receptors. Expression of IL-17RC was confirmed on RAW264.7 cells by FACS analysis. IL-17A did not affect the proliferation, but suppressed the differentiation of RAW264.7 cells in the presence of soluble RANKL into osteoclast in a dose-dependent manner. Phosphorylation of p38 MAP kinase was further enhanced in the presence of RANKL compared with control, which was reduced by IL-17A in a dose-dependent manner. These results suggest that the direct effect of IL-17A suppressed RANKL-induced osteoclast differentiation. Furthermore, it is suggested that inhibition of p38 MAP kinase phosphorylation by IL-17A may be one of the factors that suppress the differentiation of osteoclast precursors into osteoclasts. No COI.

3P-167

Effects of 365 nm LED light on cell growth of cultured RAW264.7 cells

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We studied effects of ultraviolet A (UV-A) irradiation using light-emitting diode on cell growth of RAW 264.7 cells. Cells were plated on 96 well plates at a density of 10^5–10^6 cells/ml. After 24 hr, these cells were irradiated for varied time (0–5 min) and maintained again for 0–72 hr at 37°C. Cells images were taken by a microscope, and the cell number was calculated from these images. Irradiation for 2 min significantly suppressed cell growth, and addition of N-acetyl cysteine (NAC) recovered from the cell growth inhibition and removed intra- and extracellular ROS (reactive oxygen species) induced by the irradiation. This 2 min-irradiation did not so strongly decreased glutathione and glutathione reductase activity, and did not stumulate the LDH release into medium and lipid peroxydation. Finally, to detect ROS induced in the culture medium by UV-A light iradiation for 2 min, we measured electron paramagnetic resonance (EPR) signals in the presence of spin trapping agents (TPC and DMPO) by EPR spectrometer. NaN_3 decreased the spin peaks formed by TPC and histidine decrease the peaks by DMPO. These measurements indicate that singlet oxygen ($^1O_2$) is initially induced, and $^1O_2$ reacts first with DMPO, and the resulting DMPO-$^1O_2$ intermediate is immediately decomposed to give hydroxyl radical. These results suggest that these ROS induced in cytoplasm or cultured medium inhibit the cell growth of RAW cells. No COI.

3P-168

Analysis of cold stress induced cell damage in HeLa cell

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A cell was damaged by exposure to extracellular factors such as physical and chemical factors. We focused on the cold stress as an extracellular factor to cell damage, and evaluated cell viabilities of cold stress exposed HeLa cell. Control HeLa cells were incubated at 37°C, 5% CO_2 in DMEM with 10% FBS for overnight. HeLa cells were placed under 4°C (cold treatment) for 1 and 3 days. Both cells were stained with calcein-AM (Cal) and propidium iodide (PI) and the cell viability was analyzed by flow cytometry. The viability (Cal positive/PI negative) of 3-day cold treatment cell was 10%, on the other hand, that of 1-day cell was 85%, which was the same as the viability of control cells. Flow cytometric viability of 1-day cold treatment did not decrease, however, the cell proliferation was suppressed by cold treatment and the cell growth did not recovered after the temperature setting at 37°C again. We observed time-dependent cell swelling after 24 h cold treatment. The swollen cells were not stained with acridine orange and JC-1, which was lysosome and mitochondria indicator, respectively. The swollen cell also did not stained with anti-lysosomal associated membrane protein-1. Lactose dehydrogenase activity of 1-day cold treatment extracellular medium was low. Therefore the cell membrane was not damaged during 1-day cold treatment. These results indicated that cold stress cell damage occurred on intracellular organelle. No COI.

3P-169

Mechanically induced ATP release in 3D culture of normal and cancerous mammary cell lines and effects of TGF-β1

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Cancer cells are affected by various factors from their microenvironment including mechano-signaling, such as extra cellular matrix rigidity, as well as chemo-signaling, such as growth factors. ATP, a ubiquitous cell-cell signaling molecule, is usually released by mechanical stimulation, and its receptors are expressed even in cancer cells and affect cell conditions. TGF-β1 is secreted in cancer microenvironment, and recently appeared to induce cell migration and actin remodeling via ATP release and activation of its receptors in human lung cancer cells. From these facts, we postulated that mechano- and chemo-signaling in cancer microenvironment interact via ATP signaling. Here, we used human mammary epithelial cell line (HuMEC) and breast cancer cell line (MDA-MB231) in 3D or 2D collagen-gel culture, and measured ATP release by stretch and hypo-osmotic stimulations using the real time ATP bioluminescence imaging system. Results showed both mechanical stimuli induced transient and significant ATP release from several cells with a peak concentration of nearly 10μM. It spread to the surroundings and seemed to keep higher concentration in the microenvironment. With TGF-β1 treatment for several days, cell shapes changed remarkably to elongated fibrous morphology and ATP response increased both in size and frequency. Cell morphology also seemed to change after mechanical stimuli. We believe these findings support our hypothesis. No COI.

3P-170

Analysis of the binding mode of AIF to the membrane

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Mitochondrial flavoprotein, apoptosis-inducing factor (AIF), is known as one of the key caspase-independent death effector. In response to several apoptotic stimuli, AIF is released from mitochondria and translocate to the nuclei. In static conditions, AIF is attached to the mitochondrial membrane; upon stimulation, it may need to undergo polypeptide cleavage in order to acquire solubility and pro-apoptotic properties. So far, two conflicting mechanisms have been proposed regarding to the mechanisms whereby AIF is bound to the membrane components. The one is that AIF is an integral membrane protein, where its N-terminal region (mouse, residues 67–85) penetrates into the inner membrane. The other is that AIF is a peripheral membrane protein. To elucidate exact binding mode of AIF to the membrane, we developed recombinant *E. coli* strains which overexpress two distinct forms mouse AIF, mitochondria-type AIF (delta 1–53) or cleaved-type AIF (delta 1–102). We found that more than 80% of mitochondria-type AIF was associated to the membrane fractions when the ionic strength (I) of preparation buffer was within physiological range (I = 150 mM). However, most enzyme (95%) was recovered within the soluble fractions at higher I (300 mM). It is noteworthy that cleaved-type AIF is partly associated to the membrane (about 50%) at low I, and was also dissociated by increases in I. These results suggest that the N-terminal region (residues 67–85) of AIF is not penetrated into the membrane and is indirectly involved in the membrane-binding, and that binding of AIF to the membrane are largely dependent on ionic bond. No COI.

3P-171

UV-induced apoptosis is inhibited in LPS-activated BV-2

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We previously reported the optimal dose of lipopolysaccharide (LPS) markedly extends the lifespan of mouse primary-cultured microglia by suppressing apoptotic and autophagic cell death. In the present study, we investigated the effects of LPS on apoptosis by ultraviolet (UV) irradiation in microglial cell line, BV-2. Apoptosis was observed within 1 h and more than half of BV-2 were in an apoptotic state at 3 h after UV irradiation. Procaspase-3 was cleaved to be active-form in UV-irradiated cells. By contrast, in BV-2 treated with LPS for 24 h, caspase-3 was not cleaved by UV irradiation. Treatment with LPS effectively protected BV-2 against apoptosis induced by UV irradiation. LPS treatment of BV-2 increased the $p21^{Waf1/Cip1}$ cyclin-dependent kinase inhibitor protein level at 6 h and growth arrest and DNA damage (GADD) 45α protein level at 24 h. Because p21 and GADD45α arrest the cell cycle in G1 or G2/M stage, respectively, and regulate apoptosis on stress, the protein expressions of p21 and GADD45α were suppressed by small interfering RNA. While neither caspase-3 cleavage nor apoptosis was induced in either p21 or GADD45α knockdown BV-2, caspase-3 was activated to induce apoptosis in both p21 and GADD45α double knockdown cells. These data suggest that p21 and GADD45α cooperate to regulate apoptosis caused by UV irradiation. Because microglia activated excessively may play critical roles in the exacerbation of neurodegeneration, the regulation of apoptosis in microglia could be a new and promising strategy to inhibit the deterioration of neurodegenerative disease. No COI.

3P-172

High oxygen condition facilitates the differentiation of human iPS cells into pancreatic progenitors and insulin-producing cells

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Pluripotent stem cells have potential applications in regenerative medicine for diabetes. Differentiation of stem cells into insulin-producing cells has been achieved using various protocols. However, both the efficiency of the method and potency of differentiated cells are insufficient. Oxygen tension, the partial pressure of oxygen, has been shown to regulate the embryonic development of several organs, including pancreatic β-cells. In this study, we tried to establish an effective method for the differentiation of induced pluripotent stem cells (iPSCs) into insulin-producing cells by culturing under high oxygen (O_2) conditions. Treatment with a high O_2 condition in the early stage of differentiation increased insulin-positive cells at the terminus of differentiation. We found that a high O_2 condition repressed Notch-dependent gene Hes1 expression and increased Ngn3 expression at the stage of pancreatic progenitors. This effect was caused by inhibition of hypoxia-inducible factor-1α (HIF-1α) protein level. Moreover, a high O_2 condition activated Wnt signaling. Optimal stage-specific treatment with a high O_2 condition resulted in a significant increase in insulin production in both mouse embryonic stem cells (mESCs) and human iPSCs (hiPSCs), and yielded populations containing up to 50% C-peptide-positive cells in hiPSCs. These results suggest that culturing in a high O_2 condition at a specific stage is useful for the efficient generation of insulin-producing cells. No COI.

3P-173

Analysis of biphasic Ca^{2+} uptake by the endoplasmic reticulum during Ca^{2+} oscillations in mouse eggs

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In mammalian eggs, repetitive increases in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$), or Ca^{2+} oscillations, are induced by spermatozoa, and trigger a series of events leading to egg activation. Each Ca^{2+} transient in the oscillations is due to Ca^{2+} release from the endoplasmic reticulum (ER) through inositol 1,4,5-trisphosphate receptor/Ca^{2+} channels. For comprehensive understanding of the mechanism of Ca^{2+} oscillations, therefore, the information about Ca^{2+} concentration in the ER lumen ($[Ca^{2+}]_{ER}$) is essential. In the present study, we measured the changes in $[Ca^{2+}]_{ER}$ during Ca^{2+} oscillations in mouse eggs induced by sperm or the expression of phospholipase Cζ, using a genetically coded Ca^{2+} probe, D1ER. By simultaneous monitoring of $[Ca^{2+}]_{ER}$ and $[Ca^{2+}]_{cyt}$ with D1ER and fura-2, it was revealed that, after the rapid decrease by Ca^{2+} release at each Ca^{2+} transient, $[Ca^{2+}]_{ER}$ increased with a biphasic time course, consisting of an immediate increase as fast as the decrease in $[Ca^{2+}]_{cyt}$, followed by a slow and gradual increase until the next Ca^{2+} transient occurred. Whereas the rate in the slow phase of recovery was not inhibited by thapsigargin, it was dependent on extracellular Ca^{2+} concentration, indicating that it is limited by the rate of Ca^{2+} influx, not by that of Ca^{2+} uptake by Ca^{2+} pumps on the ER membrane. These results are consistent with the idea that the interval of Ca^{2+} oscillations at fertilization is determined by the supplying rate of Ca^{2+} from outside the egg to refill the depleted Ca^{2+} stores. No COI.

3P-174

Numerical simulation of fast and slow Ca^{2+} oscillations in fertilized mouse eggs

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Many types of cell respond to external stimuli with repetitive increases in the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$), or Ca^{2+} oscillations. In mouse eggs, changes in $[Ca^{2+}]_{cyt}$ induced by the fusion with sperm show complex pattern: slow oscillations at the interval of 10–30 min, each of which consists of fast oscillations at the interval of several tens of seconds. In the present study, we examined the factors that determine the temporal characteristics of Ca^{2+} oscillations in mouse eggs, by numerical simulation based on the model by De Young and Keiser (D-K model). With the original D-K model, Ca^{2+} oscillations were generated by increasing the rate of IP_3 production, assuming the translocation of PLCζ from sperm. Although, by changing parameter(s) representing the kinetics of inhibition of IP_3 receptors by Ca^{2+}, the frequency of the oscillations was able to be adjusted similar to that of either fast *or* slow oscillations, the oscillations containing both fast *and* slow components could be reproduced only by adding the term for Ca^{2+} influx from outside the egg to D-K model. Simulated Ca^{2+} oscillations with such model agreed well with measured results not only for the changes in $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]$ in the intracellular stores, but also for the dependence of slow frequency on the extracellular $[Ca^{2+}]$. Together with the fact that the dependence of PLCζ activity on $[Ca^{2+}]_{cyt}$ had essentially no effect on the simulation results, it was suggested that slow Ca^{2+} oscillations in fertilized eggs are driven by Ca^{2+} influx, not by characteristic enzyme activity of PLCζ. No COI.

3P-175

Important role of cell membrane microdomain for Ca^{2+}-sensitization of vascular smooth muscle contraction

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There are two types of vascular smooth muscle (VSM) contractions; 1) Ca^{2+}-dependent contraction of VSM regulates physiological vascular tone and maintains blood pressure, 2) Rho-kinase-mediated Ca^{2+}-sensitization of VSM contraction contributes to vasospasm. We previously identified sphingosylphosphorylcholine (SPC) as an upstream mediator of the Ca^{2+}-sensitization. The degrees of SPC-induced Ca^{2+}-sensitization correlated well with serum and VSM tissue cholesterol (Chol) levels. Furthermore, depletion of VSM Chol destroyed Chol-enriched membrane microdomains such as caveolae and lipid rafts, and abolished the SPC-induced Ca^{2+}-sensitization. However, mechanisms by which SPC transduces the Ca^{2+}-sensitizing signals exclusively through membrane microdomains are unknown. Thus, we investigated the possible roles of cell membrane microdomain in the SPC-induced Ca^{2+}-sensitization of VSM contraction. First, we measured the interaction of SPC with human VSM cells using the surface plasmon resonance, providing the first direct evidence for Chol-dependent high affinity of D-*erythro*-SPC (*d*-SPC) for the VSM cells as compared with L-*threo*-SPC (*l*-SPC) and other sphingolipids. Secondly, we examined the interaction of SPC with model membranes for a strong linkage between Chol and the affinity of SPC for the membrane. These results support the first direct evidence that VSM cells have very high affinity for *d*-SPC, but not *l*-SPC, indicating highly structural specificity of SPC. No COI.

3P-176

Mechanisms of regulation of skeletal muscle proliferation and differentiation

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The polyamines putrescine, spermidine and spermine are low molecular weight organic polycations, well known as mediators involved in cell homeostasis. The proposed roles of polyamines are the functioning of ion channels, nucleic acid packaging, signal transduction, autophagy, DNA and protein synthesis, cell proliferation, and differentiation, as well as regulation of gene expression. Although regulation of polyamine levels is associated with muscle hypertrophy in skeletal muscle, yet the underlying mechanisms of polyamine are not well defined. Here, we studied how polyamines may affect the proliferation and/or differentiation of murine myoblast progenitor C2C12 cell line. To evaluate the role of polyamines in the proliferation process, we counted the numbers of myoblasts every 24 hours, but the polyamine did not have influence for myoblasts proliferation. On the other hand, during induction of myogenic differentiation, upon polyamine treatment of C2C12 cells the number of myotubes significantly increased. Morphologically, polyamine-treated C2C12 cells exhibit elongated cell body and become multi-nucleated myotubes. Ultrastructural analysis under transmission electron microscope revealed that polyamine-treated multi-nucleated myotubes exhibited an abundant presence of myofilaments. Therefore, our study demonstrates that polyamines may play an important role in regulating myogenic differentiation rather than myoblasts proliferation. No COI.

3P-177

Lowered extracellular pH is involved in the pathogenesis of skeletal muscle insulin resistance

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Insulin resistance in the skeletal muscle is manifested by diminished insulin-stimulated glucose uptake and is a core factor in the pathogenesis of type 2 diabetes mellitus, but the mechanism causing insulin resistance is still unknown. Our recent study has shown that pH of interstitial fluid was lowered in early developmental stage of insulin resistance in OLETF rats, known as a model of type 2 diabetes mellitus. Therefore, in the present study, we confirmed effects of the extracellular pH on the insulin signaling pathway in a rat skeletal muscle-derived cell line, L6 cell. The phosphorylation level of the insulin receptor was significantly diminished in low pH media. The phosphorylation level of Akt, which is a downstream target of the insulin signaling pathway, was also declined in low pH media. Moreover, the binding affinity of insulin to the insulin receptor was reduced by lowering extracellular pH, while the expression of insulin receptors on the plasma membrane was not affected by the extracellular pH. Finally, insulin-induced 2-deoxyglucose uptake in L6 cells was diminished in low pH media. Our present study suggests that lowered extracellular pH conditions may produce the pathogenesis of the insulin resistance in skeletal muscle cells. No COI.

3P-178

Disassembly of actin stress fibers is crucial for the growth arrest induced by cell compression.

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Adherent cells require a rigid substrate onto which they can attach for cell growth and survival. It is generally thought that mechanical force is crucial for this anchorage dependent cell growth, but the molecular mechanisms underlying the force-dependent cell growth remains to be solved. The present study examined the roles of actin cytoskeleton and tyrosine phosphorylation of focal adhesion proteins in the retarded cell growth induced by cell compression. HaCaT human keratinocytes or 3Y1 rat fibroblasts were cultured on a bi-axially stretched PDMS chamber, and tension in the cells was reduced by releasing the chamber to the original size. This effective cell compression decreased the number of EdU-positive S-phase cells, which was retarded by inhibiting actin stress fiber disassembly with the actin depolymerization inhibitor jasplakinolide. By contrast, cell growth under compression was arrested when tyrosine dephosphorylation of focal adhesion proteins was inhibited by the tyrosine phosphatase inhibitor phenylarsine oxide (PAO). Similarly, cell growth was arrested when actin cytoskeleton was disrupted by the actin-polymerization inhibitor latrunculin A, regardless of the presence of PAO. These results suggest that disassembly of actin stress fibers is crucial for the growth arrest induced by cell compression, but tyrosine dephosphorylation of focal adhesion proteins is not essential for this process. No COI.

3P-179

Role of Steroidogenic acute regulatory protein-related lipid transfer domain containing 10 (STARD10) in the regulation of PPARα activity

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Steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) domain containing 10 (STARD10) is a member of the START domain containing lipid transfer protein family. STARD10 is highly expressed in the liver, gallbladder, and intestine. We have reported that STARD10 is involeved in the regulation of the conjugation, secretion, and absorption of bile acids in the study using *Stard10* knockout (*Stard10*$^{-/-}$) mice. Gene expression assay suggested that STARD10 regulates the genes that are regulated by the transcription factor PPARα (peroxisome proliferator-activated receptor alpha). The aim of this study was to clarify the role of STARD10 in the regulation of PPARα activity. In this study, we analysed the effect of STARD10 overexpression and knockdown on the PPARα activity by luciferase reporter assay and qRT-PCR of PPARα target genes using mouse hepatoma cell line Hepa 1-6 and human colon cancer cell line Caco-2. Overexpression of *Stard10* gene enhanced the PPARα activity in Hepa1-6 cells. On the otherhand, the knockdown of *Stard10* gene using siRNA lowered the PPARα activity. Overexpression of STARD10 enhanced the gene expression of PPARα target genes in Caco-2 cells. These results indicate that STARD10 is involved in regulating the gene expression by modulating PPARα activity. No COI.

3P-180

Decrease of pyruvate kinase activity by cesium caused cesium dependent cell growth inhibition

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Distribution of Cs in the whole bodies after the intake of Cs cells had been already investigated. However, we still do not know the transport pathway of Cs into the cells and the effect of Cs on the cell metabolisms. We examined the proliferation of HeLa cells cultured in DMEM with 10% FBS at 37°C, 5% CO_2 and supplemented with 10 mM of different types of alkali metals. Among them, only Cs inhibited the proliferation of the cells. The proliferation decrease is dependent on Cs concentration. Two different methods of live and dead assay were performed (i.e., LDH assay and flow cytometric assay). The HeLa cell membrane was not damaged by 10 mM Cs. To confirm extracellular Cs uptake into the cell, intracellular cations were analyzed by capillary electrophoresis. The intracellular cation content was analyzed as magnesium-based intracellular cation ratio. The intracellular Cs was detected in Cs-treated cells but not in control cell. Pyruvate kinase activity from crude extract of HeLa cell showed dose-dependent inhibition by Cs. The intracellular pH was measured by BCECF, the pH-sensitive dye indicator. Alkalization of intracellular pH was occurred in the cells cultured with CsCl, but not in the cells cultured with the other alkali metals. Generally, intracellular pH of tumor cells was lower than normal cells. Our results suggeted that Cs added to extracellular media uptake into the cells, and inhibition of cell proliferation possibly due to decrease of glycolysis and intracellular alkalization induced by Cs administration. No COI.

3P-181

Comparative studies on the post-translational modification of recombinant and plasma-derived human serum albumin: thiol oxidation, glycation and carbonylation.

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Commercially available human serum albumin (HSA) products have been widely used in both laboratory and clinical fields. We examined the post-translational modifications (thiol oxidation, glycation and carbonylation) of recombinant albumin (rHSA), compared with those of plasma-derived albumin (pHSA). Products were obtained from Sigma Co. USA (product nos. A9731 for rHSA; A1653 and A3782 for pHSA). The rHSA is expressed in rice. For pHSA, the A1653 is an initial product obtained from large-scale pooled human sera and the A3782 is a final product which is fatty acid free and globulin free. Thiol oxidation was analyzed by an HPLC method. Glycation and carbonylation were examined by using each analytical ELISA method. Values for thiol content (%) of all products were less than 40%, and these values were quite low compared with those of healthy subjects previously reported. Dimer fraction was observed in all products. Values for glycation (%) and carbonylation (nmol/mg protein) of all products were higher than those of healthy subjects. These results suggest that the heterogeneity of amino acid modification of commercial pHSA products appears to occur during manufacturing process of HSA from large-scale pooled plasma, and kinds of product species may be also important for rHSA products. Elucidation of the exact relationship between the post-translational modification and functions of HSA requires further study. No COI.

3P-182

Systemic screening of compounds targeting aberrant protein synthesis caused by dysregulation of 2-methylthio modification in tRNA

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Genetic variations in Cdk5 regulatory subunit associated protein 1-like 1 (CDKAL1) have been associated with the development of type 2 diabetes (T2D). We have previously found that Cdkal1 catalyzes the formation of 2-methylthio modification specifically at A37 of cytosolic tRNALys(UUU). The 2-methylthio modification is critical for accurate and efficient decoding of lysine codon. In both mouse and human, the deficiency of 2-methylthio modification caused aberrant translation of proinsulin, and resulted in impairment of insulin secretion. Given the critical contribution of 2-methylthio modification in protein synthesis and T2D development, targeting aberrant protein synthesis would therefore be a beneficial strategy for treatment of T2D patients carrying risk CDKAL1 variations. However, none of current anti-diabetic drugs is developed to directly target protein synthesis. In the present study, we developed a unique luciferase-based system to screen compounds that would potentially reduce aberrant protein synthesis caused by the absence of 2-methylthio modification. Utilizing this system, we have screened a synthetic compound library and identified a number of effective compounds. The positive compounds were further examined for the effect in insulin secretion in isolated pancreatic islets and in vivo. As the results, we have identified several compounds that are beneficial for both protein synthesis and insulin secretion. No COI.

Poster Presentations
Blood

3P-183

A recombinant habutobin fragment has the inhibitory effect on collagen-induced platelet aggregation

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Four fragments of recombinant habutobin (r-habutobin) (F1, F2, F3 and F4) were produced by the truncation of habutobin cDNA in order to identify the functional domain responsible for its anti-platelet action. To examine whether the r-habutobin fragments prevent platelet aggregation, F2 and F3 r-habutobin were tested for their effects on the aggregation of washed rabbit platelets. Upon collagen stimulation of washed platelets, we assessed the effects of the r-habutobin fragments on the conformational change of glycoprotein (GP) IIb/IIIa and the expression of P-selectin using flow cytometry (FCM) with PAC-1 (which only binds to activated platelets) and P-selectin antibodies. The percent inhibition of aggregation by F3 r-habutobin was 22.6 ± 5.99% (n = 7), and that by F2 r-habutobin was 14.75 ± 13.93% (n = 4). F3 r-habutobin also prolonged the lag time of collagen-induced platelet aggregation and slightly inhibited collagen-induced ATP release from the washed platelets. These results suggest that the reduction in ATP release and the activation of GP IIb/IIIa and P-selectin on washed platelets may inhibit cytoskeletal rearrangement in the presence of F3 r-habutobin. Since a number of prolines are present in F3 r-habutobin, proline-related unique structure might play important role in the inhibition of both P-selectin expression and the activation of GPIIb/IIIa. No COI.

3P-184

Treatment with fibrinogen γ-chain peptide-coated, ADP-encapsulated liposomes as an infusible hemostatic agent

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Background: We developed fibrinogen γ-chain (HHLGGAKQAGDV, H12)-coated, adenosine-diphosphate (ADP)-encapsulated liposomes [H12-(ADP)-liposomes] that accumulate at bleeding sites via interaction with activated platelets via GPIIb/IIIa and augment platelet aggregation by releasing ADP. We evaluated the hemostatic efficacy of H12-(ADP)-liposomes in the setting of active liver bleeding in dilutional thrombocytopenic rabbits following massive transfusion. Methods: Acute thrombocytopenia (platelets<$50\times10^3/\mu L$) was induced in rabbits by repeated blood withdrawal and isovolemic transfusion of autologous washed red blood cells. Liver hemorrhage was initiated by penetrating liver injury in them. Subsequently, they received astriction treatment against the liver hemorrhage for five minutes and were intravenously administered H12-(ADP)-liposomes with platelet-poor plasma (PPP), platelet-rich plasma (PRP), PPP alone or H12-(PBS)-liposomes/PPP during the astriction. Results: Administration of H12-(ADP)-liposomes rescued 60% of the rabbits from the liver hemorrhage as well as PRP administration did 50% of them. In contrast, rabbits receiving PPP or H12-(PBS)-liposome/PPP achieved 10% or 17% survival in the first 24 hrs, respectively. H12-(ADP)-liposomes were observed at the bleeding site in the liver with thrombus formation, suggesting an induction of thrombi. Conclusions: H12-(ADP)-liposomes may be a safe and effective therapeutic tool during damage control surgery for acute thrombocytopenic trauma patients with massive bleeding. No COI.

3P-185

Effect of Ginsenosides from Panax Ginseng on protection mechanisms against oxidative stress in blood preservation

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We studied to evaluate protective effects against oxidative stress on stored blood at 4°C with ginsenosides extracted from Panax ginseng. Deformability of erythrocytes, which were stored with citrate-phosphate- dextrose (CPD) in one week, decreased. However, erythrocyte deformability of stored CPD blood with ginsenoside-Rg2 or Rh1 lesser decreased than without them. They inhibited the oxidation-induced decrease of thiol-group of membrane proteins. These two ginsenosides made lactate production to decrease, but glucose consumption not to decrease. Erythrocytes produced CO_2 by oxidative phase in Pentose Phospate Pathway (PPP). In one week, pCO_2 of CPD blood in closed tube increased, pCO_2 of CPD blood with ginsenoside-Rh1 increased more than without it. So it is suggested the ginsenosides induced PPP in the erythrocyte, then reduced glutathione regeneration increased against oxidative stress. No COI.

3P-186

Effect of lignans on protective mechanisms of erythrocyte against oxidative stress

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We are studying to evaluate the effects of iron-induced oxidative stress and protective effects of lignan, which is a kind of polyphenol from flaxseeds, against oxidative damage on redox status of erythrocyte membrane. Heparinized venous blood was obtained from healthy donors, and immediately centrifuged. After a careful removal of plasma and buffy coat, erythrocytes were purified by three cycles of resuspension and washing with isotonic HEPES-buffered saline (HBS) and resuspended at adjustable hematocrit in HBS. The erythrocytes were treated with 0–2mM $FeSO_4$/0–10mM ascorbic acid containing a lignan (Secoisolariciresinol (SECO) or Matairesinol (Mat), Lariciresinol (Lar)) and incubated at 37°C for 1 hour. Using thiol group of membrane proteins of erythrocyte as an index, we have screened lignans. (-)-SECO inhibited the oxidation-induced decrease of thiol-group of erythrocyte membrane. Iron-ascorbate treatment impaired erythrocyte suspensions viscosity. (-)-SECO partially prevented increment of viscosity. Erythrocyte suspensions were incubated at 37°C in 2-3 hours to evaluate a effect of lignans on glyco-metabolism. SECO were increased Glucose consumption at least, however lactate production did not increase. To further analyze the reducing mechanism of erythrocyte, we are now trying to evaluate oxidative phase of Pentose Phosphate Pathway. No COI.

3P-187

Dehydroepiandrosterone protected against oxidative stress on rheological function of erythrocytes

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Dehydroepiandrosterone (DHEA) has a preventive effect over nerves system, under which oxidative stress is pathologically important. We are studying to evaluate the effects of iron-induced oxidative stress and protective effects of DHEA against oxidative damage on redox status of erythrocyte membrane. Heparinized venous blood was obtained from healthy donors, and immediately centrifuged. After a careful removal of plasma and buffy coat, erythrocytes were purified by three cycles of resuspension and washing with isotonic HEPES-buffered saline (HBS) and resuspended at adjustable hematocrit in HBS. The erythrocytes were treated with 0-1mM $FeSO_4$/0–5mM Ascorbic acid containing a DHEA and incubated at 37°C for 1 hour. Iron-ascorbate treatment impaired erythrocyte suspensions viscosity. DHEA partially prevented increment of viscosity. DHEA showed the inhibitory effect from oxidation-induced decrease of membrane thiol group on erythrocyte membrane proteins. DHEA are efficacious in protecting erythrocytes against oxidative stress as reducers or radical scavengers. No COI.

3P-188

The exposure of phosphatidylserine on the surface of mouse erythroblast

Kono, Ryoma; Onji, Hiroshi; Suzuki, Yoji; Ohkubo, Nobutaka; Mitsuda, Noriaki; Aoto, Mamoru(*Depertment of Circulatory Physiology, Ehime University Graduate School of Medicine*)

In all animal cells, phospholipids are asymmetrically distributed between the outer and inner leaflets of the plasma membrane. This asymmetric distribution is disrupted during apoptosis, and exposed phosphatidylserine (PtdSer) on dying cells serves as an "eat me" signal to facilitate phagocytosis. It is disrupted in other biological systems, too. For example, when blood platelets are activated, they expose PtdSer to trigger the clotting system. It is thought that the exposure of PtdSer contributes to the cell-cell interaction. The PtdSer exposure is believed to be mediated by the activation of Ca^{2+}-dependent phospholipid scramblases that transport phospholipids bidirectionally or the inactivation of ATP-dependent flippases that produce the asymmetric distribution of phospholipid, but its molecular mechanism is still unknown.We found that PtdSer was exposed to the surface of erythroblasts which prepared from spleens of phlebotomized mice. Because the concentration of ATP was maintained highly and the concentration of Ca^{2+} was decreased in them, the exposure of PtdSer to the surface of erythroblasts was mediated by Ca^{2+}-dependent phospholipid scramblases. No COI.

3P-189

The both α_2-antiplasmin and plasminogen activator inhibitor type-1 gene deficient mice induces high IgE production

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The pathophysiological changes induced by a lack of both α_2-antiplasmin (α_2-AP) and plasminogen activator inhibitor type-1 (PAI-1) gene were investigated using double knockout (KO) mice. Plasma IgE levels in the α_2-AP/PAI-1-double KO mice increased with age and exceeded 1,000 ng/mL after 6 months of age. The plasma cells that produced IgE were detected in perivascular assembled lymphocytes. In the α_2-AP/PAI-1-double KO mice, perivascular lymphocyte infiltration was observed in the lung, liver, and kidneys and peribronchial lymphocyte infiltration was present in the lung. When the bone marrow cells from α_2-AP/PAI-1-double KO mice were transplanted into 10-Gy X xay irradiated wild-type (WT) mice, the phenotypes of the recipients were similar to those of α_2-AP/PAI-1-double KO mice. The simultaneous expression of both the α_2-AP and PAI-1 genes contribute to the maintenance of immunological functions that are related to IgE. Moreover, it is suggested that both α_2-AP and PAI-1 are involved in the recruitment of lymphocytes in the peripheral tissues. No COI.

3P-190

Essential role of Macrophage MHC receptor (MMR) in allograft rejection: generation and analysis of MMR2 knockout mice

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We previously reported mouse macrophage (Mϕ; C57BL/6; H-2D^{b}K^b)-mediated and d haplotype-specific lysis of allografts (H-2D^{d}K^d) in the rejection site and isolated two novel receptors on Mϕ or monocytes (MO) for H-2D^d and H-2K^d (mouse MHCs), MMR1 and 2. In the present study, we generated MMR2 knockout (MMR2-/-) C57BL/6 mice to examine the biological consequences of MMR1 and 2 in allograft rejection. The MMR2-/- mice showed normal body growth and fertility and had no obvious abnormalities in terms of cell number in or composition of their lymphoid tissues or in T lymphocyte responses to alloantigen or non-alloantigen. They lacked MMR2 mRNA or protein expression in their MO and failed to reject K^d-transgenic skin grafts. Surprisingly, they also lacked MMR1 mRNA and protein expression in their MO and failed to reject D^d- or D^{d}K^d-transgenic skin grafts. Meanwhile, they did reject the skin grafts from C3H [third-party], B10D.2 [allogeneic MHC class II] or BALB.B [allogeneic minor histocompatibility antigen] mice. However, H-2D^d-, H-2K^d- or H-2D^{d}K^d-EL-4 cells (lymphoma of C57BL/6 origin) intradermally or intraperitoneally injected into MMR2-/- mice were rejected by were lysed by CD8$^+$ TCR$\alpha\beta$ T cell population in a transgene-number dependent way. These results indicated that MMRs on MO/Mϕ and TCRs on cytotoxic T cell population in mice were essential for recognition and rejection of allografted skin and lymphoma. No COI.

Poster Presentations
Respiration

3P-191

Respiratory and locomotor activities in the phrenic and abdominal nerves in the neonatal rat *in vitro*

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Inspiratory active phrenic motoneurons are located in the C4-6 spinal segments among other types of motoneurons innervating forelimb muscles. Abdominal muscles have various roles such as respiration and locomotion. In the *in vitro* brainstem-spinal cord preparation from neonatal rat, phrenic nerve and abdominal muscles show inspiratory and expiratory activity, respectively. Here we examined in the isolated pons-spinal cord preparations from neonatal rat whether fictive locomotion, induced by 5–10 μM NMDA plus 10–20 μM 5-HT, occurred in the C4 ventral root (VR), phrenic and abdominal muscle nerves (ilioinguinal nerve, IIG). The hindlimb flexor muscle activity was monitored from L2VR. In control solution phrenic and C4VR showed inspiratory activity consistently, while IIG showed expiratory activity only at the beginning of the experiment. During fictive locomotion, both C4VR and IIG showed locomotor activity. Especially IIG showed the flexor activity. On the other hand, phrenic nerve did not show locomotor activity in 4 preparations and weak locomotor activity in one. We conclude that in neonatal rats, both the central pattern generator for locomotion and the medullary respiratory center provide synaptic input to the functionally proper motoneurons existing at the same segmental levels. The results also showed abdominal motoneurons have received inputs from both centers, and this preparation could be useful to explore how two centers interact with at the level of motoneurons and spinal interneurons. No COI.

3P-192

Hypoxic modulation on respiratory neuron and TRPA1 channel during perinatal period

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TRPA1 channel played a role of detection of hypoxia with its conformational change in the peripheral chemoreceptor. However, the central effects of hypoxia and TRPA1 channel on central chemoreception during perinatal stage had not been deeply understood. We examined the effects of hypoxia and TRPA1 channel on respiratory neurons using isolated brainstem spinal cord preparations. Respiratory rhythm was facilitated in hypoxia from embryonic day 18 (E18) to postnatal day 1 (P1) rats, but respiratory rhythm decreased in P2–4. The effects of TRPA1 channel agonists facilitated the respiratory rhythm in E18-P1 rats, but depressed it in P2-4 rats; that were the same as hypoxia. We investigated extracellular recordings of respiratory neurons in the rostral ventrolateral medulla (RVLM). In hypoxia, the frequency of Pre-Inspiratory (Pre-I) and tonic neurons showed depression in P2–P4; those of Pre-I and tonic neurons showed facilitation in E18-P0. The frequency of expiratory and tonic neurons was facilitated in E18-P0, and was depressed in P2–P4 by the application of TRPA1 agonist. These results suggested that hypoxia and TRPA1 agonist showed same effects on C4 rhythm, but the effecters of neurons were different between hypoxia and TRPA1 agonist. Tonic neurons might play a crucial role of the modulation of both hypoxia and TRPA1 channel on the respiratory network. No COI.

3P-193

Post-apneic respiratory responses in patients with obstructive sleep apnea

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Apnea/hypopnea (AH) stimulates chemical respiratory drive in patients with obstructive sleep apnea (OSA). Generally, chemical respiratory control is stronger during wakefulness than sleep. We postulated that this is also true of chemical respiratory drive on resumption of respiration after an episode of AH. We used diagnostic polysomnography data to analyze respiratory rate, as an index of strength of respiratory drive, in four OSA patients with AH indexes of approximately 30. We defined periods when stage 2 NREM sleep was maintained before, through and after episodes of AH as resumption of respiration during sleep (RESPs) and those when stage 2 NREM sleep before and through AH shifted to wakefulness through and after episodes of AH as resumption of respiration during wakefulness (RESPw). We measured respiratory rate, calculated from respiratory duration, and percutaneous oxygen saturation before and after AH for the first event of RESPs or RESPw after falling asleep. In the four study patients with OSA, the respiratory rate was increased and percutaneous oxygen saturation decreased for the first episode of RESPs. However, the respiratory rate was almost constant and independent of percutaneous oxygen saturation for the first such episode of RESPw. Thus, in OSA patients, respiration is controlled by severity of hypoxia during sleep and is independent of hypoxic chemoreceptive respiratory control during wakefulness. No COI.

3P-194

Neuronal mechanisms of shortening of respiratory neuron burst by eugenol

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Eugenol is contained in several plants including clove and is used as an analgesic drug. In peripheral and central nervous system, this compound modulates neuronal activity through action on voltage-gated ionic channels and/or on transient receptor potential channels. However, it is unknown whether it exerts any effects on the respiratory center neurons in the medulla. We examined effects of eugenol or carvacrol on respiratory rhythm generation in the brainstem-spinal cord preparation from newborn rat (P0–P3). The preparations were superfused by modified Krebs solution at 25–26oC, and inspiratory C4 ventral root activity was monitored. Membrane potentials of respiratory neurons were recorded in the parafacial region of the rostral medulla. Bath application of eugenol or carvacrol (0.5–1 mM) decreased respiratory rhythm accompanied with strong inhibition of burst activity of pre-inspiratory neurons. After washed out, respiratory rhythm gradually recovered but the duration of inspiratory burst was extremely shortened and this continued for more than 1 hr after washed out. The shorting of respiratory neuron burst was partially blocked by GABAA antagonist bicuculline, glycine antagonist strychnine and GABAB antagonist phaclofen. Spike train of action potentials in respiratory neurons induced by depolarizing current pulse was depressed by application of eugenol or carvacrol, with induction of only the initial spike. These results suggest that changes in both of membrane excitability and synaptic connections are involved in the shortening of respiratory neuron burst by eugenol or carvacrol. No COI.

3P-195

Cellular mechanisms of capsaicin actions on the respiratory neurons in brainstem-spinal cord preparations from the newborn rat

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Capsaicin is known as an agonist for heat-sensitive transient receptor potential vanilloid 1 (TRPV1) channel. We examined the effects of capsaicin on respiratory rhythm generation in the brainstem-spinal cord preparations from 0–3 day old Wistar rats. Preparations were superfused at a rate of 3.0 ml/min with the following artificial cerebrospinal fluid (in mM): 124 NaCl, 5.0 KCl, 1.24 KH2PO4, 2.4 CaCl2, 1.3 MgCl2, 26 NaHCO3 and 30 glucose, equilibrated with 95% O2 and 5% CO2, pH 7.4, at 26–27°C. Inspiratory activity was monitored from the fourth cervical ventral root (C4). Bath application of capsaicin induced biphasic responses in the C4 rate; initial decrease and subsequent increase, with dose-dependent manner (1–10 μM). Effects of capsaicin were desensitized in response to the repeated application. This desensitization was observed even after treatment with calmodulin antagonist, W-7 (50 μM) or in the low Ca2+ /high Mg2+ solution with 0.5 mM EGTA. Pre-inspiratory neurons were depolarized in 2 min after capsaicin application. Effects of 10 μM capsaicin were significantly attenuated after treatment with 1 μM thapsigargin that specifically inhibits the endoplasmic reticulum Ca2+-ATPase and induces the release of intracellular stored Ca2+ from the endoplasmic reticulum. Our findings suggest that activation of TRPV1 channels in the medulla had the various influences on the respiratory center and that targets of capsaicin are intracellular Ca2+ store sites of endoplasmic reticulum in addition to the cell membrane. No COI.

3P-196

Effects of riluzole on respiratory rhythm generation in the brainstem-spinal cord preparation from newborn rat

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The contributions of ionic currents play a key role in elucidating how the respiratory rhythm is generated in the brainstem. Riluzole is one of the therapeutic agents for amyotrophic lateral sclerosis (ALS) and is also known as a persistent sodium channel blocker. In the present study, we examined effects of riluzole on pre-inspiratory (Pre-I) neurons in the rostral medulla as well as on the 4th cervical ventral root (C4)-inspiratory activities in the in vitro brainstem-spinal cord preparations from newborn rats. Preparations were isolated from postnatal day 0 (P0) -P3 Wistar rats and were superfused at a rate of 3.0 ml/min with the following artificial cerebrospinal fluid (in mM): 124 NaCl, 5.0 KCl, 1.24 KH2PO4, 2.4 CaCl2, 1.3 MgCl2, 26 NaHCO3 and 30 glucose, equilibrated with 95% O2 and 5% CO2, pH 7.4, at 26–27°C. The rate of C4 inspiratory burst was inhibited in a dose-dependent manner (1–200 μM) after 15 min application of riluzole. In addition, riluzole caused a strong reduction in the drive potential of Pre-I neurons but not of inspiratory neurons. The inhibitory effects of riluzole were not reversible especially in higher doses. After washed out, C4 inspiratory burst gradually changed into episodic pattern in which one burst consisted of 2–4 short separate bursts. Our findings indicated that the burst generation of Pre-I neurons is more sensitive to riluzole than inspiratory burst generation, and thus suggested an important role of persistent sodium channels in the burst generation of Pre-I neurons. No COI.

Poster Presentations
Others

3P-197

Development of a new cancer-therapeutic method using a nano-magnetic particle for malignant pleural mesothelioma

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Background: Malignant pleural mesothelioma (MPM) is one of the worst poor-prognosis tumors of serosal surface, such as the pleura and the peritoneum. However the incidence of this tumor is increasing in the world as result of widespread exposure to asbestos. Because of lack of the established regimen for MPM, we have developed a new hyperthermia therapy using a nano-magnetic particle, EI236, which we identified. EI236 exhibits not only anti-cancer effect but also ferromagnetism, which could generate heat power in an alternating current magnetic field (AMF). Method and Results: We have experienced with use of EI236 for treating MPM cells. EI236 promoted reactive oxygen species (ROS) of MPM cells in a dose-dependent manner. We performed the electrophoresis of supercoiled plasmid DNA in the presence of various concentrations of EI236 or cisplatin. The result showed that EI236 induced DNA nicking, which was similar to that of cisplatin. EI236 exhibited potent anti-cancer effect on several MPM cells in a dose-dependent manner by MTT assay. The anti-cancer effect of EI236 was greater than that of cisplatin. EI236 promoted apoptosis of various MPM cells, and further by exposed to AMF.Conclusion: Our study show EI236 acts simultaneously as anti-cancer drug and hyperthermic effect in MPM cells, suggesting that EI236 can assist us in developing a new treatment method for MPM in the future. No COI.

Poster Presentations
Study Methodology

3P-198

Brain activation during recalling sensory by EEG measurements

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After maintaining continuous sensory stimulus, it might be able to feel like actual stimulus even if there is no stimulus. At this time, we thought that persons recall the stimulus unconsciously. The purpose of this study is to investigate whether there are feature points on EEG and to contribute to the EEG studies such as BMI in the future. To signal the light stimulus, the subjects recall the electrical stimulus. We already found that there is a linearity between the visual evoked potential (VEP) and somatosensory evoked potential (SEP) from previous studies. By using this principle, it is possible to obtain only the EEG of an association time. As a result of the estimation, like an event-related potential (ERP) was observed at about 300ms after performing a recall on the EEG. Furthermore, we estimated the signal source by sLORETA, and found that insular cortex is strongly activated. In the studies of fMRI, it was found that insular cortex has an important role in the experience of emotion such as discomfort, emotions, fear and experience of pain. Especially, the rear region of the insular cortex is strongly related to auditory, somatosensory, and skeletal muscle movement. Based on the physiological facts, in this study, we found that the feature points such as the ERP appears to EEG at recall of senses. It is suggested that this is probably related to a part of the brain that reacts when stimulated actually. No COI.

3P-199

Development and application examples of selective injection system into hippocampus CA1 via monitored theta oscillation

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Methods of cell biology and electrophysiology using dissociated primary cultured neurons allow in vitro study of molecular functions; however, analysis of intact neuronal circuitry is often preferable. To investigate exogenous genes, viral vectors are most commonly injected using a pipette that is inserted from the top of the cortex. Although there are few reports that describe the success rate of injection in detail, it is sometimes difficult to locate the pipette tip accurately within the CA1 pyramidal cell layer because the layer is only 0.1 mm thick. In the present study, we have developed a system to inject viral vectors accurately into the mouse hippocampal CA1 pyramidal cell layer using a stereotaxic injection system with simultaneous electrophysiological monitoring of theta oscillation. The pipette tip was positioned reliably based on integrated values of the theta oscillation in the hippocampal CA1 pyramidal cell layer. Using this system, transfection of exogenous genes, GFP, into hippocampus CA1 by injecting GFP-expressing lentivirus vector indicated that GFP signals were restricted in the region. Moreover, lentivirus infection of GluA1 cDNA, which is an AMPA receptor subunit, into GluA1 KO mice, which are reported to be unable to induce LTP, revealed to rescue LTP expression in the KO mice. This approach allows accurate injection of solutions and provides an efficient method of gene transfer using viral vectors into the hippocampus, which can be a useful tool for studies involving the molecular mechanisms of neuronal functions. No COI.

3P-200

Visualizing dendritic spine morphologies along single dendrites of hippocampal neuron by a high-resolution confocal microscopy with a novel clearing reagent

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Morphological changes of dendritic spines are thought to be implicated in learning and memory processes. However, it is quite difficult to examine detailed morphologies of all the spines throughout a whole single neuron by the conventional optical microscopy, since the spatial resolution or the transparency is insufficient due to the diffraction limit or due to the light scattering in the fixed brain, respectively. Previously, we found that 2,2'-tiodiethanol (TDE) rendered fixed mouse hippocampal slices optically transparent after brief immersion into a TDE solution. This allowed that the dendrites and the spines became clearly visible at the deeper region in the slices. Here, we demonstrated that the combination of a TDE solution and a high numerical-aperture oil-immersion objective lens provided high-resolution confocal images of the spines throughout the single dendrites. By measuring the lengths of the major and minor axes of the spine head, the morphologies suggested larger variations in the shape of the dendritic spine head in the apical dendrite than those in the basal dendrite. This result suggests that our technique could provide a new insight into single neuronal functions. No COI.

3P-201

Modification of a novel continuous flow-through cell separation method for the isolation of mouse dendritic cells

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We have developed a novel continuous flow-through cell separation method for the separation of human blood cells. This apparatus continuously separates cells into five fractions according to their densities by flow-through density gradient centrifugation.Mouse dendritic cells (DCs) were isolated by FACS positive selection after MACS negative selection of low-density cells recovered from the digested spleen by batch density gradient centrifugation. DCs are present in the digested spleen at about 2% with large number of lymphocytes. At the separation of the cell suspension under hypertonic osmolality of 350 mOsm/l, both densities of DCs and lymphocytes were shifted higher, while their density difference was not expanded. At the separation through a set of OptiPrep with the densities of 1.066, 1.071, 1.078, 1.085 and 1.095 g/ml, the cell suspension adjusted to 1.066 g/ml in density was pumped into the layer with the same density. Then, DCs were retained and flowed out from the same layer at a high concentration of about 80%, while all other types of cells migrated into the neighboring layers.Since the concentration of DCs by the present method was near to that by MACS negative selection, this alternative method would be time-saving and cost-effective for the cell isolation by FACS and might make the cell isolation by MACS possible. No COI.

3P-202

A new method, flow analysis, to quantify the activity of axonal transport of cultured neurons

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Takashi Katakura, Risa Isonaka, Tadashi KawakamiDepartment of Physiology, Kitasato University School of Medicine, Sagamihara, Japan-We have recently developed a new method to evaluate axonal transport. We used a public domain, Java-based program, ImageJ with KBI plugin. Enhanced video images were captured and rotated some degrees in order to hold the main orientation of the moving organelles holizontally, and then saved as Tiff-format video clips. 128×128 pixel area is cropped, in which axonal transport is displayed appropriately. Using KBI plugin, we performed Flow analysis. The activity of axonal transport is quantified as the sum of particles (organelles) which moves more than 3 pixels per second. Velocity vectors are calculated (the velocity and the degree of an angle of the moving particle within a fixed area at a given resolution pixels, such as 8, 16, 32 pixels). We can easily distinguish and sum up the number of anterograde transporting particles and retrograde transporting particles by the sign of an angle of velocity vector, where the former has a plus sign and the latter has a minus sign respectively. With 8 pix or 16 pix resolution value, the results from Flow analysis are coincided with the results obtained from our traditional quantification method. 32 pix value sometimes dismissed moving particles and thus the calculated values are not consistent with our previous results. No COI.

3P-203

Evaluation of reporter rats which conditionally express red fluorescent protein (tdTomato) under Cre-loxP system

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The Cre/loxP recombination system is one of the conditional chromosomal mimics to investigate systemic function of targeted genes, and has been adopted to examine a function of specific gene. For in vivo experiments, mouse model is currently popular and advanced genetic manipulation has progressed less far in the rat. However, rat offers potential advantages of larger body size and progressed ability to accomplish more complex behavioral task. In this study, we evaluated three founder rats which have red fluorescent protein (tdTomato) gene in the downstream of loxP-flanked STOP cassette. One of them phenotypically expressed tdTomato with the following administration of Cre recombinase. Firstly we injected AAV-Cre into striatum of F1 pups to test the conditional expression of tdTomato. Each Cre-immuno-positive cells sparsely located in the injection part and merged with tdTomato. Secondly, the fibroblasts, which were primarily cultured from the tail, also expressed cytosolic tdTomato upon transfection with Cre recombinase gene. It is suggested that the reporter rat line which conditionally expresses tdTomato is successfully established. It would facilitate the neurophysiological studies and the connectomics of identified neurons by expressing Cre under a certain promoter. All animal procedures were conducted in accordance with the guiding principles of Physiological Society of Japan and NIH. No COI.

3P-204

The development of novel electroporation for cell physiological research

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We have investigated a novel gene transfection combining electroporation techniques and droplet science with high transfection efficiency and cell viability. An aqueous droplet containing mammalian cells and foreign plasmid DNA performs a bouncing motion between a pair of electrodes by Coulomb force in dielectric oil with application of a DC electric field. When a droplet moves between a pair of electrodes for 2–5 min by application of a 1–3 kV DC electric field, the local intense electric field facilitates gene transfection during the periodic bouncing motion of the droplet. This method has several advantages compared with previous transfection techniques, including simultaneous transfection of various types of DNA into even as few as 1000 cells, transfection into differentiated neural cells, and the establishment of stable cell lines. No thermal load is applied due to the very small electric current in a DC electric field, while the droplet makes contact with the electrode that could induce a pulsed electric field. This is because the droplet electroporation electrodes for disposable 96- and 24-well plates have been improved for concurrent performance.It is possible that this electroporation technique provides a novel means for cell physiological research by high-throughput screening. No COI.

3P-205

Quasi-real time recording of biomagnetic fields using a magnetically linear MI gradiosensor

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There are numerous tissues and organs which employ electric signals, distributed over the body. For instance, neurons code information using spike activity, while muscles contract with a rise of intracellular Ca2+ triggered by action potentials. A magnetic sensor easily applicable to living systems will be a convenient tool to evaluate physiological functions non-invasively. Here we show biomagnetic field measurements using a magnetically linear magnetoimpedance (MI) gradiosensor. This magnetic sensor head is made of a single amorphous wire and a couple of fine coils mounted in the both end of this wire. As a result, applications of an excitation pulse produce very similar induction potentials in the paired detector coils, thereby subtraction of the pair of induction potentials enables to detect changes in magnetic fields less than 100 pT. Using this new MI gradiosensor, we successfully measured oscillating magnetic fields presumably underlying propagation of pacemaker electric current in gut musculatures isolated from guinea-pigs. When the direction of muscle layer was reversed against the sensor head, the direction of magnetic fields was also reversed. We simulated the oscillating magnetic fields assuming intercellular electric current and extracellular return current. Furthermore, we measured oscillating cardiac magnetic fields accompanied by ECG. Magnetic fields corresponding to premature ventricular contractions were occasionally recorded. COI properly declared.

3P-206

Physiological data recording and analyzing system to improve the score on the small bore rifle shooting target during the training and match

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To investigate the influence of the physiological parameters such as pulse rate and respiration rhythm to the score on the target of the small bore rifle practice and the match, physiological data recording and analyzing system had been developed with the microprocessors. This system includes the weak pulse laser beam floodlight to irradiate synchronously with triggering, score indicating system equipped the optical sensors on the target and microprocessor.Respiration rhythm is recorded through the thermal IC sensor located near the nostril. The pulse rate is detected with the microphone attached on the right wrist and amplified. The barrel disturbance is detected through the three dimensional accelerations IC sensor. These data are stored in the memory card on the microprocessor board and processed on the personal computer for more detail statistical analysis for relationship between the score and the physiological parameters. No COI.

Poster Presentations
Education

3P-207

The yearly changes of scores of written examinations of physiology and the lowering of the levels of academic performance of medical students in Japan

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The aim of this study is to investigate the yearly changes of scores of written examinations of physiology and to find evidence of the lowering of the levels of academic performance of medical students in Japan after the increase of the number of medical students from the point of view of scores of written examinations of physiology. Using scores of examinations of physiology from 2002 to 2012, the mean, SD, mean + SD, mean - SD, the number of the students, the number of the unsuccessful students, and the rate of the unsuccessful students were examined. The scores of examinations of physiology of medical students dropped significantly after the increase of the number of medical students. One of the causes of the lowering of the levels of academic performance of medical students in Japan is the increase of the number of medical students. No COI.