Poster Presentations

Day 1

（March 21, 12:45～14:00）

P1-001～P1-060	Ion channels, Receptors
P1-061～P1-124	Neurons, Synapses
P1-125～P1-157	Molecular anatomy, Molecular physiology
P1-158～P1-197	Organelle, Membrane transport
P1-198～P1-209	Others of Molecular anatomy, Molecular physiology, Cell biology
P1-210～P1-234	Experimental methods
P1-235～P1-355	Undergraduate Poster Presentations

P1-001

Involvement of extracellular Ca^{2+} in the heat-evoked activation of green anole TRPA1

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Transient receptor potential ankyrin 1 (TRPA1) is a Ca^{2+}-permeable nonselective cation channel expressed in nociceptors and activated by irritant compounds such as allyl isothiocyanate (AITC) and temperature. TRPA1 was initially identified as a potential mediator of noxious cold stimuli in rodent nociceptive sensory neurons while TRPA1s from non-mammalian vertebrates (snakes, green anole lizards, frogs and chickens) were recently reported to be activated by heat, but not cold stimulus. A number of studies have shown that intracellular Ca^{2+} is a key regulator of many TRP channels, including TRPA1. In the previous study, we found that extracellular Ca^{2+}, but not intracellular Ca^{2+} plays an important role for heat-evoked activation of green anole TRPA1 (gaTRPA1). In this study, we focus on extracellular Ca^{2+}-dependent heat sensitivity of gaTRPA1 by comparing gaTRPA1 with other heat-activated TRPA1s from rat snake and chicken. It was found that, rat snake and chicken TRPA1s are activated by heat with small inward and large outward currents in the absence of extracellular Ca^{2+}. These results suggest that gaTRPA1 channel, but not TRPA1s of rat snake and chicken needs extracellular Ca^{2+} for heat-evoked activation.

(COI: No)

P1-002

Annexin A2 is a modulator of maxi-anion channel (Maxi-Cl) in mouse mammary C127 cells

Islam, Md. Rafiqul[1]; Okada, Toshiaki[1]; Merzlyak, Petr[1,2]; Sabirov, Ravshan Z.[1,2]; Okada, Yasunobu[3] (*[1]Dept. Cell Physiol., Natl. Inst. Physiol. Sci., Okazaki, Japan; [2]Lab. Mol. Physiol., Inst. Bioorgan. Chem., Uzb. Acad. Sci., Tashkent, Uzbekistan; [3]Grad. Univ. Adv. Studies (SOKENDAI), Hayama, Japan*)

The maxi-anion channel (Maxi-Cl) is ubiquitously expressed in mammalian cells and has been reported as an important gateway for release of the anionic signaling molecules, ATP and excitatory amino acids, from cells subjected to osmotic perturbation, ischemia or hypoxia. The molecular nature of this physiologically and pathophysiologically significant channel is yet to be discovered, although its biophysical and pharmacological properties have been well characterized. As a part of our Maxi-Cl molecule identification efforts we have already excluded several genes (such as *Panx1, Panx2, Cx43*) from the candidates (*Am J Physiol Cell Physiol* 2012, 303: C924-C935). In the present study, we investigated a possible relation of Annexin A2 to Maxi-Cl, because its mRNA (*Anxa2*) showed differential expression between Maxi-Cl-rich C127 cells and Maxi-Cl-deficient C1300 cells as judged by microarray analysis. Using both siRNA- and miRNA-mediated transient gene knockdown strategies, we found that Maxi-Cl currents recorded in the inside-out patch-clamp mode were significantly lower in *Anxa2* -silenced C127 cells than in the mock-transfected cells. However, when Annexin A2 was heterologously overexpressed in C1300 cells, the Maxi-Cl activity could not be rescued. Thus, we conclude that Annexin A2 is a modulator but not the molecule itself of Maxi-Cl.

(COI: No)

P1-003

TRPV2 is critical for the maintenance of brown adipose tissue structure and thermogenesis in mice

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Transient receptor potential vanilloid 2 (TRPV2) is a Ca^{2+}-permeable non-selective cation channel, which plays vital roles in the regulation of various cellular functions. However, the molecular identity and function of TRPV2 remains unexplored in mouse brown adipose tissue (BAT). This study aimed to clarify the expression and function of TRPV2 in brown adipocytes. We found that functional TRPV2 was predominantly expressed in brown adipocytes. Moreover, mRNA levels of multiple genes involved in the mitochondrial oxidative metabolism were significantly lower in TRPV2 knockout (TRPV2KO) mice than wild type (WT) mice. In addition, white adipose tissue weight was significantly larger in TRPV2KO mice than in WT mice, but food intake was not different between these two genotypes. TRPV2KO BAT showed increased sizes of brown adipocytes. And TRPV2KO mice also showed cold intolerance and reduced responses to a β3-adrenergic receptor agonist, BRL37344 administration. In conclusion, TRPV2 is functionally expressed in brown adipocytes. BAT thermogenesis is impaired in TRPV2KO mice. And TRPV2 is critical for the maintainence of BAT structure and thermogensis.

(COI: No)

P1-004

Regulation of CALHM1 ion channel by N-linked glycosylation

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Calcium homeostasis modulator 1 (CALHM1) was identified as a gene linked to the pathogenesis of late-onset Alzheimer's disease. Recent studies have established CALHM1 as a pore-forming subunit of a voltage-gated ion channel, determined structure and function of CALHM1 channel, and revealed its important physiological roles. However, lacking is knowledge about other modes of regulation of CALHM1 channel including ones mediated by post-translational modifications (PMTs). When heterologously expressed, mouse CALHM1 (mCALHM1) immunosignals in western blotting appeared at three positions with one band at the mass of a mCALHM1 monomer, 37 kDa, and two additional bands at larger molecular weights, suggesting that mCALHM1 acquires some sort of PMTs. Peptide-N-glycosidase F shifted the two additional bands to 37 kDa, demonstrating that mCALHM1 is glycosylated at Asn residue(s). While mCALHM1 possesses two predicted N-glycosylation sites, point mutation studies identified a conserved Asn139 as the sole N-glycosylation site. A difference in sensitivity to endoglycosidase H of the two glycosylated forms of mCALHM1 revealed that the acquired N-linked glycan undergoes processing/conversion from high-mannose type to complex type. In the present study, we examined roles of acquisition and processing/conversion of the N-linked glycan on channel function and subcellular localization of mCALHM1 by means of chemical and enzymatic reagents and genetic mutations. Our data provide insights into a novel regulation of CALHM1 channel via N-linked glycosylation.

(COI: No)

P1-005

Oxidative stress-induced inhibition of TRPM7 is resulted from enhancement of its sensitivity to intracellular Mg^{2+}

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We recently reported that TRPM7 current was inhibited by oxidative stress [1]. 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. The TRPM7 current was inhibited by intracellular free Mg^{2+} ($IC_{50(1)}$ 6.5 μM and $IC_{50(2)}$ 467.2 μM), and oxidative stress augmented this inhibition ($IC_{50(1)}$ 1.5 μM and $IC_{50(2)}$ 27.6 μM). In the present study, we further explored the mechanisms for the inhibition by introducing mutations in TRPM7. Tetracycline-inducible HEK cell lines for stably expressing wild type (WT), phosphomimetic mutant (S1107E), and kinase inactive mutant (K1645R) murine TRPM7 were established. The current of S1107E was insensitive to $[Mg^{2+}]_i$ as reported earlier [2], and was not inhibited by H_2O_2 even in the presence of 217 μM $[Mg^{2+}]_i$ (141 ± 12 pA/pF and 124 ± 13 pA/pF, before and after an application of H_2O_2, respectively), which is consistent with our hypothesis that oxidative stress inhibits TRPM7 current by sensitizing the channel to $[Mg^{2+}]_i$. TRPM7 kinase activity does not seem to be involved in the phosphorylation of S1107, because K1645R exhibited similar $[Mg^{2+}]_i$ dependent inhibition by H_2O_2 to that observed in WT. It is suggested that phosphorylation of S1107 by an unidentified kinase other than TRPM7 confers tolerance to oxidative stress-induced current inhibition. [1] Inoue et al., Free Radical Biology & Medicine 72 (2014) 257[2] Hofmann et al., Pflugers Archiv DOI 10.1007/s00424-014-1488-0

(COI: No)

P1-006

Insights into the Gating Regulation Mechanisms of the KcsA Potassium Channel from the Measurements of the Single-Molecule Dynamics

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The KcsA potassium channel is a pH gated channel which keeps the closed state at neutral pH and undergoes gating at acidic pH. To reveal the dynamic feature of the gating, we have engaged in developing the Diffracted X-ray Tracking (DXT) method in which conformational changes of the single channel molecules were recorded as a movie. At acidic pH the channel exhibited large twisting conformational changes and vigorous structural fluctuations. On the other hand, only small fluctuations were found at neutral pH. To examine the relationship between structural fluctuations and the status of the channel, the solution pH was jumped from neutral to acidic by using the caged proton, and the opening conformational changes of the activation gate were measured. The structural fluctuation was enhanced immediately after the pH jump, and the twisting conformational change was initiated after a delay. This enhanced fluctuation was a similar degree to that observed in equilibrium acidic conditions. In addition, the open channel blocker, TBA (tetrabutylammonium), dramatically suppressed the fluctuations and stopped the twisting motions at acidic pH. These results suggest that the level of fluctuations is closely related to the regulations of the gating.

(COI: No)

P1-007

Acidic amino acids near and in Transient Receptor Potential (TRP) domain of TRPM4 channel are required for maintaining its normal Ca^{2+}-sensitivity

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Transient receptor potential melastatin 4 (TRPM4) channel is a Ca^{2+}-activated non-selective cation channel. The Ca^{2+} sensitivity is modulated by calmodulin and PI(4, 5)P_2. However, unidentified intrinsic divalent cation binding sites in TPRM4 have been presumed to exist because the Ca^{2+} sensitivity has not been abolished by the deletions of its calmodulin binding sites. The purposes of this study are to reveal the properties of the divalent cation binding sites in TRPM4 and to identify the possible amino acid residues which form the binding sites. We firstly examined the effects of divalent cations applied to the cytosolic side of the channel by using an inside-out mode patch-clamp technique. Co^{2+}, Mn^{2+}, and Ni^{2+} potentiated TRPM4 currents, but they did not evoke any currents without Ca^{2+}. These data suggest that there are at least two functionally different binding sites: one is a relatively Ca^{2+}-specific binding site and the other is a binding site for Co^{2+}, Mn^{2+}, and Ni^{2+}. Next, we explored amino acid residues responsible for the activation by Ca^{2+} using single amino acid mutagenesis. Mutations of acidic amino acids near and in the TRP domain, which are conserved in TRPM2, M5, and M8, decreased the Ca^{2+} sensitivity but hardly affected the sensitivities for Co^{2+} and PI(4, 5)P_2. These results suggest a novel role of the TRP domain in TRPM4 as a site responsible for maintaining its normal Ca^{2+} sensitivity.

(COI: No)

P1-008

Omega-3 fatty acids activate Slo1 BK channels and lower blood pressure

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Long-chain omega-3 fatty acids such as docosahexaenoic acid (DHA) found in oily fish may offer various health benefits but the underling mechanisms are only poorly understood. In vascular smooth muscle cells, large-conductance $Ca2^{2+}$- and voltage-dependent K^+ (Slo1 BK) channels provide a vasodilatory influence. We found that DHA with EC50 of ~500 nM directly and reversibly activates BK channels composed of the pore-forming Slo1 subunit and the auxiliary subunit $\beta 1$ in excised-patches, increasing currents by up to ~20-fold. The DHA action does not require voltage-sensor activation or $Ca2^{2+}$ binding but depends on an electrostatic interaction within $\beta 1$ or $\beta 4$. DHA acutely lowers blood pressure in anesthetized wild-type but not in Slo1 knockout mice. DHA ethyl ester (DHA EE), found in dietary supplements, fails to activate BK channels and antagonizes the stimulatory effect of DHA. On an equimolar basis, the stimulatory effect DHA on Slo+1 channels was greater than that of eicosapentaenoic acid, alpha-linolenic acid, arachidonic acid, or linoleic acid. Slo1 BK channels are thus receptors for long-chain omega-3 fatty acids that, unlike their ethyl ester derivatives, activate the channels and lower blood pressure.

(COI: No)

P1-009

Distribution of ASIC4 channel in the mouse brain

Hoshikawa, Mariko; Shibata, Yasuhiro; Kumamoto, Natsuko; Ueda, Takashi; Ugawa, Shinya (*Grad. Sch. Med. Sci., Nagoya City Univ., Nagoya, Japan*)

Acid-sensing ion channels (ASICs) are neuronal proton-gated cation channels expressed in mammalian central and peripheral nervous systems. Four ASIC genes (from Accn1 to Accn4) and at least six proteins (ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4) have been identified in mammalian organisms so far. In the brain, ASIC1a, ASIC2a and ASIC2b are known to be involved in a wide variety of physiopathological processes related to extracellular pH fluctuation, such as synaptic transmission and ischemic neuronal injury. However, the regional distribution and function of ASIC4 in the brain are largely unknown. To clarify them, we first performed in situ hybridization experiments using a specific cRNA probe for ASIC4. It revealed that ASIC4 transcripts were broadly expressed throughout the brain. The strong expression was observed in the olfactory bulb, piriform cortex, striatum and superior and inferior colliculi, but was hardly detected in the hippocampal structures, which is in disagreement with the related data from the Allen Brain Atlas. We then generated novel ASIC4 reporter mice using the *ASIC4*$^{tm1a(KOMP)mbp}$ targeting vector [the Knockout Mouse Project (KOMP) repository], and confirmed the proper homologous recombination by RT-PCR and Southern blot analysis. We are currently investigating the detailed distribution of ASIC4 transcripts in the brain.

(COI: No)

P1-010

Voltage-dependency of FMRFamide-gated Na^+ channels

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FMRFamide-gated Na^+ channel (FaNaC) is a peptide-gated sodium channel in the ENaC/DEG family. FaNaC is a homo-trimer, and a subunit has two transmembrane domains (M1 and M2). Although the crystallographic structure of FaNaC is not known, homology modeling based on the structure of an acid-sensing ion channel shows that N-terminal region of M2 (pre-M2) constructs the external vestibule of the channel pore. In the vestibule, there are two aspartate residues (D552, D556) which make negative-rings around the central axis of the pore. We previously showed that *Aplysia* FaNaC (AkFaNaC) expressed in *Xenopus* oocytes is inhibited by external Ca^{2+}, and that aspartate residues in pre-M2 (D552) are involved in the Ca^{2+} action (Pflugers Arch, 451:646-656, 2006; Zool Sci, 27:440-448, 2010). We also showed that D552 is a determinant of the inwardly rectifying I-V relationship of FaNaC (J Physiol Sci, 64:141-150, 2014). To examine the permeation properties as well as the gating characteristics of FaNaC, we employed a cut-open oocyte voltage clamp. We found that the gating of FaNaC is voltage dependent, showing an inward relaxation with two time constants in response to a hyperpolarizing voltage step. Relative amplitudes of exponentially increasing components but not time constants were dependent on the membrane potential as well as the concentration of Na^+. The relaxation was also affected by external Ca^{2+}, and was abolished in Ca^{2+}-free solution. We are currently examining whether the position 552 or 556 is involved in the voltage-dependency of FaNaC.

(COI: No)

P1-011

Hydrophobic layer prevents the proton permeation of voltage-gated proton channel

Kawanabe, Akira; Okamura, Yasushi (*Grad Sch Med, Osaka Univ, Osaka, Japan*)

Voltage-gated proton channel (Hv1/VSOP) has a voltage sensor domain (VSD), which is similar to the VSD (S1-S4) of classical voltage-gated ion channel, but lacks a pore domain (S5-S6). It has an ability of proton permeation through the VSD controlled by membrane voltage and pH in phagocytes and spermatozoa. We have recently revealed the X-ray crystal structure of Hv1/VSOP (Takeshita et al. 2014) and found a hydrophobic layer, which consisted of four hydrophobic residues (V112/S1, L143/S2, L185/S3 and L197/S4). These residues are not conserved in other voltage-sensor proteins. This hydrophobic layer might be important for breaking water wire and thus prevent the proton permeation in closed state. To test this idea, we analyzed the electrophysiological properties of mouse Hv1/VSOP with mutation in the hydrophobic layer heterologously expressed in HEK293T using whole cell patch clamp recording. The proton currents of three cysteine mutants at V112, L143 and L185 were similar to the proton currents of the wild-type. The cysteine mutant of L197 showed a significant change: The inward current was observed at negative voltage. The pH sensitivity and zinc dependence of gating verified that the inward current at negative voltage was proton current through the mutant proteins. These results support the idea that the hydrophobic layer serves as the barrier for proton permeation in closed state.

(COI: No)

P1-012 (AP-3)

Voltage-dependent movement of the catalytic domain of voltage-sensing phosphatase, VSP, probed by the site-specific incorporation of a fluorescent unnatural amino acid

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Voltage-sensing phosphatase, VSP, consists of a voltage sensor and a cytoplasmic catalytic domain (CD). The enzymatic activity has been shown to be coupled to the voltage sensor movement. It has been proposed that the voltage-sensor activation induces the conformation change of CD. However, the direct evidence has been lacking. To monitor the voltage-dependent conformation change of CD, we genetically incorporated a fluorescent unnatural amino acid, Anap, into CD. First, Anap was incorporated into "gating loop" which has been claimed to make large conformational change for switching enzymatic activity based on the crystallographic study of CD of VSP. Anap fluorescence was changed in a voltage-dependent manner, indicating that CD changes its conformation upon the voltage-sensor activation. Besides the conformation change, it is also possible that the voltage sensor regulates the distance between CD and the plasma membrane. Since the substrate of the enzyme is phosphoinositides which are membrane components, membrane binding of CD may be crucial for the enzymatic activity. To detect the change of the distance between CD and the plasma membrane, CD and the plasma membrane were labeled by Anap and dipicrylamine(DPA), respectively. We verified that DPA works as a FRET acceptor of Anap on VSP and are currently testing if the distance between CD and the plasma membrane is changed during the voltage-dependent phosphatase activity.

(COI: No)

P1-013

Identification of nuclear adrenergic receptor in mouse dental blast cells

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Adrenaline circulates throughout the body and shows various physiological functions by acting on its receptors in the plasma membrane of cells and regulating the intracellular signaling cascades from cAMP, IP3 or Ca^{2+}. We happened to detect the localization of β-adrenergic receptor within the nucleus of the mouse dental blast cells. Adrenaline, which is not membrane-permeable, would need a route from the extracellular fluid through the cytoplasm to reach the nuclear receptor. To address this problem, we employed a series of biochemical and physiological investigations. By modified Falck-Hillarp histochemistry, we detected the monoamine signals in the cytoplasmic and nuclear spaces of these cells. Live observation of the lipophilic dye uptake revealed that these cells showed continuous endocytotic activity, which might be an access of adrenaline to its nuclear receptor. We hereby report on the possible regulatory mechanism of nuclear adrenergic receptor activation.

(COI: No)

P1-014

Association of a nicotinic receptor gene polymorphism with spontaneous eyeblink rates

Nakano, Tamami[1]; Kuriyama, Chiho[1]; Himichi, Toshiyuki[2]; Nomura, Michio[2] (*[1]Grad Sch Front Bio, Osaka Univ, Tokyo, Japan; [2]Grad Sch Edu, Kyoto Univ, Kyoto, Japan*)

Spontaneous eyeblink rates greatly vary among individuals from several blinks to a few dozen blinks per minute. Because dopamine agonists immediately increase the blink rate, individual differences in blink rate are used as a behavioral index of central dopamine functioning. However, an association of the blink rate with polymorphisms in dopamine-related genes has yet not been found. In this study, we demonstrated that a genetic variation of the nicotinic acetylcholine receptor CHRNA4 (rs1044396) increased the blink rate while watching a video. A receiver operating characteristic analysis revealed that the blink rate predicts a genetic variation in the nicotinic receptor gene with a significant discrimination level (0.66, p=0.004). The present study suggests that an increased sensitivity to acetylcholine because of the genetic variation of the nicotinic receptor induces dopamine production by the midbrain dopaminergic neurons, resulting in an increased spontaneous eye blink rate.

(COI: No)

P1-015

TRPV1 and Nav1.8 channels expressions in nodose ganglion neurons of rats treated with capsaicin during the neonatal period

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We examined immunohistochemically the expressions of transient receptor potential vanilloid 1 (TRPV1) and Nav1.8, a tetrodotoxin-resistant voltage-gated sodium channel, in vagal primary afferent neurons in nodose ganglion (NG) of rats, which had been treated with capsaicin during neonatal period. The NG was dissociated from deeply anesthetized 6-week-old rats, which had received normal saline (CONT) or capsaicin (50mg/kg) (EXP) intraperitoneally a day after birth. The frozen serial sections were incubated with primary antibodies directed against TRPV1 and Nav1.8 channels and then double-stained with secondary antibodies. Immunofluorescence was visualized by using a spectral confocal microscope. Total numbers of neurons (per section) in CONT and EXP groups were 96 and 21, respectively, and proportion of the neurons, which showed detectable expression of TRPV1 was 47 % (CONT) and 72 % (EXP) and for Nav1.8 it was 59 % (CONT) and 49 % (EXP). In addition, 32 % (CONT) and 40 % (EXP) of neurons showed co-expression of TRPV1 and Nav1.8. Our results suggest that physiological alterations occurs in vagal afferents after neonatal capsaicin treatment, which is applied to eliminate TRPV1-expressing neurons, may involves effects related to Nav1.8 channels, which are highly co-expressed with TRPV1 channels in NG neurons and therefore its expression could be influenced by neonatal capsaicin treatment.

(COI: No)

P1-016

mASIC4 is a Zn^{2+}-sensitive constitutively active channel

Shibata, Yasuhiro; Watanabe, Masaya; Hoshikawa, Mariko; Kumamoto, Natsuko; Ueda, Takashi; Ugawa, Shinya (*Dept. of Neuroscience and Anatomy, Nagoya City Univ. Grad. Sch. Med., Nagoya, Japan*)

Acid-sensing ion channels (ASICs) are neuronal cation channels activated by extracellular acidification. There are four ASIC genes that encode at least six individual subunits with distinct and overlapping patterns of expression. ASIC1a, ASIC1b, ASIC2a and ASIC3 can be directly activated by external protons, and ASIC2b is a modulatory subunit of the family. However, ASIC4 has not yet been shown to produce or modulate proton-evoked currents. To investigate the electrophysiological properties of mouse ASIC4 (mASIC4), two-electrode voltage-clamp recordings were performed. It was revealed that expression of ASIC4 in Xenopus laevis oocytes generated small inward currents at a holding potential of -60 mV, while there was no substantial current in H2O-injected control oocytes. The ASIC4 current was insensitive to a nonselective ASIC channel blocker, amiloride, but was reversibly inhibited by 100 μM Zn2+ (IC50 = 89 μM, 95% confidence interval: 63-126 μM). The Zn2+-sensitive current was highly selective for Na+ as the reversal potential was approximately 10 mV in NaCl bathing solution (ND96). Interestingly, the ASIC4 leakage current was enhanced in the presence of extracellular protons, and the current was also blocked by 100 μM Zn2+. These results raise the possibility that ASIC4 associates with other ASIC subtypes to form heteromeric assemblies with the novel electrophysiological properties in the mammalian nervous system.

(COI: No)

P1-017

Guaiacol activates TRPV3 channels in mouse odontoblast-lineage cells

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Guaiacol is used for endodontic treatment and has high analgesic activity. Odontoblasts play important roles in nociceptive sensitivity via the activation of transient receptor potential (TRP) channels, that participate in receiving the stimuli at the dentin surface. In this study, to elucidate the pharmacological effects of guaiacol on the activation of Ca^{2+}-permeable channels in mouse odontoblast-lineage cells (OLCs), we examined the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) by fura-2 fluorescence imaging. In the presence of extracellular Ca^{2+}, we could observe a transient increase in $[Ca^{2+}]_i$ upon treatment with 0.9 μM guaiacol; however, this effect was not observed in the absence of extracellular Ca^{2+}. Repeated application of guaiacol elicited a significant desensitizing effect on Ca^{2+} entry. Increase in $[Ca^{2+}]_i$ by guaiacol was not inhibited by the antagonists of TRPV2 or TRPV4 channels, but was slightly inhibited by a TRPV1 antagonist (capsazepine). Moreover, this guaiacol-induced increase in $[Ca^{2+}]_i$ was significantly inhibited by a TRPV3 antagonist, 2, 2-diphenyltetrahydrofuran. Immunoreactivity against TRPV3 was observed in the OLCs. These results indicate that guaiacol evokes Ca^{2+} influx from the extracellular medium via Ca^{2+} channels in OLCs, and the activation of TRPV3 channels on the plasma membrane of odontoblasts is related to this guaiacol-induced Ca^{2+} influx.

(COI: No)

P1-018

X-ray crystal structure of voltage-gated proton channel

Takeshita, Kohei[1,4]; Sakata, Souhei[2,3]; Yamashita, Eiki[1]; Fujiwara, Yuichiro[2]; Okamura, Yasushi[2]; Nakagawa, Atsushi[1] (*[1] Lab. of Supramol. Crystal., Inst. for Prot. Res., Osaka Univ.; [2]Grad, sch. of Med., Integr. Physiol., Osaka Univ.; [3]Inst. for Acad. Init., Osaka Univ.; [4]JST, PRESTO*)

Voltage-gated proton channel, VSOP, has a voltage-sensor domain but lacks an authentic pore domain. VSOP is required for high-level superoxide production coupling with NADPH oxidase and so on. Our crystal structure of mouse VSOP showed a closed umbrella shape with a long helix consisting of the cytoplasmic coiled-coil and the voltage-sensing helix, S4, and featured a wide inner-accessible vestibule. We also found a Zn^{2+} ion at the extracellular region of mVSOP. The binding of Zn^{2+} suggested that the crystal structure represents the resting state, since Zn^{2+} specifically inhibits activities of VSOP. Actually, two out of three arginines as sensor residues (R204 and R207) were located lower than the conserved phenylalanine, F146, on the S2 in a charge transfer center. This makes contrast with previous structures of other VSDs in the activated state where many positive residues of S4 were located upper than the conserved Phe. Additionally, the crystal structure of mVSOP highlighted two hydrophobic barriers. Aspartic acid (D108), which is critical for proton selective permeation, was located facing intracellular vestibule below the inner hydrophobic barrier, thereby being accessible to water from the cytoplasm. Another hydrophobic layer of extracellular side probably ensures interruption of the proton pathway of mHv1 in resting state. These findings provide a novel platform for understanding the general principles of voltage sensing and proton permeation.

(COI: No)

P1-019

The performance of CB1-knockout mice in three-lever task

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Endocannabinoids play a role in synaptic plasticity through activation of presynaptic CB1 receptors. In the basal ganglia, which contribute to motor learning, CB1 receptors are abundantly expressed. So, it is likely that the endocannabinoid system plays a role in motor learning. To test this possibility, we compared the performance between wild-type and CB1-knockout (CB1-KO) mice in the three-lever operant task. In the operant box, three levers were protruded 1.8 cm into the chamber, and the right (A), center (B) and left (C) levers were positioned 2, 4 and 2 cm above the floor, respectively. One training session lasting 60 min was given once a day and five times a week. The mice were trained to press any one of the three levers for a food reward as shaping (one-lever task), and then trained to press three levers in a given sequence (ABC) (three-lever task). After the mice showed good performance of the three-lever task, the order was reversed to CBA (reverse three-lever task). We found that CB1-KO mice displayed normal performance in the three-lever task, but showed some impairment in the one-lever task, and the reverse three-lever task. In the one-lever task, CB1-KO mice showed a delay in increasing the total number of lever press during the first several sessions, and a delay in avoiding the inactive lever after the inactivation of the lever that was pressed most frequently in the previous session. Our data suggested that CB1-KO mice have difficulty in adapting motor action in response to varying demands.

(COI: No)

P1-020

Voltage-dependent single-channel gating kinetics of mouse pannexin 1 channel

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Pannexin 1 (Panx1) channels are anion-selective channels (Ma et al., Pflugers Arch, 2012) that can be activated by mechanical stimuli, membrane depolarization, caspase-mediated cleavage of its C-terminus and interaction with activated P2X7 receptor. Panx1 considered to be a conduit for ATP release, and act as a "find-me" signal that recruits macropharges to apoptic cells and a crucial paracrine regulator of muco-ciliary function in airway epithelia. Although the open probability of mPanx1 channel increases in a voltage-dependent manner, the detailed single-channel gating kinetics of mPanx1 channel is still remains unknown. In this study, we examined the single-channel conductance and opening/closing rates of mouse Panx1 (mPanx1) channel by using the cell-attached mode of the patch-clamp technique. The channel showed an outward rectification of voltage-current relationship with single-channel conductances of ~20 pS at inward current and ~80 pS at outward current. The voltage-dependency of single-channel opening/closing rates are drastically different at hyperpolarized and depolarized conditions in the boundary of the reversal potential. These results suggest that the quantity of movement per unit time and the direction of movement of charge carrier control the voltage-dependency of opening/closing rates of mPanx1 channel.

(COI: No)

P1-021

Fingerprinting catalytic activity of GPCRs on exhaustive set of G protein substrates reveals complex profiles of functional bias

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A defining feature of all known G Protein-Coupled Receptors (GPCRs) is their ability to activate heterotrimeric G proteins. It is thought that much of the GPCR signaling diversity originates from their differential coupling to an array of G protein subunits, whose unique properties underlie distinct cellular responses. However, the rules for how GPCRs engage selective G proteins from their numerous G protein substrates in response to endogenous ligands and synthetic drugs have not been defined. We developed a multi-dimensional platform for comprehensive profiling of GPCR activity on a nearly complete set of all possible G protein substrates in living cells. The key feature of the system includes quantitative analysis of both efficacy and kinetics of G protein activation using reporter-based imaging strategy yielding robust signals with high reproducibility and throughput. We found that individual GPCRs exhibited characteristic, fingerprint-like, profiles of functional selectivity among their G protein substrates varying in timing and magnitude of activation. Furthermore, synthetic agonists and intracellular molecular environment selectively impacted these fingerprints. We propose that fingerprinting individual GPCRs for their G protein preferences using this technology will enhance understanding of GPCR effects on cellular function, higher order physiology and pathophysiology, and needs to be considered in drug development campaigns to better predictive pharmacological effects of synthetic ligands.

(COI: No)

P1-022

The function of Voltage-Sensing Phosphatase in mice sperm

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Voltage-sensing phosphatase (VSP) consists of the voltage-sensor domain and phosphoinositide phosphatase domain. VSP shows phosphatase activity that is coupled to membrane potential (Murata et al, Nature. 2005). While its expression has been known in secondary spermatocyte in testis of mice, its biological function in sperm remains elusive. In the present study, we examined the biological function of VSP in mice sperms, by focusing on acrosomal reaction, capacitation, sperm motility and so on. To elucidate them, we used knockin mice (VSP-KI mice) in that endogenous VSP gene was replaced by Venus for the present study. We neither observed significant difference in the rate of acrosomal reaction nor tyrosine phosphorylation during the capacitation between VSP-KI mice and hetero mice. On the other hand, it appears that sperm from VSP-KI mice shows a different behavior in their motility from hetero and wild type (WT) mice sperm. We also examined whether endogenous VSP can regulate PIP2 levels in mice sperm. A possible mechanism underlying the regulation of sperm function by PIP2 in mice sperms is discussed.

(COI: No)

P1-023

Slow synaptic inward currents in interneurons in the CA3 area of the hippocampus

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Balance of synaptic excitation and inhibition in the hippocampal neuronal circuit plays an important role in memory, cognition, and pathogenesis of neuronal disorders. Metabotropic glutamate receptors (mGluRs) are known to mediate a slow synaptic transmission in the central nervous system (Cosgrove et al., 2011). Many studies reported that genetic disruption or reduced expression of mGluRs was causal for neuronal disorders such as schizophrenia and autism spectrum disorders (Carlisle et al., 2011; Olszewski et al., 2012), indicating that mGluRs might regulate the function of the neuronal circuits. In the CA3 area of the hippocampus interneurons express mGluRs coupled to cationic channels to mediate feedback inhibition onto the CA3 pyramidal cells (Mori et al., 2002). In the present study, we investigated intracellular signaling pathway and channels responsible for slow excitation in CA3 interneurons. So far the preliminary data were obtained as below. Electrical stimulation of the layer of CA3 pyramidal cells evoked an slow inward current (Peak amplitude, 81.13 ± 12.92 pA, n=5) in CA3 interneurons of cultured rat hippocampal slices at a holding potential of -70 mV in the presence of antagonists for AMPA/kainate receptors, NMDA receptors, $GABA_A$ receptors and $GABA_B$ receptors. The slow inward current was abolished by the bath perfusion of Ca^{2+} free solution, indicating that the inward current is of synaptic origin. Inclusion of GDP β s (1 mM) in the patch pipette on the interneuron inhibited the slow inward synaptic current.

(COI: No)

P1-024

mGluR1α-mediated excitation requires G-protein-dependent and Src-ERK1/2-dependent signaling pathways in cerebellar GABAergic interneurons

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In neurons of the various brain areas, stimulation of type I metabotropic glutamate receptors induces slow excitatory responses through the activation of transient receptor potential canonical (TRPC) channels following several signal transduction pathways. In the cerebellar molecular layer interneurons (MLIs), the underlying mechanisms of the mGluR1 α -mediated slow inward current remain unclear. We found that spontaneous firing of mouse cerebellar MLIs was facilitated by mGluR1 α activation, which caused the inward current partially through G-protein activation. Most of the mGluR1 α -mediated inward current was mediated by TRPC1 channels. Nonselective protein tyrosine kinase inhibitors, genistein and AG490, suppressed the mGluR1 α -mediated inward current significantly. Furthermore, the selective Src kinase inhibitor PP2 and the selective extracellular signal-regulated kinase 1/2 (ERK1/2) inhibitors PB98059 and SL327 decreased the inward current significantly. In contrast to cerebellar Purkinje cells, $GABA_B$ receptor activation in MLIs did not alter the mGluR1 α -mediated inward current, suggesting that there is no cross-talk between mGluR1 α and $GABA_B$ receptors in MLIs. Thus, activation of mGluR1 α in MLIs causes the slow excitatory inward current through not only G-protein-dependent but also Src-ERK1/2-dependent signaling pathways.

(COI: No)

P1-025

The kainic acid-induced synchronous oscillation in the rat barrel cortex

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How functional columns are synchronized or desynchronized is an essential problem on columnar integration. It has been reported that bath application of kainic acid induced 1-5 Hz synchronous network oscillation in layer (L2/3) 2/3 of the barrel cortex. We have recently reported that $GABA_B$ receptor-mediated presynaptic inhibition ($GABA_B$-Pre-I) of intracortical inputs was involved in inter-columnar desynchronization in the barrel cortex using dual whole-cell recordings from two L3 pyramidal cells in the mutually adjacent columns. In the present study, we investigated how the $GABA_B$-Pre-I modulates the spatio-temporal patterns of kainic acid-induced network synchronizations in the barrel cortex by using a voltage-sensitive dye imaging method in slice preparations. Bath application of kainic acid caused synchronous oscillations across multiple columns in the barrel cortex. These synchronous oscillations were mainly composed of theta and delta frequency components. An application of $GABA_B$ receptor antagonist, CGP55845, abolished the delta waves, but slightly enhanced the theta waves. These results suggest that the delta waves in the barrel cortex induced by kainic acid are generated by the activity of presynaptic $GABA_B$ receptors expressed in the glutamatergic axon terminals.

(COI: No)

P1-026

Modified autonomic regulation in mice mutated in the β4 subunit of the lh/lh calcium channel

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Genetic analyses have revealed an important association between P/Q-type calcium channel activities and hereditary neurological disorders. The aim of the present study was to elucidate the physiological importance of the β4 subunit with lethargic (lh) mutant mice. The lh mutant had a small thymus with small-to-medium-sized lymphocytes in the medulla. RT-PCR analysis revealed time-dependent changes in the expression levels of the thymus CaV2.1 and β4 subunits. Both the number and size of Purkinje cells were reduced in the lh mouse cerebellum. In addition, immunostaining with anti-CaV2.1 antibody showed that the expression level of the P/Q-type channel-forming CaV2.1 protein was reduced, which may be associated with the ataxic phenotype. ECG analysis showed that the T wave was high in 8-week-old lh mutants; this may be associated with hyperkalemia. Upon pharmacological ECG analysis, 2-3-week-old lh mutants exhibited reduced responses to a beta-blocker and a muscarinic receptor antagonist. Analysis of heart rate variability revealed that the R-R interval was unstable in lh mutants and that both the low- and high-frequency components had increased in extent, indicating that the tonus of both the sympathetic and parasympathetic nervous systems was modified. Thus, our present study revealed that the β4 subunit played a significant role in regulation of sympathetic and parasympathetic nerve activities.

(COI: No)

P1-027

Nerve injury increases expression of alpha-2/delta-1 subunit of L-type calcium channel in the rat dorsal root ganglion

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Immunohistochemistry for alpha-2/delta-1 subunit of L-type calcium channel was performed on the rat dorsal root ganglion (DRG). The immunoreactivity (IR) was detected in half of 4th and 5th lumbar DRG neurons (49%). These neurons were mostly small to medium-sized. In the ganglia, alpha-2/delta-1 subunit -immunoreactive (-IR) neurons with small cell bodies were intensely stained whereas those with medium-sized cell bodies were lightly stained. Transection of the sciatic nerve dramatically increased the number of alpha-2/delta-1 subunit-IR neurons in the DRGs. Sensory neurons mostly expressed alpha-2/delta-1 subunit-IR in the DRGs at 7 days after the nerve transection (85%). The density of alpha-2/delta-1 subunit-IR in DRG neurons was also elevated by the transection. The number of intensely stained neurons with small cell bodies greatly increased in the injured DRGs. Numerous medium-sized and large DRG neurons which were intensely stained appeared after the treatment. In addition, a double immunofluorescence study demonstrated co-expression of alpha-2/delta-1 subunit and c-Jun activating transcription factor 3 in the injured DRGs. These findings indicate that the subunit may be associated with neuropathic pain transmission.

(COI: No)

P1-028

Electrophysiological effects of volatile anesthetic sevoflurane on striatal neurons of mouse

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It has been proposed that volatile anesthetics exert their influence on nervous system by affecting synaptic transmission and excitability of neural membrane. Sevoflurane is a valuable and commonly-used agent for inhalation anesthesia because of its fast action and short recovery time. In this study we examined how the application of sevoflurane affect the excitability of striatal neurons. Electrophysiological recordings were made from medium-sized projection neurons in slices taken from mouse striatum. Bath-applied sevoflurane caused transient depolarization of membrane potential lasting several tens of seconds. The transient depolarization was obscured by blockade of glutamatergic and GABAergic receptors, suggesting the involvement of unusual synaptic transmissions. In fact, previous study demonstrated the transient increase of spontaneous EPSC frequency in this induction period of anesthesia. However prolonged application of sevoflurane seems to decrease the excitability of striatal neurons by enhanced tonic-GABA current and rise in sodium spike threshold. Furthermore, sevoflurane increased input resistance of striatal projection neurons and diminished the voltage deflections in response to hyperpolarizing and depolarizing current pulses. Therefore, the transient excitation followed by continuous inhibition may underlie the effect of sevoflurane.

(COI: No)

P1-029

Cell swelling induced in hypotonic condition causes TRPA1 activation

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Hypotonic solution causes pain sensation in nasal and ocular mucosa. However these molecular mechanisms were not still entirely known. The candidate receptors, which could be activated by hypotonic solution, are Transient Receptor Potential (TRP) channels. Although TRPV4 was reported as a osmotic sensor, this channel was not known any obious roles in sensory nerves. Therefore, the existence of the other candidates is beleaved. In this study, we tried to clarify the ability of TRPA1 to response to the cell swelling, one of physical stress. Here we shows the modulation of TRPA1 activity induced by AITC was occured by hypotonic condition with Ca-imaging method. Moreover cell swelling in hypotonic condition evoked TRPA1 single current in cell-attached mode of patch-clamp experiment when the pippet was attached to plasma membrane after cell swelling, but not before swelling. Furthermore, this single current caused by cell swelling was blocked by known TRPA1 antagonists. Since pre-application of thapsigargin did not inhibit this single current induced by cell swelling, intracellular calcium concentration did not relate to the activation of TRPA1 caused by physical stress. These findings suggest that the cell swelling cause TRPA1 activation in the cells under the hypotonic condition, resulting pain sensation in nasal and ocular mucosa.

(COI: No)

P1-030

Threshold strength of electric fields for orientation of retinal ganglion cell axons *in vitro*

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Electric fields are a predominant guidance cue directing axons of retinal ganglion cells to the future optic disc during embryonic development (Yamashita, 2013). The axons of newborn retinal ganglion cells grow along the extracellular voltage gradient that exists endogenously in the embryonic retina. The extracellular potential is generated by Na^+ transport through epithelial Na^+ channels in retinal neuroepithelial cells. To investigate molecular mechanisms of the electric effect on axon growth, the threshold field strength for axon orientation should be defined in controlled conditions. In the present study, a culture system was built to set up a uniform constant direct current (DC) electric field by continuously measuring a voltage drop between two points in the culture medium, and also by using a negative feedback circuit to regulate supplied currents. A retinal strip (1 mm in width) of a chick embryo was cultured in a constant electric field for 24 hours and the relationship between the direction of axon growth and the field strength was analyzed by staining live axons with fluorescent dye (calcein-AM). The results showed that a voltage gradient of 0.2 mV/mm was enough to direct axons towards the cathode. This field strength is far weaker than the endogenous voltage gradient (15 mV/mm). The present study suggests that the supra-threshold electric field is sufficient for the correct orientation of retinal ganglion cell axons *in vivo*.

Reference:
1) Yamashita, M. Electric axon guidance in embryonic retina: Galvanotropism revisited. ***Biochem. Biophys. Res. Commun.***, 431: 280-283 (2013).

(COI: No)

P1-031

Exploring the original function of AMPA receptor

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AMPA receptor (AMPAR) has been mainly analyzed using adult stage samples in the light of functional importance for higher brain function, such as learning and memory. However, its expression in the immature neuron suggests that AMPAR has different function in early developmental stage from in adult stage. Numbers of subunits have prevented our generating the KO mice and analyzing its contribution to the development. Here, we chose ascidian, which has only one subunit and is suitable for developmental and genetic analysis. Using morpholino oligo knockdown system, decreased AMPAR expression leaded to inhibition of sensory organ formation and developing arrest during metamorphosis. Two types of AMPARs are in the vertebrate, Ca2+ permeable or impermeable, and using both types of receptors as the situation demands enables to establish the mammalian-type learning and memory. We found that ascidian had only Ca2+ permeable AMPAR. We next examined what happened to ascidian when mammalian-like Ca2+ impermeable AMPAR is introduced. Surprisingly such AMPAR did not have channel activity, and the Ca2+ impermeable AMPAR-induced ascidian demonstrated the same abnormalities as AMPAR KD experiments. These results suggest that the evolutionarily-original function of AMPAR might be involved in a specific neuronal morphogenesis and metamorphosis, and advanced learning system using two types of AMPARs could become available only after vertebrate.

(COI: No)

P1-032

Analysis of a new CaV2.1-interacting protein, TNKS2, as a causative protein of Spinocerebellar Ataxia type 6

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Spinocerebellar ataxia type 6 (SCA6) is one of the autosomal dominant cerebellar ataxias. SCA6 is caused by a small expansion of CAG repeats in *CACNA1A*, which encodes the α_{1A} pore-forming subunit of voltage dependent calcium channel, $Ca_V2.1$ (Olga Zhuchenko et al. 1997). When *CACNA1A* is translated, this CAG repeat appears as poly-glutamine (polyQ) chain in carboxyl terminal intracellular region of $Ca_V2.1$. The general mechanism of polyQ diseases is that aggregate of the expanded polyQ protein exerts neurotoxicity. But recent study suggests that at least 54 glutamine resides are needed for aggregate form stably (Martina Stork 2005). Although other polyQ diseases can meet this condition, SCA6 has at most 40 glutamines and cannot meet this condition. However, expanded polyQ chain may form abnormal structure, therefore the interaction of proteins may be changed. To investigate whether the abnormal expansion of polyQ chain changes the interaction between $Ca_V2.1$ and cryptic molecules and exerts neurotoxicity, 2.5×10^6 clones were screened and we discovered a new $Ca_V2.1$-interacting protein, TRF1-interacted ankyrin-related ADP-ribose polymerase 2 (TNKS2 or Tankyrase 2). The interaction between TNKS2 and $Ca_V2.1$ became stronger. In human cells, current density of $Ca_V2.1$ decreased and the expression of $Ca_V2.1$ on the plasma membrane was reduced. All effects were enhanced by abnormal polyQ expansion. Our findings uncover TNKS2 as a key regulator of $Ca_V2.1$ expression on the plasma membrane and as a new SCA6-involving protein.

(COI: No)

P1-033

Regulation of cardiac Cav1.2 channel by redox via modulation of CaM interaction with channel

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Although it has been well documented that redox can modulate Cav1.2 channel activity, the underlying mechanisms are not fully clear. In the present study, we examined the effect of DTT and H_2O_2 on Cav1.2 channel activity using patch-clamp technique in guinea-pig ventricular myocytes and on CaM interaction with Cav1.2 α 1C subunit with pull-down assay. Application of 1 mM DTT in the perfusing solution decreased channel activity to 72%, while H_2O_2 increased channel activity to 303%, suggesting reduction and oxidation played opposite effect in modulation of Cav1.2 channel. Pretreatment of cardiac myocytes with 1 mM DTT and H_2O_2 significantly impact the channel activity induced by CaM (1 μM) + ATP (3 mM) (72% and 176% of control, respectively). To test the hypothesis that redox state might determine channel activity through affecting CaM interaction with the channel, we examined the effect of DTT and H_2O_2 on CaM binding to N- and C-terminal fragment of the channel which contained CaM binding sites. We found that DTT dose-dependently inhibits CaM binding to C-terminus (IC_{50} 37 μM), but H_2O_2 had no effect. Neither DTT nor H_2O_2 had an effect on CaM interaction with N-terminus. These results suggest that redox-mediated regulation of Cav1.2 channel is mediated, at least partially, by modulating CaM interaction with channel.

(COI: No)

P1-034 (AP-6)

Molecular mechanism and regulation of partial agonism of the M2 muscarinic receptor-activated K^+ currents

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Partial agonists are clinically used to avoid overstimulation of receptor-mediated signaling, as they produce a submaximal response even at 100% receptor occupancy. In addition to signaling activators, several regulators help control intracellular signal transductions. However, it remains unclear whether these signaling regulators contribute to partial agonism. Here we show that regulator of G-protein signaling (RGS) 4 is a determinant for partial agonism of the M2 muscarinic receptor (M2R). In rat atrial myocytes, pilocarpine evoked smaller G-protein-gated K^+ inwardly rectifying (K_G) currents than that evoked by ACh. In a *Xenopus* oocyte expression system, pilocarpine acted as a partial agonist in the presence of RGS4 as it did in atrial myocytes, while it acted like a full agonist in the absence of RGS4. Functional couplings within agonist-receptor complex/G-protein/RGS system controlled the efficacy of pilocarpine relative to ACh. Pilocarpine-M2R complex suppressed G-protein-mediated activation of K_G currents via RGS4. Such RGS4-mediated regulation was enhanced at hyperpolarized potentials. We also found that the relative efficacy of pilocarpine to ACh changed upon membrane voltages. Our results demonstrate that partial agonism of M2R is regulated by the RGS4-mediated inhibition of G-protein signaling. This finding helps us to understand the molecular components and mechanism underling the partial agonism of M2R-mediated physiological responses.

(COI: No)

P1-035

Methods for the functional analysis of ion channels on the contact bubble bilayer by the intra-bubble perfusion

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Planar lipid bilayers have been used as the platform for the functional measurement of ion channels. Recently, a stable planar lipid bilayer (droplet interface bilayer, DIB) can be formed easily by contacting two lipid monolayers formed on the surface of small water droplets (ca. 1 mm diameter) in the oil. Here we improve the DIB method for the time-resolved measurement of changes in the electrophysiological properties of ion channels upon instantaneous solution exchange. For this purpose, a minuscule water bubble lined with lipid monolayer is blown from a glass pipette into an oil phase. Two bubbles (each with a diameter of ca. 50 μm) are held side by side to form a bilayer, which is termed a contact bubble bilayer (CBB). The area and curvature of the CBB are under the control of the intra-bubble pressure applied by each pipette. Additionally, the lipid composition of each leaflet of the CBB is readily varied. Because of smaller area of the CBB (ca. 80 μm^2) than the conventional planar lipid bilayers (>1000 μm^2), low background electrical noise in electrophysiological measurements is attained. A rapid perfusion system is also developed by introducing additional pressure-driven injection pipettes to the bubbles. Because the volume of the bubble is small (ca. 300 pL), the solution inside the bubble is exchanged within 20 ms. Example applications of this versatile method are presented to characterize the function of ion channels.

(COI: No)

P1-036

Exploration of temperature sensitivity and new antagonists of acid-sensitive outwardly rectifying anion channel (ASOR)

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It is well known that the acid-sensitive outwardly rectifying anion channel (ASOR), which is expressed in many cell types, is involved in acidotoxic necrotic cell death. However, its physiological significance and molecular identity have not been known yet. In human epithelial HeLa cells and cultured mouse cortical neurons, whole-cell currents of ASOR were augmented by warm temperature with a threshold temperature of 32 ℃ and 26 ℃, respectively. Cell swelling induced by extracellular acidification was inhibited by cooling, and acidosis-induced necrotic cell death was rescued at low temperature in both HeLa cells and cortical neurons. These data indicate that ASOR is sensitive to temperature and that reduced temperature rescues acidotoxic cell death by preventing persistent swelling. In fact, both acidosis-induced cell swelling and necrotic cell death were inhibited by DIDS and phloretin which are known ASOR blockers. We further explored the effects of 13 known antagonists for other types of anion channels, and here found suramin and niflumic acid as new antagonists of ASOR.

(COI: No)

P1-037

Expression Analysis of TRPM7 in 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 a mouse odontoblast cell line. These results suggested that higher expression of TRPM7 play as an important role in odontoblasts. We will also show our recent result of physiological role of TRPM7 in odontoblasts.

(COI: No)

P1-038

Altered voltage sensor movements in KCNQ1 channels with a mutation causing short QT syndrome

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KCNQ1 channel is a cardiac voltage-gated potassium channel. Some of the KCNQ1 mutations can cause short QT syndrome, a rare type of cardiac arrhythmia characterized by shorter depolarization time of cardiac action potential. The mutations for short QT syndrome have been identified only in the S1 and S5 segments and the pore helix. Because the S1 is a part of the voltage-sensing domain (VSD) and the S5 closely interacts with the VSD, we hypothesized that the VSD movement is altered in the short QT mutants in favor of the open state. When the VSD movement was tracked by voltage clamp fluorometry, the wild-type KCNQ1 channel with its auxiliary subunit KCNE1 showed two components of fluorescence rise upon depolarization: The faster component corresponds to the main movement of the VSD (down state to up state) and the slower component relates to the opening of the channel (up state to open state). The short QT mutants in the S1 (S140G and V141M) with KCNE1 showed no faster component, suggesting most of the VSD was in the up state or the open state even at the resting potential. The S5 mutant (I274V) showed a smaller faster component, also indicating the up state was in favor. These results show that the channel is up-regulated due to the altered VSD movement in the short QT mutants. Interestingly, the stabilization of the up state and/or the open state was observed only in the presence of KCNE1. The prerequisite of KCNE1 implies that S140, V141 and I274 might serve as one of the interaction sites with KCNE1 for regulation of the VSD movement.

(COI: No)

P1-039

PKA-mediated facilitation of cardiac Cav1.2 channel through uncovering calmodulin binding sites by distal C-terminus of α1c subunit

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PKA-mediated facilitation of Cav1.2 channel plays an essential role in triggering myocytes contraction. However, the underlying mechanism is so far not fully clarified. Our previous studies suggest that CaM interaction determines the channel activity. In the present study, we examined the cross-talk among PKA, calmodulin (CaM), fragments of distal C-terminus of α 1c subunit (CTd) and CaM binding domain peptides (IQ and preIQ) in regulation of Cav1.2 channel in guinea pig ventricular myocytes. 10 μM of cAMP facilitated CaM-induced Ca^{2+} channel activity in the inside-out patches and 10 μM of nonspecific kinase inhibitor, K252a, abolished such a facilitation, suggesting that cAMP-mediated facilitation was through activation of inactive PKA which still attached on the channel in the inside-out patches. Ca^{2+} channel activity in the inside-out patches maintained by 1 μM CaM and 3mM ATP was inhibited by 5 μM of fragment peptide of CTd, while pre-treatment of patches with cAMP, DCT had no effect on CaM-induced channel activity. Furthermore, we found that CaM binding domain peptides, IQ and PreIQ, mimicked cAMP effect and both effects of cAMP and CaM binding domain peptides were not additive. These results suggest that PKA-mediated facilitation of Cav1.2 channel is through uncovering CaM binding sites in the C-terminus.

(COI: No)

P1-040

Mechanism of the complete block of the Kir2.1 inward rectifier K^+ channel under the external K^+-free condition

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Currents through the strong inward rectifier K^+ channels composed of Kir2 subunits are completely blocked under the external K^+-free condition, but the mechanism of this phenomenon remains to be known. We recently reported that this sensitivity to external K^+ is lost in the heterozygous M301K mutation in Kir2.1 (J Physiol Sci, 2014), which is found in the patient exhibiting cardiac and neuronal manifestations. Here, we explored the mechanism underlying the loss of Kir2.1 conductance in the absence of external K^+ using a heterologous mammalian expression system. In whole-cell recordings, Kir2.1/Kir2.1(M301K) heteromeric channels showed a linear outward conductance in the K^+-free external solution. However, K^+ selectivity and $[K^+]_o$ dependence of the conductance were not perturbed. The same findings were obtained with Kir2.1(E224G) and Kir2.1(E299S). Since with all these mutations, negative surface potential inside the cytoplasmic pore is reduced and inward rectification caused by positively-charged polyamines is diminished, we hypothesized that inhibition of Kir2.1 in the absence of external K^+ may be caused by the polyamine block of outward currents. When Kir2.1 currents were obtained from inside-out patches using the external K^+-free pipette solution, the reversal potentials were shifted to a very negative voltage, and outward currents sensitive to polyamines could be recorded. Thus, collapse of Kir2 channel pore does not occur in the absence of external K^+, but outward currents are completely inhibited by polyamine block, which increases with the K^+ driving force.

(COI: No)

P1-041

Zdhhc3/7, the members of protein-palmitoylation enzymes, inhibit the current amplitude of HCN2 channel

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Recent studies revealed that the S-palmitoylation regulates the trafficking and the function of ion channels. Previously, we reported that among hyperpolarization-activated cyclic nucleotide-modulated channel family (HCN1-4), HCN1, HCN2 and HCN4, but not HCN3, are the targets of S-palmitoylation. 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 this study, we aimed to identify subtypes of these enzymes that regulate the palmitoylation of HCN2 channel. We overexpressed each 24 subtypes of known PATs with HCN2 and found that multiple PATs of Zdhhc-family significantly increased the palmitoylation of HCN2 protein. Especially, 5 Zdhhc proteins (Zdhhc2, Zdhhc3, Zdhhc7, Zdhhc15, and Zdhhc20) augmented the palmitoylation of HCN2 protein approximately over 10-fold of control level. When Zdhhc3 and Zdhhc7 were co-expressed with HCN2 in *Xenopus* oocytes, these enzymes reduced the current amplitude of HCN2 channel, but did not affect voltage-dependent activation of the channel. Therefore, our results suggested that they might regulate the trafficking or the activity of HCN2 on the plasma membrane. Although our results demonstrated that HCN2 channel is the target for certain types of PATs, further studies are indispensable to address whether these enzymes exert physiological roles in the signal transduction system which may regulate HCN channels.

(COI: No)

P1-042

The involvement of TRPM7 in the activity of pancreatic stellate cells

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The activity of pancreatic stellate cells (PSCs) is involved in pancreatic diseases including pancreatitis and cancer. It is known that the conversion of quiescent PSCs to activated PSCs promotes proliferation of PSCs and the development of pancreatic fibrosis and lead to pancreatic diseases. Therefore, it is important to investigate the activity of PSCs for the better understanding of pancreatic diseases. Here, we focused on TRPM7, which is a ubiquitously expressed cationic ion channel. Many reports suggested that TRPM7 is involved in various cellular physiological and pathological processes including differentiation, proliferation and migration. Therefore we hypothesized that TRPM7 is involved in the conversion or proliferation of PSCs. To reveal the contribution of TRPM7 to PSCs, we prepared TRPM7 conditional knockout mouse. First, we confirmed that tamoxifen could induce the deletion of TRPM7 in PSCs in vitro using electrophysiological techniques. Next, we checked whether there are any changes in the expression of TRPM7 that accompany the conversion of PSCs. Then, we revealed that the expression of TRPM7 and α-SMA (marker of PSCs conversion) increased when Platelet-Derived Growth Factor BB (PDGF-BB) induced the conversion of PSCs. These results suggested that TRPM7 may contribute to the conversion of quiescent PSCs to activated PSCs.

(COI: No)

P1-043

Dynamic behavior of the KcsA potassium channel in membrane: Direct observation by high-speed 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 and gating-coupled clustering-dispersion of membrane-embedded KcsA channels by removing the potentially sight-obstructing cytoplasmic domain (CPD). At neutral pH, the closed channels formed self-assembled nanoclusters. At acidic pH, the open-gated channels were dispersed as singly-isolated channels. High-speed AFM revealed that the clustering-dispersion dynamics were reversible and completed within several minutes. Here, further high-speed AFM observation captured channel-channel interaction at sub-molecular resolution. When two open channels engaged in the membrane, they disengaged immediately or fused together to become apparently-closed channels. The interplay between the gating conformational change of individual channels and the clustering-dispersion dynamics provides insight into understanding membrane-mediated protein-protein interactions and functional cooperativity.

(COI: No)

P1-044

Functional analysis of CatSper channel in heterologous expression system

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CatSper channel, a cation channel expressed specifically in testis, is essential for 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). Some features of amino acid sequence, such as positively charged amino acids in the fourth trans-membrane segment (S4) and the acidic residue in selectivity filter in the putative pore forming region of S5-S6, suggest that CatSper is a voltage-dependent calcium channel. However, detailed characteristics of CatSper are still unclear since functional analysis using heterologous expression systems has been unsuccessful. In this study, we report that the region truncated just downstream of S4 of Ci-CatSper3, corresponding to the voltage-sensor domain (VSD) of other voltage-gated ion channels, has calcium permeability. With *Xenopus* oocyte expression system, we detected substantial ionic currents which were activated upon hyperpolarization. Calcium photometry revealed that Ca^{2+} permeable pathway was formed by the truncated form of Ci-CatSper3. Furthermore, introduction of mutations in S4 altered the kinetics of activation, indicating that VSD of Ci-CatSper3 itself has ion permeability. This is, to our knowledge, the first study which reports calcium permeation through CatSper channel in a heterologous expression system.

(COI: No)

P1-045

The C-linker of hERG channel interacts with EAG domain and S4-S5 linker to regulate the slow deactivation

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hERG channel is a member of the voltage-gated K^+ channel (Kv) family, and the main subunit of the rapidly activating delayed rectifier K^+ current (I_{Kr}) in human heart. This channel has six transmembrane segments (S1-S6) consisting of the voltage-sensing domain (VSD, S1-S4) and the pore domain (PD, S5-S6) like other Kv family members, and has rather large intracellular domains. This channel is well known for its slow deactivation, which has been shown to be regulated by an interaction between the intracellular domains such as the EAG domain in the N-terminal cytoplasmic region and the cyclic-nucleotide binding homology domain (CNBHD) in the C-terminal cytoplasmic region. In this study, we newly identified an interaction between the S4-S5 linker (connecting the VSD to the PD) and the C-linker (connecting the PD to the CNBHD) by using site-directed mutagenesis and two-electrode voltage clamp of *Xenopus* oocytes. When glutamic acid residues E544 in the S4-S5 linker and E698-E699 in the C-linker were mutated to lysine (K) residues respectively, deactivation kinetics of E544K mutant and E698K-E699K double mutant were much faster than that of the wild type. Interestingly, E544K-E698K-E699K triple mutant partially rescued the deactivation kinetics. Recently, the interaction between E698-E699 and R4-R5 in the EAG domain has been reported (Ng *et al.*, 2014). Taken together, our and others' results indicate the importance of the interactions among the intracellular domains in the slow deactivation of hERG channel.

(COI: No)

P1-046

Gating of the cytotoxic peptide polytheonamide B channel

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We have examined the channel properties of a cytotoxic peptide, polytheonamide B (pTB), from marine sponge by use of the planar lipid bilayer technique. The pTB channel is a monovalent cation-selective channel as well as the proton permeability. In this study voltage-dependent gating was examined. Macroscopic current of the pTB channel was recorded upon the voltage steps, and the time-dependent activation and deactivation were observed. At pH 1.5, the channel opens at positive potentials, while as the pH becomes more acidic, the channel opens more at negative potentials. The reversal of the voltage-dependent gating has never been reported for any other channels. Our results suggest that protonation of the amino acid residue(s) within the pTB regulates its gating. We will discuss the molecular mechanism underlying proton dependent gating of the pTB channel.

(COI: No)

P1-047

The stoichiometry of Kv4.2/DPP10 complex has a preference to 4:2

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Kv4 is a member of voltage gated K^+ channel family and forms a complex with various auxiliary subunits. Dipeptidyl peptidase-like protein 10 (DPP10) is a membrane protein which has one transmembrane domain. It has been shown that DPP10 increases the current amplitude, accelerates the inactivation and the recovery from inactivation of Kv4. However, it remains unknown how many DPP10s can bind to one Kv4 channel. To test whether or not the expression level of DPP10 can affect the properties of Kv4, Kv4.2 and DPP10 were co-expressed in *Xenopus* oocytes with different ratios. We observed that the electrophysiological properties of Kv4.2, such as time to peak and recovery from inactivation, gradually changed with the increase in DPP10 expression. Before examining the stoichiometry of the Kv4.2/DPP10 complex, we investigated the stoichiometry of DPP10 in the absence of Kv4.2 on the membrane using subunit-counting by single-molecule imaging. We expressed DPP10 tagged with monomeric enhanced green fluorescent protein (DPP10-mEGFP) in *Xenopus* oocytes, and observed that 60%-80% of DPP10s on the membrane exist as dimers. To evaluate the stoichiometry of the Kv4.2/DPP10 complex, we co-expressed Kv4.2-mCherry and DPP10-mEGFP with different ratios (Kv4.2-mCherry: DPP10-mEGFP = 100 : 1, 10 : 1, 1 : 1). In contrast to the Kv4/KChIP complex in our previous study, the stoichiometry data of the Kv4.2/DPP10 complex could not be explained by independent binding to the 4 sites, but it showed a clear preference to 4:2 ratio.

(COI: No)

P1-048

Conserved Trp in the adjacent S4 helices cooperatively produce the sustained-deactivation in the voltage-gated H^+ channel dimer

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The voltage-gated H^+ channel (Hv) is a voltage-sensor domain (VSD) like protein consisting of four transmembrane segments (S1-S4). The molecular structure of Hv is a homo dimer, and each channel subunit functions cooperatively. Here we show that two voltage-sensor S4 helices in dimer cooperate with each other directly via a pi-stacking interaction between two Trp at the middle. Scanning mutagenesis approach showed that the existence of Trp around the original position provided the slow gating kinetics which is a characteristic of the dimer cooperativity. Mutation cycle analysis with the dimeric/monomeric channels suggests that the two Trp in the S4 helices of dimer are energetically-coupled in deactivation but less coupled in activation. Molecular dynamics simulation showed a direct pi-stacking between the two Trp. These results provide a new aspect of the cooperative function of voltage-gated channels in which adjacent voltage-sensor helices have a physical contact and work effectively as a unit in the H^+ homeostasis.

(COI: No)

P1-049

Examination of the role of LRRC8A in the function of volume-sensitive outwardly rectifying anion channel (VSOR)

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Volume-sensitive outwardly rectifying anion channel (VSOR) is one of the volume-regulated anion channels (VRACs) and functionally expressed in almost all cell types. The roles of VSOR in cell volume regulation, proliferation, migration and cell death are well established, but its molecular identity is unclear. In 2014, two research groups independently have reported that LRRC8A is a core factor of VSOR in human cells. Then, there arise following two questions: whether LRRC8A is essentially involved in the VSOR function in mouse cells as well, and whether LRRC8A represents a missing factor in human VSOR-deficient KCP-4 cells that are derived from the parental VSOR-rich KB cells. Thus, we addressed these questions in the present study. When transfected with siRNA against LRRC8A, the VSOR current was largely reduced not only in human HeLa and KB cells but also in mouse C127 cells. The microarray data analysis showed that the expression level of LRRC8A in KCP-4 cells is not much different from that in KB cells. Also, overexpression of LRRC8A in KCP-4 cells failed to rescue the VSOR activity. These results indicate that LRRC8A is involved in the VSOR function not only in human but also in mouse cells, and loss of VSOR currents in KCP-4 cells is not due to lack or insufficiency of expression of the LRRC8A gene.

(COI: No)

P1-050

β2-adrenoceptor-mediated regulations of the spontaneous rate and ion currents in guinea-pig cardiac cells

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The β1- and β2-adrenoceptors (ARs) are functionally expressed in the heart tissues, with the predominance of the β1ARs subtype. The ratio of β1/β2AR is high in the ventricles and relatively lower in atria and sinoatrial node (SA). Compared to β1AR, β2AR-mediated functional modulation has not been fully elucidated. In the present study, the effect of β2AR stimulation on the spontaneous action potentials, on the L-type Ca^{2+} current ($I_{Ca,L}$) and on the slow component of delayed rectifier K^+ current (I_{Ks}) was examined in guinea-pig cardiac 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. In SA cells, similar to β1AR stimulation, β2AR stimulation produced an increase in firing rate of spontaneous excitation. In addition, β2AR stimulation significantly enhanced $I_{Ca,L}$ current and shifted current activation to negative potentials in SA cells. Whereas β2AR stimulation had no effect on $I_{Ca,L}$ in atrial and ventricular cells. Moreover, β2AR stimulation also increased I_{Ks} current in SA and atrial cells, but failed to enhance I_{Ks} in ventricular cells. β2AR stimulation significantly shifted I_{Ks} current activation to negative potentials. These results suggest that in guinea pig heart, β2AR stimulation has a crucial function in the regulation of spontaneous pacemaker activity and main ion currents during sympathetic excitation.

(COI: No)

P1-051

Single channel analysis of the TRPM3 channel in planar lipid bilayers

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TRPM3 is a non-selective cation channel and activated by nifedipine and neurosteroid, pregnenolone sulfate (PS). TRPM3 is expressed in various tissues, including central and peripheral nervous systems, and reported to be involved in heat sensation. However, the functional characterization of TRPM3 channels and its regulation by agonists still remain poorly understood. To investigate the single channel properties of TRPM3, we aimed to incorporate the purified TRPM3 protein in planar lipid bilayers. We investigated the TRPM3 channel activity upon different agonists' activation. Application of nifedipine resulted in dose-dependent increase of open probability of the channel. Unlike nifedipine, application of PS alone did not induce the channel openings and co-application of the PI(4, 5)P_2 or clotrimazole was required. TRPM3 channel currents demonstrated outward rectification upon activation with PS and PI(4, 5)P_2, while linear current-voltage relationship upon activation by PS and clotrimazole. In addition, we found that the channel did not exhibit strong temperature dependence. Increase in temperature up to 42 ℃ did not induce the channel openings in the absence of agonists. While, in the presence of nifedipine, TRPM3 currents demonstrated weak temperature dependence. These results indicate that TRPM3 is unlikely to represent a temperature sensor by itself and some alternative molecular mechanisms may be involved in the temperature sensation.

(COI: No)

P1-052

Expression of ion-channel proteins in the cutaneous mechanoreceptors of mice model of atopic dermatitis

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Atopic dermatitis is chronic dermatitis associated with an extremely itchy and inflamed skin. The patient's skin is commonly very sensitive to external stimuli or irritants, and scratching can increase the severity of dermatitis. Therefore, controlling the itchy skin sensation is important for the treatment of atopic dermatitis. In this study, to clarify the expression of ion-channel proteins that may cause the itchy skin sensation, we immunohistochemically examined cutaneous mechanoreceptors in a mouse model of atopic dermatitis (NC hairless mice). Atopic dermatitis was induced by repeated applications of 2, 4-dinitrofluorobenzene (DNFB) to the ear and back skin of NC hairless mice. Immunohistochemical analysis showed positive reactions for ASIC and TRP proteins in the palisade nerve endings of the auricular skin. These channel proteins may cause the itchy skin sensation that characterizes DNFB-induced atopic dermatitis in NC hairless mice. This study was supported by a Grant-in-Aid for Scientific Research (C) (No. 24590253).

(COI: No)

P1-053

Immunohistochemical distribution of interleukin-18 receptor α in the hypothalamus of male mice

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Interleukin-18 (IL-18) is a pro-inflammatory cytokine and an important mediator of peripheral inflammation and host defense response. IL-18 performs its biological activities through the IL-18 receptor (IL-18R), which consists of two chains, an IL-18-binding α chain (IL-18Rα) and a signaling β chain. IL-18R is widely expressed in the brain, however little is known about the detailed expression of IL-18R in the neurosecretory cells. In the present study, we investigated, by immunohistochemistry, the distribution of IL-18Rα in the hypothalamus of male mice. IL-18Rα-positive cell bodies and fibers were found scattered throughout the organum vasculosum of the laminae terminalis (OVLT) / preoptic area (POA) region. In the median eminence (ME), where the neurosecretory terminals are located, strong IL-18Rα-immunoreactive fibers were also detected. Outside the hypothalamus, IL-18Rα antibodies labeled fibers and cell bodies in the medial septal nucleus (MS), and the nuclei of the vertical and horizontal limbs of the diagonal band (VDB and HDB). In the MS, VDB, HDB, OVLT/POA region, and ME, it has been well known that gonadotropin-releasing hormone (GnRH) neuronal somata and/or fibers were distributed. Therefore, we performed double-label immunofluorescence for IL-18Rα and GnRH. IL-18Rα was expressed on some GnRH-immunopositive cell bodies and nerve fibers. These observations suggest that IL-18 may exert direct effects upon the GnRH neuronal system via IL-18Rα.

(COI: No)

P1-054

Involvement of ion channels in cold allodynia in a new rat model of peripheral arterial disease

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Aim of Investigation: Patients with peripheral arterial disease (PAD) often suffer from peripheral hypersensitivity to cold stimulation which precedes chronic ischemia-induced pain. However, the pathophysiology of cold hypersensitivity has not been fully understood due to the lack of an adequate animal model. In this study, to demonstrate mechanisms underlying ischemia-induced pain associated with PAD, we investigated the role of ion channels on cold allodynia in a newly developed rat model of PAD.
Methods: Under sodium pentobarbital anesthesia, the left common iliac and iliolumbar arteries were ligated respectively, through a midline laparotomy (PAD rats). Sham rats were exposed the arteries without ligation under anesthesia. Von Frey test, plantar test, acetone test were performed, respectively. We also examined alteration of paw withdrawal response to cold temperature. Histological examination of hindpaw skin was performed on day 4 and 10 after the arterial ligation. Effects of selective antagonist to cold-gated ion channels transient receptor potential melastatin 8 (TRPM8), and transient receptor potential ankyrin 1 (TRPA1) channels, were evaluated on behavioral responses to cold stimulus.
Result: Mechanical allodynia was observed for 7 days and cold allodynia was observed from day 7 to 14 after ligation. Intraplantar injection of the antagonists against TRPA1 suppressed cold allodynia in PAD rats.
Conclusions: TRPA1 may play an important role in developing ischemia-induced cold allodynia associated with PAD.

(COI: No)

P1-055

Gene expression analysis of ASIC subtypes in adult-born hippocampal neurons in mice

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Adult hippocampal neural progenitors continuously give rise to dentate granule cells throughout life. Accumulating evidence suggests that adult hippocampal neurogenesis is linked to learning and memory. ASIC1a (acid-sensing ion channel-1a) is a neuronal proton-gated cation channel expressed in mammalian central and peripheral nervous systems. In the hippocampal neurons, ASIC1a is located at the post-synaptic surface of the cells, and receives extracellular protons released from syanaptic vesicles. The resultant activation of the channel allows Ca2+ ions to enter into the neurons, contributing to synaptic plasticity and spatial memory in the hippocampus, suggesting that ASIC1a may be directly implicated in the hippocampal neurogenesis. To test this possibility, we are currently investigating the temporal expression of ASIC1a (including other ASIC subtypes) in adult-born hippocampal neurons in mice by single-cell RT-PCR, in situ hybridization, and patch-clamping.

(COI: No)

P1-056

The Gi/o coupled muscarinic receptors form signaling complex with the G protein dependent inwardly rectifying potassium channel

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Excitable cells, such as neurons and muscles, are negatively regulated by the G protein dependent inwardly rectifying potassium (GIRK) channel, which is activated by $G_{\beta\gamma}$ subunits released mostly from Pertussis toxin (PTX) sensitive $G_{\alpha i/o}$ subunits. It has also been reported that the $G_{\beta\gamma}$ subunits released from PTX resistant G_{α} subunits, such as $G_{\alpha s}$ and $G_{\alpha q}$, activate the channel only when the coupled receptors are highly expressed. It is possible that a shorter distance from the channel is the reason of coupling in the case of high surface expression. In this study, we ligated the receptor and the channel by various lengths of linkers of glycine rich amino acid residues, and examined the effects of the linker length on the channel activation. When the number of the linker residues was 100 or less, the Gq coupled muscarinic receptor type 1 (M_1R) evoked the GIRK current upon application of the agonist, whereas the GIRK channels were hardly activated when the number of the linker residues was 268 or more. The FRET efficiency between the M_1R-YFP and the GIRK-CFP was decreased in accordance with the increase in the linker size. In contrast, when the Gi/o coupled muscarinic receptors were used instead of the M_1R, increases in the linker size did not affect the efficiency of the GIRK channel activation and the FRET efficiency. Taken together, the Gi/o, but not Gq, coupled receptors are shown to stay adjacent to the GIRK channel to form the signaling complex.

(COI: No)

P1-057

LC-MS/MS analysis of an orphan metabotropic receptor Prrt3 complex

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Proline rich transmembrane receptor 3 (Prrt3) contains seven transmembrane domains like other metabotropic receptors including mGluRs and $GABA_BRs$. However, the physiological function of Prrt3 and its signaling cascade remain unknown. In our previous study, we revealed Prrt3 expression in mouse brain especially in thalamus and hippocampus, and its possible role in memory retention using Prrt3 heterozygous knock out (KO) mouse. In this study, we identified Prrt3 binding proteins using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Physiologically expressed Prrt3 protein complexes were isolated from wild-type (WT) mouse brain by immunopurification. To eliminate non-specific protein bands, Prrt3 homozygous KO mouse brain sample was used as a negative control. The specific protein bands observed in the silver staining were extracted and analyzed by LC-MS/MS. The protein bands sized between 120 and 220 kDa contained excitatory amino acid transporter 2 (EAAT2), and the band sized 40 kDa contained $G\alpha_o$ protein. The presence of these proteins was further confirmed by western blotting experiment. It is known that dopamine transporter forms a complex with GPCRs (D_2R or GPR37) to regulate the function of dopaminergic synapses. Our finding suggests a possibility that the interaction between Prrt3 and EAAT2 may play an important role in glutamatergic signaling.

(COI: No)

P1-058

Insulin transcriptionally regulated NKCC and CFTR Cl- channel expression through PI3K activation and ERK inactivation in renal epithelial A6 cells

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Insulin is known to stimulate epithelial Na+ channel-mediated Na+ reabsorption in renal epithelial A6 cells, however the insulin action on the Cl- secretion is not fully understood. In this study we investigated the insulin action on Na+-K+-2Cl- cotransporter (NKCC)-mediated Cl- secretion in renal epithelial A6 cells. Insulin treatment significantly enhanced the forskolin-stimulated Cl- secretion with an increase in apical Cl- conductance by upregulating mRNA expression of both NKCC and CFTR Cl- channel. We next examined a role of PI3K on the insulin-induced enhancement of Cl- secretion, because PI3K is a major signal molecule in the insulin cascade. LY294002 (a specific inhibitor of PI3K) decreased Cl- secretion by suppressing NKCC mRNA expression. On the other hand, we found that PD98059 (a MEK inhibitor) further enhanced the insulin-stimulated CFTR mRNA expression and the Cl- secretion in forskolin-treated A6 cells. Furthermore, insulin activated Akt as an indicator of PI3K and inactivated ERK. These observations suggest that insulin enhances forskolin-stimulated Cl- secretion through transcriptional regulation of NKCC and CFTR Cl- channel via PI3K activation and ERK inactivation in renal epithelial A6 cells.

(COI: No)

P1-059 (AP-4)

Cancer cell-specific crosstalk between Na^+, K^+-ATPase and volume-sensitive anion channel in membrane microdomains exerts anti-proliferative activity

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Na^+, K^+-ATPase is a potential target for anti-cancer therapy, because cardiac glycosides, inhibitors of Na^+, K^+-ATPase, potently block cancer cell growth. However, the mechanism underlying the anti-cancer effects of cardiac glycosides is not fully understood. In the present study, we found that ouabain, a cardiac glycoside, inhibited cancer cell proliferation via activation of volume-sensitive outwardly rectifying (VSOR) anion channel. The effects were suppressed by DCPIB, a selective inhibitor of VSOR channel, and the knockdown of Na^+, K^+-ATPase α1-isoform (α1NaK) or VSOR channel component LRRC8A (SWELL1). The disruption of membrane microdomains by methyl-β-cyclodextrin and the attenuation of the production of reactive oxygen species (ROS) by the inhibitors of NADPH oxidase (NOX) significantly suppressed the ouabain-induced VSOR activation and inhibition of cell proliferation. On the other hand, the ouabain-induced effects were not observed in non-cancer cells. These results suggest that α1NaK, NOX and VSOR channels form a signalosome in the membrane microdomains of cancer cells, and that the cardiac glycoside exerts anti-cancer activity through the cancer-specific signalosome.

(COI: No)

P1-060

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-cells play a crucial role in blood glucose regulation as they secrete hypoglycemic insulin when blood glucose levels are elevated. In β-cells, reactive oxygen species (ROS) including H_2O_2 are generated in response to many kinds of signals including blood glucose elevation. Interestingly, expression levels of catalase and glutathione reductase, are extremely low in the pancreas compared with other tissues, suggesting that ROS could function as favorable signaling molecules in β-cells.

TRPM2 is a Ca^{2+}-permeable cation channel and expressed in various tissues including brain, spleen and β-cells where TRPM2 is continuously affected by body temperature. We previously found a regulation mechanism of TRPM2 activity whereby its heat-evoked response is dynamically elevated by H_2O_2, termed "sensitization" enabling TRPM2 to be activated by body temperature with temperature threshold reduction.

In WT β-cells, H_2O_2 enhanced heat-evoked $[Ca^{2+}]_i$-increase but the effect was not observed in TRPM2KO cells. Moreover, the N-acetyl cysteine (NAC), an antioxidant compound, -sensitive fraction of insulin secretion by Wt islets was increased with temperature elevation. This temperature-dependent enhancement was not observed in TRPM2KO islets. These data suggest that endogenous redox signals in pancreatic β-cells elevate insulin secretion via TRPM2 sensitization and activity at body temperature.

(COI: No)

P1-061

The regulatory mechanisms of adiponectin in activity of hypothalamic POMC and NPY neurons

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Aim: It has been suggested that serum adiponectin secreted from adipocytes could enter to cerebrospinal fluid and regulate brain functions, in addition to regulation of insulin sensitivity and energy expenditure in the peripheral organs. We have reported that adiponectin facilitates activity of proopiomelanocortin (POMC) neuron, whereas it suppresses activity of neuropeptide Y (NPY) neuron activity in the hypothalamic arcuate nucleus. The aim of this study was to determine the regulatory mechanisms of adiponectin in the activity of NPY and POMC neurons.
Methods: The effects of adiponectin on the membrane potential and electrical firing in POMC neurons in brain slices from POMC-GFP mice were recorded by patch-clamp analysis under current clamp mode. Inhibitory postsynaptic current (IPSC) in NPY neurons of NPY-GFP mice was recorded under voltage clamp mode.
Results: Membrane potential on the ARC POMC neurons was depolarized by bath application of adiponectin, and its depolarization effect was abolished by PI3K inhibitor. IPSC amplitude on NPY neuron was significantly increased by adiponectin treatment.
Conclusion: Adiponectin activates POMC neurons via PI3K pathway and inhibits NPY neurons via facilitation of inhibitory input at postsynapses in ARC. These effects may be implicated in suppression of food intake by adiponectin.
(COI: No)

P1-062

Morphological characterisation of L5 pyramidal neurons of known synaptic properties in rat visual cortex at early developmental age

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The mammalian neocortex comprises neuronal columns that respond to specific sensory stimulus and each column has 6 horizontal cell layers segregated by cell morphology and physiological properties. This well-established laminar organisation suggests that neurons of distinct morphology are most likely to represent discrete functional classes and form specific synaptic connections. This study aims to analyse morphological variables of visual cortex layer 5 (L5) pyramidal neurons of known synaptic properties at early developmental age, and to determine whether these characteristics vary with maturation. Multineuronal whole-cell recordings were made from large and thick-tufted L5 pyramidal neurons in slices from rats aged over the first postnatal month. Altogether 30 sets of Alexa Fluor 488-labelled L5 neurons were included in this study, of which 14 were from P11-P15 rats, one at P20 and the remaining were of age P25-P29. The mean somatic area and somatic perimeter of all 61 neurons were 119.4 ± 25.3 μm^2 and 39.5 ± 4.2 μm, respectively. Intrinsic electrophysiological and synaptic properties of these neurons were shown to develop in parallel after birth. Further investigation is necessary to determine correlation (if any) between morphological maturation and synaptic dynamics of specific neuronal classes in the developing visual cortex.
(COI: No)

P1-063

Role of astrocyte-expressed FABP7 on morphology and synapse Formation of cortical neurons

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Fatty acid binding protein 7 is expressed by astrocytes in the CNS and has been associated with human neuropsychiatric disorders. Here we examined relevance of astrocytic FABP7 in regulation of neuronal dendrite morphology and synapse formation in medial prefrontal cortex (mPFC) by using *Fabp7* KO mice. Golgi staining on mPFC of *Fabp7* KO mice revealed aberrant dendritic morphology and decreased spine density of pyramidal neurons compared with wild-type (WT) littermates. Consistently, primary cortical neurons co-cultured with FABP7-deficient astrocytes exhibited aberrant dendritic morphology, and such change was also observed when neurons were grown in *Fabp7* KO astrocyte conditioned medium. Number of excitatory synapses was decreased in mPFC of *Fabp7* KO mice as well as in neurons co-cultured with *Fabp7* KO astrocytes. Whole cell voltage-clamp recording in brain slices from pyramidal cells of mPFC showed that both amplitude and frequency of action potential-independent miniature excitatory postsynaptic currents (mEPSCs) were decreased in *Fabp7* KO mice. Moreover, hyperactivity of *Fabp7* KO mice in open-field test was partially restored by transplantation of WT astrocytes into mPFC. Collectively, our study suggests that astrocytic FABP7 is important for growth of dendritic arbors and synapse formation of cortical neurons, and further for behavioral control. These findings provide new insights into links between FABP7, lipid homeostasis and neuropsychiatric disorders like schizophrenia.
(COI: No)

P1-064

Ca^{2+} current facilitation underlies short-term facilitation at inhibitory synapses between Purkinje cells

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Cerebellar Purkinje cells (PCs) form synapses not only on deep cerebellar nuclei (DCN) neurons, but also on other PCs through axon collaterals. Opposite forms of short-term plasticity take place at the PC output synapses: while PC-DCN synapses exhibit paired-pulse depression, paired-pulse facilitation (PPF) occurs at the PC-PC synapses (Orduz and Llano, 2007). To clarify a key factor determining those opposite forms of short-term plasticity, we here focused on the mechanism underlying short-term facilitation at PC-PC synapses. As a mechanism for short-term facilitation, the following candidates can be conceived: temporal summation of residual Ca^{2+}, augmentation of action potential (AP) amplitude, saturation of Ca^{2+} buffer protein, facilitated Ca^{2+} influx through voltage-gated Ca^{2+} channels, and Ca^{2+}-dependent positive modulation of vesicle release machinery. Among these, the Ca^{2+} buffer saturation hypothesis was recently shown to be unlikely because of the resistance of PPF to the genetic ablation of two major Ca^{2+} buffering proteins expressed in PCs, calbindin and parvalbumin (Bornschein *et al*, 2013). To test the other possibilities, using dissociated cerebellar cultures we performed fluorescent imaging of residual Ca^{2+} increase in PC axon terminals, and also attempted to directly whole-cell record APs and Ca^{2+} currents from a PC axon terminal. We present the data suggesting that facilitated Ca^{2+} influx into the presynaptic terminal during repetitive stimulation contributes to augmented release probability of synaptic vesicles.
(COI: No)

P1-065

Kinetics of Synaptic Vesicle Refilling with GABA

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Information transfer between neurons requires temporal and spatial balance of excitatory and inhibitory synaptic inputs. After releasing neurotransmitter, synaptic vesicles are retrieved by endocytosis and recycled to be reused for synaptic transmission. To maintain the synaptic efficacy, vesicles must be refilled with neurotransmitter during recycling. Vesicle trafficking and pools have been studied in great detail but much less is known about where and how vesicles are filled with GABA via VGAT. After exocytosis vesicles retrieved by clathrin-mediated endocytosis are rapidly acidified and recycled. Refilling of GABA might be a rate-limiting phase of recycling vesicles. The refilling speed estimated in isolated or reconstructed vesicles seems too slow to fill up vesicles within the period of recycling. Here we re-examined the vesicle refilling rate directly at GABAergic synapses in slices. We made caged GABA photolysis in simultaneous whole-cell recording from a presynaptic inhibitory interneuron and a postsynaptic Purkinje cell in cerebellar cortex. After washing out vesicular GABA in an interneuron with a whole cell pipette containing no GABA, refilling of vesicles with uncaged GABA caused a recovery of IPSCs in a Purkinje neuron within 3 min. Thus, the time course of refilling in situ was significantly faster than that reported in reconstituted or isolated vesicles (5 - 10 min).
(COI: No)

P1-066

Rational design of a novel high-affinity, ultrafast, red calcium indicator R-CaMP2

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Fluorescent Ca^{2+} reporters are widely used as readouts of neuronal activities. Here, we designed R-CaMP2, a novel high-affinity red genetically encoded calcium indicator (GECI) with a Kd for Ca^{2+} < 70 nM, and with a Hill coefficient near 1. Use of the calmodulin-binding sequence of CaMKK-α / β in lieu of a M13 sequence resulted in three fold faster kinetics than R-CaMP1.07 in rise and decay time of Ca^{2+} transients. These features allowed to resolve single action potential (AP) and fast AP trains up to near 20-40 Hz in cortical slices. *In vivo* imaging of the barrel cortex layer 2/3 neurons revealed reliable recording of single APs in R-CaMP2-expressing neurons, while synaptic Ca^{2+} transients were robustly detected in individual dendritic spines with similar efficacy as previously reported ultrasensitive green GECIs. Combining green and red GECIs, we successfully achieved dual-color monitoring of neuronal activities of distinct cell types, in the mouse cortex and in free-moving C. elegans. Together, R/G-CaMP imaging using R-CaMP2 provides a powerful means to interrogate orthogonal and hierarchical active ensembles, thus significantly enhancing our current capacity for functional mapping of neuronal circuits *in vivo*.
(COI: No)

P1-067 (AP-2)

Identification of retrograde signals required for synapse elimination in the developing brain

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Precise formation of neural circuits during development is a prerequisite for proper brain functions. Neurons form exuberant synapses with target cells early in development. Then, necessary synapses are selectively strengthened whereas unnercessary connections are weakened and eventually eliminated during the course of postnatal development. This process is known as synapse elimination. Synapse elimination is an important step to shape initial redundant neural circuits into functionally mature circuits, and the disruption is likely linked to mental disorder and brain dysfunction. While the underlying mechanism is still unclear in any systems, several lines of evidence suggest that retrograde signaling from postsynaptic cells regulates synapse elimination. However, these retrograde signals remain to be identified. We have screened retrograde molecules required for synapse elimination of climbing fiber to Purkinje cell connection in the developing cerebellum. We identified some key retrograde molecules which strengthen necessary synapses and eliminate unnecessary synapses. Here I am going to talk about the role of these retrograde molecules in synapse elimination.

(COI: No)

P1-068

Pharmacological analysis of the inhibitory actions of 5-HT on the excitatory transmission in the dentate granule cells

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The dentate gyrus in hippocampus receives serotonergic innervation from the raphe nuclei, suggesting that synaptic activities in the dentate gyrus are modulated by serotonin (5-HT). Externally applied 5-HT is known to regulate the GABAergic transmission, which indirectly affects the excitatory drive onto the granule cells. However, a possible direct action of 5-HT on the excitatory transmission in the granule cells is not well examined. We previously showed that 5-HT reduces the input resistance (IR) of granule cells, thereby affects the amplitude of EPSPs irrespective of the inputs. We also found that 5-HT specifically reduces the EPSPs evoked by the stimulation of the lateral perforant path (LPP) but not the medial perforant path and mossy cell fibers. In the present study, we examined which types of receptors are involved in the inhibitory modulation of the LPP-granule cell synapse by 5-HT. The reduction of IR by 5-HT was completely blocked by a 5-HT_{1A} antagonist, WAY100635, but the inhibitory effect of 5-HT on the EPSP amplitude was only partially suppressed. By contrast, co-application of a 5-HT_2 antagonist, ritanserin with WAY100635 blocked the inhibitory action completely. These results suggest that the activation of 5-HT_{1A} receptor inhibits excitatory transmissions in the granule cells globally, and that the activation of 5-HT_2 receptor specifically depresses the LPP-granule cell synapse.

(COI: No)

P1-069

Analysis of spontaneous slow currents in inferior olivary neurons

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Inferior olivary neurons (IONs) are the only source of climbing fibers innervating cerebellar Purkinje cells, which have important roles in cerebellar functions. To examine factors to control firing of IONs, we recorded spontaneous currents electrophysiologically. Coronal slices were prepared from C57BL6 mice, and whole cell recordings were performed from IONs. Spontaneous currents with relatively shorter duration were blocked by AMPA and $GABA_A$ receptor blockers (NBQX and bicuculline), suggesting that these were synaptic currents. In addition to the synaptic currents, we found that spontaneous currents with long durations were frequently observed in IONs. These slow currents could be further divided into two subgroups. One group was inward currents which were always followed by an outward current (bipolar currents). The bipolar currents were blocked by TTX, a blocker of the voltage dependent Na^+ channels, or carbenoxolone, a blocker of gap junction, suggesting that these were Na^+ spike from neighboring IONs via gap junctions. The other was inward currents with slow kinetics (slow inward currents). The slow inward currents were TTX resistant and suppressed by bath-applied Ni^{2+}, suggesting contribution of the T-type voltage dependent Ca^{2+} channel (T-type VDCC). To confirm this point further, we used Cav3.1, a major alpha subunit of the T-type VDCC in IONs, null mice. In these mice, the slow inward currents were totally abolished. These results suggest that activation of IONs is regulated by spontaneous generation of these currents.

(COI: No)

P1-070

Input specificity of giant miniature EPSC in hippocampal CA3 neurons

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Miniature EPSCs with exceptionally large amplitude, so-called giant minis, are recorded from hippocampal CA3 neurons. These large events are thought to originate from the mossy fiber synapses, since a lesion of the dentate gyrus decreases giant minis and large clear vesicles exist in the mossy fiber terminals. However, there is no direct evidence for the mossy fiber origin of giant minis. In this study, we examined strontium-induced asynchronous EPSCs (aEPSCs) by the stimulation of different inputs to identify the origin of giant minis. In the solution containing strontium instead of calcium ions, delayed asynchronous release follow evoked-EPSCs composed of synchronous release of multiple vesicles, offering the way to evaluate the input specific size of quantal events. Acute hippocampal slices were obtained from C57BL/6J mice (p13 - 21). Repetitive stimulation (3 pulses at 50 Hz) was applied to the mossy fiber or the associational/commissural (A/C) fiber, and the evoked EPSCs were recorded from CA3 pyramidal neurons. With the stimulation of A/C fiber, amplitudes of aEPSCs observed during 350 ms after the stimulation was not different from those of miniature EPSCs recorded during 350 ms before the stimulation ($p > 0.05$, K-S test, $n = 9$). On the other hand, giant minis (> 80 pA in this study) were observed with the stimulation of mossy fiber ($p < 0.01$, $n = 7$). The time courses of appearance of giant minis matched with aEPSCs observed in other synapses (< 400 ms). These results suggested that giant minis are exclusively originated from the mossy fiber-CA3 synapse.

(COI: No)

P1-071

Involvement of diacylglycerol kinase β in the spine formation at distal dendrites of striatal medium spiny neurons

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Spine formation, a salient feature underlying neuronal plasticity to adapt to a changing environment, is regulated by complex machinery involving membrane signal transduction. The diacylglycerol kinase (DGK) family, which is involved in membrane lipid metabolism, catalyzes the phosphorylation of a lipid second messenger, diacylglycerol (DG). Of the DGKs, DGKβ is characterized by predominant expression in a specific brain region: the striatum. We previously demonstrated that DGKβ is expressed selectively in medium spiny neurons (MSNs) and that it is highly enriched in the perisynaptic membrane on dendritic spines contacted with excitatory terminals. Moreover, DGKβ regulates spinogenesis through actin-based remodeling in an activity-dependent manner. However, the detailed mechanisms of spinogenesis regulation and its functional significance remain unclear. To address these issues, we performed Golgi-Cox staining to examine morphological aspects of MSNs in the striatum of DGKβ-knockout (KO) mice. Results show that striatal MSNs of DGKβ-KO mice exhibited lower dendritic spine density at distal dendrites than wild-type mice did. We also sought protein targets that interact with DGKβ and identified the GluA2 AMPA receptor subunit as a novel DGKβ binding partner. In addition, DGKβ-deficient brain exhibits significant reduction of TARP γ-8, which represents a transmembrane AMPA receptor regulatory protein. These findings suggest that DGKβ regulates the spine formation at distal dendrites of MSNs, presumably through GluA2 receptor-mediated mechanism.

(COI: No)

P1-072

Contextual memory encoding induces a quick change 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, 2013). In the present study, to examine whether encoding of memory strengthens the CA1 synapses, we examined the temporal change of miniature excitatory and inhibitory post-synaptic currents (mEPSC and mIPSC) in IA-trained rats. 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, IA rats showed higher mEPSC and mIPSC amplitudes from the 5 to 30 min periods after encoding session in IA but not immediately follow this test (at the 0 min). In addition, mIPSC frequency increased from 0 to 5 min periods after encoding session whereas mEPSC frequency increased at the 5 min periods after encoding session, suggesting that memory encoding induced a quick change of mEPSC and mIPSC. Moreover, bath treatment of CNQX (an AMPA receptor antagonist, 10μM) consistently blocked the mEPSC responses. In contrast, bath treatment of bicuculline methiodide (a $GABA_A$ receptor antagonist, 10μM) consistently blocked the mIPSC responses. For these results, we conclude that a quick change of AMPA receptors and $GABA_A$ receptors in hippocampal CA1 neurons plays role for encoding of contextual memory.

(COI: No)

P1-073

Simulation analysis of water and ion dynamics in astrocyte

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In response to perturbation of extracellular environment in the brain, astrocyte transport water and ions to maintain proper environment for neural activity. Extracellular K^+ concentration ($[K^+]_{out}$) in the brain increases in response to ischaemia, hypoxia, hypoglycaemia, seizures, and spreading depression and can cause significant problems in brain function. High $[K^+]_{out}$ also induces swelling in astrocytes, leading to cytotoxic edema and cell death in the brain. On the other hand, when the blood brain barrier (BBB) is disrupted, water flux to extracellular and increase of extracellular space lead to vasogenic edema. In order to prevent the harmful elevation of $[K^+]_{out}$ and increase of extracellular space, astrocytes clear excessive K^+ and water in extracellular space by redistributing K^+ and water. Despite the importance of the redistribution function for ion and water, the underlying mechanisms remain unclear. Here we report results from a simulation analysis of water and ion dynamics in astrocyte. The astrocyte models were improved from out previous astrocyte models, which were constructed by incorporating various mechanisms such as intra/extracellular ion concentrations and models of ion channels and transporters. New models of ion channels, such as volume- regulated anion channel (VRAC) were incorporated into the previous model to reproduce regulatory volume decrease, and were used to simulate not only K^+ transport but also water transport under vasogenic edema. Our simulation analysis revealed controversial mechanisms of astrocytic ion and water clearance.

(COI: No)

P1-074

Methionine ameliorates the elevated GAD67 expression in cingulate cortex and the abnormal behaviors of FABP3 KO mice

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Introduction: Fatty acid binding protein (FABP) 3 is strongly expressed in GABAergic inhibitory interneurons in cingulate cortical (CC) which is one of the important brain regions for behavioral coordination. We have so far shown that FABP3 gene ablated mice show the increase of glutamic acid decarboxylase 67 (GAD67) expression in CC and the abnormal cognitive and emotional behaviors. In order to explore the mechanism how FABP3 regulates GAD67 expression, we studied whether methionine (MET) administration, which increases DNA methylation, affects the GAD67 expression in CC of FABP3 gene ablated mice and their abnormal behaviors.
Method: Binding of methyl CpG-binding protein 2 (MeCP2) to specific GAD67 CpG-rich promoter sequence was studied by chromatin immunoprecipitation assay. Mice were treated twice a day for 6 days with MET (5.2 mmol/kg, s.c.). Expression of GAD67 mRNA was examined by qPCR.
Result: In the CC of FABP3 gene ablated mice, binding of MeCP2 to GAD67 promoter was significantly decreased compared with wild-type mice. MET administration restored the elevated GAD67 mRNA expression in the CC of FABP3 KO mice back to wild-type levels, and improved their abnormal behaviors.
Conclusion: These findings suggest that DNA hypomethylation and the associated chromatin remodeling underlie the elevation of GAD67 in CC and the abnormal behaviors of FABP3 gene ablated mice.

(COI: No)

P1-075

The ethanol metabolite acetaldehyde induces the sensation of thirst

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Thirst sensation in hangover has been considered to be due to ethanol-induced diuresis, but heavy-alcohol drinking reduces urine volume. In this study, we hypothesized that ethanol metabolite acetaldehyde is thirst-inducing factor in hangover. Male Wistar rats were used in the present study. Ethanol significantly increased water intake. Coadministration of the aldehyde dehydrogenase inhibitor cyanamide with ethanol increased both water and salt intake further and earlier. Urine volume was decreased by ethanol. Acetaldehyde with cyanamide induced water and salt intake. The elicited water and salt intake was suppressed by intraperitoneal and intracerebroventricular injections of angiotensin AT1R antagonist candesartan. c-Fos expression in the circumventricular organs and supraoptic nucleus was increased by acetaldehyde, and the increment was suppressed by central AT1R blockade. Acetaldehyde suppressed blood pressure and increased plasma renin activity. Meanwhile, intracerebroventricular acetaldehyde increased only water intake. In multi-array extracellular recordings from slice preparations of subfornical organ, acetaldehyde showed a direct effect on neuronal cell bodies. Taken together, acetaldehyde may induce thirst sensation via two distinct and previously unsuspected processes, an indirect action through the renin-angiotensin system by a depressor response and a direct action in the dipsogenic centers in hangover, independent of diuresis.

(COI: No)

P1-076

FILIP-related molecule binds to NMDA receptor and controls spine maturation and synaptic function in the hippocampal neuron

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Dendritic spines are small actin-rich structures and the primary post-synaptic sites of excitatory neurotransmission in the brain. The actin cytoskeleton is essential for spine maturation as well as for synaptic plasticity and memory formation. Non-muscle myosin IIb plays a major role for regulation of actin dynamics in the dendritic spines. However, how myosin IIb directly alters cytoskeletal dynamics through ATPase-driven contraction of actin networks and how myosin IIb function is regulated during the dendrite spine maturation are still poorly understood. We found that one FILIP (Filamin A-Interacting Protein)-related molecule, FRM, was a binding partner of myosin IIb and was expressed in the hippocampal and neocortical neurons. When endogenous FRM was knocked down in cultured hippocampal neurons, it inhibited spine shortening for spine maturation and changed the ratio of NMDA receptor expressions on spines. Additionally, we found that FRM was interacted with NMDA receptor. These data suggest that FRM is a new myosin IIb modulator that controls spine maturation and synaptic function in the hippocampus as well as in the cerebral cortex.

(COI: No)

P1-077

Kinetic Organization of Ca^{2+} Signals that Regulate Synaptic Release Efficacy

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Calcium regulation of neurotransmitter release is essential for maintenance of synaptic transmission. However, the temporal and spatial organization of Ca^{2+} dynamics that regulate synaptic vesicle (SV) release efficacy in sympathetic neurons is poorly understood. We investigate the N-type Ca^{2+} channels-mediated kinetic structure of Ca^{2+} regulation of cholinergic transmission of sympathetic neurons. We measured the effect of Ca^{2+} chelation with fast and slow buffers on exocytosis, synaptic depression, and recovery of the readily releasable vesicle pool (RRP), after both single action potential (AP) and repetitive APs. Postsynaptic potentials peaking at ~12 ms after the AP was inhibited by both rapid and slow Ca^{2+} buffers, suggesting that, in addition to the well-known fast Ca^{2+} signals at the active zone (AZ), slow Ca^{2+} + signals at the peak of Ca^{2+} entry also contribute to paired-pulse or repetitive APs responses. Following single AP, discrete Ca^{2+}-transient regulated synaptic depression in a rapid (<30 ms) and slow (<120 ms) phase. In contrast, following prolonged APs trains, synaptic depression was reduced by a slow Ca^{2+} signal regulation lasting >200 ms. Finally, after an AP burst, recovery of the RRP was mediated by an AP-dependent rapid Ca^{2+} signal, and the expansion of releasable SV number by an AP firing activity-dependent slow Ca^{2+} signal. These data indicate that local Ca^{2+} signals operating near Ca^{2+} sources in the AZ are organized into discrete fast and slow temporal phases that remodel exocytosis and short-term plasticity to ensure long-term stability in acetylcholine release efficacy.

(COI: No)

P1-078

Effects of astaxanthin on axonal transport in cultured mouse dorsal root ganglion neurons

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Astaxanthin, a non-provitamin A carotenoid, which is found in the pigment of microalgae, crustacean, salmon and so on. It is well known as an antioxidant, and it has also been reported to have anti-inflammatory and anticancer effects. We have previously demonstrated that astaxanthin suppressed against oxidative stress-induced neurite growth inhibitory. However the direct effects of AX on neuronal functions have rarely examined. Axonal transport is critical for neurogenesis and maintenance of neuronal functions. This study we investigated the effects of AX on axonal transport in cultured dorsal root ganglion neurons. Movement of organelles in neurites was observed by real-time video-enhanced microscopy. Axonal transport in anterograde and retrograde directions had not significantly changed after treatment with astaxanthin. The average velocity of particle movement and the diameter of these neurites were also not significantly changed in both directions.

(COI: No)

P1-079

Hydrogen peroxide-mediated modulation of synaptic transmission in rat spinal ventral horn neurons

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Hydrogen peroxide (H2O2) is produced at high concentrations under pathological conditions. In this study, we examined the presynaptic effects of H2O2 on the rat spinal ventral horn neurons using whole-cell patch-clamp recordings from spinal cord slices. H2O2 (1 mM, 5 min) induced biphasic changes in the frequency of miniature excitatory postsynaptic currents (mEPSC): i.e., initial augmentation and subsequent depression, lasting after 10 min washout. Subsequent depression was attenuated by GABA-A receptor antagonist, indicating that the depression is mediated by the activation of presynaptic GABA-A receptors. Actually, the frequency of GABAergic miniature inhibitory synaptic currents (mIPSC) were increased by superfusing H2O2. This action might protect neurons from an excessive excitation mediated by H2O2. Another H2O2 effect on the mEPSC frequency, initial augmentation, was suppressed by superfusing Ca2+-free solution. Furthermore, N-type voltage dependent calcium channel (VDCC) blocker inhibited completely the initial augmentation and P/Q-type blocker inhibited partially, whereas R- and L-type blockers had no effect, indicating that the augmentation is in part mediated by Ca2+ influx through the N- and P/Q-type VDCC. Meanwhile, the increase of GABAergic mIPSC frequency by H2O2 was not attenuated by Ca2+-free solution. These results suggest that the presynaptic N- and P/Q-type VDCC might represent a novel target for preventing an excessive excitation, which does not attenuate neuroprotective mechanisms such as GABA release.

(COI: No)

P1-080

Effect of long-term STN-HFS on the IPSC of Substantia Nigra pars Reticulata (SNr) Neurons in the slices from reserpinized rat

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I reported that GABAergic IPSC-LTP was induced by high frequency electrical stimulation onto subthalamic nucleus (STN-HFS) through the postsynaptic mechanism in the half of SNr neurons tested (9 out of 17 neurons) under control condition. The pared pulse ratios of 1.692 ± 0.178 before STN-HFS and 1.349 ± 0.068 at after 120 min STN-HFS were not significantly different ($p = 0.103$, $n = 14$). This IPSC-LTP induced by STN-HFS was observed in almost all neurons tested (11 out of 13 neurons) in the solution with 3 or 5 μM Sulpiride, a D_2 dopamine receptor antagonist. The normalized amplitude was 1.878 ± 0.229 at 120 min after STN-HFS. This value was significantly different from the one before STN-HFS ($n = 11$, $p = 0.004$). On the other hand, STN-HFS induced the LTD-like decrease in the amplitude of glutamatergic EPSC at SNr neurons evoked by electrical stimulation onto internal capsule in the solution with 20 μM bicuculline. As reported, this LTD-like effect on EPSC was abolished by D_1 receptor antagonist (5 μM SCH23390). Since a long time patch-recording itself might affect the amplitude of IPSC, I recorded the IPSC amplitude under voltage-clamp for 122 min without STN-HFS in some neurons ($n = 4$). As expected, an IPSC decreased in its amplitude rather than increase. Normalized amplitude was 0.856 ± 0.09. However, in the slices from reserpinized rats, an acute model rat of Parkinson's diseases, STN-HFS did not induced IPSC-LTP in 4 neurons tested. At 90 min after STN-HFS, the normalized amplitude of IPSC was 0.686 ± 0.112. This value was not significantly different from control ($p = 0.107$).

(COI: No)

P1-081

Inhibition of spontaneous GABAergic currents after increasing pre- and post-synaptic activity in neonatal rat hippocampus

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The activity-dependent plasticity of excitatory synapses is considered to be a model of learning and memory. $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs) regulate the excitatory synaptic transmission by modifying activity of the principal cells, but plasticity of the inhibitory synapses has not been sufficiently characterized. Several investigators reported that repetitive depolarization of principal cells facilitates or suppresses inhibitory synaptic transmission. Thus, direction (facilitation or suppression) and mechanism of the plasticity, remain to be established. In the present experiments, $GABA_A$ergic sIPSCs were recorded in acute slices of neonatal rat hippocampus. Using whole cell patch-clamp recording method, effect of the repetitive postsynaptic depolarization on the sIPSCs was determined. Depolarization of postsynaptic neurons alone did not cause marked alteration of the frequency or amplitude of sIPSCs. Simultaneous activation of presynaptic and postsynaptic neurons however, induced transient decrement of the frequency of the sIPSCs. In the presence of antagonists of metabotropic glutamate receptors or an antagonist of CB1 receptor, the inhibition caused by this simultaneous stimulation was suppressed. These results suggest that postsynaptic depolarization and facilitation of glutamate release from presynaptic terminal transiently inhibit the sIPSCs and that CB1 receptor probably participates in this inhibition.

(COI: No)

P1-082

Synapse-specific effects of interleukin-1β on synaptic plasticity in the mouse hippocampus

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Interleukin-1 β (IL-1 β), which is a key molecule in the inflammatory responses during infection and injury, exerts local effects on hippocampal synaptic plasticity via IL-1 receptors that are present at high levels, especially in the hippocampus. To examine the effects of IL-1 β on synaptic plasticity in different hippocampal regions, we examined long-term potentiation (LTP) in acute hippocampal slices obtained from mice, which is considered as the cellular model for learning and memory. IL-1 β (1 ng/ml) was applied for 30 min before high-frequency stimulation (HFS: 100 Hz for 1 sec × 3) to induce LTP. LTP was significantly impaired by IL-1 β application at the Schaffer collateral-CA1 synapses (138.4 ± 6.2 % vs. 119.1 ± 3.8 %, % of excitatory postsynaptic potential (EPSP) amplitude 60 min after HFS against baselines, mean ± SEM, $n = 4$ respectively, $p < 0.05$, t-test), and at the associational/commissural (A/C) fiber-CA3 synapses (160.9 ± 7.4 % vs. 134.3 ± 9.1 %, $n = 6$, respectively, $p < 0.05$), which are both dependent on NMDA receptor activation. However, mossy fiber-CA3 LTP, which is independent of NMDA receptor activation and expressed presynaptically, was not impaired by IL-1 β (155.0 ± 15.6 % vs. 161.2 ± 19.8 %, % of EPSP amplitude 30 min after HFS against baselines, $n = 8$ respectively, $p > 0.05$). Our results show different effects of IL-1 β on the LTPs at different kinds of synapses, indicating that IL-1 β has synapse-specific effects on hippocampal synaptic plasticity.

(COI: No)

P1-083

Physiological role of N-glycosylation in AMPA receptor-mediated synaptic transmission

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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. In the last meeting, we presented that the digestion of N-glycosylation of primary hippocampal cultured neurons and/or GluA1 expressing HEK293 cells by a treatment with PNGase-F changed AMPA currents from desensitization to re-sensitization, and that asparagine residues, positioned at 401 of 406, putative N-glycosylation sites, were critical sites for the expression of re-sensitization. In this meeting, we will report a physiological role of N-glycosylation in AMPA receptor-mediated synaptic transmission. First, we examined whether excitatory post-synaptic currents (EPSCs) induce the re-sensitization by treatment of acute brain slices with PNGase-F. Under the whole-cell recordings, single electrical stimulation of Schaffer collateral did not show the re-sensitization in hippocampal pyramidal neurons, however, paired pulse stimulation generated a similar re-sensitization. A mEPSC analysis revealed that PNGase-F treatment exhibited significantly longer the decay time. These results suggested that N-glycosylation modulate the synaptic transmission by altering EPSCs.

(COI: No)

P1-084

Synaptic distribution on single labeled mitral cell in the olfactory bulb

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Mitral cells are major projection neurons of olfactory bulb (OB). They receive olfactory inputs, regulate information, and send their axons to the olfactory cortex. In this study, to understand output control from the OB, we established a method for visualization of single mitral cell and examined quantitative distribution of synapses with their target neurons. Single mitral cell fluoro-labeled by virus injection was then processed for serial-sectioning electron microscopy (EM). EM-reconstructed mitral cell was obtained by approximately 300 serial thin sections each, and synapse distributions and their target neurons were analyzed. Total number of synapse on the cell body was 511: number of output and input synapse was 290 and 221, respectively. Among them, 58 % of output synapses and 76 % of input synapses made reciprocal pairs. These synapses were made by individual 261 profiles and 129 of them involved reciprocal pairs. These EM-findings for synapse on the single mitral cell have been confirmed by multiple fluoro-labeling immunocytochemistry. That is, synaptic and neuronal markers, such as parvalbumin, vesicular gamma-aminobutyric acid transporter, vesicular glutamate transporter, were expected for wider view of synaptic distributions. Then we further confirmed that light-microscopically (LM) identified sites were involved in EM-identified synapses. In conclusion, we demonstrate synapse distribution on both soma and dendrites of the single mitral cell by correlative LM and serial-EM studies.

(COI: No)

P1-085

Structural basis for cholinergic regulation in the mouse olfactory bulb

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Odor information is regulated by olfactory inputs, bulbar interneurons, and centrifugal inputs from other brain regions. Among them, centrifugal inputs have been less analyzed. Cholinergic (ACh) neurons derived from the nucleus of horizontal limb of diagonal band (HDB) are one of the major centrifugal inputs to the olfactory bulb (OB). However, little is known about how ACh neurons make synaptic connections with various bulbar neurons to regulate odor signals. In this study, we focus on ACh regulation of the OB, and analyzed the detailed distribution of ACh neurons in the HDB, and the synapses formation in the OB.

A retrograde tracer, Fluoro-Gold was stereotaxically injected into the OB, and serial slices immunostained with multiple neuronal markers to analyze cellular distribution. To clarify projection pathway of ACh neuron, the HDB neurons were fluoro-labeled by viral injection. We confirmed that the infected neuron was Ach, and then single neuron was traced three-dimensionally. Furthermore, to identify target neurons of ACh fibers in the OB, we performed fluorolabelling with various interneuron markers and observed synaptic formation of them by electron microscopy (EM).

In comparison with the other neuron, ACh neurons located rather medially in the HDB. Fluorolabelling revealed that ACh fibers were associated with bulbar interneurons throughout all layers. EM study showed that ACh fibers made asymmetrical synapses, although their post-synaptic density exhibited variable feature.

Our present study suggests that ACh neurons contribute to elaborate mechanism of olfactory processing in the OB.

(COI: No)

P1-086

Spike timing-dependent plasticity (STDP) at L2/3 intercolumner connections and its interaction with STDP at L4-L2/3 connections

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Deprivation-induced map plasticity requires standard STDP with long-term potentiation (LTP) and long-term depression (LTD) at L4-L2/3 synapses, but for spared columns to drive deprived columns effectively, horizontal connections at L2/3 between adjacent columns might also exhibit some form of plasticity during critical period. We tested this possibility and found that these connections exhibit STDP with LTP only, or both pre-before-post as well as post-before-pre timing produced potentiation in a timing-dependent manner, that is, shorter the timing differences, the larger the potentiation. To have LTP-STDP should be advantageous for the formation of horizontal connections. In addition, we found that the formation of this horizontal intercolumner connection could be facilitated by existing vertical connections between L4-L2/3 that exhibit standard STDP with LTP and LTD, just like an interaction between L4-L2/3 LTP-STDP and thalamus-L2/3 LTD-STDP during the 2nd postnatal week, as we report previously. We conclude that intercolumner STDP between L2/3 would contribute to map plasticity, and that network formation by interaction of STDP might be an important rule for shaping the neural network.

(COI: No)

P1-087

Synaptic potentiation in the central amygdala in trigeminal inflammatory pain model of rats

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Capsular part of the central amygdala (CeC), known as the "nociceptive amygdala", receives direct nociceptive inputs by way of the spino- (trigemino-) parabrachio-amygdaloid pathway (Bernard et al., 1989). The excitatory synaptic transmission from the lateral parabrachial nucleus (LPB) to the CeC neurons (LPB-CeC synapse) shows robust potentiation in various types of pain models in rodents (Veinante et al., 2013). However, such LPB-CeC synaptic potentiation has been demonstrated only in spinal pain models. We examined whether this LPB-CeC transmission is also affected in the models with trigeminal pain. We observed a marked LPB-CeC potentiation as recorded with whole-cell patch clamp in acute brain slices prepared at 6 h after upper-lip injection of 5% formalin in Wistar rats. In a similar manner to the spinal pain models, the LPB-CeC transmission in the right, but not in the left, amygdala was markedly potentiated in a manner being accompanied by decreased paired-pulse ratio, limited relation with the firing pattern of neurons and no apparent changes in NMDA/AMPA ratio. As the neurons in the spinal nucleus of the trigeminal nerve mostly project to the bilateral CeC, these results, which are the first to describe the synaptic potentiation in the trigemino-parabrachio-amygdaloid pathway, support a lateralized nature of the inflammation-induced synaptic potentiation. Supported by Kakenhi.

(COI: No)

P1-088

The formation of climbing fiber synapses with cerebellar Purkinje cell in hypogranular mice

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Cerebellar Purkinje cells (PCs) receive two kinds of excitatory input, numerous parallel fibers (PFs) and single climbing fiber (CF). While numerous PFs run in a vertical direction to sagittal plane, where PC dendrites extend, and form single or a few synapses with each PC dendrite, single CF climbs up along PC dendrite in sagittal plane and forms several hundred synapses exclusively with each PC dendrite. To evaluate the contribution of the PFs and PF synapses upon the formation of CF synapses, we observed developmental changes of CF synapses on PC dendrites under reduced PFs caused by MAM treatment from the set of serial electron-microscopic ultrathin sections including the basal of PC somata and dendritic tips. In hypogranular mice, the arbors of PC dendrites were slightly poor and shortened at adult. Moreover, PCs were innervated by multiple CFs from morphological observation. The multiple innervations are classified into two types, the distal type and the proximal type. The distal type is found in distal PC dendritic portion, i.e., spiny branchlet, whose spines were innervated by adjacent CFs. Such type was found from P12 and the number of the ectopic synapses progressively increased. The proximal type is found in proximal PC dendritic portion, which was dually innervated by associated CF and adjacent CF, or whose dendrite branch was fully governed by adjacent CF. The proximal type was found at P15 the number is not changed. Thus, the mechanism, by which multiple innervation occurs, was different between the distal type and proximal type.

(COI: No)

P1-089

SBF-SEM 3D reconstitution analysis reveals alterations in composition and morphology of mouse hippocampal mossy fiber synapses by afadin knockout

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Afadin plays roles in the formation of puncta adherentia junctions and differentiation of presynapses in hippocampal neurons. Still, little is known about the regulation of synapse composition and morphology by afadin. To elucidate this question, we performed serial block face-scanning electron microscopy (SBF-SEM) and 3D reconstitution on mouse hippocampal mossy fiber-CA3 pyramidal cell synapses (MF synapses) in an afadin-knockout background. We used hippocampal MF synapses because of their large size and distinct structure in which a mossy fiber bouton wraps postsynaptic dendritic spines. We show that puncta adherentia junctions and post synaptic densities are reduced and deformed, and that the coverage ratio of dendritic spines by a mossy fiber bouton is reduced. Moreover, the number of synaptic vesicles of the readily releasable pool is reduced by afadin-knockout implicating impaired neurotransmission. These results indicate that afadin is essential for proper composition and morphology of hippocampal MF synapses.

(COI: No)

P1-090

PACAP is involved in hippocampal neurogenesis after global ischemia

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Pituitary adenylate cyclase-activating polypepetide (PACAP) has been shown to protect neurons during CNS diseases and contributes to neurogenesis during development. However, the role of PACAP on neurogenesis during ischemia has not been understood. C57BL/6 wild-type (WT) and PACAP (+/-) mice were subjected to 12 min transient common carotid artery occlusion (tCCAO). After tCCAO, the mice were administered vehicle or PACAP38 (1 pmol) into the hippocampal dentate gyrus. Another set of mice were injected BrdU (300mg/kg, ip) before sacrifice at days 1, 3, 7, 14, 28 and 56 after tCCAO. Brains were collected followed by 4% paraformaldehyde-fixation and 15 μm sections were prepared for multiple-staining of BrdU and cell markers. The hippocampus injected with vehicle or PACAP38 were immunoblotted for collapsin response mediator protein-2 (CRMP2) to estimate axonal extension. BrdU (+) cells were observed in the subgranular zone of the dentate gyrus and increased after tCCAO. Number of BrdU (+) cells peaked at day 7 and were significantly greater in WT mice compared to PACAP (+/-) mice. BrdU (+) cells were co-labelled with nestin positive neuronal stem cells, but less in S100 and Iba-1 positive glial cells. CRMP2 signal was greater in PACAP treated mice. These results suggested PACAP contributes to neurogenesis in addition to neuroprotection during ischemia.

(COI: No)

P1-091

Oscillatory network formation and cholinergic/histaminergic activity in the cultured olfactory neurons in the slug

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Synchronous oscillatory activity in a laminar structure 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 and histamine are known to increase the oscillatory frequency in the PC, and is one of the candidates of the neurotransmitters that are involved in such higher cognitive functions. We recently found that oscillatory neuronal network was formed from dispersed cell culture of PC neurons. In the present study, we thus examined whether cholinergic and histaminergic system are present in cultured PC neuronal network or not. First, increases in neurite arborization, neurite connection and cell aggregation were observed with time in culture. Second, in calcium imaging for each PC neurons, acetylcholinesterase inhibitor or nicotine increased the number of calcium transients and induced synchronous oscillatory activity. These results suggest that acetylcholine can function as an excitatory transmitter in cultured PC neuron network via mainly nicotinic acetylcholine receptors activation. Third, histamine increased the number of calcium transients without synchronous oscillatory activity in a smaller number of PC neurons. It suggests the presence of histaminergic receptors in the cultured olfactory neuron network.

(COI: No)

P1-092

Dynamic changes of ACF7 localization during neuronal development

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Coordination between two major cytoskeletal components, microtubules (MTs) and filamentous actin (F-actin), has been shown to underlie diverse cellular functions. ACF7/MACF1 is a mammalian spectraplakin able to bind both MTs and F-actin directly. ACF7 localizes at the tip of growing MTs and coordinates MT dynamics with F-actin in fibroblasts, and is essential for proper neuronal migration and axonal projection in developing mouse brain. Interestingly, previous reports identified ACF7 as a candidate postsynaptic component by proteomic analyses of biochemically purified PSD fractions. These results indicate that ACF7 changes its localization from tip of MTs to postsynaptic sites during neuronal development.

To test this hypothesis, we performed live-cell imaging of GFP-tagged ACF7 in hippocampal neurons. In immature neurons with growing neurites, ACF7-GFP localized at the tip of MTs and showed translocation driven by MT assembly throughout the cytoplasm. However, mature neurons showed few events of ACF7-GFP translocation at the tips of MTs. Time-lapse imaging of ACF7-GFP revealed initial clustering of ACF7-GFP within dendritc shafts and subsequent translocation into dendritic spines. These results suggest that the existence of a molecular switch that converts ACF7 localization during neuronal development. ACF7 may facilitate interaction of MTs penetrating into spines with actin meshwork, and contributes to excitatory synapse development.

(COI: No)

P1-093

Investigation of the cellular structures inside neuronal compartments by two-photon fluorescent correlation spectroscopy

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Mobility of macromolecules within cells is affected by the presence of membranes, cytoskeletal polymers and nuclear chromatin. Therefore, the architecture of intracellular components in neurons, such as dendritic spines and nuclei, is a key factor for molecular interactions and is the basis for neuronal function. Morphological changes of spines during synaptic plasticity are associated with multiple cellular events that regulate the actin cytoskeleton, membranes and postsynaptic density. Similar to spines, the shape of the nucleus changes in response to neuronal activity. This morphological change affects propagation of calcium signal and transcriptional events, which may lead to intra-nuclear heterogeneity of chromatin structure. Precise measurements of molecular dynamics within specific compartments are important, but currently available techniques are not ideal for measurements within neurons with complex three-dimensional morphology. To directly obtain quantitative parameters on molecular dynamics, we are currently testing the possibility of applying two-photon fluorescence correlation spectroscopy (2P-FCS) to monitor dynamics of either biologically inert fluorescence molecules or GFP tagged proteins within spines and nuclei of cultured neurons. At the meeting, we will discuss the relationship between analytical results of 2P-FCS and molecular architecture inside spines and nuclei including organization of the actin cytoskeleton and chromatin structure.

(COI: No)

P1-094

In vivo recordings of optogenetically evoked striatum firings

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We are investigating a firing prolongation of the adult rat striatal neuron after repetitive optogenetic stimulation observed in an acute striatal slice. In this report, we tested whether the prolongation could be observed in vivo. Wistar Thy-1.2 promoter ChannelRhodopsin-2 Venus Rats were anesthetized by urethane and placed in a stereotaxic frame. A wire tetrode was coupled with a 500 μm plastic optic fiber and inserted into the striatum of the rat. We were able to observe the firing prolongation by the tetrode after repetitive LED photostimulation through the optical fiber. The striatal neurons showed a prolonged firing response of gradually increasing duration when exposed to 5 repetitive optogenetic photostimulations. This result indicates that both acute slice and in vivo striatal neurons hold their internal state in decasecond-order timescale.

(COI: No)

P1-095

Mapping of neuronal network activity in dorsal horn during the activation of low threshold afferent fibers: An application of multi-channel array system to acute slices of mouse spinal cord

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Peripheral nerve injury-induced hyperalgesia and allodynia is suggested to be associated with synaptic rearrangement of neuronal circuits in the spinal dorsal horn. However, further details of this synaptic reorganization remain to be elucidated. The present experiments used a multi-electrode array (MEA) system to analyze neuronal circuitry activity in the dorsal horn of the acutely prepared spinal slice preparation. Lumbar spinal cord slices with a dorsal root attached were prepared from an adult mouse anesthetized with ketamine and xylazine. The dorsal root was stimulated with a suction electrode and simultaneous recordings of extracellular field potential were made from 64 points on a spinal slice. Off-line analysis of the amplitude and propagation of evoked field potential (FP) was conducted. Isopotential contour maps of evoked FP were also composed. Stimulation of dorsal root at stimulus intensities of 4-5 times the lowest threshold of afferent fibers evoked negative FP. This negative FP distributed from the superficial dorsal horn into the deep dorsal horn. The amplitude of this negative FP was largest in the laminae III-IV. The negative FP recorded in the lamina II exhibited smaller amplitude and longer latency compared to that recorded in the laminae III-IV. We are currently conducting experiments in mice with partial ligation of the sciatic nerve to show differences in the evoked FP between neuropathic and control mice.

(COI: No)

P1-096

Action potential firing activates myosin II and VI in distinct dynamin isoforms-mediated synaptic vesicle recycling pathways

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Myosin II regulates presynaptic actin dynamics and VI mediates postsynaptic receptors endocytosis in the brain. We have first demonstrated role of myosin II in regulation of transmitter release, however, function of presynaptic myosin VI was not explored. Myosin IIB and VI are expressed at presynaptic terminals superior cervical ganglion (SCG) neurons. Thus, we examined synaptic vesicle (SV) trafficking and the molecular mechanism linking variation in neural activity to SV resupply. Combined genetic knockdown and direct physiological measurement of synaptic transmission from paired SCG neurons in culture show that myosin IIB and VI together cover physiological range of AP firing patterns, mediating replenishment of a shared readily releasable pool (RRP) following distinct endocytic pathways activated selectively by dynamin isoforms. Myosin VI resupplied the RRP with slow kinetics independently of firing rates but acted quickly within 50 ms after AP. Under high frequency AP firing, myosin IIB resupplied the RRP with fast kinetics in a slower time window of 200 ms. Myosin IIB mediates SV resupply from a reserved pool to the RRP in a SV recycling pathway activated by dynamin 1, while myosin VI mediates SV resupply through another pathway activated by dynamin 3. Collectively, our findings show that myosin IIB and VI work individually in distinct vesicle reuse pathways activated by dynamin isoforms, having distinct rate and time constants with physiological action potential frequency.

(COI: No)

P1-097

Analyses of bone cancer pain-related molecules in the spinal cord and the effects of irradiation on the cancer

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Bone cancer pain is a serious problem for patients. To investigate the mechanisms of this pain, we examined the related molecules and the effects of irradiation. To create cancer pain model mice, we injected osteolytic sarcoma cells into their femurs. To assess the pain levels, we used the von Frey test. We compared the expression of glial markers and a mediator of glial activation in the spinal cord between the control and the cancer pain model because glial cells contribute to cancer pain. The protein expressions of the astrocyte marker GFAP, the microglia marker Ibal and the mediator TLR-4 increased in the ipsilateral side in the cancer pain model. A decrease in the K^+-Cl^- cotransporter KCC2 and an increase in the Na^+-K^+-Cl^- cotransporter NKCC1 increased intracellular Cl^- and cause a positive shift in the reversal potential of the $GABA_A$ receptor-induced current. That process contributes to GABA-induced pain facilitation. Real-time RT-PCR analysis showed that the cancer pain decreased KCC2 and increased NKCC1 in the ipsilateral side, and that irradiation increased KCC2 and decreased NKCC1. The gramicidin perforated patch-clamp technique was used to observe the reversal potential shift. The number of neurons which induced the positive shifts in the cancer pain model was higher than that in the control. The trend of the reversal potential shifts in the cancer pain model which received irradiation was similar to that in the control. Our results may provide a clue to elucidate the mechanism that underlies bone cancer pain.

(COI: No)

P1-098

The recycling pool size estimated by different stimulation frequencies at the calyx of Held presynaptic terminal

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For sustained synaptic transmission, it is essential to maintain the recycling pool of vesicles. After exocytosis of synaptic vesicles, fused vesicle membranes are re-internalized by endocytosis, refilled with neurotransmitter and recycled for reuse, but the mechanism regulating this pool size is unclear. In hippocampal autaptic culture, Ikeda & Bekkers (2009) estimated the number of synaptic vesicles per bouton using the vacuolar ATPase blocker bafilobycin A1 (Baf A1) to block vesicle refilling. Their estimates ranged between 100 - 200 vesicles and were independent of stimulation frequency. We asked whether the recycling pool size is regulated by presynaptic activity. By blocking vesicle refilling through either bath-application of BafA1 or washout of glutamate, we found both the amplitude of evoked EPSCs (eEPSCs), and the frequency of miniature EPSCs (mEPSCs) gradually reduced. The reduction rate of eEPSCs comprised both time- and use-dependent components, the former likely caused by a passive leakage of glutamate from vesicles, and the latter by recycling of non-refilled vesicles. The recycling pool size, estimated by dividing the time integral of eEPSCs by that of a mEPSC, was approximately 500/AZ at 20 Hz stimulations, but 10 to 20 times smaller at 0.02 Hz. Neither BafA1 nor glutamate washout affected the change in presynaptic membrane capacitance following stimulation. We cautiously speculate that neuronal activity can regulate the pool size of recycling vesicles.

(COI: No)

P1-099

Role of neuronal Ca^{2+} sensor-1 in learning and memory in mice

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Intracellular Ca^{2+} plays key roles in regulating various functions in the nervous system. Neuronal calcium sensor-1 (NCS-1) is a Ca^{2+} binding protein, which mediates Ca^{2+} signals in a spacial and temporal manner. Although NCS-1-deficient *C. elegans* shows memory dysfunction, the brain functions in NCS-1 knockout (KO) mice have not been examined. Here we investigate whether NCS-1 regulates brain structure, neural functions, and physical activity. Histological analysis revealed that NCS-1 was expressed throughout the brain, but at the highest in hippocampal neurons, a memory center. Morris water maze analysis demonstrated that KO mice had lower functions of spatial learning and memory. Physical activity was not different between WT and KO mice. To understand NCS-1-mediated signaling pathway, we measured the amount of neurotrophic factors in WT and KO groups. In KO mice, BDNF, a key regulator for memory function, was significantly lower in the entire brain, although GDNF and NGF levels were not different from WT mice. Additionally, dopamine secretion was decreased in KO brain. Electron microscopy revealed that the number of large dense core vesicles, which release BDNF and dopamine, was decreased in KO group. These results suggest that NCS-1 plays an important role in spatial learning and memory through the regulation of BDNF and dopamine secretion in the brain.

(COI: No)

P1-100

Upregulation of HP1γ expression during neuronal maturation promotes axonal and dendritic development in mouse embryonic neocortex

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Immature neurons undergo morphological and physiological changes including axonal and dendritic development in order to establish neuronal networks. Since the transcriptional status changes at a large number of genes during neuronal maturation, global changes in chromatin modifiers may take place in this process. We now show that the amount of heterochromatin protein 1 γ (HP1γ) increases during neuronal maturation in the mouse neocortex. Knockdown of HP1γ suppressed axonal and dendritic development in mouse embryonic neocortical neurons in culture, and either knockdown or knockout of HP1γ impaired the projection of callosal axons of superficial layer neurons to the contralateral hemisphere in the developing neocortex. Conversely, forced expression of HP1γ facilitated axonal and dendritic development, suggesting that the increase of HP1γ is a rate limiting step in neuronal maturation. These results together demonstrate an important role for HP1γ in promoting axonal and dendritic development in maturing neurons.

(COI: No)

P1-101

Cell-type specific intracellular calcium recording in the nucleus accumbens in freely moving mice

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The nucleus accumbens (NAc), which largely receives the dopaminergic projection from the ventral tegmental area, plays a crucial role for the motivational reward and aversive response. The NAc is mainly constituted by two types of cellular groups, D1R- and D2R-MSNs (dopamine type1 and 2 receptor expressing medium spiny neurons), with differential functions and projections. To understand the function of NAc as a reward and aversive system, it is required to describe the behavior of individual cellular groups separately. However, it has been technically difficult because D1R- and D2R-MSNs are randomly distributed in the NAc. To elucidate the behavior of each cellular groups in the NAc, we developed a new fiber optical recording system for measurement of the intracellular calcium levels in deep brain structures. To express a calcium indicator in D2R-MSNs highly and specifically, we used the Knockin-mediated ENhanced Gene Expression system (KENGE-tet system; Tanaka *et al.*, 2012, *Cell Rep.*). The ratiometric calcium measurement using a forster resonance energy transfer (FRET)-based GECI, Yellow Cameleon-Nano 50, brought signals with high precision as a cancellation of artifacts by body motions of mice. We will report the particular patterns of calcium transients in the D2R-MSNs in the NAc by multiple aversive stimuli such as tail-suspension and restriction in mice.

(COI: No)

P1-102

Postnatal Development of Dendritic Structures in the Medial Prefrontal Cortex of the Marmoset

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In the primate cerebral cortex, dendritic spines rapidly increase in number after birth up to infancy or mid-childhood, and then decrease towards adulthood. Abnormalities in these processes accompany several psychiatric disorders. In this study, we examined developmental changes of basal dendrites and spines of layer III pyramidal cells in the medial prefrontal cortex (mPFC) of the common marmoset. The mPFC consists of several areas with distinct features in layer organization, histochemistry, connections, and, in humans, vulnerability to psychiatric disorders. We selected three areas for examination: granular dorsomedial prefrontal (area 8B/9), dysgranular ventromedial prefrontal (area 14r), and agranular anterior cingulate (area 24) cortices. Dendritic field areas, lengths, number of branching points, and total spine number reached a peak at 2-3 postnatal months in all three areas. However, the profiles of spine formation and pruning differed across the three areas with different degrees of granularity; the amount of spine loss from the peak to adulthood was less in areas 24 (33%) and 14r (29%) than in area 8B/9 (43%). Disturbance of this modest spine pruning in the less granular cortical areas may lead to an excessive loss of spines reported for areas 24 and 14r of schizophrenic patients.

(COI: No)

P1-103

Development of a multi-electrode array system for evaluation of human synaptic functions in neuron/astrocyte co-culture derived from human neural stem/progenitor cells

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A multi-electrode array (MEA) system enabled us to investigate synaptic functions in rodent brain slices and rodent neuron/astrocyte co-culture. It was, however, challenging to apply the human neurons/astrocytes to the MEA system, because it is not easy to prepare functional human neurons/astrocytes with simple methods. In this study, we utilized human fetal hippocampal neural stem/progenitor cells, HIP-009 cells to develop a novel MEA assay system; HIP-009 cells can differentiate into both neurons and astrocytes at an about equal ratio in the same culture. We observed that frequency and amplitude of spontaneous firings of differentiated HIP-009 cells were increased in a differentiation-time dependent manner. The electrophysiological maturation of neurons was promoted by supplementation of rat astrocyte-conditioned medium. Further analyses by using blockers for postsynaptic receptors (GABAzine, MK-801, and NBQX) revealed that the detected firings were resulted from the formation of functional synapses throughout differentiated HIP-009 neurons. In conclusion, we developed the novel in vitro assay system to evaluate human synaptic functions in mass cultures containing human astrocytes by utilizing HIP-009 cells in combination with the MEA system.

(COI: No)

P1-104

Augmentation of NMDA component of spinal monosynaptic reflex by high frequency stimulation in newborn rat

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The susceptibility of synaptic transmission to the stimulation frequency is a characteristic feature of immature animals. In an isolated spinal cord preparation of newborn rat, monosynaptic reflexes (MSRs) evoked by dorsal root stimulation, were mediated by both NMDA and AMPA glutamate receptors. In normal conditions, MSRs were constant in amplitude at 1/15 sec, and were completely eliminated by CNQX, which suggested normal MSRs were dependent on AMPA receptor. When stimulus rate was increased to 1/sec, MSR amplitudes were greatly reduced initially, and recovered later. This recovery of MSR was eliminated by APV, which suggested this recovery was dependent on NMDA receptor. In the presence of APV, AMPA component of MSRs were depressed and not recovered at 1/sec, which was presumably due to AMPA receptor desensitization. In the presence of CNQX and 0-Mg, NMDA component of MSRs were depressed initially and recovered, even though the stimulus rate was maintained at 1/sec. The stimulus intensity to induce this recovery, had to be strong enough to activate thin-fibers. This recovery was eliminated by application of spantide (16 μM), a non- specific tachykinin antagonist. It is suggested that thin sensory fibers can enhance Ia monosynaptic transmission through tachykinin receptors. Subtype of tachykinin receptors will also be discussed.

(COI: No)

P1-105

1, 8- and 1, 4-cineole presynaptically enhance spontaneous excitatory transmission in adult rat superficial dorsal horn neurons in a manner different from each other

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We have previously reported that 1, 8- and 1, 4-cineole, present in essential oils derived from eucalyptus, repeatedly increase the spontaneous release of L-glutamate onto spinal lamina II (substantia gelatinosa; SG) neurons with IC_{50} values of 3.2 and 0.24 mM, respectively, in a manner resistant to a voltage-gated Na^+-channel blocker tetrodotoxin. The present study examined a detail of the cineole actions by applying the patch-clamp technique to the SG neurons of adult rat spinal cord slices. The 1, 8-cineole activity was inhibited by TRPA1 antagonists, HC-030031 and mecamylamine, the latter of which is also known to be a nicotinic acetylcholine-receptor antagonist, but not by a TRPV1 antagonist capsazepine. On the other hand, the 1, 4-cineole activity was depressed by capsazepine but not by HC-030031 and mecamylamine. A TRPM8 antagonist BCTC, which inhibited sEPSC frequency increase produced by its agonist (-)-menthol, had no effect on the 1, 8- and 1, 4-cineole activities. 1, 8- and 1, 4-cineole reduced monosynaptically-evoked primary-afferent C-fiber but not A δ -fiber EPSC amplitudes, as with a TRPV1 agonist capsaicin and a TRPA1 agonist cinnamaldehyde, albeit with extents smaller than those of the agonists. It is concluded that the 1, 8- and 1, 4-cineole activities are mediated by TRPA1 and TRPV1 channels, respectively. This difference between the structural isomers of cineole may serve to know the property of TRP channels in the SG.

(COI: No)

P1-106

Effect of thymol on glutamatergic spontaneous excitatory transmission in adult rat spinal substantia gelatinosa neurons

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Transient receptor potential (TRP) channels expressed in the peripheral and central terminals of dorsal root ganglion neuron are involved in nociception, but the properties of TRP channels in the central terminal have not been fully examined yet. In order to know the properties of the central terminal TRP channels, we examined the actions of thymol, one of aroma-oil chemicals contained in thyme, on glutamatergic spontaneous excitatory synaptic transmission in lamina II (substantia gelatinosa; SG) neurons in adult rat spinal cord slices by using the patch-clamp technique. Superfusing thymol (1 mM) for 3 min increased the frequency of spontaneous excitatory postsynaptic current (sEPSC) with a minimal increase in its amplitude in almost all neurons examined. Seventy-eight % of the neurons also produced an outward current at -70 mV. These thymol activities were repeated at a time interval of 30 min and resistant to a voltage-gated Na^+-channel blocker tetrodotoxin. The sEPSC frequency increase was inhibited by a TRPA1 blocker HC-030031 but not a TRPV1 blocker capsazepine, while these blockers had no effect on the outward current. It is concluded that as with eugenol and carvacrol, thymol increases the spontaneous release of L-glutamate onto SG neurons by activating TRPA1 channels while producing an outward current in SG neurons without TRPA1 and TRPV1 activation. This result could serve to know the properties of central terminal TRP channels.

(COI: No)

P1-107

Localization of kirrel3 protein at synaptic sites in the mouse cerebellum

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A member of the immunoglobulin superfamily, kirrel3, plays important roles in the axonal fasciculation of the specific olfactory sensory neurons as well as in the axonal coalescence of the specific vomeronasal sensory neurons. In the brain of adult mice, kirrel3 interacts with the synaptic scaffold protein, calcium/calmodulin-dependent serine protein kinase, indicating the possible involvement of kirrel3 in synaptic function. Previously, we have reported that the kirrel3 gene was widely expressed in the cerebellum including the granule cells, Purkinje cells, and interneurons during development. In the present study, we investigated the localization of kirrel3 protein in the postnatal and adult cerebellum using immunohistochemistry. In the cerebellum, the expression of kirrel3 protein was first observed in the internal granule cell layer (IGL) at postnatal day (P) 7 and reached a maximum at P14. From P7 to P28, kirrel3 was colocalized with PSD95 at synaptic sites of IGL. From P21 to P70, the expression of kirrel3 protein was also observed in the PSD95-positive nerve plexus of basket cells (pinceau), which surrounds the axon initial segment of Purkinje cells. These findings suggest that kirrel3 may be involved in the synaptic formation/plasticity in the cerebellum during postnatal and adult stages. This work was supported by a Grant-in-Aid for Scientific Research (B) from Japan Society for the Promotion of Science (22390036).

(COI: No)

P1-108

Interleukin-18 knock out mouse induced degeneration of mitochondria in the dentate gyrus of the hippocampus

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Interleukin-18 is thought to regulate motor activity and spatial learning, and mediate inhibition of LTP in the dentate gyrus of the mouse. We investigated whether morphological changes have occurred in the dentate gyrus of the Interleukin-18 knock-out mouse (12 weeks old) by using the electron microscope. In the molecular layer, many degenerated mitochondria were found and located in the axon terminals. They were round, small (about 0.3 μm in diameter), electron dense, and showing indistinct structure of crista. The terminals containing degenerated mitochondria were small (about 0.7 μm width), and attached slender dendrites (about 0.6 μm width). These terminals contained round or pleomorphic synaptic vesicles and formed asymmetric synaptic contacts. The number of terminals contained degenerated mitochondria was smaller in the inner part than those in the outer part of the molecular layer. The granule cells were round and similar to those of the wild type mouse. In the polymorphic layer, there were a few small terminals containing degenerated mitochondria, whereas the large terminals of the mossy fibers contained non-degenerated mitochondria. These results suggest that morphological and physiological changes occur at the axon terminals of the entorhinal-dentate gyrus projections in the Interleukin-18 knock-out mouse.

(COI: No)

P1-109

Social isolation during critical period causes reduced excitatory inputs onto mouse medial prefrontal cortex neurons in adulthood

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Social experience is crucial for the functional development of medial prefrontal cortex (mPFC). Rearing mice in social isolation produces hypomyelination of mPFC in adulthood, which is paralleled by behavioral impacts including poor sociality (Makinodan et al., 2012). However, little is known about the alteration in mPFC neural circuits induced by social isolation. We studied the effects of social isolation on excitatory synaptic inputs onto layer 5 pyramidal cells of mouse mPFC in adulthood. The mouse was reared in isolation for two weeks (P21-35: early isolation or P35-49: late isolation), and then returned to its home cage and reared with its littermates. Whole-cell recordings were performed using slices prepared from P63-67. We found that the spontaneous excitatory postsynaptic current (sEPSC) frequency and miniature excitatory postsynaptic current (mEPSC) frequency were significantly lower in early-isolated mice than in grouped mice. However, there was no significant difference in sEPSC and mEPSC frequencies between late-isolated mice and grouped mice. These results show that only 2 weeks social isolation from weaning reduces excitatory synaptic inputs onto layer 5 pyramidal cells in mPFC and suggest that social experience during the critical period is pivotal in the development of mPFC excitatory neural circuit.

(COI: No)

P1-110

Pairs of stimuli enhance cell firing in hippocampal CA1 area

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In hippocampus, synaptic efficacy is regarded as the neural basis for learning and memory. To understand the mechanism of these, it is necessary to test how information is integrated in hippocampal CA1. Hippocampal CA1 neurons receive inputs from entorhinal cortex indirectly via a trisynaptic path, in which CA3 Schaffer collaterals (SC) form synapses on proximal CA1 dendrites in stratum radiatum (SR). CA1 neurons also has excitatory connections directly with entorhinal cortex via the perforant path (PP). These direct inputs synapse on distal pyramidal neuron dendrites in stratum lacunosum moleculare (SLM). 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. The functional role of the direct PP inputs is not well understood, although recent research indicates that these inputs have important effects on CA1 pyramidal cells. 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 simultaneously recorded population spike from stratum pyramidale and fEPSPs from SR in CA1, extracellularly. The results indicate that synaptic plasticity is modulated by different pairing intervals. In addition to this, we used GABAA receptor antagonist to test whether this modulation relies on inhibitory circuits.

(COI: No)

P1-111

Regulation of neuritogenesis in PC12 cells by temperature-controlled repeated thermal stimulation

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This study aimed to examine the regulation of neuritogenesis (NG) by temperature-controlled repeated thermal stimulation (TRTS) in rat neuron-like PC12 cells. Plated PC12 cells in growth or differentiation medium were exposed to TRTS using a heating palte (HP) (preset surface temperature of the HP, 39.5℃ or 42℃) for up to 18 h/day. This was followed by an evaluation of alternations in cell growth, extent of NG, or acetylcholinesterase (AChE) activity (a neuronal marker). To analyze the mechanisms underlying the effects of TRTS on these cells, its effects on intracellular signaling were examined using: the TrkA inhibitor GW441756, PKA inhibitor H89, p38 MAPK inhibitor SB203580, and MEK inhibitor U0126. While the TRTS of 39.5℃ did not decrease the growth rate of cells in the cell growth assay, it increased the number of neurite-bearing PC12 cells and AChE activity without addition of other inducers of NG. Furthermore, U0126, SB203580, and H89, but not GW441756, considerably inhibited TRTS-induced NG. These results suggested that the TRTS could induce NG and that activation of both the ERK1/2 and p38 MAPK pathways is required for the mechanism of TRTS-dependent NG in PC12 cells.

(COI: No)

P1-112

Morphological analysis of Purkinje cell-specific calcineurin B1 subunit KO mice

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A Ca^{2+}/calmodulin-dependent protein phosphatase Calcineurin (CN) is widely expressed at the central nervous system and plays important roles in various neuronal functions, such as synaptic transmission and the expression of long-term synaptic plasticity. However, little is known about how CN is associated with the formation of excitatory and inhibitory neuronal network. In the present study, we produced a novel mouse line CNB1-PCKO mouse which lacks a regulatory subunit of CN, CNB1, specifically in cerebellar Purkinje cells (PCs) and investigated the cerebellum by morphological techniques. In light microscopic analysis using with immunofluorescence, CNB1-PCKO mice showed that climbing fiber (CF) territory was proximally retracted. By neurotracing technique, some CFs showed aberrant PC wiring and caused multiple CF innervation at proximal PC dendrites. At the electron microscopic level, VIAAT-positive inhibitory terminals frequently formed asymmetrical synapses with PC spines, which in normal adult cerebellum are innervated by excitatory terminals. Postembedding immunogold microscopy revealed that such atypical inhibitory synapses on PC spines expressed both AMPA and GABAA receptors on the postsynaptic membrane. In typical PC spines contacting parallel fiber terminals, the density of AMPA receptor was significantly increased in CNB1-PCKO mice. These result suggest that CNB1 in PCs is essential for CF-PC mono-innervation, anatomical targeting of PC spines to excitatory terminals, and limiting AMPA receptor content at excitatory synapses.

(COI: No)

P1-113

Optical mapping of vagus nerve-related brainstem nuclei in the mouse embryo

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The vagus nerve (N.X) transfers autonomic input and output information to and from the brainstem, and analysis of the N.X-related brainstem nuclei is the first step to understand the functional organization of the autonomic neuronal circuits. Investigations of the neural network organization have been hampered because conventional electrophysiological means have some technical limitations. In the present study, the multiple-site optical recording technique with a voltage-sensitive dye was used to survey the functional organization of the vagal system in a mouse embryo. Stimulation of the N.X in E11 to E14 mouse embryos elicited optical responses in areas corresponding to the vagal sensory and motor nuclei. Postsynaptic responses in the first-order sensory nucleus, the nucleus of the tractus solitarius (NTS), were identified from E11, suggesting that sensory information was transferred to the brain by this stage. In addition to the NTS, optical responses were identified in the rostral and contralateral brainstem regions, which appeared to correspond to second/higher-order nuclei of the vagus nerve. Postsynaptic responses in the second/higher-order nuclei were detected from E12, suggesting that polysynaptic pathways were functional by this stage. We discuss the results of optical mapping, comparing them with previous findings obtained in chick and rat embryos.

(COI: No)

P1-114

Roles of BMP4 signaling in synapse development

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Synapse development is a process precisely regulated by both genetic programs and activity-dependent processes. Proper remodeling of synapses is required for refinement of neuronal circuits. The process of synapse turnover must be regulated by specific signaling mechanisms. BMPs are members of TGF-β superfamily. Secreted BMPs exert their functions through activation of heterotetrameric complex of BMPRI and BMPRII. After ligand binding, BMPRII phosphorylates a cytoplasmic domain of BMPRI. This phosphorylation activates BMPRI and initiates subsequent phosphorylation of the intracellular signaling molecules. Recent studies indicate a role for BMP4 signaling pathway in development of Drosophila neuromuscular junction and neural networks in the mammalian cerebellum and brain stem. Furthermore, the expression level of BMP4 in the mammalian CNS was shown to be related to learning and memory. These results indicate that BMP4 may regulate the process of synapse development and its activity-dependent modulation. Here we report that BMP4 has a function for regulating the stability of synaptic structures. We studied the roles of BMP4 in the process of synapse development by visualizing dendritic structure and distribution of synaptic molecules in hippocampal cultures taken from BMP4 conditional KO mice. Furthermore, live imaging analyses revealed spatiotemporal regulation of BMP4 exocytosis. These results suggest that BMP4-dependent mechanism of synapse remodeling is essential in proper formation of neural network in the mammalian CNS.

(COI: No)

P1-115

Dendritic spine dynamics during growth if hippocampal slice culture

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The organotypic slice culture of brain has many experimental advantages, among which is the possibility of pursuing the morphological changes consecutively for long periods. We previously analyzed the dynamics of dendritic spines in the stable culture of the mouse hippocampus after repetitive inductions of chemical LTP that led to a slowly developing long-lasting synaptic enhancement. The spines are in a stochastic equilibrium between generation and retraction. The plasticity-producing stimulus increased fluctuation keeping the equilibrium at first and then biased the equilibrium toward generation to result in a net increase in spine number. Here we analyzed the dendritic spine dynamics before maturation of culture to know whether or not the developmental synapse formation follows a course similar to the above-mentioned post-maturational (i.e. plasticity-related) synapse formation. We found that the number of spines increased through a biased fluctuation where the rate of retraction was lower than that in the mature culture. This dynamics should not be an artifact of culturing, since the cultures prepared from younger mouse pups behaved not in a culture-day-matched manner but in a cell-age-matched manner. Joro spider toxin, a blocker for the calcium-permeable AMPA receptor that is expressed in the developing hippocampus, suppressed the net spine increase through raising the rate of retraction. This work is supported by Kaken-hi to A.O.

(COI: No)

P1-116

A potential *in vitro* model system of the stress-associated memory disorder

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Previously we found in the organotypic slice culture of the rodent hippocampus that three repeated inductions, but not a single induction, of chemical LTP (cLTP) led to a slowly developing long-lasting synaptic enhancement coupled with new synapse formation. Naming this structural plasticity phenomenon RISE (*R*epetitive LTP-*I*nduced *S*ynaptic *E*nhancement), we propose that it should serve as a model system for analyzing the cellular processes underlying memory consolidation. In this study, we analyzed the effects of externally applied glucocorticoid as mimicry of stress *in vivo*. Dexamethasone (Dex; 1-100nM), when applied for a 24h period beginning 12h after the third cLTP induction, suppressed the increase in the density of dendritic spine CA1 pyramidal neurons. The analyses of spine dynamics revealed that Dex suppressed the elevation of spine generation rate that occurred in during RISE development. Dex also suppressed the enhancement of electrophysiologically-monitored strength of CA3-CA1 synapse. Dex did not induce neuronal death by this dose and period. The Dex's effect was reversed by mifepristone, a glucocorticoid receptor antagonist. Mineralocorticoid aldosterone (10nM) failed to interfere with RISE. These results endorse the usability of this *in vitro* system for analyzing the cellular mechanisms underlying the stress-associated memory disorder. This work was supported by Kaken-hi to A.O.

(COI: No)

P1-117

Cholinergic modulation of GABAergic synaptic transmission in the dorsal raphe serotonin neurons

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The dorsal raphe nucleus (DRN) is the origin of central serotonin (5-HT) system, plays an important role in the regulation of many physiological processes such as sleep/arousal, food intake and mood. The DRN has been thought to be subdivided into several clusters on the basis of differences in cellular morphology, expression of neurotransmitters such as 5-HT, dopamine, GABA and glutamate. Among these, there are many reports that GABA synapses play roles for regulation of excitability of 5-HT neurons by a form of feedback inhibition. However, the modulatory effects on GABAergic synapses at the DRN 5-HT neurons are poorly understood. In this study, we investigated modulatory effects of cholinergic receptor on GABAergic synapses in the mouse (C57/BL6, postnatal days 35-50) DRN 5-HT neurons using whole-cell recordings in the brain slices. Muscarinic receptor agonists, muscarine and carbachol decreased the amplitude of stimulation-evoked IPSCs (eIPSC) with an increase in the paired-pulse ratio, and their effect was reversibly abolished by a M2-receptor antagonist, AFDX-116. Based on these results, the activation of M2 receptor is suggested to be responsible for presynaptically decreasing the amplitude of eIPSCs. We next examined whether endogenous acetylcholine (ACh) affected GABAergic transmission in the DRN neurons. Conditioning stimulation at pedunculo pontine tegmental nucleus which has many cholinergic neurons decreased the amplitude of eIPSCs. These results suggest that the excitability of DRN 5-HT neurons may be positively controlled by disinhibition manner of GABAergic transmission.

(COI: No)

P1-118

Ischemia-induced potentiation of cortical responses to hindpaw stimulation is partly mediated by nitric oxide at the spinal cord level

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We frequently experience postischemic tingling sensation. Ischemia also produces nerve conduction block that may modulate spinal neural circuits, and tingling sensation may be induced as a result. In a mouse model, reduced mechanical thresholds for hindpaw-withdrawal reflex were reproduced after a high pressure was applied around the hindpaw. Neural activities in the spinal cord and the primary somatosensory cortex (S1) were investigated using flavoprotein fluorescence imaging. Ischemic treatment induced potentiation of the ipsilateral spinal and contralateral S1 responses to hindpaw stimulation. We also found that S1 responses elicited by vibratory stimulation applied to the hindpaw contralateral to the ischemic treatment were significantly potentiated during ischemia, suggesting that some diffusible mediators were involved in the potentiation. Nitric oxide (NO) is one of such diffusible mediators involved in synaptic potentiation. We applied L-NAME, an inhibitor of nitric oxide synthase, intrathecally at the L5-L6 intervertebral space, and the potentiation of S1 responses elicited by vibratory stimulation applied to the hindpaw contralateral to the ischemic treatment was clearly inhibited. These results suggest that NO has some roles at the spinal cord level in the induction of postischemic potentiation.

(COI: No)

P1-119

Otoferlin alters mode of exocytosis at the mouse inner hair cell ribbon synapse

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Sound encoding depends upon Ca^{2+}-mediated exocytosis at the inner hair cell (IHC) ribbon synapse in the cochlea. Otoferlin, a multi-C2 domain protein, has been proposed to regulate Ca^{2+}-triggered exocytosis at this synapse, but the precise mechanisms of otoferlin function remain unclear. In this study, we performed whole-cell patch-clamp recordings of excitatory postsynaptic currents (EPSCs) from postsynaptic spiral ganglion neurons (SGNs) in otoferlin mutant mice, in order to investigate the effect of otoferlin disruption at individual synapses with single release event resolution. Otoferlin deletion dramatically decreased the rate of spontaneous release and high potassium-evoked release, suggesting disrupted stimulus-secretion coupling in IHCs. A missense Otoferlin mutation (pachanga) also reduced the release rate but spared stimulus-secretion coupling. These findings support the proposed roles of otoferlin in Ca^{2+} sensing for fusion and vesicle supply. While both otoferlin mutant SGNs showed a decrease in the mean EPSC amplitude, large-sized and variable-shaped EPSC remained present despite the massively reduced rate of release. In addition, both otoferlin mutant SGNs exhibited a smaller fraction of multiphasic EPSCs. These findings argue for uniquantal release at the IHC ribbon synapse (Chapochnikov, Takago et al. (2014) Neuron 83:1389-1403), and we suggest a role of otoferlin in regulating the dynamics of vesicle fusion pore.

(COI: No)

P1-120

Input-selective expression of glutamate receptor GluD1 at ascending somatosensory pathway synapses in the ventral posteromedial thalamic nucleus

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Of the two members in the δ family of ionotropic glutamate receptors, GluD1 is widely expressed in higher regions of the adult brain. We have recently demonstrated that GluD1 works in concert with GluD2 for the construction of cerebellar synaptic wiring through synapse-connecting function (Konno et al., 2014). However, little is known to date regarding the expression and function of GluD1 outside the cerebellum. To address this issue, we examined the expression in the ventral posteromedial thalamic nucleus (VPM), a relay station in the trigeminal somatosensory pathway. Double-labeling fluorescence in situ hybridization addressed that GluD1 mRNA was expressed in glutamatergic thalamic neurons expressing vesicular glutamate transporter VGluT2 mRNA. VPM neurons are known to receive two types of glutamatergic inputs, one from VGluT2-positive ascending inputs from the brainstem trigeminal nuclei and another from VGluT1-positive descending inputs from the somatosensory cortex. By immunofluorescence, GluD1-positive clusters were closely apposed to VGluT2-positive terminals, but not VGluT1-positive terminals. Postembedding immunoelectron microscopy revealed that GluD1 was selectively localized on the postsynaptic membranes of dendritic protrusion surrounded by VGluT2-positive terminals. Thus, GluD1 displays input-selective expression in VMP thalamic neurons, and is selectively localized to their ascending pathway synapses.

(COI: No)

P1-121

Formation of ectopic synapses at retina in presynaptic active zone protein CAST/ELKS deletion mutant

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A photoreceptor cell is a specialized neuron which converts light into signals in the retina. The two types of cells, rods and cones, form synapses at a band known as the outer plexiform layer. In old retina, these synapses are ectopically localized in the outer nuclear layer (ONL), which may cause the loss of function. In young retina, this ectopic localization is found in some deletion mutants such as Bassoon and CAST. Here we explored the effect of the deletion of ELKS, a family member of CAST, and the deletion of both on the localization and structure of ribbon synapse and the visual processing in retina. The ELKS conditional knock out (KO) under the control of *Crx* promoter showed normal development and less effect on the mislocalization of the synapses. However, CAST and ELKS double KO (dKO) showed drastic aberrant synapse formation into the ONL. To know how the structural alteration affects the signal transduction in retina, we measured the gain of eye movement with the optokinetic response. From this test, we found serious gain reduction in dKO, however the gain was detectable indicating that the dKO was not the complete blindness. From these results, we speculate that CAST and ELKS contribute to the maturation of retinal ribbon synapse structurally and functionally.

(COI: No)

P1-122

BRAG2c, a long C-terminal splice variant, interacts with endophilin III to mediate AMPA receptor internalization

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Brefeldin A-resistant Arf-GEF 2 (BRAG2) is a guanine nucleotide exchange factor (GEF) that selectively activates ADP ribosylation factor 6 (Arf6). Arf6 is known as a small GTPase that regulates membrane trafficking between plasma membrane and endosomes. It has been reported that BRAG2 directly binds to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptors (AMPARs), and is involved in the synaptic long-term depression by regulating the endocytosis of AMPARs at hippocampal excitatory synapses. However, the molecular mechanism mediating between AMPAR endocytosis and BRAG2-Arf6 signaling remains to be elucidated. Here, we report that a long C-terminal splice variant, BRAG2c is highly enriched in the postsynaptic density (PSD) fraction compared to a short C-terminal splice variant, BRAG2b, and selectively localized at the excitatory PSD accompanied by colocalization with AMPARs in the adult mouse brain. Using yeast two-hybrid and immunoprecipitation assays, we show that BRAG2c interacts with PDZ domain of PSD-95 and SH3 domain of endophilin III. Furthermore, the blocking of the interaction between BRAG2c and endophilin III disturbed the endocytosis of AMPARs triggered by mGluR-signaling in the hippocampal primary culture neuron. Taken together, these findings unveil a novel molecular mechanism by which the BRAG2-Arf6 signaling regulates the synaptic AMPARs through the interaction with BRAG2 and endophilin III.

(COI: No)

P1-123

Sevoflurane suppresses presynaptic calcium influx leading to inhibition of excitatory neurotransmission at the hippocampal CA1 synapses

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Despite diverse effects of volatile anesthetics in the brain have been studied extensively, little is known about the effect on excitatory neurotransmission. In this study, we examined the effect of sevoflurane (Sev), one of the major volatile anesthetics, on excitatory synaptic transmission in hippocampal CA1 region. Transverse hippocampal slices were made from mice of 9 - 37 days old. Field excitatory postsynaptic potential (EPSP), paired-pulse ratio (PPR), presynaptic fiber volley (FV) were measured with extracellular recordings. In addition, fluorescent measurement of presynaptic calcium influx was used to investigate the mechanisms of presynaptic action of Sev. Sev at 5 % were mixed with 95% O_2 and 5% CO_2 and bubbled in artificial cerebral spinal fluid. Application of Sev reduced the amplitude of field EPSP to 45 % ± 8 % of control (n = 5). This effect was accompanied with concurrent enhancement of PPR to 127 % ± 5 % of control (n = 12), suggesting possible presynaptic site of action of Sev. The amplitude of presynaptic FV was not significantly affected by Sev. In contrast, fluorescent measurements revealed that presynaptic calcium influx was suppressed by Sev to 69 % ± 6 % of control, and simultaneously recorded EPSP to 44 % ± 2 % of control (n = 7). These results suggest that Sev potently suppresses excitatory synaptic transmission without affecting presynaptic action potential, but possibly due to inhibition of presynaptic voltage-gated calcium channels.

(COI: No)

P1-124

Sema7A-PlxnCl signaling is essential for triggering activity-dependent synapse formation in the mouse olfactory bulb

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Odor information detected by olfactory sensory neurons is transmitted to the brain through second-order neurons, the mitral/tufted cells. Here we report that a pair of signaling molecules, Sema7A expressed by olfactory sensory neurons and its receptor PlxnC1 expressed by mitral/tufted cells, are essential for triggering synapse formation in the olfactory bulb. In both knockout mice for either Sema7A or PlxnC1, not only synapse formation but also dendrite maturation is perturbed. The same phenotype is also observed in the knockout of cyclic nucleotide gated channels. Surprisingly, this phenotype of dendrite maturation in the channel knockout is rescued by the forced expression of Sema7A alone with the odorant receptor promoter. We can therefore conclude that Sema7A-PlxnCl signaling plays a key role in triggering the activity-dependent synapse formation and dendrite selection of mitral/tufted cells in the olfactory bulb.

(COI: No)

P1-125

c-Src dependent cell polarity in fibroblasts cultured on adhesive micropatterns

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Focal adhesions (FAs) and associated stress fibers (SFs) are specialized components contributing to cellular events such as cell migration, wound healing, adhesion of cells, etc. FAs recognize the boundary between the plasma membrane and specific extracellular matrix proteins and are involved in cell orientation and polarity. Although fibroblastic cells select specific substrates for typical cell-substrate adhesion, the mechanisms that regulate orientation and polarity are not clear. In this study, using adhesive micropatterns (MPs) in order to regulate polarized cell spreading, together with c-Src inhibitors (Src inhibitor No. 5, Biaffin), we analyzed the behavior of cultured fibroblasts during the organization of cell polarity on adhesive MPs. When normal fibroblasts attached to the MPs (width; 10 or 15 μm), phosphorylated c-Src (pY418), the active form of c-Src, was intensely detected along the inner border between the adhesive MPs and non-adhesive glass surface, reflecting the active c-Src location at the inner border. When cells were treated with c-Src inhibitor, cells were significantly elongated compared to normal cells, and aligned along the longitudinal axis of the MPs in a spindle shape with well developed SFs. However, staining of phosphorylated c-Src was not detected at the border of the MP and non-adhesive glass surface. These observations suggest that the activation of c-Src plays a key role in the recognition of the border between the adhesive MP and non-adhesive glass surface. Moreover, inactivation of c-Src causes polarized elongation of cells.

(COI: No)

P1-126

N-terminus of paxillin regulates actin stress fiber formation by binding to the active Fyn

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Rho-kinase (ROK)-mediated actin stress fiber formation plays important roles in many cellular functions, including cell adhesion and motility. We previously found the involvement of Fyn tyrosine kinase as an upstream molecule of ROK in actin stress fiber formation. However, the molecular mechanisms between Fyn and ROK have not been clarified yet. To search for the downstream molecule of Fyn, we performed pulldown assay with HaloTag constitutively active Fyn (CA-Fyn) and dominant negative Fyn (DN-Fyn) in human vascular smooth muscle cells (VSMCs), and obtained the candidate molecules which selectively bind to CA-Fyn, but not to DN-Fyn. Subsequently, matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) enabled us to identify paxillin as a novel downstream molecule of Fyn. To clarify binding site of CA-Fyn on paxillin, the recombinant Fyn and paxillin were obtained in baculovirus and E.coli expression system respectively. Surface plasmon resonance assay showed that CA-Fyn bound to N-terminus, but not C-terminus of paxillin, while DN-Fyn bound to neither of them. Colocalization of CA-Fyn and N-terminus of paxillin during the stress fiber formation in VSMCs further confirmed their binding. In addition, the overexpression of N-terminus of paxillin inhibited the actin stress fiber formation. Taken together, these results demonstrate that paxillin, as a novel signal mediator, regulates actin stress fiber formation by N-terminus binding to the active Fyn.

(COI: No)

P1-127

The morphorogical role of myosin light-chain kinase to form podosome in smooth muscle cell

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We revealed that Myosin light chain kinase (MLCK) has been essential to form podosome. Podosome is intracellular structure which is actin-filaments as a core. Vascular smooth muscle cell (VSMC) in the culture dose not little, if any, form the podosome, but it includes the protrusion by adding phorbol 12, 13 dibutyrate (PDBu) as chemical mediator driven Protein kinase C. We added PDBu to the cultures of A7r5 cells of line of VSMC as shown by the immunno-fluorescent micrograph. At first, We observed that PDBu had been applied to the medium culturing A7r5 cells by the use of the fluorescent microscope. After incubation for specified periods, the culture plates were subjected to the count how many cells formed podosome. The numbers of VSMC with podosome were increased with the elapse of time. The VSMCs with podosomes were maximal within 30 min, and ～90 % cells developed podosomes. The scanning electron micrographs were shown that the cellular surface A7r5 cell when stimulated by PDBu, the cellular surface protruded podosomes; higher magnification showed that they were composed of the legs connecting the ball-like feets to the cellular surface of A7r5. Transmission electron micrographs of the vehicle and the PDBu-stimulated cells were shown, respectively. Upon the stimulation by PDBu, the tracks of actin- bundles were disrupted, and most of them were capped by the electron dense plaque, a capping that had been not penetrated by the actin-bundle. Taken together we proposed the idea that the dense plaque had been feet, and actin-bundle been legs.

(COI: No)

P1-128

Structural change of myosin head in skeletal muscle fiber without thin filament

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Myosin converts chemical energy of ATP to mechanical work in combination with actin. The molecular mechanism of this chemo-mechanical transduction is still unknown, mainly because mechanical work significantly deforms the molecules. Therefore, it is of interest to follow the intrinsic structural changes of myosin in the absence of actin. The changes would depict a conformational path of minimal potential. We here followed intrinsic structural changes of myosin heads in sarcomere where conformational freedom of myosin would be highly restricted in a range optimized for the physiological path unlike in the purified solution system. Actin was removed from sarcomere of skinned fibers with gelsolin treatment, and helically arranged myosin heads were observed with X-ray diffraction (at BL6A of PF) following the ATP hydrolysis steps of M, M-ATP, M-ADP-Pi, M-ADP, and M, where M represents myosin. Compared with M and M-ATP (trapped with N-phenylmaleimide) states, myosin heads in M-ADP-Pi state were retracted close to the backbone of the thick filament. Retrograde binding of ADP to M to yield M-ADP did not cause this marked transition of myosin heads. Since orthograde conformational change at M-ADP state following Pi release is generally considered to be coupled with mechanical work of myosin, radial retraction and following protrusion of myosin heads would likely be the prime mover as in the case of crawling bristle grass in your gripping hand.

(COI: No)

P1-129

The effect of Nisin on keratinocyte cytoskeleton and intercellular junction

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Bacteriocins are proteinous antibacterial substances produced by bacteria. Nisin is bacteriocin produced by *Lactococcus lactis subsp. lactis*. Nisin is commonly used as a food preservative throughout the world. Bacteriocins such as nisin are thought to act only prokaryote cell and doesn't effect on normal eukaryote cell.

In this study, we investigated the effects of nisin to human epidermal keratinocyte cell line (HaCaT). Keratinocytes exposed to nisin continue to proliferate. Though, exposure of keratinocytes to nisin perturbed cobble stone-like structures. Detachment and fragmentation of the cells were increased by nisin exposure. Keratinocytes exposed to nisin showed nisin formed gaps or holes in the keratinocyte layer. As Nisin seemed to effect on cell-cell junction and cytoskeleton, we investigated the effect by immunofluorescence microscopy. Localization of desmosomal cadherin protein, desmoglein 3 (DSG3) and adherens junction protein, β-catenin was disrupted. Furthermore, intermediate filament protein, cytokeratin 5 and cytokeratin 17 decreased their localization at the cell boundary.

Taken together, our result indicates that nisin effects on not only prokaryote cells but also on normal eukaryote cells. Nisin seems safe from long history of successful usage as a food preservative. However, it doesn't mean nisin doesn't effect on eukaryote cell.

(COI: No)

P1-130

Molecular dissection of intracellular neurofilaments

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Neurofilaments (NFs) are intermediate filaments (IFs) expressed specifically in neurons and considered pertinent to support both of their morphological changes and functional structures. Our understanding of the NF dynamics in living cells, however, has been relatively poor compared to that of other cytoskeletal components.

To elucidate intracellular elementary polymerization processes, we monitored the behavior of NF medium protein (NF-M) heteropolymerizing with other IFs in cultured cells. Murine primary hippocampal neurons were infected with adenoviral vectors carrying green fluorescent protein tagged NF-M genes, and SW13vim(-) cells, which lack endogenous IFs, were coinfected with either of other viruses expressing NF light protein or α-internexin in addition to the tagged NF-M virus. We then performed fluorescence recovery after photobleaching experiments at several days after infection with highly inclined/laminated optical sheet and/or total internal reflection fluorescence microscopy, and observed different localities of NF polymerization, depending on the culturing age, cell species, and IF constituents. Our findings collectively suggest that the NF dynamics changes over time and space, reflecting the differentiation stages of neurons and their states. We will also discuss our newly developed novel microscopic methods which enable us to determine not only the protein positions but also the arrangements of NF polymers.

(COI: No)

P1-131

Mechanisms of CRMP2-induced GTP-state microtubule formation

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The asymmetric microtubule cytoskeleton is essential for axon-dendrite specification in developmental neurons and for polarized protein sorting in mature neurons. In axons of mature neurons, GTP-state microtubules (MTs) are enriched over GDP-state MTs and are preferentially searched for as landmarks by the conventional molecular motor Kinesin-1, which goes into the axon among many processes in neurons. Recently, we solved the cryo-EM structure of GTP-state MTs. They have the characteristic conformation at both the longitudinal and lateral contacts between tubulins, albeit those polymerized in vitro have the unstable lattice especially at the surface. We thus hypothesized that stable GTP-state MTs could be polymerized in vitro with the support by some MT-binding proteins/factors that we sought for. Among several candidates, CRMP2 plays the essential role at the early stages in the axonal development by promoting the axonal specification in cultured mammalian neurons. CRMP2 was also reported to interact with GTP-state tubulins and promote MT assembly in vitro. Considering that one of the functional landmarks in axons is an enrichment of GTP-state MTs, CRMP2 might promote the GTP-sate MTs in single process to give it the axonal signature, albeit the molecular mechanism of how CRMP2 induces the GTP-state MT formation is poorly understood. Here we characterize the role of CRMP2 in promoting the stable GTP-state MTs by using the several structure-based technics, such as X-ray crystallography, small angle X-ray scattering, cryo-electron microscopy, and fluorescence microscopy.

(COI: No)

P1-132

The elongation of primary cilia via the acetylation of α-tubulin in human fibroblast treated with lithium chloride

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Primary cilium is found on almost all types of cells in the human body and typically serves as the mechanical sensor of the cell. Lithium ion is known to promote the elongation of primary cilia in a variety of cell types, but it is unknown whether lithium is involved in the acetylation of α-tubulin which is important for the function of primary cilia. In order to reveal the relationship between the elongation of primary cilia by lithium and the acetylation of α-tubulin, we first observed the formation and structure of primary cilia in KD cells, a cell line deriving fibroblasts in human labium. Subsequently, by immunohistochemical and western blot analysis we elucidated that the length of primary cilia and acetylation of α-tubulin are regulated by lithium chloride (LiCl) in a time- and concentration-dependent manner. We next performed the RT-PCR, RNAi based experiments and biochemical study using an inhibitor of glycogen synthase kinase-3β (GSK-3β). We found that LiCl mobilizes the α-tubulin N-acetyltransferase 1 (αTAT1) in the signaling pathway mediating GSK-3β and adenylate cyclase III. In conclusion, LiCl treatment activates αTAT1 by the inhibition of GSK-3β and promotes the α-tubulin acetylation, and the acetylation of α-tubulin by αTAT1 facilitate the elongation of primary cilia.

(COI: No)

P1-133

Roles of a ciliary gene, Nphp2/Inv in cell cycle control

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Primary cilia are a hair-like membrane structure, presenting in almost every cell type. Disruption to the ciliary structure or its function causes multiorgan diseases known as ciliopathies such as situs inversus and cystic kidney disease. Increased BrdU incorporation has been described in cystic kidneys associated with ciliopathies. We previously reported that increased BrdU incorporation and abnormal mitotic axis formation are observed in the mutant Inv mice, and the S/G2/M population (with Fucci-Geminin positive) is increased in Nphp2/Inv-knockdown (KD) cells. Although cell cycle abnormality is well described in inv mutant, mechanisms to control cell cycle are unknown. To understand cell cycle abnormality observed in inv mutant mice, we mated inv mice with Fucci mice to analysis cyst lining cells in cell cycle. We are determining which stage in cell cycle is disturbed in Nphp2/Inv-knockdown (KD) cells. Inv /nephrocystin-2 is localized at the proximal potion of the renal cilia. However, its localization during cell cycle is controversial. We are analyzing localization of Inv /nephrocystin-2 using Fucci cells. The results will be presented.

(COI: No)

P1-134

DGKζ-interacting NAP1-like proteins regulate cell cycle and apoptosis by controlling p53 acetylation

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Diacylglycerol kinase (DGK) converts diacylglycerol to phosphatidic acid by phosphorylation. Since both of these lipids are regarded as key molecules in lipid signaling, DGK is considered to be an important regulator in lipid-mediated signal transduction. Of DGKs, DGK ζ , characterized by the presence of a nuclear localization signal (NLS), was shown to be localized to the nucleus in neurons and the transfected cells. We previously identified nucleosome assembly protein (NAP) 1-like 1 (NAP1L1) and NAP1-like 4 (NAP1L4) as novel DGK ζ interaction molecules. However, functional roles of NAP1Ls remain unknown. Since DGK ζ is shown to regulate p53 function, we examined how NAP1L1 and NAP1L4 regulate p53-mediated cell cycle and apoptosis. In WST-1 assay, knockdown of NAP1L1 promoted cell proliferation, whereas NAP1L4 knockdown inhibited cell cycle progression. In an apoptosis assay using DNA-damaging agent doxorubicin (Dox), apoptosis was enhanced by knockdown of NAP1L1, whereas NAP1L4 knockdown was resistant to apoptosis. Because acetylation status of p53 is known to regulate cell cycle or apoptosis, we next analyzed the acetylation sites of p53 under conditions of NAP1Ls knockdown. We found that knockdown of NAP1L1 increases the p53 acetylation at K382 whereas NAP1L4 knockdown augments the acetylation at K320. These results indicate that NAP1Ls regulate cell cycle and apoptosis via differential control of p53 acetylation sites.

(COI: No)

P1-135

Effects of UVA LED light irradiation on cultured RAW264.7 cells

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We studied effects of ultraviolet A (UVA) irradiation using light-emitting diode on cell growth of cultured RAW 264.7 cells. Cells were plated on 96 well plates at a density of 10^{5-6} cells/ml. After 24 hr, these cells were irradiated for varied time and maintained for 0-72 hr at 37℃. Cell images were taken by a microscope every 24 hr for 72 hr. Irradiation (365 nm) for more than 2 min suppressed cell growth. Lactate dehydrogenase release into medium was found to increase significantly at more than 3 min of irradiation, and the addition of N-acetyl cysteine as a scavenger suppressed the increase caused by the 5 min-irradiation. Formation of 8-hydroxy-2'-deoxyguanosine and malondialdehyde were also increased by the 5 min-irradiation. Finally, to detect reactive oxygen species induced in the medium irradiated by the light, we measured EPR(electron paramagnetic resonance) 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 the singlet oxygen is converted into hydroxyl radical. These results suggest that ROS induced by UVA irradiation increase membrane permeability, DNA damage and lipid peroxidation, and results in cell growth inhibition.

(COI: No)

P1-136

What happens when cell proliferation is inhibited by Cs?

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Cesium (Cs) is one of the alkali metal elements as well as potassium (K) and sodium. Distribution of Cs in the whole bodies had been investigated. However, it is not clear that how the Cs is transported through which kinds of way at the molecular levels, and that the effect of Cs on the cell metabolisms. We reported that the proliferation of HeLa cells inhibited by Cs but not other alkali metals. The proliferation decrease is dependent on Cs concentration. The cell viability was assessed by two different methods, i.e., LDH assay and flow cytometric assay, and the cell membrane was not damaged by 10 mM Cs treatment. K^+ channel blocker also inhibited cell growth. Reversible cell-cycle arrest occurs by inhibition of ATP-sensitive K^+ channels. Quinidine treatment showed cell-cycle G0/G1 arrest and inhibition of proliferation, and how was Cs treatment. Cell-cycle was evaluated by using HeLa cell with fucci-system established by Miyawaki. Quinidien treatment cell showed G0/G1 arrest but Cs treatment cell did not show the G0/G1 arrest. Cs-treated cell seemed the same as control cell-cycle. The results suggested that effect of Cs on cell proliferation was not inhibited effect but suppression effect.

(COI: No)

P1-137

D-allose inhibits cancer cell growth by an induction of thioredoxin interacting protein (TXNIP) and inhibition of Glut1 expression

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Glucose transporters are members of membrane proteins that facilitate glucose transport. Of those, glucose transporter 1 (Glut1) is responsible for the basal glucose uptake and highly expressed in erythrocytes and endothelial cells. Interestingly, Glut1 is over-expressed in many cancer cells and is play an important roles on the cell growth. Rare sugar D-allose dose-dependently induced TXNIP expression and inhibited the Glut1 expression in HuH-7, MB231 and SH-SY5Y cells. And the glucose uptake in HuH-7 cells was significantly inhibited by D-allose treatment. Both the TXNIP over-expression and D-allose treatment inhibited hypoxia-inducible factor-1 alpha (HIF-1 α) expression, that is the transcription factor of Glut-1 and is over-expressed in many cancer cells, resulting the reduction of Glut1 expression. Thioredoxin is known to increase the promoter activity of HIF-1 α via the nuclear factor-kappa B (NF- κ B), p50-RelA subunits binding. As TXNIP inhibits the thioredoxin activity, over-expression of TXNIP or D-allose treatment decreased the promoter activity of nuclear factor-kappa B and decreased the HIF-1 α expression. Along with the previously reported mechanism of D-allose that inhibits the cancer cell growth by stabilizing the cell cycle inhibitor p27 protein and inducing G1 cell cycle arrest, this study revealed a novel mechanism of the cancer cell growth inhibition by D-allose via a reduction of Glut1 expression.

(COI: No)

P1-138

Anticancer Activity of Isoamericanol from Jatropha curcas Extacts on the Human Breast Cancer Cell, MCF-7

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Various parts of Jatropha curcas trees have long been used as traditional medicine in African and Asian countries for a variety of sicknesses. There are some reports that show anticancer activity in vitro by the applications of J. curcas seed extract. J. curcas seed extract is a source of oil for biodiesel energy, but it involves a great amount of seed waste. The collaborative research with Kagawa University, Japan and Chiang Mai University, Thailand has succeeded in extracting the organic and aqueous layers with both ethyl acetate (EtOAc) and methanol (MeOH) from the waste. Furthermore, crystallization of isoamericanol from the organic layer of the MeOH extracts has been achieved. In our previous study, isoamericanol was shown to have high antioxidative activity, yet the anticancer activity of isoamericanol has never been reported. In this study, the anticancer activity of isoamericanol is tested on the human breast cancer cell, MCF-7. The inhibition of MCF-7 cell growth by isoamericanol is dose-dependent (25, 50, 100 μg/ml). We further examine the effect of isoamericanol on the cell cycle and apoptosis. Microarray analysis is also performed to identify new possible molecular pathways for anticancer therapies with isoamericanol.

(COI: No)

P1-139

Involvement of D-allose-inducible tumor suppressive factor TXNIP (thioredoxin interacting protein) *in vivo* tumor model

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D-allose, the C3-epimer of D-glucose, has an anti-proliferative effect on various cancer cell lines. We have reported that D-allose treatment caused up-regulation of thioredoxin interacting protein (TXNIP), an anti-tumor protein down-regulated in cancer cells. The anti-proliferative effect of D-allose is due to the up-regulation of TXNIP which causes cell cycle arrest at the G1/S checkpoint. Here we analyzed the signaling mechanisms of TXNIP up-regulation caused by D-allose in the hepatocarcinoma cell line HuH-7. The results suggest that both p44/p42 MAPK pathway and p38MAPK pathway participate in the TXNIP up-regulation. We further analyzed downstream molecules responsible for the TXNIP up-regulation. Moreover, we performed*in vivo* administration analysis of D-allose. The oral squamous carcinoma HSC-3 cells were used in a xenograft model with nude mice. The results show that D-allose exerts growth inhibitory effects on cancer tissues, and that TXNIP up-regulation is possibly responsible for this effect. Overall, present works would make a great contribution to the establishment of a new strategy of cancer therapy utilizing D-allose and TXNIP.

(COI: No)

P1-140

Coagulation factor IX regulates cell migration and adhesion in vitro

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Objective: Coagulation factor IX (F9) is thought to circulate in the blood as an inactive zymogen before being activated in the coagulation process. The effect of F9 on cells is poorly understood. This study aimed to evaluate the effects of intact F9 and its cleavage fragments on cell behavior.
Methods: A431 cells (derived from human squamous cell carcinoma), Pro5 cells (derived from mouse embryonic endothelial cells), Cos7 cells, and human umbilical vein endothelial cells were utilized in this study. The effects of F9 and its cleavage fragments on cell behavior were investigated in several types of experiments, including wound-healing assays and modified Boyden chamber assays.
Results: The effect of F9 depended on its processing; full-length F9 suppressed cell migration, increased adhesion to matrix, and enhanced intercellular adhesion. In contrast, activated F9 enhanced cell migration, suppressed adhesion to matrix, and inhibited intercellular adhesion. An activation peptide that is removed during the coagulation process was found to be responsible for the activity of full-length F9, and the activity of activated F9 was localized to an EGF domain of the F9 light chain.
Conclusion: Full-length F9 has a sedative effect on cells, which is counteracted by activated F9 in vitro. Thus, F9 may play roles before, during, and after the coagulation process.

(COI: No)

P1-141

Transient receptor potential cation channel 3 (TRPC3) regulates proliferation and migration via phosphorylation of STAT5 in human melanoma

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Background: It is well known that melanoma has a poor prognosis due to its rapid progression and high metastatic ability. TRPC are activated by changes of temperature or membrane voltage, resulting in activation of intracellular responses. Here, we investigate whether TRPC3 regulates cell proliferation and migration of human melanoma.
Material: C8161 cells, a BRAF wild type human melanoma cell line, were used in this study. In order to examine the role of TRPC3, lentivirus shRNA encoding either TRPC3 or scramble was used.
Result: mRNA and protein of TRPC3 were expressed in multiple human melanoma cell lines. Knockdown of TRPC3 in C8161 cells inhibited proliferation ($p<0.0001$). Pyr3, a pyrazole compound which is known to inhibit selectively TRPC3, suppressed cell proliferation (IC_{50} 12.99 μM). Both knockdown of TRPC3 and Pyr3 decreased path length of migration ($p<0.01$, $p<0.01$ respectively). Pyr3 also inhibited phosphorylation of signal transducer and activators of transcription (STAT) 5, suggesting that TRPC3-induced proliferation and migration were regulated by, at least in part, the JAK/STAT signaling pathway.
Conclusion: Inhibition of TRPC3 suppressed cell proliferation and migration, suggesting that TRPC3 could be a novel target for treating human melanoma.

(COI: No)

P1-142

The PKA- and p38-mediated phosphorylation processes are involved in the stimulatory effect of TNF-α on K^+ channel activity in human proximal tubule cells

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We previously reported that a proinflammatory cytokine, TNF-α, stimulated activity of an inwardly rectifying K^+ channel in cultured human proximal tubule cells. We also found that the stimulatory effect of TNF-α on K^+ channel activity partly contributed to the cytotoxicity of this cytokine. In this study, we investigated the mechanisms of action of TNF-α on K^+ channel activity, using the patch-clamp technique. In cell-attached patches, TNF-α (20 ng/ml) increased K^+ channel activity in a few minutes, which was blocked by an analog of the soluble TNF receptor, etanercept (10 μg/ml). Since the activity of this K^+ channel was stimulated by PKA- or PKG-mediated phosphorylation, we tested inhibitors of these protein kinases. A PKA-specific inhibitor, KT5720 (500 nM), but not a PKG-specific one, KT5823 (1 μM), blocked the effect of TNF-α. Furthermore, a specific inhibitor of p38 MAPK, SB203580 (1 μM), also blocked the TNF-α-induced activation of channel, whereas an ERK inhibitor, U0126, (20 μM) or a Jnk inhibitor, SP600125 (10 μM), failed to block it. A membrane-permeant cAMP analog, 8Br-cAMP (100 μM), stimulated channel activity in the presence of SB203580. These results suggested that the stimulatory effect of TNF-α K^+ channel activity in cultured human proximal tubule cells was receptor specific and dependent at least in part on the PKA- and p38-mediated phosphorylation processes.

(COI: No)

P1-143

Calcineurin B homologous protein 3 (CHP3) regulates phosphorylation of GSK3β

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Calcineurin B homologous protein3 (CHP3) is a EF-hand calcium-binding protein mainly expressed in heart, but its function remains largely unknown. We used adenoviral-based RNA interference system to knock down CHP3 expression in rat neonatal ventricular cardiomyocytes. Knockdown of CHP3 result in significant enlargement of cardiomyocyte size and increase the protein expression level of the pathological hypertrophy marker ANP. Furthermore, the phosphorylation level of GSK3beta was dramatically elevated. On the contrary, CHP3 overexpression results in increment of GSK3beta phosphorylation induced by insulin stimulus. Co-immunoprecipitation experiments demonstrated the interaction of CHP3 with GSK3beta. These results suggest that CHP3 serves as a novel regulatory factor for GSK3beta, which modulates cardiomyocyte hypertrophy.

(COI: No)

P1-144

Oxidized S100A4 inhibits the activation of protein phosphatase 5 through S100A1 in MKN-45 gastric carcinoma cells

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S100 proteins bind to numerous target proteins, as well as other S100 proteins and activate signaling cascades. S100 proteins can be modified by various post-translational modifications, such as phosphorylation, methylation and acetylation. In addition, oxidation is important for modulating their activities. Previous studies have shown that S100A1 interacts with S100A4 in vitro and in vivo. Due to this potential cross-talk among the S100 proteins, the aim of the present study was to examine whether S100A4 modulates the activity of S100A1. S100A4 was readily oxidized and formed disulfide-linked dimers and oligomers. Although non-oxidized S100A4 bound to protein phosphatase 5 (PP5), the Cu-oxidized S100A4 failed to bind PP5. Instead, the Cu-oxidized S100A4 directly interacted with S100A1 and prevented PP5 activation. Hydrogen peroxide induced S100A4 oxidation in MKN-45 gastric adenocarcinoma cells and decreased S100A1-PP5 interaction, resulted in the inhibition of PP5 activation by S100A1. These data indicate that oxidized S100A4 regulates PP5 activity in a unique manner under oxidative stress conditions.

(COI: No)

P1-145

Knockdown of DEAD-box protein 5 (DDX5) represses NF-kB transcriptional activation

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DEAD-box protein 5 (DDX5) is one of the DEAD-Box families that has a helicase activity. Recent studies suggest that DDX5 also serves as a regulator of transcription factors. Diacylglycerol kinase (DGK) converts diacylglycerol to phosphatidic acid in phosphoinositide turnover. We identified DDX5 as a new binding partner of zeta type DGK (DGK ζ). Transcription factor nuclear factor-kappa B (NF-kB) is known to play a crucial role in various processes, such as immune response, inflammation, cell proliferation, and oncogenesis. We previously reported that DGK ζ knockdown facilitates inhibitor of kappa B (IkB) degradation and phosphorylation of NF-kB p65 subunit, thereby upregulating of NF-kB transcriptional activity. In this study, we examined how DDX5 affects NF-kB pathway. To this end, we knocked down DDX5 in HeLa cells and performed immunoblot analysis and luciferase assay. We found that in these cells phosphorylation levels at Ser536, Ser468, and Ser311 of NF-kB p65 subunit were attenuated compared with wild-type cells. On the other hand, IkB level remained unchanged. Luciferase assay revealed that DDX5 knockdown represses NF-kB transcriptional activity. Collectively, these results suggest that DDX5 knockdown has no effect on IkB degradation, but attenuates phosphorylation of the p65 subunit, thereby downregulating NF-kB transcriptional activity.

(COI: No)

P1-146

Role of Steroidogenic acute regulatory protein-related lipid transfer domain containing 10 (STARD10) in hepatic inflammation

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Steroidogenic acute regulatory protein related lipid transfer (START) domain containing 10 (STARD10) is a member of the START domain containing lipid transfer protein family. We have previously shown that STARD10 is highly expressed in the liver and involved in regulating expression of PPAR α -target genes. Since the activation of PPAR α negatively regulates NF-κ B activity that promotes inflammatory gene expression, STARD10 may exert anti-inflammation activity. The aim of this study was to clarify the role of STARD10 in inflammation in the liver. We examined the effect of STARD10 using *Stard10* knockout (*Stard10*$^{-/-}$) mice. *Stard10*$^{-/-}$ mice fed with high fat diet gained weight in a manner similar to WT mice. However, the liver of *Stard10*$^{-/-}$ mice was smaller in size and accumulated significantly less cholesterol and triglyceride than that of WT mice. Sizes of individual lipid droplet of hepatocytes of *Stard10*$^{-/-}$ mice was significantly smaller than those of WT mice. These results are consistent with the down-regulation of PPAR α target gene such as *Mogat*, which is involved in triglyceride synthesis, was down-regulated in *Stard10*$^{-/-}$ mice. Gene expression levels of IL-1 β and TNF- α were increased in *Stard10*$^{-/-}$ mice, suggesting that STARD10 regulates these genes through inhibition of NF- κ B activity. These results indicate that STARD10 is involved in the regulation of lipid storage and inflammatory responses in the liver through PPAR α -dependent mechanism.

(COI: No)

P1-147

Analysis of interdomain interactions in PLCζ by the combined expression of split mutants

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Phospholipase C ζ (PLC ζ) is a strong candidate for mammalian sperm-derived factor that triggers Ca^{2+} oscillations required for the egg activation at fertilization. The PLC ζ protein consists of EF-hand domain in the N-terminus, X and Y catalytic domains, and C-terminal C2 domain. Although the three-dimensional structure obtained by computer modeling predicts the contacts between domains at EF/C2, X/Y, and Y/C2 interfaces, the functional significance of these putative interactions for the catalytic activity has yet to be elucidated. In the present study, we constructed several truncated mutants of human PLC ζ by splitting at the linkers between two flanking domains, and evaluated the Ca^{2+} oscillation-inducing activity by injecting their cRNAs into mouse eggs. While none of the mutants examined induced Ca^{2+} oscillations on its own even at the high level of expression, the pairwise expression of complementary split mutants, such as EF-X and Y-C2, or EF-X-Y and C2, generated the normal pattern of Ca^{2+} oscillations, suggesting the combinatorial contribution of the interdomain interactions among four domains, for PLC ζ to adopt the active conformation. It was also shown that the linker region between X and Y domains is not necessary for the PLC ζ activity, since the pair of EF-X and Y-C2 mutants lacking XY linker could, if less effectively, induced Ca^{2+} oscillations. Results of experiments for other combinations of split mutants, as well as for some circularly permutated mutants, will also be presented to discuss the structural requirements for the catalytic activity of PLC.

(COI: No)

P1-148

FABP7 is involved in epigenetic modification of mouse caveolin-1 gene promoter

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Introduction: Intracellular lipid dynamics are closely associated with the epigenetic status such as DNA methylation and histone modification. Fatty acid-binding protein 7 (FABP7), which binds to PUFAs, is expressed by astrocytes in developing brain. We have so far shown that FABP7 is involved in the lipid raft function in the astrocytes through its gene regulation of caveolin-1, a scaffold protein of lipid raft. In this study, we sought to examine whether the epigenetic modification of caveolin-1 gene promoter was dependent on FABP7 levels.
Methods and Results: Immunostaining analysis showed that FABP7 was localized in both cytosol and nucleus in the mouse astrocytes. In qPCR, caveolin-1 gene (Cav-1) expression was decreased in FABP7-KO astrocytes compared with wild-type (WT) astrocytes. Luciferase reporter assay using FABP7-transfected-NIH-3T3 cells revealed that the activation of an approximately 200bp upstream region from Cav-1 transcriptional start codon was dependent on the FABP7 levels. Furthermore, CHIP assay revealed that the level of H3K27 acetylation in Cav-1 promoter was increased by FABP7-transfection in NIH-3T3 cells. In bisulfite sequencing analysis, FABP7-KO astrocytes contained significantly higher methylated CpG sites in Cav-1 promoter than WT.
Discussion: FABP7 may have the role in the regulation of histone acetylation and DNA methylation possibly through its effect on the cellular lipid metabolism.

(COI: No)

P1-149

The difference of properties of hypotonic swelling among different cell species

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We already reported that the rate of maximum swelling after hypotonic challenge differed among different cell species. For example, HeLa cells showed cell swelling 1.6 times higher than isotonic condition by hypotonic challenge. SH-SY5Y cells showed cell swelling only 1.1 times higher than control condition. We focused on the difference of hypotonic swelling between HeLa cells and SH-SY5Y cells. Both HeLa cells and SH-SY5Y cells are suspended by trypsin treatment and cell volume was measured by flowcytometry. HeLa cells showed rapid increase of cell volume after hypotonic challenge. This hypotonic swelling was suppressed by bumetanide, NKCC blocker. Isotonic cell volume did not changed by bumetanide. SH-SY5Y cells showed little swelling by hypotonic challenge as reported last year. SH-SY5Y cells showed cell shrinkage by applying bumetanide in isotonic condition. By applying hypotonic solution with bumetanide SH-SY5Y cells showed cell swelling which is almost same absolute value as the hypotonic challenge without bumetanide. We found that SH-SY5Y cells showed apparent cell swelling like HeLa cells if we plotted the ratio of cell volume with bumetanide. We also examined the expression of NKCC by RT-PCR in HeLa cells and SH-SY5Y cells. We found that both cell lines showed NKCC expression and the signal of SH-SY5Y was higher than HeLa cells. These data suggest that in SH-SY5Y cells NKCC might transport Na^+, K^+, Cl^- together with water into the cells even in isotonic condition and cell volume reached maximum value with small increase by hypotonic challenge.

(COI: No)

P1-150

The multifunctional anion transporter SLC26 gene family as potential candidate for oxyanion transport

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Toxic anions have attracted great interest in academia and industry during the last decade. However, regarding the toxic anion pathway inside the human body is poorly understood. The SLC26 gene family is a multifunctional anion transporter gene family that possesses a wide variety of anion transporting properties. Which makes it a potential candidate as a toxic anion transporter. This research involves SLC26 gene family localization and in vitro assessment of Na_3VO_4 and Na_2CrO_4 toxicity in human cells. Human cells (HeLa, HEK293, CACO2, and SH-SY5Y) were incubated for 3 days with Na_3VO_4 and Na_2CrO_4 concentration of 300 μM, 30 μM, 3 μM, 100 μM, 10 μM, and 1 μM respectively. Results show that there is a specific pattern for the SLC26 gene family localization and expression levels in human cells. Which leads to a possibility of different toxicity levels of Na_3VO_4 and Na_2CrO_4 against different human cells. These findings could be useful information to predict the toxic anion pathway inside human cells.

(COI: No)

P1-151

Possible Roles of sodium ion / proton exchanger 1 (NHE1) on the conversion of latent form of TGFβ to active form

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We already have the implications for participation of TGF β in glioma invasions toward non-cancerous brain parenchyma, and premetastatic niche formations on lymph node metastases of head and neck squamous cell carcinomas. Since upregulations of mRNA or TGF β precursor protein were observed in either cases, we expect them as at least a part of mechanisms for progression of the malignancy or as a possible target for anti-metastasis therapy. Importantly, TGF β is secreted in inactive form, namely latent from. Therefore, measurement of TGF β activities are indispensable to assess whether the factor really functions or not, and the mechanisms convert TGF β from latent to active has physiological as well as pathophysiological significance. NHE1 acts as an ion transporter excretes proton toward extracellular space owing to the concentration gradient of sodium ion as a driving force. Simultaneously, NHE1 also acts as an anchor of actin-cytoskeleton on plasma membrane via its intracellular domain. We have also found aberrant overexpression of NHE1 in gliomas as well as squamous cell carcinomas, and furthermore, observed decreases of TGF β activities in conditioned culture media prepared from NHE1 knockdown cells or NHE1 inhibitor treated cells in preliminary experiments. We would like to discuss as to how the participations of NHE1 in TGF β conversion is possible.

(COI: No)

P1-152

Evaluation of anti-angiogenic drug for endothelial cells derived from glioma stem cells

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Central nervous system (CNS) is mainly composed of neuron, astrocyte and oligodendrocyte. They are derived from neural stem cells (NSCs), which have the characteristics of self-renewal and multipotency. In glioblastoma (GBM), which is the most malignant brain tumor, the existence of glioma stem cells (GSCs) in GBM is reported in 2004. GSCs have similar features with NSC in terms of self-renewal and multipotency. GSCs also have high tumorigenesis and therapeutic resistance. GBM is one of the vascular rich tumors, and neovascularization is normally performed by recruiting endothelial cells from brain vessels. Vascular endothelial growth factor (VEGF) is a critical regulator of this process. In addition, recent study says that GSC can transdifferentiate into endothelial cells and vascular pericytes to support tumor environment. In our research, 15 percent of tumor vessels include the GSC derived endothelial cells, but the mechanism and features are little known. Here, we report two kinds of tumor angiogenesis from the point of VEGF pathway. And focusing on the effect of anti-angiogenic drugs for these two angiogenesis, we suggest new therapeutic target leads to development of new anti-angiogenic drugs.

(COI: No)

P1-153

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

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Pain is a harmful sensation that usually arises from noxious stimuli. Transient Receptor Potential Ankyrin 1 (TRPA1), a member of TRP subfamilies, is one of those targets for studying the pain mechanism. TRPA1, a sole member of TRPA subfamily is known to be activated by various stimuli such as noxious cold (potentially in rodents), pungent natural products (like cinnamaldehyde; CA) and environmental irritants (like acrolein). Since TRPA1 is an attractive target for pain therapy, many TRPA1 antagonists have been developed and some of them function as analgesic agents. Here, I show that HC-030031 (HC), one of the most potent mammalian TRPA1 antagonists, did not inhibit heterologously expressed western clawed frog TRPA1 (fTRPA1). In a heterologous expression system with *Xenopus* oocytes, HC failed to inhibit fTRPA1 activation elicited by CA (a TRPA1 agonist) but inhibited CA-evoked currents of human TRPA1 (hTRPA1) with a dose-dependent manner. Chimeric studies between fTRPA1 and hTRPA1 as well as point mutant channel analyses revealed that one specific amino acid residue located within the transmembrane domain was partially involved in the inhibitory action of HC. These findings are based on species differences in sensitivity of TRPA1 antagonists and provide novel insights for the structural-function relationship of TRPA1.

(COI: No)

P1-154

Visualization of fluctuating motions of the selectivity filter in the potassium channel: A computational study

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Ion channels are membrane proteins that allow ions to permeate through them and generate electrical signals. Since the determination of the x-ray crystal structure of the KcsA potassium channel, ion permeation through the K^+ channel have been visualized by computer simulation using the molecular dynamics (MD) method. Simulations revealed the motions of ions in the selectivity filter (SF), the most constricted part of the channel, connecting the extracellular solution and the wide cavity being located on the intracellular side. Partially dehydrated ions in SF are coordinated by the carbonyl backbone of SF. The electrical interaction between the ions and the backbone is so strong that it could alter the conformation of the backbone, however, the extent of its change is not known. Here, we performed the MD simulation of the outward current through the Kv1.2 channel and observed the fluctuation of the channel. The fluctuation of the backbone was found to be suppressed due to the interaction with the permeating ions. There are four threonines in SF, whose hydroxyl groups usually fluctuate vigorously in the cavity. When an ion enters into the cavity and comes close to SF, the ion interacts with one of them first, and then coordinates to all four sidechains to make a stable complex. An analysis of the relation between the fluctuations of hydroxyl groups and the current shows that there exists a moderate correlation between them. Thus, the current measured by the single channel recordings reflects the fluctuation of the channel to some extent.

(COI: No)

P1-155

Functional expression of $P2X_7$ receptors in odontoblasts

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Extracellular purine nucleotides activate receptors of the P2 receptor family that are subdivided into two structurally distinct subfamilies: the inotropic P2X and G-protein coupled P2Y receptors. Although immunohistochemical expression of the P2X receptor subtypes-$P2X_2$, $P2X_4$, $P2X_6$, and $P2X_7$-in odontoblasts has been reported, their physiological and detailed pharmacological properties remain unclear. We thus examined the functional expression of $P2X_7$ receptors in mouse odontoblasts. Currents induced by $P2X_7$ receptor activation were recorded by whole-cell patch-clamp recording with a holding potential of -70 mV. Extracellular application of adenosine 5'-triphosphate dipotassium salt (K^+-ATP), a nonselective agonist for P2 receptors, evoked inward currents with an amplitude of -2.9 ± 0.6 nA (n = 3). These currents showed a significant desensitizing effect by repetitive application of K^+-ATP. Extracellular application of 300 μM 2'(3')-O-(4-benzoylbenzoyl)adenosine 5'-triphosphate triethylammonium salt (BzATP), a selective $P2X_7$ agonist, also evoked inward currents with an amplitude of -2.8 ± 0.8 nA (n = 6). KN-62 (10nM), a selective $P2X_7$ antagonist, significantly suppressed the BzATP-induced inward currents to 81.9 ± 9.7 % (n = 6). In addition, application of 300 μM BzATP induced a positive shift in the reversal potential. The estimated permeability of BzATP-induced currents was 11.6, compared with that without BzATP (1.0). These results indicate that odontoblasts express $P2X_7$ receptors.

(COI: No)

P1-156

Modeling effect of age-related changes in ionic systems on action potential of pulmonary vein myocardium

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The pulmonary vein contains a myocardial layer that is capable of generating spontaneous or triggered action potentials, which is considered to play a central role in the generation and maintenance of atrial fibrillation. The pulmonary vein myocardial layer is extending from the left atrium, but has less negative resting membrane potential due to a lower density of the inwardly rectifying K^+ current. Although electrophysiological and pharmacological characteristics of the pulmonary vein myocardium are reported in various literatures, a comprehensive understanding of the spontaneous action potentials generated in the myocardial layer is yet to be assessed. Here, we integrated electrophysiological properties of the pulmonary vein myocardial layer on the basis of the Kyoto model. Based on the preceding research which reported that approximately half of the isolated pulmonary vein myocardial layer exhibited spontaneous action potential and the remaining half were quiescent, we constructed various combinations of the pulmonary vein myocardial models in order to represent the variation of the action potentials. On the basis of the transcriptome data from young and aged myocardial tissues, we expanded the combinations to represent the "aged" pulmonary vein myocardial layer. As a result, we predicted that the spontaneous action potentials, including burst-like action potentials, are more likely to be observed in "aged" combinations than "young" combinations.

(COI: No)

P1-157

Water flow in mantle cavity of bivalve observed by high-field MRI

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Water flow in the mantle cavity of bivalves (*Mytilus galloprovincialis*) was measured by phase-contrast magnetic resonance imaging (PC-MRI), and transient changes in water velocity were imaged by using the inflow effect of T_1-weighted MRI. All experiments were done by 7 T high-field MRI. During steady ventilation, water velocity in the inhalant aperture, lower mantle cavity, interlamellar space and the exhalant aperture were 40-20 mm s^{-1}, 10-20 mm s^{-1}, 5-10 mm s^{-1} and 50 mm s^{-1}, respectively. Spontaneous opening of the shells caused a quick increase of the flow in the mantle cavity within 1 min. A high correlation was detected between the area in the inhalant aperture and that in the exhalant siphon. However, the flow in the interlamellar cavity showed a low correlation with that in the inhalant aperture. The flow in the right and left interlamellar cavities changed independently. These results suggested that the mussel could control flow in a local area of the gill by changing activities lateral cilia in the demibranches. In conclusion, a combination of PC-MRI and the inflow effect of T_{1w}-MRI allowed us to perform quantitative flow analysis in all of the cavities in the mussel.

(COI: No)

P1-158

Analysis of Atg9-containing membrane structures: ultrasturecure and function in autophagy

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Atg9 is a multispanning membrane protein that is conserved from yeast to human. Atg9 is essential for autophagy and considered to be directly involved in the early step of autophagosome formation. We have previously reported that yeast Atg9 is localized on the cytoplasmic small vesicle, an Atg9 vesicle. Atg9 vesicles are derived from the Golgi apparatus and transport vesicle- tethering proteins to the autophagosome formation site. In mammalian cells, Atg9 was reported to be localized to the trans-Golgi network, late endosomes, and recycling endosomes. In order to characterize the mammalian Atg9-containing membranes, we examined the localization of Atg9 in HEK293 cells that stably express Atg9-GFP. Atg9-GFP was partially localized to static membrane structures, which are colocalized with a recycling endosome protein, transferrin receptor. Atg9-GFP was also observed as multiple puncta that move rapidly throughout the cytoplasm. We succeeded in isolation of these cytoplasmic small membranes. The immunoprecipitated membranes did not contain common organelle marker proteins. Electron microscopic analysis revealed that these are small vesicles resembling the yeast Atg9 vesicle. These results suggested that Atg9-specific membrane structures also exist in mammalian cells. Proteomic analysis of these membranes will lead to a better understanding of the function of Atg9 vesicles.

(COI: No)

P1-159

AQP11 null mice enhance autophagy activity in the proximal tubule before polycystic kidney disease

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Water channel AQP11 is expressed in the various tissues. In the kidney, AQP11 is selectively expressed in the proximal tubule. AQP11 null mice are dead from polycystic kidney disease within two months after birth. The intracellular vacuoles and the cysts were reported to be caused by ER stress as documented by the microarray data of the kidneys from P7 mice. The alternation of gene expression that related autophagy was also performed by our microarray in the kidney from P3 and P28. Based on these results, we initiated the study on autophagy in the kidney of AQP11 null mice. We generated GFP - LC3 (microtubule associated protein light chain 3) transgenic mice in the background of AQP11 null to visualize and monitor the activity of autophagy. Specifically, the expression of GFP fluorescence was analyzed in the frozen sections of the kidney. The primary cultured cells from the proximal tubule were also employed to quantify the autophagy activity. The expression of GFP-LC3 was increased in the vacuolated proximal tubule of P7 AQP11 null mice, which become more intense at P14 when the cysts were formed. The autophagy was absence in the other segments of the nephron. The primary cultured cells from the proximal tubules as proved by AQP11 expression revealed that the number of GFP fluorescence was 2 fold more in AQP11 null than that of wild type, although there were no morphological and viability differences. Our results suggested that autophagy may play an important role for the survival of vacuolated cells and later cyst formation in AQP11 null mice.

(COI: No)

P1-160

Analysis of accumulated proteins in abnormal lysosomes of brain tissue in cathepsin D-deficient mice

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Cathepsin D, a major lysosomal aspartic proteinase, is ubiquitously expressed. Cathepsin D knockout (CD-/-) mice die at ca.26 days after birth with massive neurodegeneration, intestinal necrosis, and lymphopenia. In neurons of CD-/- mice, abnormal lysosomes with typical hallmarks for neuronal ceroid lipofuscinosis, autophagosomes and autolysosomes accumulate in neurons. We previously found that in such lysosomes subunit c of mitochondrial ATP synthase accumulates, which is one of substrates of CD. However, there are many other candidates for CD substrates in the brain. In the present study to comprehensively identify proteins accumulating in the lysosomes of CD-/- mice, we modified the subcellular fractionation of lysosomes from mouse brains and investigated the comportments of the lysosomes of CD-/- mice through mass spectrometry analysis.

(COI: No)

P1-161

Membrane dynamics in yeast lipophagy

Tsuji, Takuma; Takatori, Sho; Fujimoto, Toyoshi (*Grad. Sch. Med. Nagoya Univ., Nagoya, Japan*)

Triglycerides and sterol esters stored in lipid droplets (LDs) are used for energy production but they are also utilized to generate membranes and signaling mediators. Due to the physiological importance of these processes, how lipid esters in LDs are mobilized has been a focus of intensive studies. A large portion of lipid esters are hydrolyzed by cytosolic enzymes, but it is becoming clear that an autophagic process called lipophagy is also involved in degrading LD-laden lipid esters. However, details of lipophagy are not characterized yet.

In the present study, in order to understand how lipophagy is executed, we examined the membrane dynamics of the yeast lipophagic process by utilizing quick-freezing and freeze-fracture replica labeling electron microscopy (QF-FRL). By taking advantage of the merit of QF-FRL, which can define distribution of membrane molecules, both proteins and lipids, at the nanoscale, we could observe that distribution of phospholipids changes significantly in relation to LDs, the vacuole (lysosome), and autophagic bodies (i.e., structures in the vacuolar lumen). Implication of the observation with regards to the mechanism of lipophagy will be discussed.

(COI: No)

P1-162

Biogenesis of nuclear lipid droplets

Ohsaki, Yuki; Cheng, Jinglei; Kawai, Takeshi; Fujimoto, Toyoshi (*Nagoya Univ. Grad. Sch. Med., Nagoya, Japan*)

The lipid droplet (LD) is thought to be formed at the endoplasmic reticulum (ER), and is composed of a core of neutral lipids and a phospholipids monolayer. So far LD has been considered a cytoplasmic organelle that plays different physiological roles including as a platform of protein degradation for example. However, LDs are also observed in the nucleus of certain cell types such as hepatoma cells. In the present study, we addressed the mechanism that nuclear LDs (nLD) are generated. nLDs were devoid of perilipin-2 and virtually absent in adipocytes and steroidogenic cells although abundant cytoplasmic LDs (cLD) are present, indicating that nLDs and cLDs form differently. Interestingly, nLDs were associated with polymyelocytic leukemia (PML)-nuclear bodies (NB) and with intranuclear membranes extending from the nuclear envelope. The nLD-PML body complex was labeled for ubiquitin, SUMO1 and p53, suggesting that nLDs function as a site of protein modification and transcription control. The number of nLDs was reduced by knocking down PML isoform-II (PML-II), whereas overexpression of PML-II enhanced the nLD formation, probably by increasing the intranuclear membrane that harbors lipid-ester synthesizing enzymes. These results indicated that nLDs is a unique structure correlated with the PML-NB function.

(COI: No)

P1-163

Subcellular distribution of PI(3, 5)P_2 revealed by quick-freezing and freeze-fracture replica labeling

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Our knowledge about the lipid distribution is limited because of the difficulty in visualizing lipids. To overcome this, we have developed a unique, electron-microscopic method, named quick-freezing and freeze-fracture replica labeling. This method physically fixes membrane lipids and enables to determine their precise localization.
In this study, we applied the method to phosphatidylinositol-3, 5-bisphosphate (PI(3, 5)P_2), which is important for the endolysosomal function and implied in the pathogenesis of motor neuron diseases. We utilized recombinant ATG18p from *S. cerevisiae* as a probe and blocked its binding to PI3P in the presence of an excess amount of the $p40^{phox}$ PX domain, which only binds to PI3P and not to PI(3, 5)P_2.
In *S. cerevisiae*, the PI(3, 5)P_2 labeling was observed in vacuole upon hyperosmotic shock. Interestingly, the labeling was concentrated in a vacuole domain where transmembrane proteins were excluded, whereas PI3P existed in the entire vacuole. The domains were frequently invaginated toward the lumen and coincided with membrane contact sites either between neighboring vacuoles or between the vacuole and the nucleus.
In HeLa cells, PI(3, 5)P_2 was labeled in intracellular vesicles with tubular extensions, which morphology suggests them to be endosomes. This is the first nanoscale demonstration of the PI(3, 5)P_2 distribution. Our approach should help analyze the function of PI(3, 5)P_2 in both physiological and pathological contexts.

(COI: No)

P1-164

UBXD8 deletion in hepatocytes induced more evident abnormalities in female than in male mice

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Background: Using hepatoma cell lines, we found that UBXD8 is engaged in transporting ubiquitinated ApoB from LDs to proteasomes and that knockdown of UBXD8 causes aberrant accumulation of ApoB in the ER lumen facing the LD. To address the function of UBXD8 in liver in vivo, we generated hepatocyte-specific UBXD8 knockout (U8-LKO) mouse and examined the phenotype in detail.
Method: Mice with the floxed exon1 of the *Ubxd8* locus were crossed with mice expressing the albumin promoter-driven Cre recombinase to generate U8-LKO mice. U8-LKO mice and their age-matched littermates (control) were fed either a normal diet or a high-fat diet for 26 weeks starting at 4 weeks old.
Results: (1) After 26 weeks of a normal diet feeding, histological analysis of liver sections did not reveal any difference between U8-LKO and control mice, and any sign of hepatic damage or steatosis was observed. (2) When mice were fed a high-fat diet for 26 weeks, 40% of female and 80% of male of the control mice showed microvesicular steatosis primarily in the perivenular area (zone 3), whereas 60% of female and 27% of male of the U8-LKO mice showed macrovesicular steatosis mainly in the periportal area (zone 1). (3) The serum TG level in U8-LKO mice on a high-fat diet was significantly lower than that in control mice on a high-fat diet (female: 39 vs. 59 mg/dl, male: 44 vs. 65 mg/dl).
Conclusion: Female U8-LKO mice exhibited abnormalities in more indices than male U8-LKO mice. The result showed the importance of examining mice of both sexes, especially when studying genetically engineered mouse models for the first time.

(COI: No)

P1-165

Ring-shaped Golgi apparatus observed in the epithelial cells of rat thyroid follicles

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As we previously reported, the Golgi apparatus of pituitary gonadotropes is spherical in shape, which possibly reflects the highly isotropic arrangement of microtubules from the central microtubule organizing center (MTOC) characteristically seen in a poorly polarized endocrine cell. In contrast, epithelial cells of the thyroid follicle are well polarized and could transport membrane carriers/vesicles bidirectionally toward both the apical and basal cell surfaces. In the present study, we immunocytochemically examined the overall shape of the Golgi apparatus and the intracellular organization of the microtubule network in thyroid epithelial cells as a representative polarized cell. The overall shape of the Golgi in the thyroid epithelial cells was just like as a ring located above the nucleus, of which outer and inner surfaces were cis- and trans-sides, respectively. The MTOC immunolabeled with anti-γ tubulin antibodies, was located just beneath the apical plasma membrane, around which dense network of microtubules was observed. From the apical network of microtubules, bundles of microtubules extended along the lateral cell surface toward the bottom of the cell. Some bundles of microtubules also ran through the inner area of the ring-shaped Golgi and reached the basal cytoplasm along the outer surface of the nuclear envelope. These findings suggest that the bidirectional movements of membrane carriers/vesicles along microtubules arranged parallel to the apico-basal axis possibly determine the characteristic ring-shaped Golgi apparatus in the highly polarized epithelial cells of thyroid follicles.

(COI: No)

P1-166

Effects of Brefeldin A on Localization of Alkaline Phosphatase in McA-RH 7777 Rat Hepatoma Cells

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Alkaline Phosphatase (ALP) in McA-RH 7777 (rat Hepatoma cell) translocates from Golgi area of cytoplasm to the plasma membrane at the cell borders by cell to cell contact between adjacent cells. In the present study, we investigated changes on location of ALP by Brefeldin A in culture of McA-RH 7777 cells to examine whether vesicular transport are involved with such translocation of ALP. McA-RH 7777 cells were seeded in culture slides and cultured. After synchronized, cells were cultured for several minuites α-MEM containing Brefeldin A. Afterwards, they were fixed in Zamboni solution for 10 min at room temperature and reacted with mixed solution containing anti-GM130 and anti-ALP antibodies for double staining. After cells were reacted with mixed solution containing FITC-labeled and rodamine-labeled secondary antibodies, they were examined under a confocal scanning laser microscope. In McA-RH 7777 cells cultured in α-MEM containing Brefeldin A, many granules were showing immunofluorescence for GM130 (cis-side Golgi marker) were scattered throughout the cytoplasm. Immunofluorescence for ALP was also observed on small granules scattering throughout the cytoplasm. ALP was furthermore localized in whole plasma membrane although immunofluorecence was particularly strong at the borders between adjacent cells. The present study suggests when vesicular transport is inhibited, it makes Golgi complex disassembled and scattered throughout the cytoplasm but does not subject translocation of ALP to the plasma membrane although ALP loses directivity for transferring to local plasma membrane of the cell border.

(COI: No)

P1-167

Effects of arginine methylation via PRMT1 on Golgi body

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Cumulative of reports have shown the importance of ER stress in pathology of neurodegenerative diseases, such as Alzheimer's disease, Parkinson disease, etc. These studies indicate that the cellular events in response to ER stress should relate to the pathology of neurodegenerative diseases. To this aim, we investigated the altered genes in SK-N-SH cells under ER stress and found that Protein arginine N-methyltransferase 1, PRMT1, is up-regulated in SK-N-S H cells under ER stress. Based on this result, we addressed the following is sues; 1: Can ER stress increase the protein level of PRMT1? 2: What kind of ER stress pathways is involve in the expression of PRMT1? 3: Can ER stress a ffect the localization of PRMT1? Our results elucidated that several ER stress pathways induced PRMT1 expression, some of which affected the subcellular localization of PRMT1. Next, to examine the function of PRMT1 in the ER stress response, we downregulated the expression of PRMT1 by RNAi. When we analysed the organelle localization and function in the PRMT1 knockdown cells, the localization of Golgi apparatus was altered and the induction of GRP78 by tunicamycin was severely impaired. These results suggested a novel pathway via protein methylation that mediates organelle stress to the nucleus, possibly involved in the pathogenesis of neurodegenerative diseases.

(COI: No)

P1-168

The observation of mitochondria in spermatogenesis by FIB-SEM tomography

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Sperms are motility cells with a flagellum. The structure of sperm is constructed by head, midpiece and tail. We are able to observe specialized mitochondria that are coiled around the flagellum in the midpieace. In *Drosophila*, recently study reported that the morphogenesis of mitochondria drives the elongation of the sperms. But, it is not revealed if other animals have same mechanism. The reason why the study of morphogenesis of mitochondria is not proceeding is the resolution of the optical microscopy. It is difficult for the resolution of the optical microscopy (about 200 nm) to observe the morphology of the mitochondria that size is 300-1000 nm. Recently, FIB-SEM tomography is developed to solve these problems. FIB-SEM, is the instrument combine with the scanning electron microscopy (SEM) and the focus ion beam (FIB), is able to fabricate the sample by gallium ion and observe the surface in the same chamber. In FIB-SEM tomography, we are able to observe the 3D nanoscopic structure of the internal portion by repeats of surface removals with nm order and observations of the cross-section surface. In this study, we investigated the 3D structure of mitochondria in the spermatogenesis by the FIB-SEM tomography. And result, we found mitochondria became adhering tightly to the flagellum after contact with the flagellum. This result suggests that morphogenesis of the mitochondria is carried autonomously out before cell shape change in the spermatogenesis.

(COI: No)

P1-169

Effects of estrogen on mitochondrial elongation through MIEF1 in human breast cancer cell line

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Background: Mitochondria are dynamic organelles whose morphology is controlled by balancing between fission (fragmentation) and fusion (elongation). It is reported that estrogen affects the mitochondrial morphology in breast cancer cell line. Mitochondrial elongation factor 1 (MIEF1) is a mitochondrial outer membrane protein which suppresses dynamin-related protein 1 (Drp1)-mediated fission, leading to mitochondrial elongation. However, the precise role of MIEF1 in mitochondrial elongation regulated by estrogen is still unknown. Therefore, we examined the effect of estrogen on MIEF1 expression in MCF7, human breast cancer cell line.
Materials and methods: MCF7 cells were treated with 17 β-estradiol (E2) for 12 hours. MIEF1 mRNA and protein expressions were examined by RT-PCR and western blot, respectively. Immunohistochemistry was done by using anti-MIEF1 antibody (5.2 μg/ml, Proteintech) and anti-OxPhos V antibody (4 μg/ml, Invitrogen), a mitochondrial inner membrane protein.
Results: MIEF1 mRNA and protein expressions were increased by E2 treatment. Before E2 treatment, immunohistochemical expression of MIEF1 was diffuse, round and small dotted shapes in the cytoplasm. After E2 treatment, MIEF1 expression was changed to clear, and long cluster ones.
Conclusion: These findings suggested that MIEF1 may play an important role in mitochondrial elongation depending upon estrogen treatment in breast cancer cell line.
(COI: No)

P1-170

Cobalt inhibits the movement of motile mitochondria in the axons

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Cobalt is an important element necessary to form vitamin B12 in the human body. However, an overdose of cobalt can cause neurotoxicity and the mitochondria are the main target of cobalt toxicity. In the present study, we investigated the effect of cobalt on the axonal mitochondrial dynamics in primary cultures of rat dorsal root ganglia (DRG). Mitochondria in the axons were visualized by the transfection of lentivirus vectors containing the mitochondrial-targeted DsRed2 sequence. DRG cultures at four weeks were transfected with Mito-DsRed2 and incubated another 2-3 weeks. To observe the mitochondrial dynamics, we used time-lapse imaging. 200 mitochondrial time-lapse images were taken every 6 seconds before and after cobalt chloride treatment. The exposure duration to cobalt chloride was 24 hours. The concentrations of cobalt in replacement mediums were 200 μM, 400 μM, 600 μM or 800 μM. The exposure to cobalt inhibited the movement of motile mitochondria and the effects of cobalt was prominent from 600 μM. In addition to the cobalt effect on the motile mitochondria, mitochondrial fragmentations were observed in the axons. The mechanisms of cobalt neurotoxicity have yet to be identified however, it is possible that a high concentration of cobalt has a harmful influence on mitochondrial transport in the axons.
(COI: No)

P1-171

Rupture of vesicles and nuclear membrane under various stress on cells

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It is known that many kind of stress like ionophore administration cause cell death with vesicle and nuclear membrane rupture. We previously found that ion replacement of extracellular fluid could suppress amphotericin B-induced cell death with lysosome stabilization. In this study, we examined vesicle behavior and enzyme release after various stress including ionophore, povidone-iodine, and temperature stress. With ionophore administration, nuclear membrane rupture occurred after vesicle disappearance, and lysosomal enzyme was dispersed in entire cytosol. This rupture was inhibited with proteinase inhibitors.
(COI: No)

P1-172

Continuous stress induces multiple organelle dysfunction and subsequent cell death in rat melanotroph

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Continuous stress (CS) induces cell death of pituitary melanotrophs (MT) in rat. We used a rat CS model in which rat is kept in a cage with 1.5 cm of water for 5 days, and observed the morphological characteristics during the degeneration process under electron microscopy. The degenerating MT are classified into three types by morphological characteristics; (I) dilation of ER lumen, (II) vesicle accumulation, and (III) mitochondrial clustering. In type (I), MT initially had dilated ER and swelled mitochondria and subsequently the MT showed a degenerative morphology with a brighter cytoplasm and ruptured plasma membrane. In type (II), MT initially had dark cytoplasm and normal ER, and some autophagy related structures including mitophagy were also seen. Along with an increase of the cytoplasmic electron density, these MT were filled with huge number of vesicles such as endosomes and lysosome. In type (III), MT also had dark cytoplasm and normal ER initially, but unlike type (II), contained large mitochondria, which assembled and formed a huge mitochondrial cluster. The electron density of cytoplasm gradually decreased, although the mitochondrial cluster remained. Eventually these MT were degenerated with ruptured plasma membrane. Those observations suggest that CS elicits multiple organelle dysfunctions and causes multiple cell death in MT.
(COI: No)

P1-173

Three dimensional analyses of peroxisomes by SBF-SEM: peroxisomes proliferate by budding

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Purpose: To visualize the newly formed peroxisomes in a proliferate sate in hepatocytes in three-dimensions and at the nanometer resolution, we used serial block face-scanning electron microscopy (SBF-SEM), which images resin-embedded specimen by SEM with a sequential removal of the block surface by diamond knife microtome.
Method: Rats were fed with a diet containing peroxisome proliferator di-ethylhexyl phthalate (DEHP) for 3, 5 and 20 days. Liver tissues were fixed and histochemically stained by alkaline DAB reaction for catalase, and then subjected to SEM after embedded in resin. They were observed by SBF-SEM by back-scattered electron mode.
Result: Peroxisomes were observed as electron-dense particles in each section. Treating with DEHP, their number was gradually increased until 20 days in the hepatocytes. The three dimensionally reconstructed peroxisomes showed an almost spherical in the shape, which diameters were in a range from 0.15 to 1.37 μm. Some peroxisomes had small buds, and were observed to produce new microperoxisomes (ϕ = 0.1 μm) by fission from "mother" peroxisomes (ϕ = 1 μm).
Conclusion: Using SBF-SEM combined with alkaline DAB reaction, the ultrastructures of peroxisomes were observed at nanometer resolution. The induction of peroxisome proliferation by DEHP showed an increase of the number of peroxisomes. Precise observation suggested that small peroxisomes were newly produced by budding/fission from preexisting peroxisomes, not from the endoplasmic reticulum.
(COI: No)

P1-174

Molecular mechanism for endocytosis of TASK1 channels in adrenal medullary cells and PC12 cells in response to extracellular stimuli

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TASK channels belong to a family of K2P channels, which are involved in multiple physiological functions. We have recently elucidated that activation of muscarinic receptors or a decrease in external pH in rat AM cell induces secretion of catecholamines through the inhibition of TASK1-like channels. Additionally, we indicated that TASK1 channels in rat AM and PC12 cells are translocated from the cell membrane to the cytoplasm in response to NGF and muscarine. Here, we explored the molecular mechanism for this internalization of TASK1 channels in rat AM and PC12 cells. We first examined the effects of various inhibitors on receptor endocytosis. Both NGF- and muscarine-induced internalization of TASK1 channels were remarkably suppressed by chlorpromazine, suggesting that TASK1channels were internalized in a clathrin-dependent manner. Next, we investigated this signalling mechanism. Pharmacological and biochemical studies revealed that NGF-induced endocytosis of TASK1 channels was mediated by both PLC and PI3 kinase pathways that converge on PKC with the consequent activation of Src kinase. On the other hand, the muscarine-induced endocytosis of TASK1 channels was mediated by PLC, and subsequently PKC and Src kinase. However, the PI3 kinase pathway was not involved in muscarine-induced endosytosis. These results indicated that both NGF and muscarine induces the internalization of TASK1 channels in a clathrin-dependent manner, but NGF and muscarine induce endocytosis of TASK1 channels through different signaling pathways.
(COI: No)

P1-175

Dissociation of the effect of Rho agonist on endocytosis from that on cell fusion in RAW 264.7 cells

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We have previously proposed that actin superstructure, termed the zipper-like structure promotes the generation of large osteoclast in RANKL-induced osteoclastogenesis using RAW264.7 cells. Actin cytoskeletal dynamics in osteoclasts is regulated by factors including Src, Arp2/3 and small GTPase Rho. On the other hand, phagocytic activity is reported to increase during LPS-induced osteoclast fusion. Both phagocytosis and endocytosis involve actin reorganization that shares common molecules with cytoskeletal dynamics described above. Here we examined whether endocytosis is involved in osteoclast fusion. Endocytosis was estimated by the uptake of rhodamine-dextran (MW 10,000) by RAW 264.7 cells. Mononuclear precursor cells showed higher endocytosis than the fused multinucleated cells. Mononuclear cells took up dextran beneath the plasma membrane in 5 min and transferred it around the nucleus in 15 min. Dextran taken up by multinucleated cells concentrated at the ventral plasma membrane in 16 h. Rho agonist, Rho activator II had little effects on these endocytic events. The same treatment with Rho activator II inhibited the generation of the zipper-like structure and produced smaller osteoclast-like cells. The zipper-like structure was free from endocytic vesicles. The results suggest that actin reorganization involved in osteoclast fusion is distinct from that involved in endocytosis.

(COI: No)

P1-176

Scavenger receptor-mediated gliding on the dendritic membrane of MARCO cell

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Scavenger receptors were initially identified by their ability to bind to low-density lipoprotein. MARCO is a type II transmembrane protein of the class A scavenger receptor family, and is expressed primarily on macrophages and dendritic cells. Their functions were described to be similar to those of scavenger receptor AI. However, the receptor-mediated cellular dynamics still remain unclear. In this study, using the MARCO receptor gene-transfected Chinese hamster ovary cells (MARCO cell) we investigated the role of MARCO receptors in the membrane trafficking. Unlike the ordinary CHO cells, the MARCO cells were able to exhibit a highly active movement of lamellipodia which were adhesive to some type of nanoparticles. In addition, the MARCO cells formed remarkable dendritic structures in their foot trace of migration. Some nanoparticles (such as latex beads, ZnO nanoparticles, nanodiamonds, and quantum-dots) can adhere to the dendrites. Then the dendrite carried the particles by some gliding activity to the cell body. Finally, the particles were endocytosed at the cell body or at the wavefront of lamellipodia. By immunostaining, we showed that the actin filaments and some myosin molecules are present in the dendrites but microtubules are not. Cytochalasin D clearly inhibited the gliding movement of nanoparticles along the dendrites, but colchicine did not. Our results collectively suggest that MARCO receptors capture nanoparticles, and carry them along the dendritic shaft by an actin-driven gliding mechanism.

(COI: No)

P1-177

Epidermal fatty acid binding protein (EFABP/FABP5): a potential regulator of M cell differential transcytosis

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Fatty acid binding proteins (FABPs) belong to the group of conserved multigene lipid binding protein family. Results of our previous studies implied Epidermal FABP (EFABP/FABP5) association of differential M cell transcytosis of intestinal antigens. In our 16S rRNA gene microbiome analysis, *Lactobacillus acidophilus* was revealed in testinal lumen of EFABP null mice and was not in wild type mice. Dendritic cell (DC) specific intercellular adhesion molecule 3 (ICAM3)-grabbing nonintegrin (DC-SIGN) is the major molecule to recognize*L. acidophilus*. Membranous fraction of intestinal homogenate western blot analysis showed both DC-SIGN and DC-SIGN neckless isoform expression in EFABP null mutant, while only DC-SIGN expression was observed in wild type mice tissue sample. Double-overexpression of DC-SIGN and DC-SIGN neckless in Caco2 cells significantly decrease microbeads engulfing. Thus, EFABP associates differential transcription of DC-SIGN isoforms, then differential engulfing of antigens. According to the results of our previous studies, EFABP-Galectin4 complex might work as a M cell transcytosis enhancer. Microbeads engulfing time-lapse observation of EFABP-Galectin4-DC-SIGN triple-overexpression Caco2 also supported EFABP functional association of transcytosis. Further examinations were carried out to clarify precise mechanisms of EFABP induced M cell transcytosis.

(COI: No)

P1-178

RhoC GTPase regulates phagosome formation through mDia1 promoting actin assembly during FcγR-mediated phagocytosis in macrophges

Egami, Youhei; Kawai, Katsuhisa; Araki, Nobukazu (*Sch. Med., Kagawa Univ., Miki, Kagawa, Japan*)

Phagosome formation is a complicated process that requires precisely regulated actin reorganization. Here, we demonstrate that RhoC GTPase is a crucial regulator of Fc γ R-mediated phagocytosis in macrophages. Our live-cell imaging analysis revealed that RhoC is specifically recruited to the phagocytic cups along the surface of IgG-opsonized erythrocytes (IgG-Es). RhoC silencing by RNA interference (RNAi) or the expression of GDP- or GTP-bound mutant of RhoC inhibited the rate of phagocytosis of IgG-Es. During the phagocytosis, actin-driven pseudopod extension to form phagocytic cups was severely impaired in cells expressing GTP-bound mutant RhoC-G14V, which increases cortical F-actin. mDia1, a Rho-dependent actin nucleation factor, and RhoC were colocalized at the phagocytic cup. In addition, coexpression of mDia1 along with GTP-bound RhoC-G14V or expression of constitutively active mDia1 had a drastic inhibitory effect on the uptake of IgG-Es. These data suggest that RhoC regulates phagosome formation by actin cytoskeletal remodeling via mDia1.

(COI: No)

P1-179

Rab10-positive macropinosome-like structures provide a novel endocytic pathway

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Macropinocytosis is the most effective way for cells to ingest large amounts of extracellular fluid. Its processes consist of membrane ruffling, circular ruffle (macropinocytic cup) formation and then separation form the plasma membrane as macropinosomes by cup closure. Generally macropinosomes fuse with lysosomes to degrade its contents. Recently we found that Rab10 is localized in some of macropinosome-like structures including circular ruffles in RAW264 macrophage cells. In this study, we characterized Rab10-positive macropinosome-like structures. It was observed by live cell imaging using a confocal microscope that most of Rab10-positive macropinosome-like structures disappeared in 1-5 minutes after the onset. It was frequently found that tubular structures extended from Rab10-positive macropinosome-like structures towards the perinuclear region. It was previously reported that PI(3, 4, 5)P_3 accumulates in the membrane of the formation of cup, and PI3K inhibitor, LY294002 prevents cup closure. However, formation Rab10-positive macropinosome-like structures and tubulation were not prevented by LY294002. Moreover, PI(3, 4, 5)P_3 probe, Akt-PH was not recruited to Rab10-positive macropinosome-like structures. These results suggested that Rab10-positive macropinosome-like structures may be a novel endocytic pathway that is distinct from canonical macropinocytosis.

(COI: No)

P1-180

Proteome analysis of brush border membrane fraction of small intestinal of ezrin knock-down mouse

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Ezrin is an actin binding protein which cross-links membrane proteins and actin cytoskeleton directly or indirectly through PDZ domain-containing scaffold protein. It is mainly expressed at the brush border membrane (BBM) of gastrointestinal tract, and is involved in the construction of microvilli structure and functional expression of membrane transporters at the cell surface. It was reported that the loss of ezrin disrupted the formation of apical membrane complexes. To precisely study the roles of ezrin on the expression of membrane proteins at the BBM, here we prepared the BBM fractions of small intestines from wild-type and ezrin knockdown ($Vil2^{kd/kd}$) mice, and analyzed them by LC-MS/MS and compared their proteomic patterns. In the jejunum, and ileum of $Vil2^{kd/kd}$ mice, the villus structure was maintained and the proteomic analysis showed that the expression of NHERF1 was down-regulated at the BBM. NHERF3 (PDZK1) was also down-regulated at the BBM of their jejunum. In addition, the expression of PEPT1 (Slc15a1) and SMCT1 (Sodium monocarboxylate transporter 1) (Slc5a8), which contain a PDZ domain-binding motif, was down-regulated at the BBM. Multidrug resistance protein 1 (MDR1) was up-regulated at the BBM of their jejunum and ileum in the $Vil2^{kd/kd}$ mice. On the other hand, CFTR and NHE3 were not detected in the BBM fraction by mass spectrometry although these proteins were assumed to be assembled with ezrin and located at the BBM.

(COI: No)

P1-181

The expression and localization of VAMP5 protein in the kidney

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Vesicle-associated membrane protein 5 (VAMP5) is a member of the SNARE protein family, which is generally thought to regulate the docking and fusion of vesicles with their target membranes. It has been reported that the mRNA of VAMP5 is preferentially expressed in cultured skeletal muscle cells. But the detailed expression and function of VAMP5 protein was unclear. Our study showed the expression of VAMP5 was detected in various organs by western blotting and immunohistochemistry. In this study, we found that VAMP5 was also expressed in kidney. The localization of VAMP5 in kidney was vasa recta, where the columns of capillaries in the medulla. We are now investigating the function of VAMP5 in kidney.

(COI: No)

P1-182

Function of a t-SNARE protein SNAP23 in exocrine and endocrine pancreas

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Fusion between secretory granules and plasma membranes is crucial for the exocytosis of hormones and enzymes in diverse tissues. Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) are essential for the secretory granule fusion. Synaptosomal-associated protein of 23 kDa (SNAP23), a ubiquitously expressed homologue of SNAP25, is known to be a target-SNARE protein, and forms a ternary complex with the other SNARE proteins VAMP and Syntaxin to fuse the membranes. Though the function of SNAP25 in the release of neurotransmitter has been clearly defined *in vivo*, the function of SNAP23 *in vivo* is largely unknown. To determine the function of SNAP23, we generated SNAP23 knockout (KO) mice. SNAP23 homozygous mutant mice (-/- and geo/geo) were embryonic lethal before 8.5 dpc. Thus, SNAP23 is essential for embryonic development. To know the function of SNAP23 in secretory cells, we generated pancreatic exocrine- or endocrine-specific knockout mice by Cre-loxP system. The exocrine-specific KO mice showed decreased fusion of zymogen granules, but the endocrine-specific KO mice showed increased fusion of insulin granules. These results suggested that SNAP23 has opposite roles in the secretion mechanisms of exocrine and endocrine pancreas.

(COI: No)

P1-183

ADAMTS9 /GON-1 regulates insulin secretion and insulin signaling

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ADAMTS9 is a metalloprotease that cleaves components of the extracellular matrix and is also implicated in transport from the ER to the Golgi. It has been reported that an ADAMTS9 gene variant is associated with type 2 diabetes. However, the molecular mechanisms of ADAMTS9 on the beta cell and peripheral tissues are unknown. First, we investigated how GON-1, the *C. elegans* homolog of ADAMTS9, is involved in the type 2 diabetes by using *C.elegans*. INS-7 and DAF-28 encode insulin-like proteins that are secreted from neurons in the wild type background. INS-7 and DAF-28 were accumulated in neurons in *gon-1(tm3146)* mutant background. To investigate the role of GON-1 in peripheral tissues, we examined the subcellular localization of DAF-16, the *C. elegans* homolog of FOXO. DAF-16/FOXO was present in both the nucleus and the cytoplasm in wild-type animals. DAF-16/FOXO was exclusively localized to the nucleus in peripheral tissues in *gon-1* mutant background. Next, we investigated how ADAMTS9 is involved in the type 2 diabetes by using mammalian cell lines. Glucose-stimulated insulin secretion was gradually inhibited by depletion of ADAMTS9 in the INS-1 cells, a glucose-sensitive pancreatic beta-cell line. Depletion of ADAMTS9 decreased insulin-stimulated glucose uptake in differentiated 3T3-L1-derived adipocytes and differentiated C2C12-derived skeletal muscle cells. Translocation of GLUT4 to the plasma membrane was impaired by depletion of ADAMTS9 in differentiated 3T3-L1. Our data suggest that ADAMTS9/GON-1 is involved in insulin secretion from insulin secretory cells and insulin signaling at the peripheral tissues.

(COI: No)

P1-184

Regulation of CFTR Cl channels by adenosine 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 transepithelial ion transport through cystic fibrosis transmembrane conductance regulator (CFTR) Cl channels in pancreatic duct cell monolayer. However, mechanism of the regulation has not been extensively investigated.
Objectives: The present study aimed to clarify the regulation of Cl channels by adenosine in pancreatic duct cells.
Methods: We measured whole-cell current in human pancreatic duct cells (Capan-1) using gramicidin-perforated patch techniques.
Results: The application of adenosine induced a sustained inward current at 83 mV with K_d value of 10 μM. BAY 60-6583, an adenosine A_{2B} receptor agonist, increased the inward current, which was inhibited by a CFTR Cl channel inhibitor (CFTRinh-172). The current response to BAY 60-6583 was observed in 68% of the cells tested. When chloride was substituted with equimolar glutamate in the bathing solution, the reversal potential of the current-voltage curve significantly shifted from 31 to 5 mV, indicating that membrane conductance was chloride selective. The intracellular chloride activity, calculated with the Nernst equation using extracellular chloride activity and the reversal potential, was 51 mM. The inward current induced by BAY 60-6583 was also observed in a bathing solution in which sodium was replaced with *N*-methyl-D-glucamine.
Conclusion: These results indicated that the adenosine A_{2B} receptor mediated the increase in anion transport through CFTR Cl channels in Capan-1 cells.

(COI: No)

P1-185

Bicarbonate transport in interlobular pancreatic ducts isolated from cystic fibrosis mice

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Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in CFTR gene. Pancreatic dysfunction in CF is characterized by low volume and less alkaline pancreatic juice. In this study, we analyzed HCO_3^- transport in interlobular pancreatic ducts isolated from deltaF mouse, a cystic fibrosis mouse model in which F508del mutation was introduced in the mouse Cftr. Interlobular pancreatic duct segments (diameter: ~100 μm) were isolated by microdissection. The bath and lumen were perfused separately with HCO_3^--buffered solutions. Intracellular pH (pH_i) was measured at 37°C in duct cells loaded with BCECF. In isolated pancreatic ducts from DF/DF mice, basal level of pHi was significantly ($p<0.05$) higher (by ~0.06 pH unit) compared with ducts from wild-type (wt/wt) mice. Stimulation with forskolin (1 μM) caused significant ($p<0.05$) elevation of pHi by 0.049 ± 0.008 unit (mean ± SE, n=6) in DF/DF ducts but not in wt/wt ducts. Under stimulation with forskolin, $NH4^+$ pulse (20 mM, 2 min) followed by Na^+ removal from bath and lumen caused intracellular acid-loading to pH 6.8~6.9. Under high-K^+ (70 mM) in the bath, restoration of Na^+ to the luminal solution caused significantly ($p<0.05$) faster increase of pH_i in DF/DF ducts compared to wt/wt ducts. In summary, higher level of pHi in DF/DF ducts is consistent with impaired HCO_3^- secretion across the apical membrane. The present data also suggest that the activity of Na^+-dependent HCO_3^- absorption across the apical membrane is enhanced in DF/DF ducts.

(COI: No)

P1-186

Antioxidant signaling involving the microtubule motor KIF12 is an intracellular target of nutrition excess in beta cells

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Beta cell injury due to oxidative stress is a typical etiology of diabetes caused by nutritional excess, but its precise mechanism remains largely elusive. Here, we demonstrate that the microtubule motor KIF12 mediates an antioxidant cascade in beta cells as an intracellular target of excess fat intake or "lipotoxicity." KIF12 knockout mice suffer from hypoinsulinemic glucose intolerance due to increased beta cell oxidative stress. Using this model, we identified an antioxidant signaling cascade involving KIF12 as a scaffold for the transcription factor Sp1. The stabilization of nascent Sp1 appeared to be essential for proper peroxisomal function by enhancing Hsc70 expression, and the pharmacological induction of Hsc70 expression with teprenone counteracted the oxidative stress. Because KIF12 is transcriptionally downregulated by chronic exposure to fatty acids, this antioxidant cascade involving KIF12 and Hsc70 is proposed to be a critical target of nutritional excess in beta cells in diabetes.

(COI: Properly Declared)

P1-187

Modeling Analysis of Glucagon-like peptide-1 (GLP-1)-induced Inositol 1, 4, 5-trisphosphate Receptor (IP3R)-Mediated Ca^{2+} liberation in Pancreatic β -Cells

Takeda, Yukari; Noma, Akinori (*Department of Bioinformatics, Ritsumeikan University, Kusatsu, Japan*)

Upon elevation of plasma glucose concentration, pancreatic β-cells generate bursts of action potentials and produce cyclic changes in intracellular calcium concentration ($[Ca^{2+}]$) regulating pulsatile insulin release. GLP-1 increases cAMP levels and synergistically enhances glucose-dependent insulin secretion. Further rise of $[Ca^{2+}]$, in forms of Ca^{2+} transients and oscillations, achieved by mobilization of intracellular stores through IP3R was suggested to be part of the fundamental mechanisms by which cAMP effectors amplify insulin release in murine β-cells. The molecular mechanisms as well as the intracellular conditions to evoke GLP-1-induced Ca^{2+} liberations, however, have not still been well elucidated. Here we developed a mathematical model of IP3R and reconstructed GLP-1-induced Ca^{2+} transients and oscillations in a simplified cellular model. Simulation studies and mathematical analyses were then applied to investigate the mechanisms of the IP3R-mediated Ca^{2+} mobilizations in pancreatic β-cells. Results indicated that Ca^{2+} transients and oscillations were produced by positive feedback involving Ca^{2+}-dependent activation of the channel. A slower rate of Ca^{2+}-dependent inactivation was revealed to provide a remarkable contribution to determine the time course of the decay in Ca^{2+} transients. Interestingly, Ca^{2+}-dependent inactivation of the channel was the key to driving and pacing Ca^{2+} oscillations whereas fast rate of Ca^{2+}-dependent activation amplifies the signal.

(COI: No)

P1-188

Analysis of changes in the Ca^{2+} concentration in the endoplasmic reticulum during Ca^{2+} oscillations in mammalian eggs

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Repetitive increases in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$), or Ca^{2+} oscillations, are induced in mammalian eggs by the fusing 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. To understand the mechanism of Ca^{2+} oscillations, therefore, the information about the relation between the Ca^{2+} concentration in the ER ($[Ca^{2+}]_{ER}$) and $[Ca^{2+}]_{cyt}$ is essential. In the present study, we measured the changes in $[Ca^{2+}]_{ER}$ during Ca^{2+} oscillations in mouse eggs induced by the sperm-borne egg-activating protein, phospholipase C ζ, using a genetically coded Ca^{2+} probe, D1ER. By simultaneous monitoring of $[Ca^{2+}]_{ER}$ and $[Ca^{2+}]_{cyt}$, it was revealed that the typical time course of the change in $[Ca^{2+}]_{ER}$ at each Ca^{2+} transient consists of four consecutive phases: a fast decrease, a flat bottom, a fast increase, and a much slower increase, each of which corresponds to a fast increase, a slow decrease, and a fast decrease, and a much slower increase in $[Ca^{2+}]_{cyt}$, respectively. The rates of the fast increase and decrease in $[Ca^{2+}]_{ER}$ were both affected by the ER Ca^{2+} pump inhibitor, thapsigargin. The rate of the slow increase was not inhibited by thapsigargin, but was dependent on the extracellular Ca^{2+} concentration. Results of the experiments to elucidate the effect of Ca^{2+}-buffering proteins in the ER on the Ca^{2+} dynamics will also be discussed.

(COI: No)

P1-189

Down-regulation of Ca^{2+}-activated Cl^- channel TMEM16A by the inhibition of histone deacetylase in human breast cancer cell line YMB-1

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The Ca^{2+}-activated Cl^- channel TMEM16A plays an important role in facilitating cell growth and metastasis of TMEM16A-expressing cancer cells. Histone deacetylase HDAC inhibitors (HDACis) are useful agents for cancer therapy, but, it remains unclear whether ion channels are epigenetically regulated by them. Utilizing real-time PCR, Western blot and whole-cell patch clamp assays, we found a significant decrease in TMEM16A expression and its functional activity induced by vorinostat, a pan-HDACi in TMEM16A-expressing human breast cancer cell line YMB-1. Pharmacological blockade of HDAC3 by 1 μM T247, a HDAC3-selective HDACi elicited a large decrease in TMEM16A expression and functional activity in YMB-1, and pharmacological blockade of HDAC2 by AATB (300 nM) elicited partial inhibition of TMEM16A expression (about 40 %). In addition, siRNA-induced inhibition of HDAC3 elicited a large decrease in TMEM16A transcript in YMB-1. Taken together, in malignancies with a frequent gene amplification of TMEM16A, HDAC3 inhibition is suggested to exert suppressive effects on cancer cell viability via a downregulation of TMEM16A.

(COI: No)

P1-190

A ventricular cell model refined on Ca^{2+}-induced Ca^{2+}-release

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The graded Ca2+ release via ryanodine receptors (RyRs) is dependent on the extent of activation of the L-type Ca2+ channel current (ICaL) within the dyadic junction. However, this local control of Ca2+ release is still not achieved in most of cardiac cell models developed on the desk-top computer level. Mostly, the Ca2+-induced Ca2+-release occurred in an all-or-none manner because a single common pool was assumed for both ICaL and RyR Ca2+ fluxes. We adopted the model of Ca2+ induce Ca2+ release (CICR) based on local control theory (Hinch et al. 2004) in the guinea pig ventricular cell model. This CICR model was improved by incorporating the experimental large Ca2+ gradient recorded near the Ca2+ releasing site, and by removing the RyR inactivation. The new cell model demonstrated that CICR was terminated through the local Ca2+ depletion in the sarcoplasmic reticulum. Furthermore, the graded Ca2+ release during voltage clamp pulse was observed in the presence of local Ca2+ accumulation in junction space. The detailed mechanism of induction decay of CICR and comparison of the new ventricular cell model with previous animal ventricular cell models will be presented.

(COI: No)

P1-191

An activator of TRPM7, naltriben, accelerates Mg^{2+} influx in rat ventricular myocytes

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To estimate the Mg^{2+} influx rate, we measured cytoplasmic free Mg^{2+} concentration ($[Mg^{2+}]_i$) in rat ventricular myocytes with a fluorescent indicator furaptra (mag-fura-2). $[Mg^{2+}]_i$ was first lowered by depleting the cells of Mg^{2+}, and was subsequently recovered to the basal level in Ca^{2+}-free Tyrode·s solution containing 1 mM Mg^{2+}. The time course of the $[Mg^{2+}]_i$ recovery was generally well described by a single exponential function, and was analyzed as the Mg^{2+} influx rate. The rate of Mg^{2+} influx was, on average, 0.27 ± 0.04 μM/s with the initial $[Mg^{2+}]_i$ at 0.35 ± 0.02 mM (n=10). We studied the effect of naltriben, a δ opioid receptor antagonist, recently identified as an activator of the TRPM7 channel [1]. Application of naltriben (50 μM) significantly increased the rate of Mg^{2+} influx, 0.57 ± 0.12 μM/s, with similar initial $[Mg^{2+}]_i$ at 0.37 ± 0.02 mM (n=7). In the presence of 50 μM naltriben, the $[Mg^{2+}]_i$ recovery often had a transient overshoot; $[Mg^{2+}]_i$ reached the level higher than the basal level before it slowly decreased to the basal level. In combination with our previous results that inhibitors of the TRPM7 channel slowed the rate of Mg^{2+} influx [2], the present results suggest a major role of the TRPM7 channel as a physiological Mg^{2+} influx pathway in cardiac myocytes.

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(COI: No)

P1-192

Role of an intestinal ion transport in salt-sensitive hypertension

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The specific mechanisms by which high-salt diet lead to the elevation of blood pressure have been elucidating. High oral salt intake are firstly sensed by the brain, to activate renin-angiotensin-aldosterone system and enhance the production of endogenous ouabain. The secretion of cardiotonic steroid (CTS) from adrenals is chronically enhanced, and then effects on target peripheral organs by both inhibiting the pump activity of Na^+/K^+-ATPase (NKA) and activating the intracellular signaling pathway via NKA. In the proximal renal tubules (PRT), it enhances the trafficking of basolateral NKA and luminal Na^+/H^+ exchanger (NHE) 3 to stimulate natriuretic response, which is suppressed in Dahl salt-sensitive (DSS) hypertensive rats. There is many similarities in the ion transport mechanism between the intestine and the PRT including the trafficking of NHE3. As the first step to elucidate whether the intestine is one of the targets of CTS, in the present study, we compared the effect of high-salt diet on intestinal NKA activity between DSS hypertensive rats and salt-insensitive Sprague-Dawley (SD) rats using Mucosa-submucosal preparations mounted on the Ussing chamber. Short-circuit current and tissue conductance were measured as indices of transepithelial ion transport and permeability. High-salt diet increased ion transport stimulated by ouabain less in DSS hypertensive rats than in SD rats. The more selective study to measure basolateral NKA activity will be performed by treating ionophore to permeabilize in the absence of mucosal Na^+.

(COI: No)

P1-193

Volume-sensitive anion channels in melanoma cells before and after tumor formation

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Melanoma is one of the most aggressive malignancies commonly associated with poor prognosis for patients; it is characterized with a high level of drug resistance and successful escape from apoptosis. Volume-sensitive outwardly rectifying anion channel (VSOR) is known to play a key role in cell proliferation and in apoptotic cell death. However, it is poorly characterized in melanomas. We studied the phenotype of VSOR in two melanoma cell lines: parental B16 and KML (patent UZ IAP 02729), which was obtained by continuous culture of excised primary lung tumors from mice intravenously injected with B16. When inflated by using slightly hypertonic pipette solutions, both cell types responded with swelling accompanied with robust activation of anionic currents exhibiting the VSOR phenotype. The macroscopic current density, degree of outward rectification and sensitivity to DCPIB were indistinguishable in both cell types. However, VSOR in KML cells became activated about twice as faster, had higher selectivity to glutamate over chloride and a significantly smaller single-channel amplitude. We hypothesize that modulation of the biophysical properties of VSOR of melanoma cells by their history of tumor formation in vivo might be due to differential expression of auxiliary components of the whole channel complex.

(COI: No)

P1-194

Volume-sensitive anion channel regulates butyrate-induced apoptosis

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Butyrate is present in colonic epithelium at millimolar concentrations and involved in keeping colonic homeostasis. Although it has been demonstrated that excess of butyrate triggers cell death in colonic epithelial cells, the mechanism is poorly understood. Recently, volume-sensitive anion channel is reported to be involved in a variety of cell death. In the present study, therefore, we investigated whether the volume-sensitive anion channel contributes to the butyrate-induced cell death in mouse colonic epithelial MCE301 cells. Whole-cell patch-clamp recordings demonstrated that volume-sensitive currents after cell swelling exhibit outward rectification, time-dependent inactivation on more depolarized potentials, and anion selectivity ($I^->Br^->Cl^->F^-$). The volume-sensitive anion currents were sensitive to Cl^- channel blockers, DCPIB (2.5 μM) and NPPB (10 μM). Flow cytometry using annexin V-FITC and propidium iodide indicated MCE301 cells treated with butyrate (8 mM) for two days were in late apoptosis. Interestingly, butyrate-induced late apoptosis was inhibited by Cl^- channel blockers. In the cells, apoptotic volume decrease and caspase 3/7 activation were observed 16 h after the butyrate application, and these effects were also suppressed by Cl^- channel blockers. Our results suggest that the volume-sensitive anion channel is essential in the butyrate-induced apoptosis in mouse colonic epithelial MCE301 cells.

(COI: No)

P1-195

Hypotonicity-activated cation currents in the principal cells of isolated rat Kidney cortical collecting Ducts

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We have previously demonstrated that the hypotonicity-induced Ca^{2+} entry was inhibited by a voltage-gated Ca^{2+} channel inhibitor, Nicardipine in the principal cells of rat cortical collecting ducts (CCDs). However, electrophysiological properties and molecular identity of the hypotonicity-induced Ca^{2+} entry pathway are still unknown. In this study, we performed whole-cell voltage clamp recording to confirm whether a cation conductance is activated in response to the hypotonicity in the principal cells of rat CCDs. To minimize K^+ and Cl^- currents, whole-cell recordings were carried out using NMDG-methansulfonate pipette and Na-gluconate bath solution. When exposed to hypotonic solution, whole-cell current amplitude at -80 mV was gradually increased. The activation of whole-cell currents was also observed using the Na^+ free bath solution containing 5 mM Ca^{2+}. The hypotonicity-activated wholec-cell current was inhibited by the application of Nicardipine but not influenced by either Gd^{3+} or amiloride. Although RT-PCR analysis showed the presence of transcripts of T-type Ca^{2+} channel $\alpha 1_G$ subunit, but not L-type Ca^{2+} channel $\alpha 1_C$ subunit in rat CCD, a T-type Ca^{2+} channel blocker, Ni^{2+} did not change the hypotonicity-induced current activation. These data suggest that a Nicardipine sensitive cation conductance, which is activated by hyotonicity is present in the principal cells of rat CCDs. We will further characterize the current to elucidate the molecular identity of this current.

(COI: No)

P1-196

Effects of extracellular phosphates on voltage-gated H+ channels in RAW-derived osteoclast-like cells

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Osteoclasts are highly differentiated bone-resorbing cells and play a significant role in bone remodeling. In the resorption pit, formed between the plasma membrane of osteoclasts (the ruffled membrane) and the bone surface, the concentrations of Ca^{2+} and inorganic phosphates (Pi) are increased, according to degradation of hydroxyapatite by a large amount of protons secreted from osteoclasts. The rise in the extracellular Ca^{2+} level inhibits osteoclastic bone resorption, but the effects of extracellular Pi on osteoclast functions, particularly on H^+ fluxes in the membrane, are largely unknown. We investigated the effects of extracellular Pi on the voltage-gated H^+ channels in osteoclast-like cell generated from a macrophage cell line (RAW264) using the whole-cell recordings. In the presence of extracellular Na^+, Pi (1-20 mM) increased the H^+ currents reversibly under the condition where the Na^+/H^+ exchanger was inhibit by its blocker, amiloride. The enhancement was observed even in the absence of intracellular ATP. The reversal potential of the H^+ channels shifted slightly to more positive voltages by Pi, suggesting that the potentiation was not due to increases in the driving force for protons. In the absence of Na^+, 20 mM Pi increased the H^+ currents at pH 5.5-6.5. These data suggest that extracellular Pi might modify the H^+ channel properties in both presence and absence of extracellular Na^+, leading to increases in the channel activities in osteoclasts.

(COI: No)

P1-197

An acid-inducible proton influx mechanism in the plasma membrane of osteoclasts

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Osteoclasts dissolve bone tissue by secreting acids and proteolytic enzymes from the plasma membrane facing bone tissue (the ruffled membrane) into the resorption pit. Consequently, the plasma membrane faces to highly acidic extracellular environments (~pH 4), and the pH gradient across the plasma membrane could generate a large driving force for protons entering into the cells. However, the proton influx mechanism in osteoclasts exposed to strong acids is largely unknown. In murine osteoclasts derived from RAW264, we identified inward currents activated by decreasing the extracellular pH lower than 5.5. The currents were characterized by a high proton-selectivity, a slight inward rectification and insensitivities to amiloride and ruthenium red, blockers for acid-sensitive cation channels, ASIC and TRP channels, and, to DIDS, a blocker for Cl^- channels. The acid-inducible proton influx decreased the intracellular pH near the plasma membrane, which was monitored by the reversal potentials of voltage-gated proton channels coexisted in the same membrane, even when V-ATPases and Na^+-H^+ exchangers were functional. These results suggested that osteoclasts may possess a proton-selective pathway which could mediate proton influx upon severe extracellular acidification. The acid-inducible proton influx may regulate the pH of the resorption bit by balancing the rates of proton pumping out by V-ATPases or may modify osteoclast functions through intracellular acidification.

(COI: No)

P1-198

Regulation of vessel formation by synthetic peptide derived from activator of G-protein signaling 8

Mamun, Abdullah; Hayashi, Hisaki; Suzuki, Hiroko; Sakima, Miho; Sato, Maki; Nishimura, Naoki; Inukai, Yoko; Iwase, Satoshi; Sato, Motohiko (*Dept Physiol, Aichi Med Univ, Nagakute, Japan*)

Heterotrimeric G-proteins are essential signal transducers involved in many human diseases. Previously, we identified an ischemia-inducible G-protein activator, activator of G-protein signaling 8 (AGS8) from angina model of the rat heart, which bound heterotrimeric G-protein $\beta\gamma$ subunit ($G\beta\gamma$). AGS8 is involved in hypoxia-induced apoptosis of cardiomyocytes and vessel formation of endothelial cells. Previously, we have developed a synthetic peptide (AGS8 peptide) for AGS8-specific signal intervention, based amino acid sequences of biding domain of AGS8 to $\beta\gamma$. AGS8 peptide successfully protected cultured cardiomyocytes from hypoxia-induced apoptosis. Here, we examined the effects of AGS8 peptide and Gallein, a small compound designed as universal $G\beta\gamma$ signal inhibitor, on tube formation of HUVEC. AGS8 peptide inhibited vascular endothelial growth factor (VEGF) induced tube formation of HUVEC in vitro (20 μg/ml: 46.4% ± 4.2, $p<0.01$ vs negative control, mean ± SEM). AGS8 peptide also inhibited VEGF-induced phosphorylation of VEGF receptor type 2 (Tyr 996; 26.7 ± 3.2 %, $p<0.05$, Tyr 1175; 19.8 ± 4.5 % vs negative control, $p<0.05$). In contrast with AGS8 peptide, Gallein did not blocked VEGF-induced tube formation and phosphorylation of VEGF receptor. These data indicate an advantage of specific intervention of AGS8-mediated signal by synthetic peptide and a potential of AGS8 as a therapeutic target for cardiovascular diseases.

(COI: No)

P1-199

Activator of G-protein signaling 8 is required for angiogenesis in vascular endothelial cells

Hayashi, Hisaki; Mamun, Abudullah; Suzuki, Hiroko; Sakima, Miho; Sato, Maki; Nishimura, Naoki; Inukai, Yoko; Iwase, Satoshi; Sato, Motohiko (*Dept Physiol, Aichi Med Univ, Nagakute, Japan*)

We have previously identified receptor-independent G-protein regulator, activator of G-protein signaling (AGS) 8, from a rat heart model of repetitive transient ischemia. AGS8 expression was up-regulated in rat ischemic heart, and directly interacted with G-protein $\beta\gamma$ subunit ($G\beta\gamma$). AGS8 played a pivotal role in the hypoxia-induced apoptosis of cardiomyocytes by regulating $G\beta\gamma$ signaling. Since collateral arteries were significantly developed in the experimental model, we hypothesized that AGS8 was involved in vascular formation. Here, we analyzed roles of AGS8 in vascular endothelial cells (ECs). AGS8 knockdown by siRNA inhibited VEGF-stimulated tube formation of HUVEC on matrigel (27.0 ± 4.8 % of control, $p<0.01$, mean ± SEM). MTT assay revealed that AGS8 knockdown also inhibited VEGF-induced cell proliferation (30.5 ± 3.7 % of control, $p<0.01$, mean ± SEM). AGS8 knockdown significantly suppressed VEGF-induced phosphorylation of VEGF receptor type 2 (VEGFR2), ERK1/2 and p38/MAPK. Interestingly, FACS analysis demonstrated that AGS8 knockdown reduced VEGFR2 localization on the cell surface. Our data first indicate that G-protein regulator is involved in VEGF-mediated angiogenesis by influencing distribution of VEGFR2 and activation of VEGFR2 signal. Therefore, AGS8 is a potential therapeutic target of pathological angiogenesis in ECs, as well as of hypoxia-induced apoptosis in cardiomyocytes.

(COI: No)

P1-200

Analysis of temporal-information coding mechanism of MAPK signaling

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Signaling by the conserved MAP kinase family is a major mechanism through which eukaryote cells respond properly to various extracellular stimuli and induce adaptive responses, such as gene expression, control of cell cycle, growth, and differentiation. Mechanisms and regulation of MAPK signaling have been elucidated in detail, and it is becoming possible to predict kinase activation in sillico, however, how cells induce appropriate cellular function according to the context of their surrounding environment (or stimulation type) remains unclear. Moreover, because living cells under physiological conditions are normally exposed to fluctuating environment, how cells interpret and process those temporally changing information and activate pertinent adaptive response also remains elusive. For this, we took advantage of real-time imaging of kinase activity within living cells/ cells of living animals to analyze its regulatory mechanism in situ. We performed control systems type of analysis that consist of application of a set of defined multiple stimuli to cells (input) followed by measurement of kinase response (output), from which systems properties are deduced. From this, we found that MAPK signaling exhibit a complex, non-linear response to the duration of stimulation to cells. Our results suggested that MAPK signaling deciphers the different temporal pattern of input stimulation. Furthermore, a combination of imaging analysis with mathematical modeling revealed that the complex input-output relation of the kinase signaling can be explained by a fairly simple regulatory mechanism.

(COI: No)

P1-201

This poster presentation was withdrawn.

P1-202

Mechanosensitive ATP release from hemichannels causes acceleration of wound healing in keratinocytes via Ca^{2+} influx through TRPC6 channels

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The cutaneous wound healing (gap closing) was accelerated by a mechanical stimulation, stretch, and impaired in TRPC6-KO mice. Here we elucidated how the mechanical force and TRPC6 channels contribute to the wound healing. HaCaT keratinocytes were cultured on an elastic chamber and treated 1 day with hyperforin, known as a traditional herbal medicine and also a TRPC6 activator. At 3 h after making scratching, ATP release and intracellular Ca^{2+} response by stretch were determined by live-imaging using luciferin-luciferase luminescence and fluo-8 fluorescence, respectively. ATP release was observed only from foremost cells of leading edge of wounded area and it caused Ca^{2+} waves spreading to the behind cells. The Ca^{2+} response and the acceleration of wound healing were inhibited by a diphosphohydrolase, apyrase, a P2Y antagonist, suramin, a hemichannel blocker, CBX and a PIP_2 analog, diC8-PIP_2. In addition, hemichannel permeable dye calcein entered to only ATP-releasing cells. These results suggested that stretch-accelerated wound closure was due to ATP release via hemichannels from the foremost cells and subsequent Ca^{2+} waves in the behind cells mediated by TRPC6 activation.

(COI: No)

P1-203

Dipalmitoleoyl-phosphatidylethanolamine attenuates insulin signaling by enhancing PP2A and PTP1B activities

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The phospholipid phosphatidylethanolamine is implicated in the regulation of a variety of cellular processes. The present study investigated the effect of phosphatidylethanolamines such as 1, 2-diarachidonoyl-*sn*-glycero-3-phosphoethanolamine (DAPE), 1, 2-dilinoleoyl-*sn*-glycero-3-phosphoethanolamine (DLPE), 1, 2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), and 1, 2-dipalmitoleoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) on protein phosphatases, Akt1/2 activity, GLUT4 mobilizations, and glucose uptake into cells. Of the investigated phosphatidylethanolamines, DLPE and DPPE significantly enhanced activities of protein phosphatase 2A (PP2A) and protein tyrosine phosphatase 1B (PTP1B). DPPE inhibited insulin-induced phosphorylation of insulin receptor, insulin receptor substrate 1 (IRS-1), Akt1/2, ERK1/2, and mammalian target of rapamycinm (mTOR) in differentiated 3T3-L1-GLUT4myc adipocytes. DPPE also inhibited insulin-stimulated GLUT4 translocation to the cell surface and reduced insulin-stimulated glucose uptake into adipocytes. Taken together, the results of the present study indicate that DPPE serves as an enhancer of PP2A and PTP1B, causing reduction of Akt1/2 activity as a result from inhibiting insulin receptor and IRS-1 or mTOR, and then leading to suppression of GLUT4 translocation to the cell surface and glucose uptake into adipocytes.

(COI: No)

P1-204

The voltage-dependence and mechanism of RGS4-mediated regulation on the M2 muscarinic receptor-activated K^+ currents

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The regulator of G-protein signalling (RGS) proteins are a family of well-known GTPase-activating proteins that negatively regulate G-protein cycle. We have found that cardiac predominant subtype, RGS4, plays an important role in modulating the M2 muscarinic receptor (M2R)-activated G-protein-gated inwardly rectifying K^+ (K_G) currents. However, the mechanism of RGS4-mediated regulation still remains unclear. Here we show that RGS4 is essential for the voltage-dependent response of K_G currents upon M2R agonists. In rat atrial myocytes, M2R partial agonist pilocarpine-evoked K_G currents showed a decrease in current amplitude during membrane hyperpolarization. In a *Xenopus* oocyte expression system, we observed a similar voltage-dependent response of pilocarpine-evoked current in the presence of RGS4, while it lacked such voltage-dependent property in the absence of RGS4. We found that RGS4 suppressed the pilocarpine-evoked K_G currents in a pilocarpine concentration-dependent manner. Such RGS4-mediated regulation was enhanced at hyperpolarized potentials. We also found that the relative efficacy of pilocarpine to ACh changed upon membrane voltages. Charged residues of M2R modulated the voltage-dependence of RGS4-mediated regulation on K_G currents. These findings help us to understand the molecular components and mechanism underlying the RGS4-mediated regulation on the M2R-activated physiological responses.

(COI: No)

P1-205

Cell line dependency of cesium ion induced suppression of cellular proliferation

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Since aftermath of the 2011 Tohoku earthquake and tsunami, cesium (Cs) as a radioisotope became popular, but on the other hand, intracellular reaction of Cs^+ ion is still little known. It is already known that Cs is similar to potassium (K). And it is also well known that K^+ channel plays an important role in tumor cell proliferation. We previously demonstrated that Cs^+ inhibited HeLa cells proliferation. In this study, we established a new primary culture system of rat airway fibroblast cell (RAWF). RAWF was obtained from rat trachea by enzymatic digestion using protease type XIV in 4℃ for over night. EC50 of Cs^+ inhibiting cell proliferation was 4 ± 1 mM (mean ± SD) on HeLa cells, on the other hand, 19 ± 9.5 mM on RAWF. This imply as a possibility that Cs^+ has a stronger cell proliferation inhibiting effect on carcinoma cells than on normal cells. In addition, we will report the inhibiting effect of Cs^+ on the cell proliferation of some other cell lines such as B16, SH-SY5Y and so on.

(COI: No)

P1-206 (AP-1)

TRPM2 protects mice against polymicrobial sepsis by enhancing bacterial clearance

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TRPM2 is an oxidative stress-activated nonselective Ca^{2+} permeable channel abundantly expressed in macrophages to regulate production of inflammatory mediators. However, the role and mechanism of TRPM2 in polymicrobial sepsis remains unclear. Using CLP-induced polymicrobial sepsis model, Trpm2-KO mice had increased mortality compared with wild-type (WT) mice. The increased mortality was associated with increased bacterial burden, organ injury, and systemic inflammation. TRPM2-mediated Ca^{2+} influx plays an important role in LPS or CLP-induced HO-1 expression in macrophage. HO-1 up-regulation decreased bacterial burden both in WT BMDMs and in CLP-induced septic WT mice. Disruption of TRPM2 decreased HO-1 expression and increased bacterial burden in BMDMs. Interestingly, pretreatment of Trpm2-KO BMDMs with HO-1 inducer markedly increased HO-1 expression and decreased bacterial burden. Moreover, pretreatment of Trpm2-KO mice with HO-1 inducer reversed the susceptibility of Trpm2-KO mice to sepsis by enhancing bacterial clearance. In addition, septic patients with lower monocytic TRPM2 and HO-1 mRNA levels had a worse outcome compared with septic patients with normal monocytic TRPM2 and HO-1 mRNA levels. TRPM2 levels correlated with HO-1 levels in septic patients. Our data demonstrate a protective role of TRPM2 in controlling bacterial clearance during polymicrobial sepsis possibly by regulating HO-1 expression.

(COI: No)

P1-207

Morphological studies on cell membrane permeability of amphiphilic gold nanoparticles in cultured Schwann cells and dorsal root ganglion cells

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Gold nanoparticles (AuNP) have been the focus of much attention as an attractive material for medical uses, such as a tool for photothermal therapy, biosensing devices and drug delivery carriers. Gold nanoparticles are usually taken into the cells by endocytosis. Niikura and colleagues have developed amphiphilic gold nanoparticles with cell membrane permeability. The gold nanoparticles were coated with ethyl ester-headed polyethylene glycol ligands (C2-Ester). To verify the cell membrane permeability of the C2-Ester AuNP into the cells, the morphology of the cultured Schwann cells and dorsal root ganglion (DRG) cells was observed by electron microscopy. The gold nanoparticles coated with C2-Ester were localized not only in the endosomes and multivesicular bodies but also in the cytosol of Schwann cells and DRG cells. The uptake of C2-Ester AuNP in the DRG cells was less than in the Schwann cells. The presence of the C2-Ester AuNP in the cytosol is evidence that the AuNP was taken up by the cell membrane permeability. Our finding regarding the advantages of ester ligands will be applicable in exploring how they deliver the various functional nanoparticles into cells. However, electron microscopy did not provide evidence in the present study indicating that the C2-Ester AuNP arrives in a nuclear pore and nucleus. The matter of how to develop amphiphilic AuNP that can reach into the nucleus is yet to be resolved.

(COI: No)

P1-208

Expression analysis of metallothionein genes and application to the production of heterologous proteins in *Tetrahymena thermophila*

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Metallothioneins (MTs) are a family of low molecular weight (>10 kD), cystein-rich, absence of aromatic amino acids, heavy metal-binding proteins. The expressions of MTs genes are induced by the presence of heavy metal such as zinc, copper, and cadmium. MTs play important roles in the maintenance of homeostasis and in the detoxification of heavy metals. Recently several studies suggested a disturbance of the zinc and copper metabolism related to autism spectrum disorders. The zinc deficiency and an excess of copper level are observed in children diagnosed with autism spectrum disordered. For the experiment of toxic compounds, *Tetrahymena* is excellent eukaryote model organism. Using *T. thermophila* the expression level of copper-inducible metallothionein genes in response to zinc and copper has been carried out by RT-PCR. *T. thermophila* was grown in PPYG medium at 25℃. Cells were treated with 2-500 μM $CuSO_4$, 2-870 μM $ZnSO_4$ for 24h. Total RNA was isolated using RNeasy Kit (Quagen) from the cells (5-8 × 10^5). AMV reverse transcriptase was used to synthesize cDNA. Template cDNA was amplified with MTT2 primers. The 5′ region of a copper-inducible metallothionein gene (MTT2) acts as a promoter, a region 1456 bp upstream of the start codon in MTT2 was amplified and ligated into a vector (pMTT2^p-EGFP). This recombinant vector introduces by electroporation into T. thermophila. MTT2 promoter, strongly induced by copper, might be effective to produce the higher level of MTs in various tissues.

(COI: No)

P1-209

Effects of food additives on human neuroblastoma-derived cells

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Introduction: Several reports have described the effect of food additives on cell activities. However, the details of such effect were unclear. Thus, we investigated the effects of food additives used alone or in combination on human neuroblastoma-derived cells, focusing particularly on morphological changes observed in these cells.
Materials and Methods: Tumor cells derived from human neuroblastoma (NB-1) were cultured in medium containing a single food additive or multiple food additives (aspartame, tartrazine, sodium benzoate, sodium nitrite). The morphology of these cells and the fine structure of cell surface were observed by scanning electron microscope. The effects of these food additives on degeneration or death of cells were examined by double staining using fluorescein diacetate and propidium iodide.
Results and Discussion: In this study, there was marked decrease in numbers of cell processes incubated with sodium nitrite alone, sodium benzoate alone, or multiple food additives. A statistically significant decrease in the cell survival rate was apparent in cells cultured with sodium nitrite alone, sodium benzoate alone, or multiple food additives, in comparison with cells of control. From these data, it was suggested that food additives may exert major influences on functional properties of neuroblastoma-derived cells.

(COI: No)

P1-210

Development of three color variants of super-brilliant luminescent proteins for multicolor, real-time bioluminescent imaging

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Since bioluminescence is free from auto-fluorescence, it has been used for quantitative analysis of gene expression and in vivo imaging. Furthermore, it is free from potential phototoxicity and is compatible with optogenetic tools. However application of bioluminescent imaging has been limited mainly by two drawbacks. Firstly, the light output from the bioluminescent protein was much dark. Secondly, its color variants have been limited, precluding multicolor imaging. In recent study, we addressed the first limitation by developing a super brilliant yellow luminescent protein, Nano-lantern (Saito et al., Nat. Commun. 2012). In this study, we have overcome the second barrier. We report the development of cyan and orange variants of Nano-lantern, both of which are even brighter than the original yellow Nano-lantern by 1.5-2.3 times. Fusions of these multicolor Nano-lanterns with a variety of subcellular localization tags showed correct localization, demonstrating their utility as imaging probes. In addition, expansion of the color palette of Nano-lanterns also enabled expression analysis of multiple genes at single cell level in embryonic stem cells, which are known to be very sensitive to phototoxicity. Furthermore, by combining split luciferase complementation with Ca^{2+}-sensing peptide (CaM-M13), we demonstrated simultaneous measurement of Ca^{2+} dynamics in the nucleus and mitochondria. These data indicates our multicolor Nano-lanterns will be excellent imaging tools for in vivo imaging, stem cell study and so on.

(COI: No)

P1-211

Special-purpose simulators for biological research: intracellular calcium dynamics

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Recent advances in live imaging have enabled visualization of spatiotemporal dynamics in living cells. However, mechanisms that orchestrate cellular dynamics have not been well elucidated. Computer simulations are necessary to elucidate these mechanisms because they allow us to predict dynamics of hypothetical models. The predicted dynamics are to be used for improving the models, compared with in vivo dynamics. Despite such importance, computer simulations are not widely used in biological research. A major reason for such limited usage is the difficulty in setting up simulations. Although many high-performance simulators are currently available for biological research, setting up simulations requires some programming-like skills in these simulators because they are designed as a general-purpose simulator. To accelerate the use of computer simulations in biological research, we are developing special-purpose simulators.

In this poster, we present a special-purpose simulator for intracellular calcium dynamics. This simulator does not require any programming-like skills for setting up, but does require inputs of several parameter values through a graphical user interface. In this simulator, a calcium-induced calcium release mechanism is modeled by Nagumo equation, a reaction-diffusion equation where its reaction term is modeled as a cubic polynomial. Numerical solutions are obtained by using explicit method. We are planning to develop a variety of special-purpose simulators so that biologists can find suitable ones for their research.

(COI: No)

P1-212

CellCompiler: Multiscale biological function model simulator which can use complex calculation schemes

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We have developed a code generator software called CellCompiler, which can automatically generate programs for biological function simulations. The code generation system requires three inputs, namely a CellML or PHML file describing a biological model, a TecML file describing various calculation schemes to discretize and solve the biological model, and a RelML file relating the CellML and TecML file. The biological model may be a combination of multiple models with different temporal or spatial scales. TecML uses recurrence relations to describe various solution schemes. In the case of multiple models with temporal scale variety, it can represent a combination of multiple temporal scale calculations. As an example, consider a coupled pharmacokinetic model coupled with a cellular electrophysiology model. The two models have different time scales, hours for the pharmacokinetic model and milliseconds for the cell model. For this, the code generator creates a double loop; an inner loop containing the excitation propagation of the cell model and an outer loop containing the drug absorption. The code generator also allows users to automatically perform parameter studies to analyze the effect of different model parameters to simulation results.

(COI: No)

P1-213

Simultaneous measurements of sound evoked electrical activities and calcium responses from deep brain regions by photometric patch electrode

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Intracellular calcium increase associated with neural activity is essential for supporting neural functions such as neural plasticity. It is therefore important to know how calcium changes to elucidate underlying mechanisms of calcium regulation. Signaling molecules are generally monitored by optical methods. However, application of two-photon microscopy in the deep brain tissue imaging is impossible due to light scattering. We overcame this problem by using photometric patch electrode (PME) recording system that utilizes a patch electrode as a light guide and is enabled us to excite and obtain fluorescence from a target neuron simultaneously with electrical recording. We measured calcium sensitive Oregon Green BAPTA-1 fluorescence signal simultaneously with the field current in response to sound stimulus from various auditory nuclei of young chicken; Field-L (avian auditory cortex), inferior colliculus (IC), and nucleus magnocellularis (NM, avian cochlear nucleus). We found distinct calcium fluorescence signals in ascending order across the nucleus from NM to Field-L. Neurons in NM practically suppressed calcium increase during orthodromic excitation. NM is a relay nucleus and receives inputs of high frequency activity of auditory nerve fibers and has high rate of spontaneous and driven firing activity; thus calcium influx is likely suppressed otherwise neurons are poisoned to apoptosis.

(COI: No)

P1-214

Quantification of the axonal transport activity of cultured neurons by flow analysis (II)

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We used KBI Flow Analysis plugin on Image J in our study. We defined previously the activity of axonal transport as a sum of particles which moves more than 0.3 pixels per frame (0.1 μm/s) within a limited area and a limited time. Our present study revealed that the greater part of the apparent velocity calculated by Flow analysis after the fixation of observed neurons by 4 % paraformaldehyde at the end of each experiment is less than 0.3 pixels per frame. The value less than 0.3 contains artificial errors derived from diffused reflection and such, which should be eliminated. The activity value based on our definition is well coincided with the value, Transported organelles (% of control) obtained from our traditional method. Another problem, where the velocity estimation of the particle sometimes fails because of the high speed of the moving particles, can be solved by applying an appropriate small value, 2 or 4 pixels but 8, to stepXy. We can also distinguish and sum up the number of anterograde transporting particles and retrograde transporting particles separately by an angle of calculated velocity vectors. The next step of our study is to write a series of macro programs in order to automate huge numbers of calculations.

(COI: No)

P1-215

Magnetic Resonance Imaging using human fetuses preserved in formalin solution

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To understand the mechanism of development and disorder during childhood, the morphological changes during fetal period has been focused and recently some fetal brains have been imaged by magnetic resonance imaging (MRI). Those images were quite fine, however, obtained by isolated brain samples; there is no definitive imaging protocol for whole body samples. The Kyoto Collection is the world largest collection of human conceptuses, and contains almost 40,000 embryos and 5,500 fetuses, stored at the Congenital Anomaly Research Center, Kyoto University Graduate School of Medicine. The fetuses have been fixed and preserved in the formalin solution. To establish the protocol suitable for MRI using whole-body fetuses, here we imaged 12 fetuses using MRI system with 3T magnet (SIEMENS MAGNETOM SKYRA). For magnetization-prepared rapid gradient-echo (MP-RAGE) sequence, no treatment were not required for fetuses in formalin solution to obtain fine images; but less signals could be obtained from the specimens in T2-weighted and diffusion-weighted imaging (DWI). To increase the signals obtained from water, the fetuses were soaked in phosphate buffered saline for 3-14 days and the T2 and DWI signals were improved after the substitution. The specimens with long-term preservation in formalin solution were available for T2 and DWI as well as MP-RAGE, and it implies the possibility for diffusion tensor imaging (DTI) using abundant specimens from the Kyoto Collection.

(COI: No)

P1-216

Thiel's fixation method to prepare cadavers for surgical training

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Following the release of "Guidelines for Cadaver Dissection in Education and Research of Clinical Medicine, " we began extensive surgical training at Ehime University in 2012. In addition, a surgical training center was established at Ehime University in December of 2013. The use of conventional 10% formalin-fixed cadavers for surgical training is unsuitable because their tissues are much harder than those of living bodies. Therefore, as a substitute for formalin when preparing cadavers for surgical training, we performed Thiel's fixation method. We purchased "Thiel's fixation method liquid" from A. S. CHEMICAL Co., Ltd. (Concord, ON, Japan) and added two types of blood, resolvent and formalin (3.9%), to it. We then fixed the cadavers by injecting the total solution via the femoral artery. Cadavers fixed using Thiel's method retained the softness of a living human body; these cadavers were useful for surgical training (e.g., laparoscopic surgery). However, there were some problems, including individual differences in the fixed state of the abdominal organs and occasional insufficient fixation of the brain. It is necessary to perform suitable cadaver fixation for the purpose of surgical training by adjusting the formalin content in the fixation liquid and improving the infusion method.

(COI: No)

P1-217

Anatomical observation of cadavers embalmed with 10% N-Vinyl-2-Pyrrolidone

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N-Vinyl-2-Pyrrolidone (NVP) solution was used for embalming cadavers as a substitution fixative of formalin (FA), as reported in the 116th Annual Meeting of the Japanese Association of Anatomists (Fujikura et al, 2011). Formerly, the effect of NVP as a fixative was studied histologically, using the animal tissue (Fujikura et al, 2008, 2009). At present, we are continuing observation of cadavers fixed with NVP of various concentrations to pursue an optimal condition. In the 119th Annual Meeting of the Japanese Association of Anatomists (2014, Tochigi), we reported the observations on cadavers embalmed with different NVP solutions: the final concentration in the tissue was 4.0, 4.2, 5.4, 5.5, 10.2, 10.5, and 21.5%. In that observation, the 4 cadavers containing lower concentration of NVP were very soft and vulnerable, but the ligaments were easily identified through the transparent connective tissue. On the contrary, the connective tissue was too hard and opacified in the cadaver fixed at the highest concentration of NVP, though the original shape of the organs was well preserved. These observations revealed that the cadavers became harder with increasing final tissue concentration of NVP, and that 10% NVP was suitable for dissection by students. In the present study, we report the observation of the cadavers fixed with 10% NVP, especially on the several joints, hearts and brains.

(COI: No)

P1-218

Usefulness of the cadavers embalmed by the saturated salt solution method for surgical training: The evaluation of surgeons and feasibilities of clinical procedures

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Background: For surgical training (ST) courses using cadavers performed to advance a surgeon's techniques without any risk to patients, the new embalming methods to make cadavers the more soft and safe are desired. The aim of this study is to evaluate the suitability of cadavers embalmed by the saturated salt solution (SSS) method for ST.
Methods: Six cadavers were embalmed by three methods: formalin solution, Thiel's solution (TS), and SSS methods. Fourteen surgeons evaluated the three embalming methods. Furthermore, seven trauma surgeons and two orthopedists operated these cadavers by 21 procedures. In addition, ultrasonography, central venous catheterization, and incision with cauterization followed by autosuture stapling were performed.
Results: The surgeons evaluated the cadavers embalmed by the SSS method to be highly equal to those embalmed by the TS method. Ultrasound images were clear in the cadavers embalmed by both TS and SSS methods. Central venous catheterization could be performed in a cadaver embalmed by the SSS method and then be affirmed by X-ray. Lungs and intestines could be incised with cauterization and autosuture stapling in the cadavers embalmed by TS and SSS methods.
Conclusion: Cadavers embalmed by the SSS method are sufficiently useful for ST not less than ones embalmed by the TS method. The SSS method is considered to have a beneficial feaure that it is simple and low-cost.

(COI: No)

P1-219

Comparison of embalming methods from the aspect of suitability for surgical training: On their antiseptic effect and cadaver fixation

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Objective: To compare the cadavers embalmed by the several new embalming methods for surgical training from the aspect of their antiseptic effect and hardness of cadaver tissues.
Methods: Four cadavers were prepared by conventional formalin fixation method (formaldehyde: FA 3.7%), Thiel method (FA 1.8%), Preserve(R)fixation method (FA 0%), Saturated salt solution (SSS) method (FA 0.75%). Bacterial and fungal culture tests, dissection and histological observation by Hematoxylin-Eosin staining were performed.
Results: Each method performed much the same antibiotic effect immediately after injection. The FA embalmed cadaver seemed too rigid for surgical training. Thiel embalmed cadaver had the greatest joint ranges of motions. The internal organs of this cadaver were damaged gradually after opening of the body cavities. The hardness of the cadaver embalmed by Preserve(R)fixation showed the same tendency. The hardness of the cadaver embalmed by SSS method was intermediate.
Discussion: Although the Thiel embalmed cadaver is so close to a living body and suitable for a clinical training orientation, the improvement of ex-post treatment may be required for long-term use. Preserve(R)fixation method will be improved by adjustment of the composition, especially alcohol concentration. Although the SSS method is poorly understood, it may be relatively suitable for surgical training as it stands.

(COI: No)

P1-220

Quantitative imaging by a newly endoscopic system for pathological malignancy status based on endogenous fluorescence

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Background: We found that a nitrosamine-induced esophageal tumor model rat showed strong endogenous fluorescence, which was highly related with emergence of atypical cells, hyperplasia and tumorous changes in epithelia. Hence, the aim of this study was to clarify the origin of fluorescence and to quantitatively visualize the pathological status with the endogenous fluorescence as a clue using a fluorescence multi-spectral imaging (FMSI) system.
Methods and Results: We obtained fluorescence multi-spectral images of mucosal membrane of extracted esophagus from the rats that were administrated N-nitroso methyl butylamine(NMBA) (15 mg/L) in a drinking water for 1-16 weeks. The FMSI showed fluorescence with a peak of 630 nm (excitation: 405 nm) in areas where atypical cells exist, and intensity of the fluorescence was positively correlated with the time period of NMBA administration. HPLC revealed that an origin of the endogenous fluorescence was protoporphyrin IX and other porphyrins (e.g. uroproporphyrinogen) were not detected. For in-vivo realtime quantitative imaging, we developed an endoscope-based FMSI system equipped with spectral unmixing mechanism. The novel endoscopic system made it possible for us to detect early lesions by transesophageal approach.
Conclusion: The newly developed endoscope-based FMSI system can be a promising tool for the detection of precancerous lesions based on endogenous fluorescence.

(COI: No)

P1-221

Directly observed membrane disruption and resealing during centrifugation of sea urchin eggs by centrifuge polarizing microscope

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Large plasma membrane disruptions (PMDs) rapidly invoke a localized exocytotic reaction that adds a 'patch' of internal membrane to the plasma membrane at the PMD site, a calcium-dependent resealing mechanism. We have used sea urchin eggs as a model system to define the mechanistic basis of this fundamental cell survival response. Here we directly observed plasma membrane tears that occur in sea urchin eggs during centrifugation with a special centrifuge polarizing microscope (CPM). Dilute suspensions of unfertilized eggs were layered in a centrifuge chamber above an osmotically matched dense solution containing Percoll, forming a density gradient that allowed the eggs to slowly settle to an equilibrium position. Centrifugation at speeds of up to 8,000 rpm for 20 min, separated the eggs into two parts. One part was filled with yolk granules and internal vesicles, the second part was filled with clear cytoplasm. These membrane tears by shear forces did not show variously shaped surface projections involved in exocytosis at the PMDs. These cell separations depended on the presence of calcium. However, sea urchin eggs were broken by this centrifugation in the absence of calcium. The part filled with yolk granules and internal vesicles repaired the PMDs made by a two- photon laser, but the part of the eggs containing clear cytoplasm did not repair.

(COI: No)

P1-222

The 3D-atlas of adult zebrafish

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Zebrafish is an experimental model animal, which is used to study the development of the tissue and the body. Zebrafish embryos have clear bodies, and allow us to perform hole mount microscopy to know the 3-dimentional (3D) structures of the tissues and whole body. When zebrafishes grow up into adults, they are observed by sectioning and microscopy in general. Sections of adult zebrafish provide the 2-dimentional (2D) information, but lack the 3D-information of a tissue or a whole body. For the 3D analysis of adult zebrafishes, we utilized the serial sectional images, and reconstructed in a personal computer with a free softwere, OsiriX. XY resolution of the original 2D image is $4.7 \times 4.7\,\mu$m. XYZ resolution of reconstructed 3D image $18.8 \times 18.8 \times 20\,\mu$m. The image quality, including resolution, brightness and contrast was enough to observe various organs, for example brain, skeletal system, vasculature and gastrointestinal system. Zebrafishes are often used in the phenotype analysis after gene manipulations. Our atlas of adult zebrafish should be useful as the basic knowledge for such analysis.

(COI: No)

P1-223

Advances in open-skull surgery for *in vivo* imaging by biocompatible materials

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To understand the mechanisms of learning and memory, it is important to observe how neural circuits are activated, modified, and maintained in the living mouse brain. Two-photon microscopy is a useful tool for observing the neural circuits that extend across brain regions *in vivo*; this method can penetrate deep into thick specimens, achieve less invasive optical sectioning, and provide 3D images reconstructed from these sections. However, the quality of *in vivo* images is dependent on the transparency of the cranial window, which is in turn affected by the experimentalist's technical skill in the open-skull surgery that replaces the cranial bone with cover glass. In addition, even after a successful surgery, the cranial window tends to become cloudy several days later. Consequently, it becomes difficult to observe the neural circuits *in vivo* over long periods.

Here, we report new methods for keeping cranial windows clear using two biocompatible materials. The anti-thrombogenic biocompatible material Lipidure® kept the cranial window clear for long periods. Another biocompatible material, Cocktail X, increased the fluorescence signals emitted from neurons and allowed sharp visualization of fine structures at deep regions. These biocompatible materials should be useful for studies of changes in neural circuits at the synapse level over long periods.

(COI: No)

P1-224

Evaluation of *in vivo* two-photon microscopy by imaging of embedded fluorescent beads in mouse brain

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Morphological changes in post-synaptic structures (dendritic spines) are thought to be involved in synaptic plasticity, which is implicated in information processing by the neural network. Therefore, in order to understand brain functions, it is important to visualize synapses in the living mouse brain. Because of its high resolution and deep imaging capability, *in vivo* two-photon microscopy has been used to observe dendritic spines under live conditions. In our previous study, we found that penetration depth could be improved by changing the diameter of the irradiation excitation laser. However, the resolution under these conditions was not determined, because the details of the focal spot size of the excitation light were not measured precisely. In general, the resolution of a laser scanning microscope is reversibly correlated with the focal spot size. This size is sometimes evaluated by measuring full width at half maximum (FWHM) of a structure with a known shape (e.g., a fluorescent bead) that is smaller than the diffraction limit. In this study, we injected fluorescent beads into the living mouse brain, and succeeded in *in vivo* two-photon imaging of single beads at various depths in the brain. We estimated the resolutions by measuring FWHM from single-bead images.

(COI: No)

P1-225

Direct measurement of the binding rate constant of kinesin to microtubules in living cells

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It has been established that conventional kinesin (kinesin-1, KIF5 in mammalian cells) selectively moves along a specific subset of microtubules in living cells. For example, KIF5 is specifically recruited to the microtubules in the axon initial segment in neurons, which would enable efficient transport into the axon. However, the mechanism of this selective binding is still controversial. Some groups have proposed that acetylation or other post translational modifications of tubulin serve as the cue for selective binding. We are proposing that conformational differences between the GTP-form and GDP-form of microtubules provide the cue. To test this idea, it would be important to examine whether kinesin binding to specific subsets of microtubules is enhanced, inhibited or both. Here, we measured the binding rate constant of kinesin to microtubules in living cells and in vitro using single molecule fluorescence microscopy. To our surprise, the binding rate constant of KIF5 to the track-microtubule in vivo was nearly ten times higher than that in vitro, suggesting that mechanisms exist in the cell to recruit KIF5 specifically to some subset of microtubules by accelerating the binding reaction.

(COI: No)

P1-226

Quantitative measurement of ATP concentration inside single mammalian cells

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Despite the fact that adenosine triphosphate (ATP) is required for a wide variety of intracellular processes, it is not clear how the synthesis and consumption of ATP is balanced inside living mammalian cells. Since the energy required at each subcellular domains or organelles could vary from site to site or change over time, subcellular localization and fluctuation of ATP concentration are important. We previously developed a new fluorescent ATP indicator protein named "QUEEN" that can be used to quantitatively measure absolute ATP concentrations inside living bacterial cells. Here, we developed an improved version of QUEEN suitable for application in mammalian culture cells. We expressed this indicator in mammalian cultured cells and successfully measured the ATP concentration inside different organelles. In addition, we have also examined the time-dependent change of ATP concentration in response to exogenous perturbations.

(COI: No)

P1-227

High-resolution imaging of live cells and tissues by scanning ion conductance microscopy

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Scanning ion conductance microscopy (SICM), introduced by Hansma in 1989, is a technique of scanning probe microscopy and uses a microglass pipette as a sensitive probe. Because it can obtain contact-free images of the sample topography, SICM is expected to be used for studying the surface structure of soft biological samples under liquid conditions. We previously showed that hopping mode SICM is useful for imaging complicated surface structures of fixed cells and tissues in liquid conditions. The present study was performed for the assessment of the usefulness of SICM for observing biological samples without fixation. We succeeded in obtaining SICM images of live cultivated cells without any severe sample damages, which might be caused by the probing tip. The minimum data acquisition time per image (128 by 128 pixels) was about 8～10min. The movement of cellular processes were clearly seen on the surface or the periphery of the cells, indicating that SICM is useful not only for observing fixed cells, but also for analyzing the movement of live cells. In this study, we will also show the applicability of SICM to the study of the surface structure of live tissue samples (e.g., the epithelial surface of the urinary bladder).

(COI: No)

P1-228

Application of Sihler's staining in histology

Hayashi, Hiroyuki[1]; Kimura, Akihiko[2]; Kanemura, Naohiko[1]; Nishihara, Ken[1]; Gomi, Toshiaki[2]; Naruse, Hideo[2] (*[1]Saitama Prefec. Univ., Saitama, Japan; [2]Tokyo Ariake Univ., Tokyo, Japan*)

Sihler's staining is a technique for staining nerve endings in muscle. This technique involves decalcification of the specimen and it renders soft tissue transparent. This study was conducted to determine if this staining could be used to study both the macroscopic anatomy and histology of the same specimens. An experiment was performed using ICR mice. The femoral region was separated starting at the hip joint. Specimen was fixed in 10% neutral buffered formalin. The specimen was then rinsed with water and subjected to Sihler's staining. The specimen was stored in 100% glycerol. Nerves were studied macroscopically and photographed. Afterwards, glycerol was washed off with water and specimens were embedded in paraffin. Specimens were sectioned, stained with eosin, and then examined using microscopy. Sihler's staining resulted in hematoxylin staining of nerve fibers innervating the leg, facilitating their macroscopic observation. After nerves were examined macroscopically, tissue sections were prepared. The result was a staining technique that did not require a special decalcification step and that allowed ready sectioning of bone. However, the hematoxylin staining of nerve fibers that were verified macroscopically faded, precluding a clear depiction of their histology. This was because the staining of nerve fibers faded during the preparation of tissue specimens. A remedy to this problem will result in Sihler's staining being a useful staining technique to study both the macroscopic anatomy and histology of the same specimens.

(COI: No)

P1-229

Real time measurement of pharmacokinetics of an ototoxic drug in the microspace of in vivo inner ear by a diamond microelectrode

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A loop diuretic, bumetanide, often damages inner ear and causes dizziness and deafness. To develop the therapeutic strategies that reduce such side effects and design the analogs nontoxic for hearing, real-time monitoring of the pharmacokinetics local environments of in vivo inner ear is necessary. Several methods are available to determine the concentrations of bumetanide in body fluids such as HPLC, and mass spectrometry. These measurements require large amounts of samples taken from body, and thereby enable to detect the dynamics in the microspace continuously. Also, some of them represent a low sensitivity. To resolve these problems, we utilized boron doped diamond (BDD) microelectrode. The electrode was inserted into the inner ear fluid of living guinea pig. On the chronoamperometry, we successfully observed increase of the oxidation current elicited by bumetanide in several seconds after injecting the drug of 30 mg intravenously. Calibration curve demonstrated that the peak current approximately corresponded to 532 μg/dl. Converse response was detected in an inner-ears potential that mirrors hearing level and was measured by a glass microelectrode. Our BDD microelectrode system can be applied to monitor other drugs in numerous tissues and organs and helpful to promote the pharmacological researches.

(COI: No)

P1-230

The development of PET imaging for detecting AMPA receptors trafficking during acquisition of fear memory

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When experiencing or learning, some plastic changes occur in synapses and drive AMPA receptors (AMPA-Rs) into postsynaptic membrane. Moreover, interruption of AMPA-Rs trafficking into synapse disrupts newly learning. These studies establish that AMPA-Rs work as a key molecular machinery underlying experience and learning. Recent studies using postmortem brains revealed the quantitative alteration of AMPA-Rs may relate to some variety of mental disorders including schizophrenia, depression and ASD. Among these studies, membrane numbers of AMPA-Rs seem to account for these disorders. However, with the current techniques, we cannot observe the behavior of AMPA-Rs in living human brains. Our study aim to develop new PET probes to detect the membrane numbers of AMPA-Rs in living human brains. We disclosed that animals experiencing inhibitory avoidance (IA) task increase the membrane surface expressions of AMPA-Rs up to 1.5-fold of control animals in the hippocampus. To detect these changes in vivo, we developed new method using LC/MS-MS. Briefly, we administered compounds previously known to bind to AMPA-Rs specifically to adult rats intravenously. 24 hours later, we dissected hippocampus and measure these compounds in the hippocampus using LC/MS-MS. Then we analyze the correlation between biochemical data and MS data. As a result, some compounds increased in IA+ animals compared to control animals. These results indicate that these compounds detect the increase of surface expression of AMPA-Rs in vivo.

(COI: No)

P1-231

A new method to isolate basophils from peripheral blood without dilution or hemolysis by the flow-through density gradient centrifugation

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We have developed a novel flow-through density gradient cell separation method. This system continuously separates a large number of cells into five fractions according to their densities. As the blood contains a huge number of red blood cells (over 1,000 times of the number of leukocytes), the pretreatment with dilution and hemolysis is usually essential for harvesting leukocytes. However, we separated basophils, which had the fewest number among leukocyte population, from 20ml of human peripheral blood in 3 hours without the pretreatment. A set of isosmotic Percoll media with the densities of 1.050, 1.074, 1.079, 1.090, 1.095 and 1.104g/ml was prepared, and introduced into the channel to form a density gradient. Then the anti-coagulated blood was continuously fed into inlet 1, through which Percoll medium with the density of 1.050g/ml was flowing. Harvested fractions with the density of 1.079g/ml and 1.090g/ml were washed, and the cell pellets were re-suspended into 1ml of the density medium with 1.050g/ml. This cell suspension was fed through inlet 1 and separated under the same condition, again. Separated cells in the density of 1.079g/ml and 1.090 g/ml by the second run contained basophils at about 72% and neutrophils at about 92%, respectively. The red blood cell counts were about 6% in each fraction. Without diluting the blood sample shortened the time required for cell separation and the repeating operation made possible to remove most of red blood cells.

(COI: No)

P1-232

Novel mouse xenograft model for noninvasive *in vivo* imaging of human tumor cell and tissue in the auricle

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We developed methods of preparing xenograft model and imaging GFP-expressing cells to observe noninvasively cells in mouse auricles. We selected the ear auricle of mouse for observation of tumor cells because of very thin (about 150-200 μm) and limited hypodermal tissue. We have developed a novel xenograft model which has tumor in auricle. We injected five kinds of human cancer cell lines into the ear auricle of SCID mice; breast cancer cell lines named KPL4-EB1-GFP and MDA-MB-231, MDA-MB-231-EB1-GFP and MDA-MB-231-GFP-tub, and glioma cells line U87MG. Tumor was successfully formed 100% of injected mice at incitation of $> 4.6 \times 10^6$ cells in all cell lines. The tumor and cells in auricle were noninvasively imaged by spinning disk confocal (CSU) system equipped with automatic positioning stage, piezo actuator for objective and an EMCCD camera. We imaged GFP fluorescence in the MDA-MB-321-GFP-tub cells in tumor of ear auricle without injuring mice. The individual two cells in tumor were distinguished faintly with bright background of tumor fluorescence. We also took a montage view of tumor cover wide area (3 × 2 mm). The shape of a tumor appeared faintly at the depth > 40 μm, suggesting the shape is background of a tumor located deeper. There are several bright spots in the diameter of about 20 μm in the enlarged image, indicating those are single cells. We could successfully perform real time observation of GFP fluorescence in the breast cancer cells in noninvasive condition by a CSU system.

(COI: No)

P1-233

Thermo stabilized super-active TALEN mediated highly efficient and homogeneous gene knock-out in mammalian embryos

Ikeda, Kazuho; Terahara, Yoko; Sumiyama, Kenta; Okada, Yasushi (*RIKEN, Osaka, Japan*)

Gene editing *in vivo* has become possible by the development of artificial nucleases that can be designed to cut the genome DNA selectively at the target site in the genome. TALENs are highly specific artificial nucleases, has been proven to be useful for the genome editing in lower vertebrates such as zebrafish and *Xenopus*. However in mammalian cells and embryos, TALENs often show poor activity, which limited its applications. To overcome this limitation of TALEN, we introduced amino acid substitutions into TALE DNA binding domain which might participate in conformational stability under high temperature. Several mutations were introduced, and some successfully showed significantly higher activity at 37 °C both *in vitro* and *in vivo*. TALENs made from this high activity mutant (named "super-active" TALEN) showed significantly higher rate of genome editing in zebrafish eggs. Finally we have demonstrated that our super-active TALEN efficiently introduced site specific mutations in mouse embryos. Interestingly, genome analyses detected only less than four different mutant alleles in each baby. In the case of CRISPR/Cas9 mediated gene editing, however, more than eight alleles were often detected. These results suggest that super-active TALEN shows its activity at two-cell stage, earlier than CRISPR/Cas9, thus super-active TALEN might serve as an effective tool for the genome editing in mammalian cells and embryos.

(COI: No)

P1-234

In vitro analysis of thermo stabilized super-active TALEN

Terahara, Yoko; Ikeda, Kazuho; Miyashita, Naoyuki; Okada, Yasushi (*Quantitative Biology Center, RIKEN, Osaka, Japan*)

Gene editing *in vivo* has become possible by the development of artificial nucleases that can be designed to cut the genome DNA selectively at the target site in the genome. TALENs are highly specific artificial nucleases, has been proven to be useful for the genome editing in lower vertebrates such as zebrafish and Xenopus. However in mammalian cells and embryos, TALENs often show poor activity, which limited its applications. Recently we have developed "super-active" TALEN by introducing amino acid substitutions into specific residues of TALE DNA binding domain, and demonstrated that super-active TALEN can mediate efficient genome editing in mouse embryos and zebrafish eggs.

To evaluate how our "super-active" mutations actually affect enzymatic properties, we have produced active TALEN proteins in *E. Coli* expression system and measured their activities *in vitro*. Our data suggested that super-active TALEN is more stable at 37 °C, which might cause high activity. In addition, all-atom molecular dynamics simulations confirmed the stabilization of the conformation. The mutated residues apparently suppressed the intramolecular fluctuations. Our *in vitro* and *in silico* based approaches provide us a new insight for further improvement of TALEN techniques, including even more active TALEN, other gene editing enzymes, and new biological tools such as sequence specific DNA binding probes.

(COI: No)

P1-235

On the relationship of the distributions of the cutaneous nerves between the musculocutaneous nerve and the radial nerve

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It has been explained in many textbooks that the musculocutaneous nerve is the ventral nerve and the radial nerve is the dorsal nerve. However, we encountered a special case of the total defect of the superficial branch of the radial nerve, which was compensated by the lateral antebrachial cutaneous nerve from the musculocutaneous nerve. Now we show the details of the distribution of the cutaneous nerves in the hand region and the formation of the brachial plexus of this case. To compare with the normal state, we also examined the distributions of the cutaneous nerves in the hand in some normal cases. On the consequence, the lateral antebrachial cutaneous nerve generally communicated with superficial branch of the radial nerve at the radial side of the distal part of the forearm and distributed in some area of the dorsal side of the wrist region, while the radial nerve distributed into the palmar side of the thumb. Thus, the fibers in the musculocutaneous nerve was not only the ventral components and the radial nerve also contained not only the dorsal components. These indicated that the peripheral nerves should be considered as the road to the fibers, therefore, the cutaneous fibers to the dorsal hand could run in the musculocutaneos nerve substituted for the radial nerve.

(COI: No)

P1-236

Transposition of innervation to brachial flexors: musculocutaneus nerve variations

Shionoya, Kento[1]; Hayashi, Mari[1]; Hayashi, Shogo[2]; Qu, Ning[2]; Hatayama, Naoyuki[2]; Kawata, Shinichi[2]; Itoh, Masahiro[2] (*[1]Sch. Med. Tokyo. Med. Univ., Tokyo, Japan; [2]Depart. of Anat. Tokyo. Med. Univ., Tokyo, Japan*)

Backgrounds: A musculocutaneous nerve (MCN) generally innervates the brachial flexors (BF), i.e. the coracobrachialis (CB), biceps brachii (BB) and brachialis (B). As variations of MCN, the absence of MCN and the communicating branch (Com) with median nerve (MN) are known. Furthermore, the cases that MN innervates to some BF have been also reported as the translocation of BF innervations.
Methods: Sixty two upper limbs were observed. In each limb, the variations of MCN and the nerves innervating the BF were recorded.
Results: In all cases, the BF branches came out in the order of the CB, BB, and B. Sixteen limbs had the Com between the MCN and MN. The absence of MCN was observed in one limb. Translocation of the BF innervations was observed in one limb. In this case and all previously reported cases of the translocation, the Com between MCN and MN were observed. Furthermore, in all cases of the translocations, no BF branch arose from the MCN distal to the Com.
Discussion: Depending on these characteristics of the BF branches and the origins of the Com, MCN variations could be classified theoretically into five patterns. Although all patterns were not covered in the present upper limbs, these patterns were covered including the previous reported cases without exception.
Conclusion: These results suggest that the translocation of BF innervations closely relates to the communication between MCN and MN.

(COI: No)

P1-237

Sex difference in the location of the obturator nerve leaving pelvic cavity

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Background: To prospect location of the obturator nerve (ON) from the body surface is difficult. The aim of this study is to evaluate the location of the ON and its sex difference.
Methods: Twenty-seven bisected pelvises of human cadavers (10 males and 17 females) were observed. After the external obturator muscle was exhibited on the pelvic surface, the following measurements were carried out; the distance between pubic tubercle and the ON (D1); the angles between the inguinal ligament and the ON, at pubic tubercle (A1); the minimum distance between the ON and the inguinal ligament (D2); and the angles formed by inguinal ligament and a line which links pubic tubercle and ischial tuberosity (A2).
Results: The ONs were located on anterolateral part of obturator foramen in all cases. D1s were partially correlated with D2s ($r=0.45$, $p=0.017$). The significant sex differences in A1s (94.0 ± 3.36 degree in males and 86.4 ± 4.08 degree in females, $p<0.0001$) and A2s (106.6 ± 5.37 degree in males and 95.3 ± 3.47 degree in females, $p<0.0001$) were detected.
Conclusion: In females, ONs tended to leave pelvises more smaller angle than those of males. This difference may reflect sex difference in the form of pelvis.

(COI: No)

P1-238

Constantly existence of the sensory branch in the nerve to the pyramidalis muscle and gender difference of the course in the nerve

Haba, Daijiro[1]; Watanabe, Yuko[2]; Arakawa, Takamitsu[3]; Kageyama, Ikuo[4]; Kumaki, Katsuji[5]; Miki, Akinori[3] (*[1]Kobe University School of Health Science, Kobe, Japan; [2]Kobe University Graduate School of Medicine, Kobe, Japan; [3]Kobe University Graduate School of Health Science, Kobe, Japan; [4]Department of Anatomy, Faculty of Life Dentistry, The Nippon Dental University at Niigata, Japan; [5]Nigata University of Rehabilitation, Nigata, Japan*)

The pyramidalis (Py) is often absent in human-being. It is very important to investigate distribution patterns of the nerve to the Py to study morphogenesis of its muscle. Sensory branches from the nerve to the Py were constantly observed. Ten cases out of six cadavers were studied. In all cases, the sensory branches from the nerve to the Py innervated to the tendon of Py, the rectus abdominis, internal oblique and the linea alba were found. Additionally, gender difference in the course of the ilioinguinal and genital branch of the genitofemoral nerves was observed. The nerve to the Py appeared from the superficial inguinal ring went downward and upward immediately in male cases. Namely, it made U-shaped course in male. However, the course of the nerve to the Py in females was straight. We suggest the U-shaped course of the nerves was based on the results of the descensus testis. It is important to investigate the distribution of sensory branches and gender differences on the course of the nerve to the Py in the absent case of the Py.

(COI: No)

P1-239

Anatomical variations of arterial supply to the spleen in rabbits

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The morphology of the spleen differs depending on each species. The rabbit that is widely used as an experimental animal is popular recently among companion animals. The rabbit has the flat, elongated spleen with the longitudinal hilus running along the visceral surface. However, the arterial distribution to the spleen has not been studied in detail so far. Thus, we studied anatomical variations of arterial supply to the rabbit spleen. Twenty-six male and 5 female New Zealand White rabbits, weighting 2.5-3.0 kg, were used. In the cadaver fixed with formalin, a cannula was inserted into the thoracic aorta to be injected with colored latex. After further fixation for more than 7 days, we observed the macroscopic arterial supply to the spleen. The splenic artery arises as the first independent branch of the celiac artery, and runs toward the splenic hilus to provide the splenic branches, whose number is predominantly 5 or 6, to the paremchyma of the spleen. The splenic branch often arises as a trunk (predominantly 1 ranging from 0 to 4) in common with the short gastric arteries (predominantly 4 ranging from 3 to 6) that distribute to the greater curvature or the visceral surface of the stomach. The terminal branch of the splenic artery continues as the left gastroepiploic artery toward the greater curvature. The results demonstrate that the pattern of arterial distributions to the spleen varies depending on each specimen, suggesting that such variations must be considered at experimental and veterinary surgical treatments in rabbits.

(COI: No)

P1-240

Distribution of artery in the maxillary sinus

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A dental implant is the advanced dental care at present, and is developed new methods. When especially the bone resorption in an upper molar part is remarkable, the maxillary sinus (MS) floor augmentation which increases the thickness of a bone required for an implant operation is performed. However, the detailed study of the distribution of artery in the maxillary sinus was not performed.
Methods: We used 13 maxillary sinuses in 42 cadavers which had the thickness of bone at the upper molar area. Then the height of the artery/groove from the bottom of MS and thickness of the artery were measured and then the branches from the infraorbital artery were dissected.
Results and Discussion: The height of the blood vessel from the bottom of MS runs to anteroposterior direction among blood vessels macroscopically observable as a blood vessel or it's groove on the lateral wall of MS was 6.2mm of average. The artery running anteroposterior direction on the lateral wall of MS was formed Posterior superior alveolar artery from a posterior wall and the anterior superior alveolar artery from an anterior wall as the past report. Additionally, we checked that the median superior alveolar artery (The name of his artery is not describe in the textbook.) and malar artery (The name of this artery is not described in human anatomical textbook.). From the above results, we suggest naming it the "intrasinual (arterial) arch".

(COI: No)

P1-241

A rare case report of inferior vena cava duplication

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The inferior vena cava (IVC) is the largest vein in the body, and functionally important for the majority of venous return from the lower extremities and abdomen, and drains into the right atrium. The double IVC or duplication of IVC is a well-known congenital anomaly and the incidence has been reported to be 0.2%-3%. Most duplicated IVC cases are clinically asymptomatic. However, since it has been reported that duplicated IVCs tend to be found in patients with hydroneprosis and various other conditions, they may have significant clinical implications, such as deep venous thrombosis and unexpected hemorrhage. Here, we report a case of an anatomic variant of IVC duplication that was found in the cadaver of an 81-year-old Japanese female during a student dissection course at Aichi Gakuin University School of Dentistry. We will describe the detailed anatomical features of this case, and will also discuss about this from the developmental aspect, since IVC duplication is caused by abnormal connections and regressions of subcardinal veins during embryonic development of the IVC. The authors have no conflicts of interest to declare.

(COI: No)

P1-242

The continuity of the marginal artery system distributing the stomach and duodenum

Kurachi, Moegi; Kudoh, Hiroyuki; Sakai, Tatsuo (*Juntendo Univ., Tokyo, Japan*)

The gastric arteries (GA), the gastro-omental arteries (GOA) and the pancreaticoduodenal arteries (PDA) run along the wall of stomach and duodenum to give off terminal branches (Vasae rectae) supplying them. The situation is similar to the outermost arcades of jejunal / ileal arteries and the marginal artery of the colon. We hypothesize that they constitute a continual marginal artery system in the abdominal gastrointestinal tract. The aim of this study is to examine the continuity of the marginal arteries in the stomach and duodenum region. 5 human abdominal gastrointestinal tracts were investigated. The marginal arteries sent off series of the terminal branches to a longitudinal band-like area on the wall of stomach and duodenum. The longitudinal band supplied from GOA was situated along the greater curvature of stomach, and continued to the band on the anterior surface of duodenum supplied by the anterior PDA, which continued to the band on the jejunal wall. The other band of stomach supplied by GA was located along the lesser curvature. The distributing area of the supraduodenal arteries connected the band on lesser curvature and the band on posterior duodenal wall supplied by the posterior PDA. The posterior duodenal band terminated at the Treitz ligament. These result suggested that the gastroduodenal artery and its terminal branches, the supraduodenal arteries, constitute the marginal artery system of the duodenum. The marginal artery system is thought to be formed as double stream until the Treitz ligament.

(COI: No)

P1-243

A case of duplicated inferior vena cava

Suzuki, Hiroyuki[1]; Sutou, Satoko[1]; Sugimoto, Shun[1]; Suga, Ryota[1]; Kurisaki, Tomohiro[2]; Aochi, Hidekazu[2]; Takano, Kazuhiro[2]; Fujita, Keiko[2]; Nagashima, Masabumi[2] ([1]*Saitama Med. Univ., Saitama, Japan;* [2]*Dept. Anat., Saitama Med. Univ., Saitama, Japan*)

A case of duplicated inferior vena cava (IVC) along with other anatomical vessel variations in a female cadaver is reported.

Duplicated inferior vena cava (D-IVC) is congenital venous anomaly has an incidence rate ranging from 0.3 % to 3 %. According to Adachi (1940), it has an incidence of 1.4 ± 0.34 %. More recently, diagnostic imaging has revealed that D-IVC tends to be found in patients with hydronephrosis and various other conditions. The clinical importance of this anomaly lies in three principal areas: the potential for misdiagnosis on imaging; technical difficulties during retroperitoneal surgery and their significance in relation to the etiology and management of venous thromboembolism. In this anomaly, the right and left iliac veins drain into ipsilateral vena cavas that ascend on either side of the abdominal aorta until they form a confluence at the level of the renal veins. During the anatomical practice at Saitama Medical University in 2014, a case of D-IVC was observed in a 93-year-old Japanese female cadaver. Bilateral inferior vena cava, left sided IVC receiving a left renal vein, and transiliac vein were found through dissection. In this study, we will discuss the literature with respect to embryological studies, morphological classifications, and disease correlations.

(COI: No)

P1-244

Observation of the lenticulostriate arteries

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The importance of the lenticulostriate arteries (LSA) was pointed out because the arteries distributed to the internal capsule, caudate and lentiform nucleus. Recently, with the development of 7.0-T MRI (although it has not been spread) the imaging of the arteries has been reported (Kang et al, 2009). We think, however, the observation results with the gross anatomy method on the arteries are very necessary, because it is the basic data of the arteries and could to offer consultations for the MRI imaging. For the purpose we investigated the LSA with twenty-four Japanese brain specimens (fixed with 15% formalin and preserved in 50% alcohol for 6 months), to report the branch number, branch pattern, the distance between the confluence point of the internal carotid artery with the posterior communicating artery to the origin point of the LSA.

(COI: No)

P1-245

Thoracic insufficiency syndrome in an elderly woman

Taniguchi, Jumpei; Kaidoh, Toshiyuki; Okazaki, Kenji; Nakane, Hironobu; Naguro, Tomonori; Mukuda, Takao; Koyama, Yuka; Kameie, Toshio; Inaga, Sumire (*Fac. Med., Tottori Univ., Yonago, Japan*)

During the demonstration of an anatomical dissection for medical students at Tottori University, a narrow left thoracic cavity and flattened left lung was observed in an elderly woman who had died of pneumonia. Autopsy findings also included left scoliosis and deformity of the upper four ribs on the left side. These findings in the thorax and the left lung suggested that this patient had thoracic insufficiency syndrome (TIS), which is characterized by the inability of the thorax to support normal respiration or lung growth. Further histological examination of the lung tissues showed an absence of alveoli in the left lung. Therefore, the hypoplastic left lung was thought to have had negligible respiratory function. This suggests that hypoplastic lung tissue in TIS does not recover throughout life without appropriate treatment. The right lung, however, showed compensatory enlargement and had well-developed alveoli, although filled with inflammatory cells. Although the right lung had provided adequate respiratory function over the lifetime of the patient, pneumonia in the one normally functioning lung proved fatal in this elderly woman. This finding suggests that early surgical treatment is desirable to promote development of the thorax and lung on the affected side. Medical professionals should be aware of the potentially fatal consequences of pulmonary infections in limited functioning lung tissue in elderly TIS patients.

(COI: No)

P1-246

Two atypical cases of vertebral arteries

Aoyama, Masaya; Iwahashi, Yumi; Ueyama, Takashi; Ito, Takao; Yamamoto, Yuta; Tsuruo, Yoshihiro (*Wakayama Med. Univ., Wakayama, Japan*)

In general, the vertebral arteries arise from the subclavian arteries, and enter the transverse foramens of the sixth cervical vertebra and pass through the transverse foramen of C1 (the atlas). They travel medially and posteriorly along the posterior arch of C1, penetrate the dura mater and enter the foramen magnum. We encountered two cases of vertebral arteries with anomalous origins, and courses in a Japanese 93-year-old (case 1) and a 101-year-old (case 2) Japanese female cadavers during the student dissection practice at Wakayama Medical University at 2012. In case 1, the left vertebral artery directly branched from the aortic arch and entered the left transverse foramen of the fourth cervical vertebra. The right vertebral artery branched from the right subclavian artery and entered the right transverse foramen of the fourth cervical vertebra. In case 2, the left vertebral artery ran medially into the vertebral canal immediately though the transverse foramen of the axis instead of passing through that of the atlas (a C2 segmental type of vertebral artery). The vertebral artery on the right side and the cervical nerves on both sides were in a conventional position.

(COI: No)

P1-247

The relationship between a maxillary sinus and superior alveolar nerves and vessels demonstrated by cone-beam CT combined with μ-CT and histological analyses

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Background: There is no available detailed data on three-dimensional courses of human superior alveolar nerves and vessels. This study aimed to clarify the relationship between a maxillary sinus and superior alveolar nerves and vessels using cone-beam computed tomography (CT) combined with μ-CT and histological analyses.
Methods: DICOM data obtained from the scanned heads/maxillae of cadavers for undergraduate dissection practice and skulls using cone-beam CT (MercuRay; Hitachi) were reconstructed into the three-dimensional (3D) images using a software (INTAGE Realia; KGT). The 3D images were compared with μ-CT (Elescan; Nittetsu Elex) images and histological sections.
Results: Cone-beam CT clarified the relationship between a maxillary sinus and the superior alveolar canals/grooves. The main anterior superior alveolar canal/groove ran anteriorly through the upper part of sinus, and terminated at the lower part of piriform aperture. The main posterior one ran through the lateral lower part of sinus and communicated with the anterior one. Histological analysis demonstrated the existence of nerves and vessels in these canals/grooves.
Conclusions: The cone-beam CT is suggestive to be the useful method to clarify the superior alveolar canals/grooves including nerves and vessels at the level of histological section.

(COI: No)

P1-248

Development of the skeletal model with facial and masticatory muscles to reproduce a three-dimensional positional relationship between these muscles

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Objectives: This study aimed to dissect the precise courses of facial and masticatory muscles and develop the skeletal model with muscles to reproduce a three-dimensional positional relationship between these muscles.
Methods: During the anatomical dissection courses held in the Nippon Dental University at Niigata and Niigata University, we investigated a three-dimension positional relationship between facial and masticatory muscles using human cadavers. Based on these findings, we made the prototype of facial and masticatory muscles using epoxy putty.
Results: There were variations in the courses of facial muscles between cadavers and even in the same cadaver. Some muscles changed their courses depending on distributions of blood vessels. Finally, we succeeded to make the prototype of facial and masticatory muscles to reproduce a three-dimensional positional relationship between these muscles based on anatomical knowledge.
Conclusions: Although there was moderate regularity in the regional distribution of facial muscles, different facial muscles compensated their spread each other, resulting in variations in the courses of facial muscles. The skeletal model with facial and masticatory muscles based on this knowledge is useful to understand a three-dimensional positional relationship between these muscles.

(COI: No)

P1-249

Comparative anatomical analysis of the itch neural circuit in mammals

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Recently, the spinal gastrin-releasing peptide (GRP)-receptor has been identified as an itch-specific mediator in the somatosensory system. We focused on GRP as a marker of itch neural circuit and demonstrated the expression of GRP in the small-sized dorsal root ganglion and trigeminal ganglion (TG) neurons, and axon terminals in the superficial layers of the spinal dorsal horn and trigeminal sensory nucleus caudalis (Vc) in male rats. In order to compare the GRP distribution in different mammalian species, we used male mouse (rodent), male suncus (insectivore), and male monkey (primate) by staining with toluidine blue and immunohistochemistry. Morphometric analysis showed that GRP was expressed in 7% of mouse, 12% of rat, and 9% of suncus TG neuron. GRP was found in small-sized TG neurons in mouse, rat, and monkey but in various types of suncus. GRP terminated the superficial layers of the spinal dorsal horn and Vc in mouse, rat, suncus, and monkey. These findings indicated that GRP is common mediator to mammalian sensory neurons.

(COI: No)

P1-250

Comparative study of the innervation pattern to the plantaris muscle between human and non-human primates

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In phylogenetically, the plantaris muscle (PM) constantly exists in non-human primates, though this muscle often lacks in human. To elucidate its phylogeny, it might be useful to examine and compare detailed innervation patterns in the human and non-human primates. We compared innervation pattern of the PM in specimens of human (6 sides; 1 side of these lacked the PM), chimpanzee (2 sides) and rhesus monkey (1 side). Epineurium of the tibial nerve was peeled in all specimens. In the rhesus monkey and chimpanzees, the nerve to the PM (NP) formed a common trunk with the nerve to the flexor digitorum fibularis muscle (NF). But, in one chimpanzee, an additional muscular branch to the soleus was found (NS2), which formed a common trunk with the NP. In all human cases, the branch equivalent to the NS2 of the chimpanzee existed and formed a common trunk with the NP. The NP forming the common trunk with the nerve to the gastrocnemius (NG) and soleus (NS) was found only in one human case. In the case in which the PM lacked, the NS2 formed the common trunk with the NG and NS in addition to the NF. These results suggest that in the human case in which the PM is lacking, muscular component of the PM might be mingled with the bipennate muscle part of the soleus innervated NS2 in the human to adapt increasing antigravitational activity during the evolutionary change.

(COI: No)

P1-251

Comparative anatomy of the teres major muscle in a rough-toothed dolphin (*Steno bredanensis*)

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In most textbooks (Romer & Parsons 1977; Stark 1982), the teres major muscle (tm) in mammals is described to be differentiated from the latissimus dorsi muscle (ld). However, Koizumi (2012, 2013) has clarified that the tm or its relevant muscles in monotremes and monitors had a close relationship with the subscapularis muscle (sb), not with the ld. This fact was confirmed in humans (Kato 1989). On the other hand, the tm of dolphins has been reported to receive the branch of the thoracodorsal nerve that innervated the ld. (Sekiya 2011; Takakura 1997). Therefore, in this study we have clarified the innervation of the tm in both arms of a rough-toothed dolphin. Results: the scapular spine and infraspinous fossa cannot be distinguished from each other and formed a flat surface. From the caudal one-third of this surface the tm was originated and inserted into the humerus joining with the ld. The thoracodorsal nerve and the several subscapular branches were branched off from the posterior cord, formed by the ventral rami of the lowest four cervical nerves (C5-8) and the first thoracic nerve (Th1). The tm was supplied by two different nerves. One was from the subscapular branches and the other from the thoracodorsal nerve. Observing the intramuscular distribution, the subscapular branches distributed into the cranial two-thirds of the tm and the branch from the thoracodorsal nerve supplied the caudal one-third of the tm. Based on this observations we will discuss the dual origin of the tm and the relationship among tm, ld and sb in dolphins.

(COI: No)

P1-252

Effects of decrine of mechanical stress on structure of tibial articular cartilage and growth plate in rats

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Purpose: Articular cartilage and growth plate have same embryological origin, but their difference in reactions of these cartilage to decrease in mechanical stress hasn't been reports. This study aimed to compare and investigate differences of reactions of the articular cartilage, the growth plate and epiphyseal cancellous bone to decreasing in mechanical loads.
Materials and methods: Five weeks old rats (wistar strain, male) were used as materials. They were divided into tail-suspended group (TS) and control (CO). Furthermore, TS was divided into three groups (TS1, TS2 and TS3), CO was also divided into similar groups (CO1, CO2, CO3). TS1, TS2 and TS3 were tail-suspended for 1, 2 and 3 weeks, respectively, and CO were fed normally in same periods as TS. They were killed under euthanasia, knee joints were excised. Those structures were observed histologically and their histomorphometrical data were measured.
Results: Thickness of middle layer of articular cartilage decreased at middle and posterior portions and thickness of cartilage also decreased wholly, in CO. On the other hand, little changes of thickness of that were recognized in even TS3. Many TRAP positive cells were found at subchondral bone in TS, compared to CO. Size of chondrocytes slightly decrease in growth plate of TS, but thickness of each cell layers was same as CO.
Conclusion: It was suggested that mechanical loading might be important factor for decrease in thickness of the articular cartilage with growth, and this was defer from in the case of growth plate that related to bone growth.

(COI: No)

P1-253

Immunohistochemical localization of ectonucleotide pyrophosphatase/phosphodiesterase-1(ENPP-1) and tissue-nonspecific type alkaline phosphatase (TNALP) in bone

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Introduction: Ectonucleotide pyrophosphatase/phosphodiesterase-1 (ENPP-1) is an enzyme which synthesizes a pyrophosphate - an inhibitor of mineralization -, *e.g.*, from extracellular adenosine nucleotide. In contrast, tissue-nonspecific alkaline phosphatase (TNALP) divides a pyrophosphate into two PO4-. Both molecules are involved in bone mineralization, so that, in this study, we have examined the immunolocalization of TNALP and ENPP-1 in bone.
Materials and Methods: Male C57BL/6 mice with the age of around 8-weeks old (control group) and the mice injected with hPTH (1-34) with a regimen of 80μg/kg/day, twice/day (PTH group) were fixed, and their femurs were embedded into paraffin for histochemical detection of ENPP-1 and TNALP.
Results and Discussion: In the femoral metaphyses, TNALP-immunoreactivity was seen mainly in osteoblasts and overlying preosteoblasts, while ENPP-1 positivity was observed in osteoblasts and osteocytes embedded in the bone matrix. Unlike the control group, PTH-administered femora showed many trabeculae surrounded with a thick layer of preosteoblasts. An intense TNALP reactivity was detected in the thick layer of preosteoblasts and plump osteoblasts. Interestingly, ENPP-1 immunoreactivity was observed in some groups but not all of osteoblasts, as well as osteocytes. Thus, TNALP tends to localize preosteoblasts and osteoblasts, while ENPP-1 is seen in mature osteoblast and osteocytes.
(COI: No)

P1-254

Ultrastructural phenotypes of preosteoblasts and bone marrow stromal cells in tibial metaphyses in mice

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Purpose: Preosteoblasts are identified as osteoblastic precursors, which are localized over the mature osteoblasts and able to proliferate. Scott has verified ultrastructures of preosteoblasts by using 3H-thymidine electron microscopic autoradiography, but their ultrastructural phenotypes are still veiled. In addition, it is difficult to distinguish preosteoblasts from bone marrow stromal cells. In this study, we examined ultrastructures of preosteoblasts in the murine metaphysis.
Materials and Methods: Eight weeks-old ICR mice were perfused with a mixture of paraformaldehyde and glutaraldehyde solution, and then tibiae were extracted for additional immersion with the same fixatives. The specimens were decalcified with 5% EDTA and embedded into epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate prior to TEM observation.
Results and discussion: Bone marrow stromal cells showed flattened or spindle shapes, possessing lysosomes, lipid droplets and Golgi apparatus. In contrast, there were at least, in part, two phenotypes of preosteoblasts: one is a cell with well-developed rough endoplasmic reticulum (rER) and Golgi apparatus, which suggested their potential to synthesize extracellular matrix. The others lacked abundant rER, but included many vesicles and Golgi apparatus. Thus, our ultrastructural study suggests, at least, two distinct phenotypes of preosteoblasts in murine metaphyses.
(COI: No)

P1-255

Disrupted alveolar bone surrounding tooth germs in transgenic mice overexpressing parathyroid hormone-related peptide (PTHrP)

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Purpose: Parathyroid hormone-related peptide (PTHrP) has been reported to play a pivotal role in the development of the tooth germs, as well as bone and cartilage development. In order to verify to which tissues PTHrP predominantly affect the biological function, we have examined tooth germs and surrounding alveolar bone in mandibles of PTHrP overexpressing transgenic (Tg) mice.
Materials and Methods: Tg mice overexpressing PTHrP were generated by inserting the PTHrP cDNA downstream type I collagen promoter specific to osteoblasts. Mandibles of E18 fetuses embedded in paraffin were histochemically examined for tissue nonospecific alkaline phosphatase (ALP), tartrate-resistant acid phosphatase (TRAP), and ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1).
Results and discussion: PTHrP Tg mice showed no obvious histological abnormality of the teeth germs of molars, which revealed normally-developed enamel organs encompassed by dental follicules. But instead, there was only a few surronding alveolar bone and a huge amount of ALP-positive preosteoblastic cells throughout accompanied with a few blood vessels. ENPP1-positive mature osteoblasts and TRAP-reactive osteoclasts were shown to focally accumulate the alveolar bone surfaces. Meckel's cartilage did not seem to be enlarged. Thus, our histological findings suggest that, unlike previous reports, PTHrP would regulate cell proliferation/differentiation in alveolar bone rather than tooth germs at the fetal stage.
(COI: No)

P1-256

Ultrastructural observation on osteoblasts and osteocytes in c-fos deficient mice

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Purpose: Mature osteoblasts synthesize bone matrices, with being differentiating into osteocytes. Unlike wild-type mice, *c-fos* deficient (*c-fos*$^{-/-}$) mice lack osteoclast, and therefore, cell coupling between osteoclasts and osteoblasts does not take place. Taken together, we have attempted to verify the ultrastructural features of osteoblasts and osteocytes in the circumstance lacking cell coupling from osteoclasts.
Materials and Methods: Twelve weeks-old *wild-type* and *c-fos*$^{-/-}$ mice were perfused with an aldehyde solution, and then, femora were extracted. The femoral specimens were decalcified with 5% EDTA and embedded into epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate prior to TEM observation.
Results and Discussion: *Wild-type* mice showed mature osteoblasts with abundant rER and Golgi apparatus throughout the plump cell bodies, while *c-fos*$^{-/-}$ mice did not have such mature osteoblasts. But, instead, *c-fos*$^{-/-}$ trabecules localized bone marrow cells and fibroblast-like cells with many vesicles extending their cytoplasmic processes. Despite no mature osteoblasts, *c-fos*$^{-/-}$ specimens had osteocytes including fewer cell organelles with the nucleus becoming more prominent, and the lacunar walls showed lamina limitans, electron dense organic materials. Taken together, cell differentiation into mature osteoblasts appears to be disturbed due to a lack of osteoclasts, and the osteocytes in *c-fos*$^{-/-}$ mice seem to be previously embedded in bone matrix during their modeling period.
(COI: No)

P1-257

Generation of *tardbp* deficient zebrafish using CRISPR/Cas9 system

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Transactive response DNA binding protein-43 (*tardbp*) gene is one of responsible genes of Familial Amyotrophic Lateral Sclerosis (FALS), which is characterized by the loss of upper and lower motor neurons. In this study, we knocked out zebrafish *tardbp* and its paralogue *tardbp*-like (*tardbpl*) genes using the CRISPR/ Cas9 system to analyze the mechanisms of neural degeneration in ALS patients and relationship between the neurogenesis of motor neurons and angiogenesis. Although *tardbpl* gene lacks the glycine-rich domain where many ALS associated mutations are reported, it is upregulated to compensate *tardbp* function in its mutant. So we designed and synthesized sgRNAs for the targeted sequence of both *tardbp* and *tardbpl* genes. Genome DNAs were extracted from the sgRNA-injected embryos at 1 day post fertilization (dpf) and then analyzed the activity for genome modification by Heteroduplex Mobility Assay (HMA). Furthermore, we analyzed the vascular morphogenesis in *tardbp* and *tardbpl* mutant using *Tg(fli1a:EGFP)*^{y1} embryos, in which the endothelial cells specifically expressed the EGFP. As a result, we succeeded to confirm the activity of each sgRNA for targeted genome editing by HMA analysis and observe aberration of intersegmental vessel in sgRNAs and Cas9 mRNA injected embryos at 2dpf. In future, we will generate homozygotic *tardbp* and/or *tardbpl* mutant zebrafish and elucidate mechanism of neural degeneration in ALS and the involvement of neurogenesis in angiogenesis.
(COI: No)

P1-258

Cyclin B3 is involved in leg regeneration of the cricket *Gryllus bimaculatus*

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Several animals such as planarian, cricket and newt have remarkable regenerative capacity to restore the lost part of limb completely although regenerative capacity of higher vertebrate including human is limited. Almost all genes are conserved in the genomes between both regenerative animals and non-regenerative animals, suggesting that regenerative capacity of human could be regained by modifying gene(s) function. The cricket *Gryllus bimaculatus* is an emerging model animal for regeneration biology. When we amputate leg of cricket nymph, cricket regenerates the lost part of leg through several molts. Our previous studies showed that tumor suppressor Hippo pathway suppresses cell proliferation during regeneration. In this study, we focus on the function of cyclin B3, which is a target of Hippo pathway, during regeneration. We cloned partial fragments of *Gryllus* homologue of *cyclin B3* (*cycB3*) and performed RNAi. The amount of *cycB3* mRNA was decreased by RNAi, but amount of *cycB* mRNA was not decreased as revealed by quantitative PCR. In *cycB3*(RNAi) cricket, lost part of leg was regenerated but the size of regenerate was smaller than that of control. These results suggest that cyclin B3 may promote cell proliferation during regeneration to restore the lost part. We cloned partial fragments of *Gryllus* homologue of *cycB*. We will discuss cooperative functions of *cycB3* and *cycB* during regeneration.
(COI: No)

P1-259

Chronological changes in prosaposin receptors immunoreactivity in rat brain after birth

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Prosaposin (PSAP) is the precursor of saposins A-D. Many reports suggest that PSAP is a neurotrophic factor in vivo and in vitro that induces differentiation and prevents death in a variety of neuronal cells. We previously reported the chronological changes in PSAP immunoreactivity and in the mRNA expression of PSAP in developing rat brain using in situ hybridization. Abundant PSAP expression in the perinatal stages indicates a potential role for prosaposin in early rat brain development (Xue et al. 2011). Recently, the G protein-coupled receptors GPR37 and GPR37L1 were recognized as PSAP receptors. In the present study, we examined changes in immunoreactivity against the PSAP receptors GPR37 and GPR37L1 in rats. In rat brain at 1, 2, or 4 weeks after birth, many neurons in the cerebral cortex showed intense or weak immunoreactivity against GPR37 and GPR37L1. In particular, in rat cerebral cortex at 1 week after birth, neurons in the lower layers showed intense immunoreactivity. Also, in the hippocampus and dentate gyrus, many neurons with receptor immunoreactivity were observed.

(COI: No)

P1-260

Deletion of ATF6α enhances Kainate-induced neuronal death in mice

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High level of glutamate results in neuronal degeneration/death in various pathological conditions including epilepsy and stroke. We previously reported that ORP150, a molecular chaperone in the endoplasmic reticulum (ER), protected hippocampal neurons against glutamate-induced neuronal death (Kitao et al., 2001). However, the role of ATF6 α , a transcriptional factor important for the chaperone expression, was not clear yet in such situations. We, therefore, analyzed the activating status and the role of ATF6 α in a mouse model of glutamate-induced neuronal death. When kainate (KA), a strong agonist of glutamate receptor, was injected into the CA3 region of the hippocampus in wild-type (WT) mice, enhanced expression levels of ORP150 and GRP78, both are downstream gene products of Atf6 α , were observed, suggesting activation of ATF6 α in this model. We then estimated the level of neuronal damage in both WT and Atf6 α -/- mice. Higher levels of neural degeneration and neuronal death were observed in Atf6 α -/- mice, while no significant difference were observed in glial cell activation between two genotypes. Further analysis revealed that expression level of c-fos, a marker of neuronal activity, was higher in Atf6 α -/- mice after KA injection. Injection of thapsigargin, an inhibitor of calcium uptake into the ER, also caused higher level of neuronal death in Atf6 α -/- mice. These results suggest that ATF6 α plays important roles for neuronal survival after KA injection through the regulation of calcium response and neuronal activity.

(COI: No)

P1-261

Changes of serotonin-positive neuron in rat medulla oblongata during hypoxia

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Hypoxia activates the neurons in ventral respiratory group (VRG) and dorsal respiratory group (DRG) in medulla oblongata to increase tidal volume and respiratory frequency. It is reported that concentration of serotonin (5-HT) rise in the VRG and DRG during hypoxia. Thus we immunohistochemically examined changes of serotonergic neurons in medulla of rats exposed to hypoxia (10% O2) for 1, 2, 4 and 6 hr. Using antibodies against Fos, 5-HT, tryptophan hydroxylase 2 (TPH2) and Ser-19 phosphorylated TPH2 (pTPH2). In the rat exposed to hypoxia, Fos-labeled neurons were observed in the paragigantocellular reticular nucleus, lateral part (PGRNl) in the VRG, and nucleus of the solitary tract, gelatinous part (NTSge) and medial part (NTSm) in the DRG. In PGRNl, 5-HT-immunoreactivity in the nerve fibers were significantly increased in rats exposed to hypoxia for 2, 4 and 6 hr in rostral part, and 1, 2, 4 and 6 hr in caudal part compared with control ($p<0.05$). In the NTSge and NTSm, 5-HT-immunoreactivity were increased in 2 hr ($p<0.05$). In these areas, 5-HT-immunoreactive nerve fibers were observed close to the Fos-immunoreactive cells by double immunofluorescence. On the other hand, nerve cell bodies immunoreactive for TPH2 and pTPH2 were distributed in nucleus raphe magnus, nuculeus raphe obscurus, nucleus raphe pallidus and gigantocellular reticular nucleus pars α . The number of the immunoreactive neuron in these nuclei did not change by hypoxic exposure. In conclusion, it is suggested 5-HT is increased in VRG and DRG during hypoxia to modulate respiratory drive.

(COI: No)

P1-262

A gap between adjacent surfaces deteriorates depth perception based on binocular correlation computation

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The visual system computes depth from binocular disparity. The initial encoding of disparity is achieved by computing cross-correlation between left-eye and right-eye images. When luminance contrast of either image is reversed (binocularly anti-correlated), neurons signaling the cross-correlation show inverted disparity tuning. It remains elusive whether the correlation-based signals are exploited by the brain to produce depth perception. We previously showed that anti-correlated stereograms (aRDSs) evoke reversed depth, suggesting that the brain does use the signals (Tanabe et al., 2008). However, Hibbard et al. (2014) found no reversed depth for stimuli that had a gap between a patch and its surround but were otherwise similar to ours. Here we examined effects of a gap between the two surfaces on reversed depth. Subjects were shown a concentric-bipartite RDS and reported whether the center patch was nearer or farther than the annular surround. The patch was either a contrast-matched RDS (cRDS) or an aRDS with crossed or uncrossed disparities, while the surround was always a cRDS at 0 disparity. Most subjects (8 out of 12) perceived reversed depth for aRDS patches with a gap of <0.175 deg. Reversed depth diminished as the gap became wider, and disappeared when a gap was 0.7 deg wide. A small gap thus profoundly affected reversed depth, suggesting that correlation-based depth signals are integrated over a spatially limited range of visual field.

(COI: No)

P1-263

Gradual loss of visual ability in the animal model of retinitis pigmentosa

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The Royal College of Surgeons (RCS) rat is an animal model of retinitis pigmentosa, losing the visual ability gradually over time, and has been used for the various recovery studies. However, the time course of the visual loss has not been examined quantitatively in a behavioral test because visual degradation is possibly progressive before RCS rats complete the learning of the behavioral task. Moreover, it remains unknown how the visual responses of neurons in the geniculocortical pathway diminish with time. To answer these questions, we established a new method of task-training enabling one-week learning of the two-alternative forced-choice visual grating detection task. Rats were trained at 4 weeks of age, and measured the contrast threshold from 6 to 11 weeks of age every day. We found that the visual ability of pattern vision was diminished from 7 to 8 weeks of age, and thereafter, fell to an unmeasurable level. To examine the neuronal basis of the grating detectability degradation, we conducted the extracellular recordings from the dorsolateral geniculate nucleus (dLGN), the primary visual cortex (V1), and the superior colliculus (SC) of awake RCS rats at various ages, finding that the visual responses to grating stimulus in these regions were decreased in a similar time course to the behavioral performance. Now, we are conducting the additional measurement of behavioral and neuronal visual sensitivity to brightness vision using a high luminous flash stimulus.

(COI: No)

P1-264

Schizophrenia-relevant symptoms were displayed in *Zinc Finger Protein 521 (ZFP521)* knockout mice

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Zinc finger protein 521 (ZFP521) in the mice, also known as ZNF521 in humans, is a nuclear protein. ZFP521 regulates the differentiation of several kind of stem cells in a wide range of tissue, such as osteoblast formation and adipose commitment and differentiation. In the field of neurobiology, it is reported ZFP521 is an essential factor for transition of epiblast stem cells into neural progenitors *in vitro*. However, the role of *ZFP521* in the brain *in vivo* still remains elusive. To elucidate the role of ZFP521 in the mouse brain, we generated *ZFP521* knockout (*ZFP521*$^{-/-}$) mice and analyzed them in detail.

Although *ZFP521*$^{-/-}$ mice were smaller than *ZFP521*$^{+/+}$ and *ZFP521*$^{+/-}$ littermates, they had no apparent defect in the body. They displayed abnormal behavior, such as hyper-locomotion, lower anxiety, impaired learning and deficits in prepulse inhibition, which correspond to the symptoms of schizophrenia. The border of the granular cell layer of the dentate gyrus in the hippocampus of the mice was indistinct and granular neurons were reduced in number. Furthermore, Sox1-positive neural stem cells in the dentate gyrus and cerebellum were significantly reduced in number. Taken together, these findings indicate that ZFP521 affects the formation of the neuronal cell layers of the dentate gyrus in the hippocampus, and thus *ZFP521*$^{-/-}$ mice displayed schizophrenia-relevant symptoms.

(COI: No)

P1-265

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. Using Ftsj1 knockout mice, we demonstrate that Ftsj1 methylates cytosolic transfer RNAs (tRNAs) at position 32 and 34. While the FTSJ1 KO mouse developed normally, we observed a decreased protein synthesis level in hippocampus of FTSJ1 KO mice using puromycin-mediated in vivo pulse-labeling technique. Especially, there was a marked decreased of synaptic proteins including glutamate receptors and signaling molecules. The decreased protein synthesis level resulted in the electrophysiological and morphological abnormalities in hippocampal neurons of FTSJ1 KO mice. There results suggest that the accumulation of hypomodified tRNAs disturbs neuronal protein synthesis, which ultimately contributes to the development of mental retardation in Ftsj1-deficient mice and human.

(COI: No)

P1-266

Spatiotemporal recalibration of inferred motion in monkeys

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Even when a moving object is temporally occluded behind a stationary object, we can precisely predict when and where it reappears. Previous studies suggest that both the parietal cortex and the cerebellum are involved in the inference of visual motion. As a step toward understanding the neural mechanism, we have developed a behavioral paradigm that requires spatial and temporal recalibration of inferred motion. Experiments were conducted on two Japanese monkeys. A target spot moved obliquely at 20°/s. After 500 ms, it was occluded behind a stationary rectangle that was visible throughout the trial. Monkeys were trained to make a predictive saccade to the target that reappeared on the other side of the rectangle. In the spatial adaptation paradigm, the location of target reappearance was displaced by 5° horizontally. In the temporal adaptation paradigm, the target reappearance was delayed or preceded by 200 ms. These trials were presented in separate blocks. After 600 spatial adaptation trials, saccade endpoints were shifted by 3.7 ± 0.5° (SD, n = 8), while saccade timing remained unchanged. Likewise, after 600 temporal adaptation trials, saccade timing altered by 107 ± 17 ms (n = 8), while saccades remained accurate. Although adaptation of predictive saccades did not transfer to visually-guided saccades, adaptation of visually-guided saccades altered the metrics of predictive saccades. These results suggest that the spatial and temporal aspects of inferred motion might be subject to separate recalibration mechanisms, which appear to be different from the saccade adaptation mechanism in the medial cerebellum.

(COI: No)

P1-267

The secondary auditory cortex receives topological projections from the ventral division of the medial geniculate body in mice

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It is generally known that the belt region including the secondary auditory cortex (AII) receives thalamic inputs from the dorsal division (MGd) of the medial geniculate body (MGB) that is not structured tonotopically. Recently, however, a robust tonotopic structure was revealed in AII in mice. Here, we verified the possibility that the mouse AII receives topological projections directly from the ventral division of MGB (MGv) which is structured tonotopically. We identified the precise location of AII in C57BL/6 mice using flavoprotein fluorescence imaging. When 5-60 kHz tones were presented, a clear tonotopic gradient traveled ventrally. Next, we injected Alexa Fluor 488- or 555-conjugated CTB, a retrograde tracer, into a 5 or 35 kHz area in AII to investigate the location of neurons projecting from MGB to AII. Three days after injection, we performed cardiac perfusion and prepared coronal sections. Immunostaining of non-phosphorylated neurofilament (NNF) in adjacent slices was used to parcellate subdivisions of MGB. We obtained three positive results as follows. First, majority of neurons projecting to AII were located inside MGv. Second, neurons projecting to MGv were localized in the caudal part of the MGv. Finally, neurons projecting to the 5 kHz or 35 kHz area were different in location from ventrorostral to dorsocaudal. These results strongly suggest that the caudal part of MGv has a distinct tonotopic gradient, and the tonotopic gradient of AII reflects topological projections originating in the caudal part of MGv.

(COI: No)

P1-268

Effects of D/L-Valine on tongue movement in the isolated brain stem-spinal cord intact tongue preparation

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The tongue is composed of sensory system as a taste, and motor system such as mastication, swallowing and vocalization. Sense of taste on the surface of the tongue and sent to the brain can feel only sweetness, sourness, saltiness, bitterness and umami. In the rat, at first the gustatory nerve connects to 1) the solitary tract nucleus, toward reticular formation; 2) the solitary tract nucleus, toward parabrachial nucleus and reaches taste area in cortex. We designed the preparation remained taste circuit keeping sensory-motor connection, so we produced isolated brain stem-spinal cord intact tongue preparation including solitary tract nucleus, parabrachial nucleus, facial nucleus. Moreover, we examined the effects of sweet amino acid D-valine and bitter amino acid L-valine on tongue muscle activity as a tongue movement. The tongue movement was recorded by bipolar-tungsten electrode inserted to tongue muscle. Application of D-valine to tongue increased tongue movement after 5-10minutes from application, but L-valine inhibited tongue movement or showed long delayed effect in postnatal 0-2-day-old rat. We also examined developmental changes of D-/L-valine effects. In the embryonic day 16 (E16), the tongue movement was irregular and D-/L-valine effects were invisible, but in the embryonic day 18 (E18), the tongue movement detected clearly and the effects of that were seen. These results suggested that we succeeded the useful preparation for analysis of taste circuit, and we showed difference influence of between bitter and sweet sense.

(COI: No)

P1-269

Developmental changes in synaptic plasticity of the hippocampal CA1 neurons by contextual memory

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Neural mechanisms of brain keep changing during the course of a lifetime. Although our previous study showed a developmental relationship between contextual learning and dorsal hippocampal ACh levels (Takase et al., Sci Rep, 2014), it has not revealed the developmental changes of post-synaptic currents by contextual learning. In this study, we compared miniature excitatory and inhibitory post-synaptic currents (mEPSC and mIPSC) between contextual learning trained and untrained rats in 3-weeks, 4-weeks, 6-weeks and 8-weeks-old. As a learning model, we employed inhibitory avoidance (IA) task, and acute brain slices were prepared for patch clamp analysis. For untrained rats, 6-weeks and 8-weeks-old revealed higher mEPSC frequency than 3-weeks-old. Whereas, after employing IA task, 4-weeks-old trained rats showed higher mEPSC amplitude than that of untrained rats. In addition, IA trained rats showed higher mIPSC amplitude than untrained rats in 4-weeks, 6-weeks and 8-weeks-old. These results suggest that hippocampal CA1 synapses change with development of brain and contextual learning has a strong effect on CA1 synapses in 4-weeks, 6-weeks and 8-weeks-old rats. Thus, we conclude that the strengthening of hippocampal CA1 synapses by contextual memory may differ in developmental stages.

(COI: No)

P1-270

Changes in hippocampal CA1 neuronal activity and sympathetic nerve activity during fear conditioning in rats

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Hippocampus has been implicated in the emotional responses of sympathetic nerve acidity to fear, however there has been lack of direct evidence on the changes hippocampal neuronal and sympathetic nerve activity and on functional relationships between those activity during development of fear. The aim of the present study was to measure relationship between hippocampal neuronal activity and sympathetic nerve activity during fear conditioning. Wistar male rats were instrumented chronically with multiple electrodes for hippocampal CA1 neuronal activity and bipolar electrode for renal (RSNA) and lumbar sympathetic nerve activity (LSNA) and electroencephalogram. We gave each rat 5-sec tones co-terminating with a 1-sec, 5-mA foot shock twice a day that was carried out over 3 days. During the fear conditioning over 3 days, changes in hippocampal CA1 neuronal activity, RSNA, LSNA were measured simultaneously during quiet awake state in the rat's home cage. Heart rate decreased progressively due to the fear conditioning over 3 days. Hippocampal CA1 neuronal activity and RSNA increased due to fear conditioning, while LSNA decreased during fear conditioning. These data suggest that there is a neuronal positive coupling between hippocampal neural activity and RSNA during fear conditioning in rats.

(COI: No)

P1-271

Slow depolarization induced by dopamine in spinal motor neurons

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Dopamine plays important roles as a slow neurotransmitter in the nervous system. Dopamine was reported to have an excitatory effect on motor coordination (Whelan et al., 2000). The cellular mechanisms of dopamine action on the motor neurons and the origin of dopamine are not clarified yet. We studied the effect of exogenous dopamine on the membrane properties of the motor neurons in the organotypic slice culture of rat spinal cord prepared from P0 rat. Bath application of dopamine (10 μM) induced slowly developing depolarization in a motor neuron under current-clamp (peak amplitude of the depolarization, 11.2 ± 3.4 mV, n=4) whether tetrodotoxin (1 μM) was present in the bath solution or not, indicating that a postsynaptic mechanism is involved in the dopamine- induced depolarization. Then the effect of dopamine on the membrane conductance of a motor neuron under voltage clamp was studied with a potassium gluconate-based solution in the patch pipette. In the presence of tetrodotoxin (1 μM), the membrane currents were measured at different holding voltages (from -90 to +40 mV) under voltage-clamp before application of dopamine and after the inward currents reached a plateau level. The relationship between the change of the membrane currents and the holding potentials revealed that the change of the membrane currents was due to the reduction of the outward currents with a reversal potential, close to an equilibrium potential for potassium, -86 mV, indicating reduction of a potassium conductance.

(COI: No)

P1-272

Involvement of 5-HT_6 receptor in local feedback inhibition of the dorsal raphe serotonergic neurons

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In the dorsal raphe nucleus (DRN), many GABAergic neurons project to serotonergic (5-HT) neurons and regulate their activity. However, the mechanisms how such local inhibition is maintained remain unclear. In this study, we examined the roles of 5-HT receptors in the local GABAergic inhibitory circuits regulating 5-HT neuronal activity. In the organotypic raphe slice cultures, a $GABA_A$ receptor antagonist, bicuculline, increased 5-HT release. Similarly, an atypical antipsychotic, olanzapine, which potently antagonizes some 5-HT receptors, increased 5-HT release, and this effect was occluded in the presence of bicuculline. Among 5-HT receptors to which olanzapine has higher affinity, a 5-HT_6 receptor antagonist, SB399885, but not 5-HT_{2A} and 5-HT_{2C} receptor antagonists, significantly increased 5-HT release. Like olanzapine, SB399885 did not show further increase in 5-HT release in the presence of bicuculline, suggesting the involvement of GABA inhibitory inputs. Moreover, in acute raphe slice, both olanzapine and SB399885 significantly decreased spontaneous firing of the DRN Gad2-positive neurons, in which 5-HT_6 receptor mRNA was expressed. These results suggest that 5-HT_6 receptor plays an important role for maintaining activity of DRN GABAergic neurons as a feedback regulation of DRN 5-HT neurons.

(COI: No)

P1-273

Synchronized high frequency oscillation and the theta oscillation between the hippocampus and the amygdala after fear conditioning correlates freezing behavior

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Memory consolidation process occurs during rest stage including slow-wave sleep by sharp wave-ripple complex (SWRs) that observed in CA1 region of the hippocampus (HPC). The SWRs interaction of BLA and HPC to fear conditioning was unknown. To find out about this issue, we investigated the relationship of the freezing behavior reflecting fear memory and the synchronization of the high frequency oscillations from HPC and BLA during rest time. After recovery from the electrode implantation, rats were placed into the test box for foot shock. After the foot shock, rats were returned to their home cage, and local field potentials of HPC and BLA were recorded for 40-50 minutes. After 1 hour from foot shock, rats were placed into the test box again and record the freezing behavior. The synchronized high frequency oscillations (100-250 Hz) indicating SWRs between HPC and BLA were observed during the rest time at the home cage after foot shock. The synchronized theta oscillation with the synchronous events was observed in the BLA. This synchronized theta oscillation power showed a negative correlation to the freezing behavior. These results suggest that the HPC ripple affects the theta oscillation of the BLA and is involved in fear memory consolidation.

(COI: No)

P1-274

Multiple free-radical scavenging activity of alpha lipoic acid derivatives: An ESR study

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Objectives: α-lipoic acids are illustrated to have antioxidant activity in various ischemia/reperfusion models, but few studies reveal against which free radical species they have radical scavenging activity. In this study, using electron spin resonance spectrometry (ESR), we directly evaluated spectra of free-radical scavenging activity of α-lipoic acid derivatives and estimated IC_{50}.

Methods: We evaluated the following recently synthesized water-soluble α-lipoic acid derivatives: dihydrolipoate (DHL)-taurine-Zn complex, DHL-penicillamine-Zn complex, DHL-glutamate-Zn complex, DHL-norleucine-Zn complex, DHL-anthranilate-Zn complex, DHL-histidine-Zn complex, and DHL-Zn complex. Direct free radical scavenging activity was evaluated for the following free radical species; hydroxyl radical, superoxide anion, *t*-butyl peroxyl radical, ascorbyl free radical (AFR), 1, 1-diphenyl-2-picryl hydrazyl radical, and nitric oxide. *g*-CYPMPO, DMPO and c-PTIO were used as spin traps. Peroxidation in brain homogenate was evalutated by TBARS assay.

Results and Conclusion: All α-lipoic acid derivatives examined indicated concentration-dependent radical scavenging activity against all radicals examined except AFR. Although IC_{50} varied among radical species, antioxidant activity of α-lipoic acid derivatives is, at least, partially attributable to their direct free radical scavenging activity against multiple free radical species.

(COI: No)

P1-275

Changes in the neurons of the spinal cord in a chick model of spina bifida aperta

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Spina bifida aperta (SBA), a neural tube defect that occurs during embryonic development, is one of the most common human congenital defects of the central nervous system. It involves protrusion of the spinal cord and/or meninges through a defect in the vertebral arches and skin. Depending on the position of the lesion, SBA causes postnatal physical disabilities, including paralysis of the legs, a lack of bowel and bladder control, and hip, knee, and foot abnormalities after birth. Although the etiology of SBA remains unknown, the pathogenic mechanism is generally thought to be a disorder of neurulation, with a failure of neural plate closure. The leg dysfunction that occurs in this model was reported to be the result of a decrease in the number of interneurons at the spinal segments, which modulate the motor neurons that innervate dysfunctional muscles (Mominoki et al., 2006). These findings prompted us to further investigate abnormalities in the spinal motor neurons. We created SBA chicks by incising the roof plate of the neural tube in the embryo and studied the pathological changes in the spinal cord. Histological analyses revealed large spinal neurons, most likely motor neurons, at the level of the lesion in the SBA chicks with an irregular configuration compared to the normal control chicks. The number of large neurons did not differ between the SBA and control chicks, but the large neurons were densely packed.

(COI: No)

P1-276

Prosaposin and its receptors in the facial nucleus after facial nerve transection

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Prosaposin is the precursor protein of four small lysosomal glycoproteins known as saposins (saposin A-D). In addition to its role as a precursor protein, prosaposin acts as a neurotrophic factor. Both saposins and prosaposin are widely expressed in various tissues, although the brain, skeletal muscle, and heart cells predominantly contain unprocessed prosaposin rather than saposins. Prosaposin and prosapotide, a peptide containing the neurotrophic activity domain of prosaposin, promote neurite outgrowth, elevate choline acetyltransferase activity in neuroblastoma cells, and prevent programmed cell death in cultured neurons. We previously detected increases in prosaposin immunoreactivity and the expression of prosaposin mRNA in the rat facial nerve nucleus following facial nerve transection. Prosaposin mRNA expression increased not only in facial motoneurons, but also in microglia during facial nerve regeneration. In the present study, we examined the change in immunoreactivity of the prosaposin receptors GPR37 and GPR37L1 in the rat facial nucleus following facial nerve transection. In the facial nucleus on the transected side, many small cells, most likely glial cells, with strong GRP37L1 immunoreactivity were observed.

(COI: No)

P1-277

The new spontaneous mutant mouse allele for *dystonia musculorum*

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The spontaneous mutant mouse showing twisting body and limbs was found in the colony of Riken BRC (Riken *dt* mouse). Linkage mapping analysis suggested the chromosome 1 is mutated and this mouse is the new candidate allele for *dystonia musculorum* (*dt*) with abnormal *dystonin* (*Dst*) gene. To confirm this hypothesis, Riken *dt* mouse was mated with the newly created *Dst* gene trap mouse (Dst^{Gt}), in which actin-binding domain-containing isoforms are disrupted. Dst^{Gt} homozygotes show *dt* phenotype as Riken *dt* mouse but heterozygote is normal. Several littermates obtained from the heterozygous parents of Riken *dt* and Dst^{Gt} mice showed twisting body and limbs. These littermates also show the abnormal neurofilament staining on the sections in the nervous system. These results strongly suggested that Riken *dt* mouse is new *Dst* allele.

(COI: No)

P1-278

Netrin-5 is highly expressed in neurogenic regions of the adult brain

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Mammalian netrin family proteins, netrin-1, -3, and -4, are involved in targeting of axons, neuronal migration, and angiogenesis and act as repulsive and attractive guidance molecules. Netrin-5, a new member of the netrin family, has homology to the C345C domain of netrin-1. Unlike other netrin proteins, murine netrin-5 consists of two EGF motifs of laminin V domain (LE) and the C345C domain, but lacks the N-terminal laminin VI domain and one of the three LE motifs. Interestingly, netrin-5 is strongly expressed in the olfactory bulb, rostral migrate stream (RMS), the subventricular zone (SVZ), and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus, where neurogenesis occurs in the adult brain. Doublecortin (DCX)-positive neuroblasts co-express netrin-5 in both the SVZ and RMS, whereas GFAP-positive astrocytes do not. In the SGZ, DCX-positive neuroblasts co-express netrin-5, indicating that netrin-5 expression occurs in at least type 2b to type 3 cells. In type 1 cells, GFAP does not show co-localization with netrin-5 in the SGZ. Overall, these expression patterns of netrin-5 support the hypothesis that this molecule may play a fundamental role in adult neurogenesis. In addition, we also show developmental expression pattern of netrin-5.

(COI: No)

P1-279

Response of human mesenchymal stem/progenitor cells (hMSCs) on ischemic hippocampal homogenate

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Cerebrovascular disease is a devastating disease and places third position of the death in Japan. Observations made on animal models suggest that a potential therapy for disorders of the disease is the administration of adult hMSCs. However, it is still unclear how the hMSCs response in the implanted brain. To solve the issue, we conducted hMSCs culture incubating with ischemic brain homogenate (ibCM) or non-ischemic brain homogenate (bCM). Global ischemia was induced by 15 min transient common carotid artery occlusion. One day after, the hippocampus were removed and homogenated with α-MEM based medium (CM). The supernatant was diluted to 0.5mg/ml with 1% FBS containing CM, and applied to sub-confluent cultured hMSCs. The cells were collected and analyzed the gene and protein expressions by omix analysis at 1day. Furthermore, the total and dead cell numbers were counted. MSCs were immunostained 24-factors which were selected from the gene expressions. No differences were observed the cell numbers between ibCM and bCM exposure. ibCM hMSCs increased 98 genes and decreased 78 genes more than 2-fold. Immunocytostaining was confirmed an increment of CXCL1, CCL2, IL-6, Mn-SOD, HuD, thioredoxin1, amyloid β in ibCM hMSCs. Semi-quantification with chemokine protein array also determined an increase of them. These results suggest that transplanted hMSCs could communicate with host tissues/cells mediated by chemokines.

(COI: No)

P1-280

Oct-3/4 induces expression a DNA-repair enzyme O6-methylguanine-DNA methyltransferase (MGMT) through its epigenetic effects in glioblastomas

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Alkylating agents, such as temozolomide, are the most effective agents for the treatment of 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 for alkylating agents. MGMT expression is controlled by a methylation/demethylation of the cytosine phosphate guanosine (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. However, little is known regarding the MGMT expression in glioblastoma. We investigated whether Oct-3/4 involves in the sensitivity of temozolomide through the expression of MGMT in this study. Oct-3/4 overexpression resulted in decreased susceptibility to temozolomide thata accompanied upregulated expression of MGMT mRNA. As analyzed by genomic sequencing of bisulfate-modified DNA, Oct-3/4-expressing cells showed enhanced demethylation of CpG islands. In glioblastoma patients, Oct-3/4 expression were well correlate with the expression of MGMT mRNA and CpG-demethylation status of its promoter region. These results suggest that Oct-3/4 promotes the resistance against temozolomide of glioblastoma cells by upregulating MGMT expression through the epigenetic change of MGMT promoter region.

(COI: No)

P1-281

Analyses of the recruitment mechanism of TGFβ activity in the invasive glioma tissue

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Insidious invasions of glioma cells from primary tumor lesion toward surrounding normal brain parenchyma cause untreatable situations at the recurrence after surgical resection. 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 (TypeIII TGF β receptor) positive while CD309/VEGFR2 negative blood vessels, implying dominant contribution of TGF β 1 rather than VEGF in insidious glioma invasions. The most promising candidate for the source of TGF β might be the primary tumor mass. The mRNA levels of TGF β 1 were markedly higher in the primary tumor tissues than in non-tumor counter hemispheres. Primary tumor lesions are constituted not only but with significant amount of tumor associated macrophage-like cells (TAM). In comparison of the mRNA levels of primary cultured TAM and C6 cells, former was the dominant. However, since conversion from the latent form to the active form by protein processing is essential for TGF β to act, measurement of TGF β activity is indispensable to know the molecular mechanism of recruitment of the TGF β in glioma invasions. We are on the way to determine the dynamics of the activity through assessments of culture media and ECMs where latent form of TGF β deposits. We would like to discuss about therapeutic possibility of the source of TGF β and the molecular mechanisms of the recruitment of the activities.

(COI: No)

P1-282

Quantitative analysis of axonal elongation and dynamics of neuronal mitochondria by using confocal time-lapse imaging

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Neuronal migration and axonal maturation occur during the construction of neural network. The process of axonal elongation needs proper transport and localization of proteins, lipids, and organelles. It is known that mitochondria produce ATP and localize at growth cones, and inhibiting mitochondrial transport and localization causes abnormal formation of axons. However spatial and temporal relation between mitochondrial dynamics and morphological development of axon is poorly understood. In order to study this relationship, we performed confocal time-lapse imaging using dissociated hippocampal neurons expressing mitochondrial marker (mitochondrial outer membrane protein Omp25C tagged with EGFP; OMP-EGFP) and DsRed2. By analyzing 12-hour-time-lapse imaging with 5 min intervals quantitatively, we could investigate the spatiotemporal relationship between axonal elongation and mitochondrial dynamics. The distribution of neuronal mitochondria changed following axonal elongation but mitochondria showed a tendency to position in the proximity of growth cones. This result suggests the presence of a mechanism that regulates positioning of mitochondria close to the advancing growth cones, which may consume more energy than other parts of the growing axons.

(COI: No)

P1-283

Molecular mechanism of phagosome formation by Rac1switching control both in space and time

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Rac1, a G-protein molecular switch, controls actin organization and mediates actin-based cell motilities such as pseudopod extension, ruffling and phagocytosis. However, significance of Rac1 switching between ON and OFF in Fc γ R-mediated phagocytosis is unknown. Therefore, we elucidated the roles of Rac1 activation/deactivation in the process of phagosome formation using quantitative assay, live-cell imaging, immunofluorescence and scanning electron microscopy of RAW264 macrophages expressing YFP-fused Rac1 mutants. Also, optogenetics of photoactivatable Rac1 (mCherry-LOV-Rac1-Q61L) was applied to reversible control of the molecular switch. As well as dominant negative Rac1-T17N, constitutively active Rac1-Q61L inhibited phagocytosis of IgG-opsonized erythrocytes (IgG-Es). Live-cell imaging and scanning EM of Rac1-Q61L-expressing cells demonstrated that ruffle-like pseudopodia were formed around IgG-Es bound on the cell surface, however, formation of phagocytic cups grasping IgG-Es was restrained. Immunofluorescence microscopy showed that phosphorylation of myosin light chain was reduced by activated Rac1, indicating activated Rac1 inhibits myosin II. Furthermore, optogenetic analysis revealed that Rac1 activation extends pseudopodia around IgG-Es, and its deactivation circularly constricts the pseudopodia to grasp IgG-Es. These findings suggested that Rac1 activation is crucial for pseudopod extension through actin polymerization, but subsequent deactivation is also required for contractile activities by myosin II to shape phagocytic cups.

(COI: No)

P1-284

Sequential recruitments of Rab35, Rab8 and Rab10 during macropinosome formation

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Macropinocytosis is a fluid-phase endocytic process that forms relatively large vacuoles called macropinosomes. Its processes consist of membrane ruffling, circular ruffle (macropinocytic cup) formation and then separation from the plasma membrane as macropinosomes by cup closure. In this study, we elucidate whether several Rab proteins (Rab1, Rab8, Rab10, Rab12, Rab13, Rab15, Rab35) are involved in macropinocytosis by live cell imaging. RAW264 macrophage cells were transiently expressed GFP-fused Rab proteins and observed using by a confocal microscope. As a result, Rab8, Rab10 and Rab35 were recruited to local sites of macropinosome formation. Rab35 localized in ruffle membranes and dissociated from the membrane during macropinocytic cup formation, whereas Rab8 and Rab10 were transiently recruited to the membrane of macropinocytic cup and then disappeared. We compared the timing of recruitment of Rab8 and Rab10 by co-expression experiments. Rab8 was slightly earlier recruited to the cup than Rab10. Moreover, dominant negative mutant of Rab10 or Rab35 inhibited macropinocytosis. These results suggested that sequential recruitments of Rab35, Rab8 and Rab10 play crucial role in macropinosome formation.

(COI: No)

P1-285

Time-lapse imaging of endosome acidification using pH-responsive fluorescent organosilica nanoparticles

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Cellular phagocytosis plays an important role in life activities such as uptake and degradation of pathogens. After phagocytosis the inside of endosome is acidified by proton pumps to degrade the contents. The detailed analysis of the endosome acidification is important in understanding the life phenomenon.

To understand the process from phagocytosis to acidification, we performed time-lapse imaging of the endosome acidification of the macrophage.

We prepared pH-responsive fluorescence organosilica nanoparticles. A pH-responsive fluorescence dye, AcidiFluor™ ORANGE was attached to the surface of thiol-organosilica nanoparticles containing FITC.

We added the nanoparticles and observed the phagocytosis and endosome acidification of the RAW264.7, a macrophage cell line, with time-lapse microscope. We measured two kinds of fluorescence intensities, inside of nanoparticle and AcidiFluor™ ORANGE on the surface of particles, in single cell at the same time. The ratio analysis was performed using these two kinds of fluorescence over time.

The kinetics of the uptake and endosome acidification varied according to a cell, and the special formed cell that did not show the acidification was observed.

We succeeded in time-lapse imaging of endosome acidification, and were able to observe the fluorescence change due to endosome acidification quantitatively and sequentially. The variations of the cell function such as phagocytosis and endosome acidification might be important to understand the life phenomenon.

(COI: No)

P1-286

Intracellular dynamics of estrogen-related receptors

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Estrogen-related receptor (ERR) is a member of the nuclear receptor superfamily and has highly homology with estrogen receptor (ER) α. They have three subtypes (α, β, and γ) that are widely expressed throughout the body including placenta, uterus, and brain. Although endogenous ligands of ERRs have not been identified to date, ERRs regulate the transcription of target gene by their constitutively active structure. The transactivity of ERRs is repressed by binding with diethylstilbestrol (DES), a potent synthetic estrogen. Therapeutic treatment of DES in the pregnancy has been known to be associated with abnormality of reproductive development of the offspring. In this study we analyzed intracellular dynamics of ERRs treated with DES to elucidate molecular mechanism of ERR action. Using live-cell imaging with fluorescent protein labeling, we found that all subtypes of ERRs were mainly localized within the nucleus. Upon DES treatment, the expression of each subtype of ERRs changed from diffuse to punctate pattern in the nucleus. Fluorescent Recovery After Photobleaching (FRAP) analysis revealed the reduction of intranuclear mobility of all subtypes of ERRs after DES treatment. These results show the relationship between the inactivation of ERRs by DES and the cluster formation of ERRs concomitant with mobility reduction. We hypothesize that DES-bound ERRs recruit cofactors to form a protein complex that induces transcriptional repression. Detailed quantification of FRAP analysis has been examined.

(COI: No)

P1-287

Preparation and application to in vivo noninvasive imaging of near-infrared organosilica nanoparticle

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Recently, in vivo imaging with near-infrared fluorescence is used in many fields including molecular imaging. We prepared near-infrared fluorescent nanoparticle (NIR-NP). NIR-NP enables to trace its in vivo behavior through the body. As its property, we can noninvasively observe how nanoparticle reaches the target. NIR-NP was made from mercaptopropyltrimetoxysilane and near infrared fluorescent dye. NIR-NP was evaluated using electron microscopy and in vivo imaging system. We administrated NIR-NPs to mice, then measured the distribution and fluorescent intensity. The accumulations of NIR-NP were detected highly sensitively. Then, we applied NIR-NP to tumor-bearing mice. NIR-NPs were detected in tumor tissue due to EPR effect. In addition, endogenous phagocytes or RAW 264.7, mouse leukemic monocyte macrophage cell line, were labeled with NIR-NP, and they were applied to tumor-bearing mice. Accumulations of the labeled cells to tumor tissue were detected. In addition, biodistribution and intensity of fluorescence were changed and decreased according to time. These results suggested that labeled cells were excreted through a certain pathway. Biodistribution and excretion of labeled cells are unclear. NIR-NP and its application to in vivo imaging are useful to research on the mechanism of the excretion of labeled cells. We believe that understanding of excretion pathway of nanoparticle and labeled cells can be breakthrough of the nanomedicine.

(COI: No)

P1-288

Development of organosilica nanoparticle for photodynamic therapy (PDT) and single cell analysis of PDT effect

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PDT is a local treatment using photochemical reaction of the photosensitizer and excitation light. It has some merits such as less damage to normal tissue, less pain in the treatment. Nanoparticle improve the PDT effect because nanoparticle can target to tumor tissue. We have developed functionalized organosilica nanoparticle for PDT. We performed single cell imaging and quantitative analysis of the cell death to evaluate PDT effect. RAW267.4 cells, a macrophage cell line, were incubated with the particles for PDT overnight. The culture medium containing propidium iodide (PI) was exchanged to detect cell death. The cells were irradiated the excitation light of the ultraviolet lamp. Fluorescence intensities of the particle in all cells and of the PI in killed cells were analyzed. 13 kinds of particles for PDT were evaluated. Some they showed better cytotoxic activity under an excitation wavelength of 650 nm. The single cell imaging and analysis demonstrated time course of cell death and heterogeneous response against PDT. Some cells showed cell death but some didn't be dead in spite of nanoparticle component cell (PDT resistant cell). So cell death wasn't dependent on the quantity of nanoparticle uptake. We speculated that the PDT effect vary by the cellular localization of the nanoparticle. Further experiments will be required to improve PDT effect and to understand the mechanism of PDT resistant.

(COI: No)

P1-289

IL-6 modulates the proinflammatory nature of rat primary cultured microglia

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Accumulatingevidences have shown that neuronal injury affect neuroprotective or neurotoxic actions of microglia by modulating their cytokine and growth factor release. The cytokines and growth factors may affect microglial cells in an autocrine manner. Among the cytokines, interleukin-6 (IL-6), consisting of 184 amino acids, may be one of the most abundantly produced cytokine by activated microglial cells. Yet, the effects of IL-6 on microglial cells are still to be elucidated. To understand how microglia respond to IL-6, we examined the effects of IL-6 on primary cultures of rat microglia where the cells were treated with IL-6 (10 ng /ml) and incubated for various periods. Incubation with IL-6 for 30 min - 5h caused phosphorylation of STATs 1 and 3 as revealed by immunoblotting. After incubation with IL-6 for 2, 5 or 24 h, total RNA samples of the microglial cells were collected for quantitative RT-PCR (qPCR). qPCRshowed that microglial cells increased the expression of mRNA encoding IL-18 but decreased IL-4 at 2 h after IL-6 addition. At 5 h, they increased mRNA encoding IL-1b and IL-18 and suppressed those for IL-4 and IL-10. At 24 h, these changes almost disappeared. Although IL-6 has been recognized as a pleiotropic cytokine, its action on microglial cells appeared to be predominantly proinflammatory. The proinflammatory actions of IL-6 may be mediated by phosphorylated STATs.

(COI: No)

P1-290

Circadian rhythm orchestrates the synaptic homeostasis via microglia

Miyanishi, Kazuya; Choudhury, Mohammad Emamussalehin; Yano, Hajime; Tanaka, Junya (*Department of Molecular and Cellular Physiology, Ehime University of Graduate School of Medicine*)

Circadian rhythms are a 24-hour oscillation process, sustained by a molecular clock and provide a temporal matrix that ensures the coordination of homeostatic processes of animals. Like other immune cells involving the homeostasis, microglia also posses clock genes and in addition to immune surveillance, microglia removes damaged neurons and dysfunctional synapse in brain. To gain insight into possible roles of circadian rhythm in the modification of synaptic structures, we sampled prefrontal brain tissues from rats at ZT0 (Zeitgeber time, light on) and also at ZT12 (lights off). Interestingly, the increased expression of CD68, F4/80, CX3CR1, interferon regulatory factors and Matrix metalloproteinases where as decreased expression of some metabotropic glutamate receptors in the cerebral cortex at ZT0 compared to ZT12. To go further, we focused on primary microglial culture where glutamate stimulates IRF8 and noradrenaline (which peak during active period of circadian cycle) abolishes the glutamate effects. Additionally, glutamate increased the expression of IRF8 that was abolished by NA. Taken together, our data of study on nocturnal mammals provide evidence that the roles of microglia in circadian rhythm; microglial cells eliminate glutamatergic input from the thalamus during the sleep period that is abolished by activated NA neurons during the wake period.

(COI: No)

P1-291

Toll-like receptor 3 mediated activation of microglia; an analysis of its signaling pathway

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Microglial cells rapidly become activated in response to endogenous ligands for Toll-like receptors (TLRs) that are produced in pathologic brains. The activated microglia are supposed to aggravate neuropathologic processes in various neurological disorders. Therefore, signaling pathways from TLRs responsible for the activation of microglia have been intensively investigated, whereas the majority studies have addressed TLR-mediated MyD88-dependent pathway. In this study, we investigated the response of rat primary microglial cells to poly I:C (pIC), a synthetic TLR3 ligand. TLR3 links to only MyD88-independent pathway. pIC induced iNOS expression and subsequent NO production more weakly than LPS, a ligand for TLR4 that links to both MyD88-dependent and independent pathways. Immunoblotting study showed that pIC induced strong STAT1 phosphorylation and elevated IRF1 expression in microglial cells 3 h after addition of the ligand. LPS weakly caused the similar changes but CpG did not exert any effects. Furthermore, pIC alone induced phosphorylation of MSK1 one h after addition of pIC. Unlike LPS, pIC did not promote NFkappaB translocation into nuclei. However, enhancement of MSK1 phosphorylation might be related to phosphorylation of NFkappaB p65, leading to the binding to the enhancer regions of the target genes. These results suggest a possibility that TLR3 activates microglial cells through a pathway distinct from TLR4-employed one.

(COI: No)

P1-292

Glutamate and noradrenaline modulates phagocytosis of rat primary cultured microglial cells

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Microglial cells have been demonstrated to express a variety of receptors for neurotransmitters. Among the receptors, adrenergic and glutamatergic receptors have been well investigated on their expression and functions. In this study, we have addressed the effects of noradrenalin (NA) and glutamate (Glu) on microglial phagocytic activities with the use of rat primary cultured microglial cells. Microglial cells were cultured in serum-free DMEM containing supplements such as insulin and transferrin. When evaluating their phagocytosis activity, the cells were incubated with PKH26 microparticles possessing red fluorescence for 45 min, followed by fixation and staining with FITC-labeled Phalloidin. The area with red fluorescence within microglial cells was defined as an index for their phagocytic activity. Consequently, microglial cell were found to promote their phagocytic activity in response to Glu, and NA abolished the promoting effect of Glu when NA and Glu was simultaneously added to microglial culture. Na alone did not show any significant effects. Glutamatergic agonists AMPA, NMDA and kainite exerted similar promoting effects to Glu. We have recently found some experimental evidence that microglial cells in the normal and pathologic brains are engaged in the control of synaptic transmission through phagocytic elimination of synapses. Collectively, Glu and NA are involved in the regulation of the synaptic transmission through the control of microglial phagocytic activities.

(COI: No)

P1-293

Expression and interaction between CD38 and TRPM2 in microglia

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CD38 and cyclic ADP-ribose (cADPR) formation have been identified in the hypothalamus and are critical for Oxytocin (OT), but not arginine vasopressin (AVP) secretion, with profound consequential changes in social behaviors in mice. However, expression and role of CD38 in glial cells still remains elusive. In the present study, we examined the immunolocalization of CD38 and TRPM2 which was reported to bind to CD38. In the hypothalamus, CD38 immunoreactivity was found more commonly in OT neurons than AVP neurons. In the CD38-deficient hypothalamus and posterior, stronger staining of OT was observed, suggesting accumulation of OT due to lack of the releasing process, as reported previously. Co-expression of CD38 with glial cells showed that CD38 was rarely expressed in glial fibrillary acidic protein (GFAP)-positive astrocytes. However, expression of CD38 protein in microglia was detected and more expression of CD38 in microglia was observed in the lipopolysaccharide-injected mouse brain. The up-regulation of CD38 was also observed in primary cultured microglia. Expression of TRPM2 was also confirmed and partially merged with CD38. Knocking down of TRPM2 significantly changed the expression level of CD38. These results suggest that CD38 and TRPM2 interact each other and may play an important role in microglial function.

(COI: No)

P1-294

Inhibitory effects of noradrenaline and a hypnotic bromvalerylurea on LPS-induced proinflammatory activation of microglia

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Activated microglia in the pathologic brain presumably aggravate neuropathological processes by releasing potentially neurotoxic substances such as proinflammatory cytokines and reactive oxygen/nitrogen species. Therefore, some intervention suppressing the aggravating activation in an appropriate manner, it would be a promising novel treatment for the brain diseases. In this study, we compared inhibitory effects of noradrenaline and a hypnotic bromvalerylurea (BU), both of which can inhibit LPS-induced NO release by rat primary cultured microglial cells to the similar extent. Noradrenaline (NA) suppressed LPS-induced nuclear translocation of NFkappaB and subsequent STAT1 phosphorylation. However, NA did not inhibit IL-6-induced STAT1 phosphorylation. By contrast, BU did not suppress NFkappaB translocation, but inhibited STAT1 phosphorylation. Simultaneous addition of NA and BU to LPS-treated microglial cells caused multiplier inhibitory effects on NO-release. Although these results suggest that NA and BU inhibit activated microglial cells through distinct manners, it is not yet well elucidated the mechanisms underlying inhibitory actions of BU. The suppressive action of BU may depend on its on JAK1, because BU suppresses interferon-gamma-induced STAT1 phosphorylation in peripheral macrophages. We are currently conducting studies to elucidate the distinctions between NA and BU actions more clearly.

(COI: No)

P1-295

Noradrenalin suppresses proinflammatory reactions of LPS-treated microglial cell

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Noradrenaline (NA) has been well-known of its anti-inflammatory effects on LPS-terated microglial cells. The aim of this study was to elucidate the mechanisms underlying the suppressive NA effects using rat primary cultured microglial cells. NA, an a1 agonist, phenylephrine (Phe) and a b2 agonist, terbutaline (Ter) suppressed LPS-induced elevated expression of mRNA encoding inducible nitric oxide synthase (iNOS) and other proinflammatory mediators by rat primary microglia. Both an a1-selective blocker terazocine and a b2-selective blocker butoxamine overcame the suppressive effects of NA. NA prevented LPS-induced translocation of NFkB into nuclei. LPS decreased IkB followed by phosphorylation of signal transducer and activator of transcription 1 (STAT1) and elevated expression of interferon regulatory factors (IRFs) 1 and 8. NAinhibited LPS-induced these changes. When NFkB expression was knocked down with siRNA, LPS-induced STAT1 phosphorylation and upregulated IRF1 expression was largely abolished. NA did not suppress IL-6 induced STAT1 phosphorylation. These results suggested that one of the critical mechanisms underlying the anti-inflammatory effects of NA may be the inhibition of NFkB translocation. Since NA, Phe and Ter exerted almost the same effects and H89 did not show significant antagonistic effects, the suppressive effects of NA might not be dependent on specific adrenergic receptors and cAMP-dependent signaling pathway.

(COI: No)

P1-296

Effects of IL-18 on rat mixed glial cell culture

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Reactive phenotypes of glial cells including astroglia, oligodendrocyte progenitor cells or NG2 glia, and microglia have been repeatedly documented in the ischemic penumbra of the stroke brain. The reactive phenotypes may be induced by some diffusible factors from the ischemic core lesions, where many bone marrow-derived macrophages accumulate. We have addressed interleukin-18 (IL-18) among the many kinds of factors produced in the ischemic core of rat brains, whose middle cerebral artery was transiently occluded for 90 min. Although IL-18 is quite abundantly produced in the ischemic core, it is still to be elucidated what kinds of roles IL-18 play, what kinds of cells are the targets of IL-18, or how IL-18 works in the ischemic brain. To solve these questions, we have investigated the effects of rat recombinant IL-18 on rat primary mixed glial culture. The culture was started from the whole forebrains of neonatal rats and maintained for 10-13 days. Then, the culture was incubated with IL-18 (0, 1, 5, 25 ng/ml) for 24 h followed by fixation for immunocytochemical staining, collection of protein and total RNA samples, for immunoblotting and RT-PCR, respectively. In response to IL-18, mRNA expression that encodes type I interferon, nestin, NG2, olig2 and hepatocyte growth factor (HGF) was increased in a dose-dependent manner. These results suggest a possibility that IL-18 may be responsible for activation and increase in the number of oligodendrocyte progenitor cells.

(COI: No)

P1-297

Na^+/H^+ exchanger isoform 1 is involved in regulation of microglial cell volume

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Severity of brain edema is one of the critical factors to determine the outcome of stroke patients. Na+/H+ exchanger isoform 1 (NHE1) has been implicated in homeostasis of cell volume by introducing Na+ into the cytoplasm that lead to cell swelling. We have shown that treadmill exercise during acute phase after transient middle cerebral artery occlusion ameliorated brain edema that accompanied downregulated NHE1 expression. The treadmill exercise increased blood corticosterone concentration and the administration of anti-glucocorticoid and anti-mineralocorticoid agents abolished favorable effects of exercise. In this study, we have addressed the effects of NHE1 expression on glial cell volumes, because there should be almost no viable neurons in the ischemic brain tissues. Incubation of rat primary mixed glial cell culture with low concentrations of corticosterone lead to decreased NHE1 expression and showed resistance to incubation with culture medium diluted with water as revealed by LDH assay. Immunocytochemical staining demonstrated that microglial cells expressed NHE1 the most remarkably in the mixed glial cell culture. NHE inhibitors reduced individual cell size in pure microglial cell culture based on cell area determination of microglial cells that had been stained with Hoechst 33258 and FITC-labeled phalloidin. These results suggest that inhibition of NHE1 activities in microglial cells in the ischemic brain may lead to amelioration outcomes of stroke.

(COI: No)

P1-298

Activated microglia in the substantia nigra pars reticulata and globus pallidus of rats with 6-OHDA-induced Parkinsonism

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Activation of microglial cells in the substantia nigra pars compacta (SNpc) and the globus pallidus in a 6-hydroxy dopamine (6-OHDA)-induced rat Parkinsonism model. In this study, we have conducted immunohistochemical analyses on activation of 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 characteristically expressed CD68, a marker for phagocytes, suggesting that the cells are engaged in phagocytosis. Immunoreactivity of glutamate receptors (mGluR1, NMDAR2D) as well as PSD95 (a marker for post-synapse) was reduced in the region of the SNpr where activated microglia were markedly accumulated. 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. Rat primary microglial cells incubated with glutamate enhanced phagocytic activity by microglial cells. These results suggest that disinhibited glutamatergic neurons in the subthalamic nuclei caused microglial in the SNpr and GP. Then, activated microglia may be engaged in elimination of glutamate receptors in the SNpr and GP, leading to the suppressed activity of GABArgic neurons. Microglia may partially suppress the effect of DArgic neuronal degeneration on the motor symptoms of Parkinsonism.

(COI: No)

P1-299

Treadmill exercise after middle cerebral artery occlusion ameliorates brain edema

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Rehabilitation may be the most effective therapy for stroke, yet mechanisms underlying the curative effects are to be elucidated. In this study, the effect of treadmill exercise on ischemic brain edema was investigated. Wistar rats were subjected to transient (90min) middle cerebral artery occlusion (MCAO). The area of the lesion was measured with magnetic resonance imaging (MRI) on 1 day-post reperfusion (1dpr), and only rats with substantially large ischemic lesion were grouped into exercise and non-exercise ones. Treadmill 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. Consequently, the exercise 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. As revealed by quantitative real-time RT-PCR, the exercise prevented the elevation of mRNA encoding aquaporin 4 (AQP4) and Na+/H+ exchangers (NHEs). These results suggest that the treadmill exercise increases glucocorticoid level in the circulation, leading to suppression of AQP4 and NHEs expression that results in the amelioration of brain edema.

(COI: No)

P1-300

Early-life stressed mice easily induced the epilepsy by application of pentylenetetrazole

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Epilepsy is potentially triggered by neuronal remodeling. For example, activity of newly formed synapses in CA3 or CA1 becomes a cause of epilepsy (Ben-Ari et al., 2008). Previously reported, decrease in stability of mushroom spines in somatosensory cortex and hypersensitivity is detected in early-life stressed mice (Takaturu et al., 2009). This model also showed the increase in growth of mushroom spines which potentially compensate the loss of spines. Thus, this early-life stressed mice possibly be suffered the epilepsy more easy compared with those in control mice. In this study, we prepared the maternal deprivation (MD) mice as follows. MD mice were separated from their mother from post-natal day 2 (P2) to P14, for 3 h every day. The separated MD mice were placed isolated from one another in a locally-made incubator with regulated humidity and temperature. Then we injected pentylenetetrazole (PTZ; 20mg/kg or 30mg/kg) intraperitoneally every other day in adulthood (P56-84) for a month. After injection, we observed the behavior of mice for 30 minutes in the home cage. To estimate behavioral scale, we use Racine scale (RS) (Racine et al., 1972). The behavioral test showed that high RS was recorded in MD mice in early phase after starting to injection compared with those in control mice. This result indicated that MD mice may be vulnerable to epilepsy more than control mice. We are going to observe the concentration of glutamate and GABA in hippocampus by in vivo microdialysis and investigate the mechanism of epilepsy by using molecular biological experiment.

(COI: No)

P1-301

Homeostasis of glutamatergic synapses is disrupted by early-life-stress

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Stress during early life stage induces several neuropsychological disorders in adulthood. The disorders which related with early-life stress should be induced by functional alteration of the glutamatergic system. However, their underlying mechanisms have not yet been fully understood. In this study, we used maternal deprivation (MD) mice as an early-life-stress model, and studied the changes in the glutamatergic system in adulthood. The glutamate concentration and neuronal activity in the somatosensory cortex (SSC) increased under basal condition in MD mice compared with those in control mice. Stressful physical stimulation (SPS) increased the concentration of corticosterone in the control mouse SSC, but not that of glutamate even under the application of SPS. On the other hand, in the MD mice, although the basal concentration of corticosterone in the SSC was increased, no SPS-induced increase was observed. On the other hand, the concentration of glutamate extremely increased during SPS. It was significantly high for 30 min after stimulation. The expression level of α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid / N-methyl-D-aspartate receptor in the MD mice was also changed compared with those in control mice after SPS. These findings indicate that early-life stress disrupts the homeostasis of glutamatergic synapses.

(COI: No)

P1-302

Odorant X-induced analgesia is not stress-induced analgesia in mice

Yamaguchi, Ran[1]; Ishikawa, Sodemi[2]; Tashiro, Shogo[2,3]; Kajiya, Katsuko[1]; Kanmura, Yuichi[3]; Kuwaki, Tomoyuki[2]; Kashiwadani, Hideki[2] (*[1]Dept Biochem Sci Tech, Facul Agri, Kagoshima Univ, Kagoshima, Japan; [2]Dept Physiol, Grad Sch Med Dent Sciences, Kagoshima Univ, Kagoshima, Japan; [3]Dept Anesthesiol, Grad Sch Med Dent Sciences, Kagoshima Univ, Kagoshima, Japan*)

Previously, we found that an odor molecule (odorant X) showed significant analgesia in mice. The odorant X-induced analgesia was not observed in olfactory-deprived mice, indicating that olfactory input evoked by odorant X trigger the analgesia. Furthermore it was not observed in orexin peptide deleted mice or in orexinergic neuron ablated mice. These results indicated that orexinergic transmission is essential for the odorant X-induced analgesia. However, it has not yet revealed whether the odorant X-induced analgesia is one of the stress-induced analgesia or not. To address the issue, we first examined the elevation of plasma ACTH, one of the stress hormones in odor exposed mice. ELISA analyses revealed that the increase of plasma ACTH was not observed in odorant X exposed mice but evident in TMT, one of the predator odors triggering the stress-induced analgesia in rodent. Next to examine the aversion to odorant X, we performed odor preference/avoidance test using two-chambered odor exposure apparatus. Time spent exploratory behavior for odorant X-perfused chamber was comparable with that for odorless air-perfused chamber, indicating that mice did not show the aversion to odorant X. These results indicated that odorant X-exposure did not induce the acute stress for mice which could trigger the stress-induced analgesia.

(COI: No)

P1-303

The analgesic effect of odorant-X is concentration-dependent

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Recently, we found that odorant-X(one of the terpenoid) exposure induced analgesic effect in mouse. Because the analgesic effect was not observed in anosmic mouse, the analgesic effect was driven by odorant X-evoked olfactry input. In addition, we found that the analgesic effect was mediated by hypothalamus orexinergic neurons. However, it has not yet been examined the analgesia threshold or the concentration-response relationship of odorant X. To address the question, we measured the thermal pain threshold under several concentrations of odorant X using classical hot plate test(54.5 degree). The analgesic effect of odorant X vaporized from 10% odorant X solution tended to be attenuated compared to that from 100% odorant X solution, however, the analgesic effect was still significant. The gas from 1% solution did not show the significant analgesic effect. These data indicate that the analgesic effect of odorant X exposure depends on the concentration of odorant X. In our presentation, we will also discuss the difference between detection threshold and analgesia threshold of odorant X.

(COI: No)

P1-304

Prolactin reduces maternal behaviour impairment by lack of CIN85

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Cbl-interacting protein of 85 kDa (CIN85) is a scaffold/multi-adaptor protein implicated in the regulation of receptor endocytosis, cell division 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 the 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. Moreover, despite of the fact that no defect in the mammary glands of CIN85 KO mother mice was found, milk was not detected in the stomachs of most pups. 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. We therefore injected PRL (0.05 μg/g bw/day, ip) to pregnant CIN85 KO mice in mid to the last day of pregnancy. It could partially rescue the defect in maternal behavior of the next generation.

Our findings indicate a loss of CIN85 function leads to a neglect-like behaviour of the next generation due to aberrant dopamine-PRL signaling.

(COI: No)

P1-305

Lack of CIN85 causes impairment of maternal behaviour by disruption of fetal environment

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Cbl-interacting protein of 85 kDa (CIN85) is a scaffold/multi-adaptor protein implicated in the regulation of receptor endocytosis, cell division 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 the 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. Importantly, when measuring the plasma levels of prolactin (PRL) on delivery day, we detected significantly decreased PRL levels in CIN85 KO mice compared to heterozygote and wild-type mice. We therefore have transferred wild type (WT) embryos into the oviduct of KO mice. As a result, many of the mice that were born in the embryonic transfer were neglected. Our findings indicate a loss of CIN85 function leads to neglect behaviour in the next generation due to aberrant environment in fetal period.

(COI: No)

P1-306

Developmental sex differences of the synaptic input onto tuberoinfundibular dopaminergic neurons

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Dopamine neurons located in the ARC are known as tuberoinfundibular dopaminergic (TIDA) neurons. A part of their functions is to inhibit prolactin (PRL) release from the anterior pituitary as a PRL inhibitory factor. Since the basal activity of neurons are controlled by synaptic inputs and there is a sex difference in the release of PRL, we examined the synaptic inputs onto TIDA neurons. Transgenic mice carrying GFP under the control of the rat tyrosine hydroxylase gene (Matsushita et al, 2002) were used in the present study. We first confirmed that the expression of GFP was reliable marker for TIDA neurons by immunocytochemistry. Next, we investigated that the developmental changes in the synaptic input onto TIDA neurons. In male and female mice at the age of 2 and 4 weeks, whole-cell voltage-clamp techniques in acute slice were applied. TIDA neurons were identified as GFP-positive cells by fluorescence microscopy. The frequency of miniature excitatory postsynaptic current (mEPSC) in female mice at the age 4 weeks was significantly lower compared to that in male mice at the same age. On the other hand, the mean amplitude of mEPSC was not affected between sexes at this age. There were no significant changes in the frequency or the mean amplitude of mEPSC between female and male mice at the age 2 weeks. These results suggest the presence of the sex difference in the controlling mechanism for TIDA neurons.

(COI: No)

P1-307

Postnatal changes of excitatory synaptic inputs in the rat masseter motoneurons

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Feeding behavior of mammals dramatically changes from suckling to mastication during early postnatal period. The postnatal development of oro-facial structures during this period is accompanied by developmental changes of central neural mechanisms involved in controlling jaw movement. However, whether the synaptic inputs to trigeminal motoneurons change during postnatal development is still unclear. In this study, we examined the developmental changes of miniature excitatory postsynaptic currents (mEPSC) in the rat masseter motoneurons (MMNs) during early postnatal period. Whole-cell patch-clamp recordings were made from dextran tetramethylrhodamine-lysine (DRL)-labeled MMNs obtained from P2-5 (n = 7) and P10-15 (n = 9) Wistar rats. After bath applications of strychnine, SR95531 and tetrodotoxin, mEPSCs were observed in P2-15 MMNs. Subsequent addition of CNQX almost completely abolished the mEPSCs. The amplitude of the mEPSCs significantly increased from 17.0 ± 1.7 pA at P2-5 to 21.1 ± 0.84 pA at P10-15 ($p<0.05$). Furthermore, the decay time constant of the mEPSCs was significantly reduced from 2.9 ± 0.24 ms at P2-5 to 1.6 ± 0.16 ms at P10-15 ($p<0.01$). In contrast, there were no significant differences in the frequency between P2-5 and P10-15. These results demonstrate the developmental increase in mEPSC amplitude and decrease in mEPSC decay time during postnatal ages. It is possible that the postnatal development of the synaptic inputs to the MMNs contributes to the transition from suckling to mastication.

(COI: No)

P1-308

Glycine-activated outward currents in neurons of the hippocampus

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Glycine receptors are widely expressed throughout the central nervous system such as spinal cord, brain stem, but their physiological roles in the brain and their endogenous ligands have not been clearly identified yet. In the hippocampus glycine activates pyramidal cells in the area of CA1 and CA3 (M. Mori, 2002). Extracellular electrical stimulation revealed no glycinergic synaptic responses in the CA3 pyramidal cells. Here we sought for the roles of glycine receptors in the hippocampus, using organotypic rat hippocampal slice culture, prepared from P6 rats. Pressure application of glycine (0.3 mM in an application pipette; 50 μm away from the soma of the neurons studied) activated an outward current in the neurons but not in the glial cells identified by their failure to generate action potentials at a holding potential of -70 mV. The glycine-activated currents were blocked by the bath-perfusion of a glycine receptor antagonist, strychnine. Peak amplitudes of glycine-activated current density in interneurons were larger than those in pyramidal cells (CA3 pyramidal cell, 17.5 ± 3.24 pA/pF, n=5; interneurons, 36.0 ± 13.2 pA/pF, n=5). We found that the variance of the glycine-activated currents density in interneurons was much more than that in CA3 pyramidal cells, suggesting that this significant variation of the glycine-activated currents in interneurons could be derived from diversity of the type of interneurons.

(COI: No)

P1-309

An old hypnotic bromvalerylurea ameliorates 6-hydorxydopamine-induced rat Parkinsonism

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Damaged neurons express damage-associated molecular patterns (DAMPs) such, which can activate microglia to display proinflammatory reactions that further aggravate neuronal damage. Therefore, such vicious cycles should be prevented either by inhibiting neuronal damage or microglial activation. We have attempted to suppress the activation of microglia to ameliorate neuronal damage. Recently bromvalerylurea (BU), an outdated hypnotic/sedative, was found to suppress nitric oxide (NO) release by lipopolysaccharide (LPS)-activated microglial cells in a concentration-dependent manner. Inducible nitric oxide synthase (iNOS) expression by LPS-activated microglial cells was suppressed at mRNA and protein levels as revealed by real-time RT-PCR and immunoblotting. A rat Parkinson's disease (PD) model was induced by administrating 6-OHDA into the right striatum to cause a substantial loss of dopaminergic neurons in the substantia nigrapars compacta. BU dissolved in drinking water was administered to the PD model rats at a dose of 50 mg/kg body weight/day. BU administration prevented dopaminergic neuron loss and microglial activation. As revealed by quantitative real-time RT-PCR revealed, BU suppressed expression of mRNA encoding IRF1, IRF8 and IL-6, all of which may be involved in microglial activation. Furthermore, BU ameliorated motor function of the rats as revealed by Rota-rod test. Thus, BU may be a promising agent for the treatment of PD by suppressing microglial activation.

(COI: No)

P1-310

Single-cell imaging mass spectrometry revealed lower abundance of palmitoleic acid in breast cancer stem cells

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Imaging of biomolecules has brought significant progress in microanatomy. We have developed an imaging mass spectrometry (IMS) protocol that enabled visualization of lipids. Previously, we found abnormal lipid metabolism in human breast cancer lesions. To further characterize particular rare cellular populations, we conducted IMS analyses of individual cells isolated by fluorescence-activated cell sorting. As a target, we focused on breast cancer stem cells (CSCs), which are thought to cause cancer relapse and drug resistance.

We dispersed surgically-resected breast cancer tissues, sorted $CD45^-/CD44^+/CD24^-$ CSCs, and analyzed them by time-of-flight secondary ion mass spectrometry-type IMS. To validate the results, we analyzed bulk cells by liquid chromatography tandem mass spectrometry (LC-MS/MS).

We visualized simultaneously 4 fatty acid species and phosphoric acid in sorted CSCs. Integrated ion intensity of palmitoleic acid was significantly smaller in CSCs as compared with that of $CD45^-/CD44^-/CD24^+$ non-stem cancer cells; the tendency was identical in 2 cases. This finding was supported by the results of LC-MS/MS analysis in 3 cases.

Our novel method successfully showed the distribution of lipids within unique microanatomical components in human clinical specimens. The abnormal lipid metabolism in breast CSCs identified in this study may have a future application as an anti-cancer therapeutic target specific to CSCs.

(COI: No)

P1-311

Possible participation of sodium ion / proton exchanger1 (NHE1) in lymph node metastasis of head and neck squamous cell carcinoma

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Metastases of head and neck squamous cell carcinomas to the draining lymph nodes precede to distant metastases, and dramatically affect in the prognosis. Thus, prevention of the lymph node metastases is expected as promising therapeutic target for this disease. We had already established highly effective lymph node metastasis model of squamous cell carcinoma by using human metastatic squamous carcinoma cell line SASL1m. By xenografting this cell line to KSN nude mice tongues, we obtained almost 100% of metastases to the submandibular lymph node which corresponds to the draining lymph node for the tongue. We found protein expression of NHE1 is enhanced in SASL1m cells compared with non-metastatic parental cell line SAS. NHE1 acts as a regulator of intracellular pH by excreting proton by exchanging with sodium ion, and has potential to make tumor microenvironment acidic. Simultaneously, NHE1 plays a role as an anchoring point of actin cytoskeleton to the cellular plasma membrane, and participates in cellular motility, polarity and invasive activity. We are exploring possible roles of upregulation of NHE1 in squamous cell carcinoma metastases to the draining lymph nodes by using stable NHE1 knockdown SASL1m cells. The knockdown cells exhibit reduced metastatic rates to the lymph node in the model system described above, and also severely reduced in vitro invasive activities. We would like to discuss about the possibilities of NHE1 as a therapeutic target of this disease.

(COI: No)

P1-312

The KCNK13 channel current is increased by the activation of either the Gi/o- or the Gq-coupled receptor

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The KCNK13 channel, a member of the two-pore-domain potassium channel family, is known to be activated by arachidonic acid and inhibited by halothane. In addition, we have previously observed that the KCNK13 channel is activated by the Gi/o-coupled $GABA_B$ receptors ($GABA_BR$) in mouse cerebellar Purkinje cells, but not in oocytes. Here we examined whether or not the activation of the $GABA_BR$ potentiates the KCNK13 channel in human embryonic kidney 293 cells by patch clamp recording. We confirmed that $GABA_BR$ positively regulates the KCNK13 channel. We also observed that the activation of the Gi/o-coupled muscarinic type2 receptor potentiates the KCNK13 channel and the potentiation is inhibited by pretreatment with Pertussis toxin. Furthermore, we observed that the KCNK13 channel is potentiated by the Gq-coupled metabotropic glutamate receptor type1a or muscarinic type 1 receptor (M_1R). The effect of the M_1R was suppressed by application of the PLC inhibitor (U73122, 10 μM) but not by the infusion of 5 mM BAPTA in pipette solution, suggesting that downstream of PLC, excluding the increase in the intracellular Ca^{2+}, potentiates the KCNK13 channel. These results demonstrate that the KCNK13 channel is positively regulated by either the Gi/o- or the Gq-coupled receptor.

(COI: No)

P1-313

Physiological significance of the novel spliced isoform of two-pore domain K^+ channel $K_{2P}5.1$

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The two-pore domain K^+ (K_{2P}) channel, $K_{2P}5.1$ (also known as TASK-2/KCNK5) is one of the background K^+ conductance, is activated by extra- and intracellular alkalization and contributes to the setting of the resting membrane potential in various types of cells. We recently identified the novel splice variants of $K_{2P}5.1$, $K_{2P}5.1B$ from mammalian spleens. They were lacking the N-terminus of the original $K_{2P}5.1A$, however, conserved the C-terminus, which is essential for the forming of functional dimers. In the human embryonic kidney HEK293 cell heterologous expression system, the cellular distribution of CFP-tagged $K_{2P}5.1A$ and/or YFP-tagged $K_{2P}5.1B$ showed $K_{2P}5.1B$ inhibited the trafficking of $K_{2P}5.1A$ to the plasma membrane. Using a fluorescence imaging system, alkaline pH-induced hyperpolarization by the activation of native human $K_{2P}5.1A$ ($hK_{2P}5.1A$) was significantly suppressed and the influx of Ca^{2+} was simultaneously decreased in $hK_{2P}5.1B$-overexpressing human leukemia K562 cells. Recent researches highlighted the potential role of $K_{2P}5.1$ in the pathogenesis of autoimmune diseases such as rheumatoid arthritis and multiple sclerosis. We also found a significant increase in $K_{2P}5.1$ expression in the splenic $CD4^+$ T-lymphocytes from a mouse model of chemically-induced inflammatory bowel disease. The mRNA splicing mechanisms underlying the transcriptional regulation of $K_{2P}5.1B$ may implicate for a new therapeutic strategy in autoimmune and inflammatory diseases.

(COI: No)

P1-314

Morphological analysis of small intestinal organoids

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In the small intestine, the epithelial cells are regulated its differentiation along crypt-villus axis, and specialization along proximodistal axis. Recently, Sato and Clevers have been established the method of primary mouse small intestinal epithelial tissue culture (organoid). By using the organoid technique, we investigated whether specialization of epithelial cells along proximodistal axis reflect the organoids derived from different region of small intestine. Crypts were isolated from mouse jejunum and ileum segments, and then cultured for several weeks. The organoids from each segment were observed histologically and identified cellular population. The organoids were composed of central cyst structures and surrounding crypt-like structures. In the epithelium, four types of mature cells were presented; enterocytes, goblet cells, enteroendocrine cells and paneth cells. However, it seems that cellular population of organoid differed with small intestinal segment, we need further examination such as ultrastructural observation and analysis of the epithelial function. The author has no financial conflicts of interest to disclose concerning the presentation.

(COI: No)

P1-315

Ephrin-B1 and EphB2 expression in the stratified squamous epithelium of the skin and mucosae

Kohara, Yukari; Ogawa, Kazushige (*Fac. Life Environ. Sci., Osaka Prefect. Univ., Izumisano, Japan*)

Eph receptors and ephrin ligands are membrane proteins that regulate cell adhesion and proliferation. Recently it has shown that EphAs and ephrin-As expressed epidermal keratinocytes modulate proliferation, migration and differentiation (Lin et al., 2012) while expressions of EphBs and ephrin-Bs are almost unknown. We previously reported ephrin-B1 and EphB2 localizations in the stratified squamous epithelium of the nonglandular part of the rodent stomach. In this study, we screened ephrin-B1 and EphB2 expressions in stratified squamous epithelia of diverse tissues and organs (tongue, esophagus, nonglandular part of the stomach, palm, sole, anus, lip in adult ICR mice). RT-PCR analysis showed that all members of EphBs and ephrin-Bs were expressed in all tissues examined. Immunofluorescence staining revealed that (1) ephrin-B1 was expressed in keratinocytes of the basal and spinous layer of the epithelia in the mucosae and skins, (2) ephrin-B1 was highly expressed in thick regions of the epithelia, where connective tissue papillae were developed, (3) ephrin-B1 were expressed higher in keratinocytes of the basal layer adjacent to the top of the papillae in the dorsum of tongue and these cells were Ki67-negative, (4) EphB2 was expressed in keratinocytes of the basal layer clearly in the mucosae such as the esophagus and nonglandular stomach. These findings may indicate that ephrin-B1expression pattern is almost the same among stratified squamous epithelia except for the tongue while EphB2 expression pattern differs between the skin and mucosae.

(COI: No)

P1-316

Prosaposin and its receptors in the kidney

Watanabe, Haruka[1]; Shimokawa, Tetsuya[1]; Nabeka, Hiroaki[1]; Msi, Khan[1]; Doihara, Takuya[1]; Yamamiya, Kimiko[1]; Hamada, Fumihiko[2]; Matsuda, Seiji[1]; Kobayashi, Naoto[3] ([1]*Anat Embryol, Ehime Univ Grad Med., Ehime, Japan;* [2]*Anat, Oita Univ F Med., Oita, Japan;* [3]*Education C, Ehime Univ Grad Med., Ehime, Japan*)

Prosaposin (PSAP) is the precursor of saposins A-D. Accumulating documents suggest that PSAP is a trophic factor *in vivo* and *in vitro* that induces the differentiation and prevents the death of a variety of cells. Recently, the interaction of PSAP with polycystic kidney and hepatic disease gene 1 was reported. The two proteins regulate cellular proliferation and apoptosis (Sun et al., 2010). These findings prompted us to further investigate the distribution of PSAP in the kidneys. We generated a specific antibody to PSAP and examined the spatiotemporal distribution of PSAP-immunoreactive (PSAP-IR) cells in the kidney. PSAP-IR cells were rarely observed in the renal glomerulus. Strongly immunoreactive cells were observed in the proximal tubules, while weak immunoreactivity was detected in the distal tubules. The background levels of immunoreactivity were also observed in the proximal tubules; this artificial reaction must be examined carefully.

(COI: No)

P1-317

Prosaposin and its receptors in the spleen

Takezawa, Mitsuaki[1]; Shimokawa, Tetsuya[1]; Nabeka, Hiroaki[1]; Msi, Khan[1]; Doihara, Takuya[1]; Yamamiya, Kimiko[1]; Hamada, Fumihiko[2]; Kobayashi, Naoto[3]; Matsuda, Seiji[1] ([1]*Anat Embryol, Ehime Univ Grad Med., Ehime, Japan;* [2]*Anat, Oita Univ F Med., Oita, Japan;* [3]*Education C, Ehime Univ Grad Med., Ehime, Japan*)

Prosaposin (PSAP) is a trophic factor and activator of sphingolipid hydrolase in lysosomes. PSAP is the precursor of four small heat-stable glycoproteins called saposins (saposin A-D), which are required for the hydrolysis of a variety of sphingolipids by specific lysosomal hydrolases (O'Brien et al. 1988). PSAP is found in several organs and is secreted into biological fluids such as milk, cerebrospinal fluid, and seminal fluid, suggesting that PSAP serves not only as a precursor for saposins inside lysosomes but also as a secretory protein without undergoing proteolysis (Hiraiwa et al. 1992, 1993). We generated a specific antibody against PSAP and examined the spatiotemporal distribution of PSAP-immunoreactive (PSAP-IR) cells in rat spleen. PSAP-IR cells were distributed in both the red and white pulp of the spleen. To identify PSAP-IR cells, double and triple immunostaining was performed using antibodies against PSAP, CD68, and CD1d. These results suggest that antigen-presenting cells in these lymphatic tissues contain abundant PSAP (Shimokawa et al. 2013). In the present study, we examined the distribution of immunopositive cells for the prosaposin receptors GPR37 and GPR37L1 in rat spleen. Receptor-immunoreactive cells were observed mainly in the red pulp of the spleen.

(COI: No)

P1-318

Molecular basis of intercellular adhesion in mesangial cells

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Mesangial cells provide the mechanical support for glomerular capillaries by generating an inwardly directed counter force and regulating the glomerular wall tension by contraction and relaxation. Interaction between mesangial cells and endothelial cells are often observed by EM. However, molecular basis of intercellular junction between two cells is not fully clarified. In this study, we demonstrate the intercellular adhesion molecules expressed in mesangial cells. Recently, we have found that filamin, one of actin-binding proteins, is expressed in both normal and proliferating mesangial cells. Filamin is located in the whole cell body of mesangial cell. Therefore, filamin staining is useful for visualizing mesangial cells. Capillary endothelial cells is detectable by ICAM-2 staining. N-cadherin and catenins are located at the intercellular junctions between mesangial cells. On the contrary, spot signals for l-afadin at the tips of mesangial processes including filamin are observed beneath the glomerular capillary. Immunoelectron microscopy demonstrates that l-afadin is located at cell-cell contact between mesangial cell and capillary endothelial cell. The signals for those adhesion molecules are dramatically decreased during mesangiolysis induced by injection of Thy1.1 monoclonal antibodies. Proliferating mesangial cells observed in the expanded mesangial area at day 5 after antibody injection do not express N-cadherin, catenins and l-afadin. The data suggest that l-afadin is involved in the heterologous interaction between mesangial cell and capillary endothelial cell.

(COI: No)

P1-319

The role of αSMA in renal fibrosis

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Renal fibrosis is the final common pathway of a wide variety of chronic kidney diseases, irrespective of the initial causes of nephropathy. The key player causing fibrosis is the myofibroblast. TGF-β1 overproduced in kidney transforms fibroblasts to myofibroblasts, which induce type I collagen accumulation (fibrosis). They are morphologically characterized by having αSMA formed the stress fiber. Significance of αSMA expression remains unknown. In the present study, the role of αSMA in renal fibrosis was investigated by transfecting αSMAsiRNA into NRK49F cells (rat renal fibroblasts). Immunofluorescence for αSMA exhibited that the stress fibers of αSMA were formed by TGF-β1, whereas it was not found in the cells transfected αSMAsiRNA (the siRNA cells). Also it was shown by Western blotting that αSMA expression was accelerated by TGF-β1 and it was suppressed in the siRNA cells. In transmission electron microscopy, TGF-β1 was exhibited to induce building up of stress fibers and dilatation of RER, while building up of stress fibers was repressed but dilatation of RER was similarly recognized in the siRNA cells. In culture of NRK49F cells in type I collagen gel, TGF-β1 markedly evoked gel contraction, whereas the siRNA cells were leaded to suppression of TGF-β1-stimulated gel contraction. Accumulation of type I collagen was induced by TGF-β1, and not affected by αSMA knock down. Dilatation of RER may correspond to increased accumulation of type I collagen. In conclusion, it was demonstrated that αSMA stress fibers caused gel contraction, but did not influence accumulation of type I collagen.

(COI: No)

P1-320

Study of protective effect against oxidative stress on rheological dysorder of erythrocyte

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We study to evaluate the effects of iron-induced oxidative stress and the protective effects of dehydroepiandrosterone (DHEA) against oxidative damage on rheological properties of erythrocytes. Human erythrocytes were incubated for 1 hour at 37℃ with 0-2 mM $FeSO_4$ in the presence of ascorbate. For evaluations of erythrocyte membrane damage. Thiol content of membrane proteins were measured by Ellman's method. A cone-plate viscometer, and high-shear rheoscope were used to evaluate the rheological parameters in Fe^{2+}/ascorbate-treated erythrocytes. 1) Fe^{2+}/ascorbate treatment impaired erythrocyte deformability and erythrocyte suspension viscosity, with increasing membrane protein oxidation. 2) DHEA partially prevented Fe-ascorbate-induced deformability impairment and decrease viscosity of erythrocyte suspensions, by reason of decreasing its oxidative damage. DHEA is efficacious in protecting erythrocyte's against iron-mediated oxidative injury, which can be attributed to its potent reductant and radical scavenging abilities.

(COI: No)

P1-321

Simulation of changes in ionic mechanisms underlying contraction of ventricular cells during embryonic development

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The heart develops and gains new functions while continuously pumping blood, and heart abnormalities progress to congenital heart malformations; therefore, the developmental program of the heart, including the expression of the genes responsible for various ionic channels, is likely to be tightly regulated. The quantitative changes in ionic channels, pumps, exchangers, and sarcoplasmic reticulum Ca^{2+} kinetics are responsible for the changes in electrophysiological properties of the developing cardiomyocytes. Previously, we demonstrated that the developmental changes in action potentials of ventricular myocytes were well represented, as Na^+ current (I_{Na}) increased before the disappearance of and funny current (I_f), followed by a 10-fold increase in inward rectifier K^+ current via simulation; briefly, the relative conductances of the 9 components were switched between early embryonic (EE) and late embryonic (LE) values and simulated the 512 combinations of the model. Here, we constructed a model to represent "middle" embryonic (ME) stage of guinea pig ventricular cell on the basis of experimental data. We then shifted relative current densities of the 9 components among EE, ME, and LE stages, in order to compare the changes in excitation-contraction coupling mechanisms with the simulated results when all components are shifted equally between EE and LE stages without assuming ME stage.

(COI: No)

P1-322

Augmentation of Na^+/K^+-ATPase expression by aerobic training in male rat skeletal muscle

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Aerobic exercise facilitates oxidative phosphorylation and glycolysis of skeletal muscle. Thyroid hormone (TH) controls metabolic activity in a wide range of tissue including skeletal muscle. To examine the relationship between aerobic training and thyroid hormone action in the skeletal muscle, we have studied whether TH signaling pathway is activated by training with different intensity. We previously reported that adult male rats received 30 min/day aerobic treadmill training showed the suppression of TSH level, increase of TRβ1 mRNA and protein levels, and augmentation of Na^+/K^+-ATPase β expression by T3. In the present study, we constructed a series of reporter plasmids containing truncated mutants of Na^+/K^+-ATPase β promoter region and showed that the region -466/-378 bp function as a novel thyroid hormone response element (TRE) in L6 myoblast-derived cells. Liquid chemiluminescent DNA pull down assay, which is in vitro DNA-protein binding assay, showed that TRβ1 bound to the Na^+/K^+-ATPase promoter region. Chromatin immunoprecipitation assay in L6 cells showed that TRβ1 bound to the nucleotide sequence containing typical TRE. These results indicate that aerobic training alters TH signaling at least in part, and such TH signaling alteration may contribute metabolic adaptation in skeletal muscle through the alteration of sensitivity of TH-target gene.

(COI: No)

P1-323

Contractile function of reconstituted cardiac tissue is facilitated by mechanical stretch

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In the field of cardiac regenerative medicine, development of cardiac tissue with sufficient contraction is needed. To this aim, we developed a three dimensional cell culture system and explored optimal conditions for vigorous contraction. Cardiac cells obtained from ventricle of neonatal rats were cultured in a ring shaped gel (outer diameter: 16 mm, inner diameter: 8 mm) including collagen. The gel construct was cultured in a CO_2 incubator in static condition for seven days. Subsequently, the gel construct was transferred to a mechanical stretching device installed in the incubator and cultured for additional seven days. We explored optimal conditions by manipulating collagen concentration and frequency of the mechanical stretch. Tissue formation and contraction of the ring culture were observed under light-field microscope. Histological analyses were carried out in hematoxylin-eosin stained- and phalloidin stained-paraffin sections. Intense contraction was correlated with formation of concentric, fibrillar aggregation of cardiac cells. In those tissues that showed intense contraction, long axis of the cells was aligned in circular direction of the ring. Optimal concentration for collagen was about 0.7 mg. In the ring culture underwent 10% stretch at frequency of 0.5 and 1 Hz, spontaneous, macroscopic contraction was evident. In contrast, ring cultures kept in static condition only showed microscopic contraction. This result suggests that contractile function of reconstituted cardiac tissue was facilitated by mechanical stretch.

(COI: No)

P1-324

Relation between the Hemodynamic Macroscopic and the Microscopic Parameters: Model Study

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A model consisted of a circulation model and a ventricular cell model is useful to understand the relation among the cellular characteristics and the circulation properties, however, several scale factors can be found unknown, if we try to construct such model. Specifically, there are very few experimental data for the scale factor that converts cellular contraction force to the heart wall active stress, and the scale factor that converts cellular passive elastic force to the heart wall passive elastic stress. In this research, a circulation model, which consists of a cardiac contraction model, Negroni Lascano 2008 model, a cardiovascular hemodynamics model, Heldt 2008 model, and a left ventricular geometry model, Laplace's law model was used to determine the ranges of the unknown scale factors. To calculate the possible ranges of those parameters, we used the experimental hemodynamics data, which includes several end-diastolic and end-systolic pressure and volume data, and rates of the left ventricular volume change, left ventricular wall thickness change, left ventricular pressure change at the moment of the maximum LV volume change. We also assumed that the left ventricular wall passive elastic stress is negative at the same moment. Finally, we found that the scale factor that converts positive cellular passive elastic force to heart wall passive stress and a proportion of contractile force to the active force lies on small ranges which implies that the reliability of these values are fairly high.

(COI: No)

P1-325

Thiamine pyrophosphate preserved cardiac function against ischemia-reperfusion injury

Yamada, Yuki; Kusakari, Yoichiro; Ikegami, Taku; Kudo, Yuka; Minamisawa, Susumu (*Dept Physiol, Jikei Univ, Tokyo, Japan*)

Background: Thiamine (vitamin B1) deficiency was recognized as a cause of Beriberi (*Kakke*; a neurological disease and heart failure). Dr. Kanehiro Takaki who founded Jikei University, eliminated Beriberi from the Imperial Japanese Navy with an improved diet (thiamine supplementation). Thiamine pyrophosphate (TPP), a thiamine derivative, is an active form of thiamine. However, the effect of TPP on ischemia/reperfusion (I/R) heart has not been elucidated.
Aim: The present study was to investigate the effect of TPP on cardiac function after I/R injury.
Method: Male Sprague-Dawley rats (around 10 weeks old) were used of this study. Hearts were extracted and quickly put into Langendorff perfusion. A balloon was inserted into the left ventricle to measure left ventricular developed pressure (LVDP) by calculating the difference of systolic and diastolic pressure. After 5 min perfusion of Tyrode's solution with or without 100 μM TPP, the hearts were treated with mild ischemia (20 min global ischemia followed by 30 min reperfusion: mild I/R) or with severe ischemia (40 min global ischemia followed by 60 min reperfusion: severe I/R).
Results: In mild I/R, LVDP after 30 min reperfusion in TPP solution were significantly higher than that in control solution (74.6 ± 7.5 mmHg in TPP, 50.7 ± 3.8 mmHg in control, n=3 each, $p<0.05$). Furthermore, in severe I/R, LVDP after 60 min reperfusion in TPP solution were dramatically higher than that in control (60.9 ± 7.1 mmHg in TPP, 13.2 ± 5.2 mmHg in control, n=5 each, $p<0.001$).
Conclusion: TPP has a cardioprotective effect against I/R injury.

(COI: No)

P1-326

Time course of changes in sympathetic nerve activity and arterial pressure during development of obesity in Zucker fatty rats

Shiwa, Yuki; Yoshimoto, Misa; Okano, Rika; Miki, Kenju (*Dept Physiol, Nara Womens Univ, Nara, Japan*)

Obesity-associated arterial hypertension has been implicated in activation of the sympathetic nervous system, activation of the renin-angiotensin system, and sodium retention. However, there has been lack of direct evidence on the changes in sympathetic nerve activity during the development of obesity. In the present study, potential contribution of sympathetic nerve activity to the development of hypertension was assessed in Zucker fatty rats. Male Zucher fatty (fa/fa) rats were chronically instrumented with bipolar electrodes for measurements of renal (RSNA) and lumbar sympathetic nerve activity (LSNA), and a telemeter was used for measurement of arterial pressure (AP). The time course of changes in AP, heart rate (HR), RSNA, and LSNA were measured continuously and simultaneously from 8 to 12 weeks of age. Body weight progressively increased from 256 ±2g at 8th weeks and to 428 ± 7g at 12th weeks. AP remained constant around 100mmHg. HR gradually decreased throughout the experimental period. RSNA and LSNA did not appear to increase in association with the increase in body weight over 8-12 weeks of age. These results suggest that the progressive increase in body weight over 4 weeks is not directly related to the changes in RSNA, LSNA and HR, and such that AP in Zucker fatty rats.

(COI: No)

P1-327

Effects of peripheral chemoreceptor denervation on cardiovascular and sympathetic responses to obstructive sleep apnea in conscious rats

Shizuka, Kataoka; Yoshimoto, Misa; Mizukami, Yuri; Kajihara, Chisato; Miki, Kenju (*Dept Physiol, Nara Wemans Univ, Nara, japan*)

Potential role of peripheral chemoreceptor in regulating responses of systemic arterial pressure, heart rate, and sympathetic outflows to obstructive sleep apnea was studied. Wistar male rats were chronically instrumented with electrodes for measurements of renal (RSNA) and lumbar (LSNA) sympathetic nerve activity, and electroencephalogram, electromyogram, and electrocardiogram and with catheter for measurement of systemic arterial pressure and with a tracheal balloon for induction of apnea. The tracheal balloon was inflated for 40 seconds during non-rapid eye movement sleep. Systemic arterial pressure, RSNA, and LSNA increased after onset of the tracheal balloon inflation while heart rate decreased. Bilateral carotid body resection attenuated significantly the magnitude of the decrease in heart rate observed in the intact rats, however it exerted minor effects on the responses in arterial pressure and RSNA and LSNA to the obstructive sleep apnea. These data suggest that peripheral chemoreceptor may play a critical role in the bradycardia in response to obstructive sleep apnea, and a minor role in the increases in systemic arterial pressure, RSNA and LSNA in conscious rats.

(COI: No)

P1-328

Suppressive effects of estradiol replacement on stress-induced pressor response mediated by renal sympathetic nerve in ovariectomized rats

Nagatomo, Yu; Tazumi, Shoko; Ito, Risa; Yoshimoto, Misa; Takamata, Akira; Morimoto, Keiko (*Dept. Environm. Health, Facult. Human Life & Environm., Nara Women's Univ., Nara, Japan*)

We examined whether chronic estrogen replacement has suppressive effects on psychological stress-induced pressor responses by attenuating the renal sympathetic nerve (RSN) and renin-angiotensin system (RAS) in ovariectomized rats. Female Wistar rats aged 9 wk were ovariectomized. After 4 wk, the rats were assigned either to a placebo-treated (Pla; n=6) group or a group treated with 17 β-estradiol (E2; n=6) subcutaneously implanted with either pellet. Two wk later, the rats were denervated renally and implanted with radiotelemetry devices for blood pressure (BP) and heart rate (HR) measurements. These rats underwent cage-switch stress at 2 wk after the renal sympathetic denervation. The stress elevated the BP and HR rapidly and continuously both in the Pla and E2 groups. However, these responses to the stress were attenuated significantly in the E2 group compared with the Pla group. Simultaneously, the stress induced elevations of plasma renin activity and angiotensin II concentration in Pla group, but not in E2 group. In addition, the renal sympathetic denervation attenuated the pressor response in the Pla group, but not in E2 group. Therefore, the denervation abolished the difference in the pressor responses between the two groups. These results suggest that estrogen replacement attenuates psychological stress-induced pressor response by suppressing RSN-RAS activation in the ovariectomized rat.

(COI: No)

P1-329

Influence of high-cholesterol on arrhythmogenicity in mouse atrium

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Background: Changes in cardiac structures due to inflammation and electrophysiological properties may play an important role to generate chronic atrial fibrillation (Af). High cholesterol (Chol) causes systemic inflammation, however the influence of high-Chol on Af is not clarified. We hypothesized that high-Chol induces fibrosis via inflammation in atrial tissue to ameliorate Af.
Methods: Wild type mice (WT) and mice knocked-out the anti-inflammatory mediator IL-10 (KO) were given normal diet (ND) or high-fat diet (HFD) to organize four groups (WT-ND, WT-HFD, KO-ND, KO-HFD). Body and heart weight, serum Chol level, cardiac function on ultrasound cardiography (UCG), ECG parameters, and duration of Af induced by transesophageal pacing were compared. RNA was obtained from mouse atrium to quantify by real-time PCR.
Results: In both WT and KO mice, HFD increased body weight and serum Chol. UCG revealed no significant differences in cardiac function including left atrial diameter. P wave duration on ECG was significantly longer in KO-HFD than in WT-ND. Af sustained significantly longer in KO-HFD than in other three groups. In KO mice, α-SMA and the K^+ channel significantly increased, where the Na^+ channel decreased. Gap-junctional protein Cx40 significantly decreased in mice given HFD.
Conclusion: Chronic inflammation increases fibrosis, impaires electrical conduction by reducing the Na^+ channel, and shortens refractory period by increasing the K^+ channel. In addition, high-Chol decreases Cx40, which also reduces conduction property in atrium. The combination of chronic inflammation and high-Chol ameliorates Af.

(COI: No)

P1-330

Role of apelin in human atrial tissue with persistent atrial fibrillation

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The aim of this study was to identify a group of genes abnormally expressed in cardiomyocytes with atrial fibrillation (Af), and to examine the role of genes involved in pathogenesis of Af. We analyzed expression profiles of mRNA from patient s right atrial appendage with persistent Af (n=10) and normal sinus rhythm (NSR, n=10) by use of gene microarray platform covering a total of 18855 human genes. We found that 149 mRNAs were differentially expressed between persistent Af and NSR cardiomyocytes, where 6 genes were classified as hormone-related genes. Among them, apelin was significantly highly expressed in Af cardiomyocytes, which was further comfirmed by quantitative real-time PCR. Protein expression of apelin was also markedly increased in Af cardiomyocytes. By exposing Ca^{2+} ionophore A23187, isoproterenol and apelin, neonatal rat cardiomyocytes showed up-regulation of apelin and down-regulation of apelin receptor (APJ). Apelin was highly expressed in cardiomyocytes whereas APJ was highly expressed in fibroblast. Treatment of cardiomyocytes with apelin did not change expression of ion channels ($Ca_V1.2$, KCNJ2, KCNJ3). On the other hands, α-SMA, a marker of myofibroblast, was markedly increased by apelin in a dose-dependent manner. Our data provide first evidence that Ca^{2+} overload and/or β-adrenoceptor stimulation in cardiomyocytes increase expression of apelin which triggers neighboring myofibroblast activation leading to formation of Af substrate in the heart.

(COI: No)

P1-331

Subtype-specific down-regulation of Ca^{2+} and cAMP signaling proteins in diabetic mouse atria

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Diabetes mellitus (DM) is one of high risk factors for atrial fibrillation (AF) and its prevalence in aged population. Atrial structural and electrical remodeling underlies progression of AF. Electrical remodeling is characterized by refractoriness due to a shortening of APD in atrial myocytes. In addition to K^+ channels, Ca^{2+} handling and β-adrenergic receptor (β-AR)/cAMP signaling proteins are major determinants of APD. In order to clarify the mechanism linking DM and AF, we examined the age-dependent changes in expression levels of Ca^{2+} and cAMP signaling proteins in atria of DM mice.

Methods: DM was induced in C57BL/6J mice (male, 8-week-old) by a single injection of streptozotocin (STZ, 180 mg/kg, i.p.). Hyperglycemia was confirmed by a blood glucose test. Four weeks after injection of STZ, hearts were excised from mice under deep anesthesia. Gene expression levels were quantified by qRT-PCR.

Results & Discussion: In atria of DM mice, mRNA levels of L-type Ca^{2+} channel CaV1.2 and CaV1.3, junctophilin-2 (JPH2), SERCA2, adenylate cyclase 6 were significantly lower than vehicle controls (n=6). The level of ryanodine receptor 2 (RyR2) was also lower in DM mice. In contrast, gene expression levels of βAR1 and βAR2 tended to be higher in atria of DM mice than controls, while mRNA levels of βAR3, M2R, AC1, AC5, and IP_3R2 were not different between the two groups. These results indicate that DM causes subtype-specific down-regulation of Ca^{2+} and cAMP signaling proteins, which may lead to a shortening of APD and raise the risk for AF.

(COI: No)

P1-332

Comparison of effects of eugenol on respiratory activity in the brainstem-spinal cord preparation from newborn rat and in the arterially perfused preparation from juvenile ratjuvenile rat

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Eugenol is contained in several plants including clove and modulates neuronal activity through actions on voltage-gated ionic channels and/or on transient receptor potential channels. We have reported effects of eugenol on respiratory rhythm generation in the *in vitro* brainstem-spinal cord preparation from newborn rat (P0-P3). Here we compared effects of eugenol in the *in vitro* preparation with those in the decerebrate and arterially perfused *in situ* preparation from juvenile rat (P12 - P15). In the *in vitro* preparation from newborn rats, bath application of eugenol (0.5-1 mM) decreased respiratory rate accompanied with inhibition of pre-inspiratory neuron burst. 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 washout. In contrast, in the in situ preparation, eugenol (1 mM) induced gradual decrease in the amplitude (but not the rate) of integrated phrenic nerve activity followed by complete disappearance within 10 min. Phrenic nerve activity gradually recovered at 25-30 min after washout with burst duration similar to control values. Thus, we found noticeable difference in effects induced by eugenol between two types of preparations; shortening of burst duration in the *in vitro* preparation but decrease of burst amplitude in the*in situ* preparation. We discuss possible mechanisms of these different effects.

(COI: No)

P1-333

Interaction of testosterone with thyroid hormone on sex differentiation of brain in PD7 female rat pups

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Sex differentiation of brain is determined by sex steroids during the perinatal critical period, ED18 - PD5, in the rat. However, it is still unknown what regulates the steroid sensitivity. In this study, we examined the interactive effects of testosterone (T) and thyroid hormone (T3) in PD7 female rat pups, after ceasing the critical period, on adult ovarian functions and sexual behaviors. Three groups of females were treated with one of following combinations, TP+T3: T propionate (TP, 250 μg / 0.05 mL sesame oil, sc) and T3 (30 μg / 500 nL saline, iv), TP+S: TP and saline, and O+T3: sesame oil and T3. After maturation, observations of vaginal smear revealed that PD7 TP treatment (TP+T3 and TP+S) induces acyclicity. At 9 weeks old, all females were ovariectomized and implanted sc with a T capsule, and weekly tested for olfactory preference (estrous and male odors) and male sexual behavior 3 times. TP+T3 and TP+S, but not O+T3, females showed frequent mount, whereas only TP+T3 females showed masculinized preference, i.e. preferring estrous odor to male odor. After intervening 4 weeks following removal of T capsules, female sexual behavior was tested under estrogen and progesterone priming. TP+S and O+T3 females showed almost 100% lordosis quotient (LQ = # lordoses / # mounts x 100), while TP+T3 females had significantly lower LQ. These demonstrate that T3 is involved in both masculinization and defeminization via the interaction with androgen. We suggest that T3 is one of determinants opening the critical window for brain sex differentiation.

(COI: No)

P1-334

Oxidative stress and anti-oxidative responses during hyperthermia in mice brain and the effect of daily exercise

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Recent studies have shown that hyperthermia induces inflammatory response in rodents. Hypothalamic damage has long been speculated as the mechanism involved in heat stroke, although there are few evidences. In the present study, we assessed oxidative stress in the brain during hyperthermia in mice. In addition, we examined the effect of daily exercise. Male mice (n=20) were used. One group had a voluntary running wheel in a cage (Ex), the other not (NEx). After the 6-w period, each group had a 3-h exposure at 39.5℃ (H) or remained at the housing temperature (C). At the end of the exposure, the animals were sacrificed, and the basal parts of the brain tissues were obtained. The protein expressions of SOD1/2, UCP2, NF-κ β and 4-HNE were analyzed by Western blotting. Body temperature in both Ex-H and NEx-H groups became body temperature of >41℃, although the NEx-H group reached the level earlier. SOD2 were much greater in the Ex-C group than in the NEx-C group. In addition, SOD2 in the Ex-H group was greater than that in the Ex-C group. The expression of 4-HNE was smaller in the NEx-H group than in the NEx-C group, but NF-κ β was greater. Hyperthermia may activate anti-oxidative responses, decreasing 4-HNE, one of reactive oxidative species. However, such change was not observed in the Ex groups. The reason may be greater basal ability of anti-oxidative responses or smaller heat stress due to augmented thermoregulatory ability in the Ex groups.

(COI: No)

P1-335

Roles of lysyl oxdase like factor2 (LOXL2) in the recruitment of TGFβ activities on premetastatic niche formation on lymph node metastasis of squamous carcinoma cells

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TGF β plays multiple roles in a variety of physiological processes such as tissue remodeling, wound healing and reorganization as well as pathological events such as tumor metastases. This cytokine is secreted from many cells in inactive latent form, and be activated on various biological demands. We had already established highly effective lymph node metastasis model of squamous cell carcinoma by using human metastatic squamous carcinoma cell line SASL1m. By xenografting this cell line to KSN nude mice tongues, we obtained almost 100% of metastases to the submandibular lymph node which corresponds to the draining lymph node for the tongue. Simultaneously, we found the formation of premetastatic niches prior to the establishment of macrometastases to tongues in this model. We also found upregualtions of several genes including TGF β and LOXL2 in SASL1m cells by gene expression profiling analyses compared with non-metastatic oral cancer cell ACC2. LOXL2 acts as a deaminase on amino group of lysin residue, and participates in the remodeling of extracellular matrices (ECM) by cross-linking of ECM proteins such as collagen or elastin. We identified the expression of LOXL2 protein and confirmed TGF β activities in SASL1m cell culture media, and are exploring the possible interactions of LOXL2 and the recruitment of TGF β activities on premetastatic niche formations on the squamous carcinoma cell metastases to draining lymph nodes.

(COI: No)

P1-336

Tumor-associated vascular endothelial cells express CD90 in rat experimental gliomas

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We have found specific expression of CD90 by tumor-associated endothelial cells (TECs) in experimental GBs (EGBs) that had been prepared by transplanting C6 glioma cells into the rat forebrains. Vasculatures in the normal brain parenchyma are surrounded by astrocytic endfeet, whereas those in the EGBs were not. CD90+ cells in the EGBs expressed an endothelial marker von Willebrand factor (vWF) and NG2 chondroitin sulfate proteoglycan (NG2). herefore, the CD90+ cells were judged as TECs. To elucidate the origin of the CD90+ TECs, C6 glioma cells were transplanted into the forebrain of the rats that had been subjected to bone marrow transplantation with the use of green rat marrows as donors. As a result, only a few number of EGFP+/CD90+ cells were found in the EGB mass. Furthermore, there was a very few number of CD90+ circulating monocyte-derived cells labeled with intravenously injected red fluorescent Rhodamine 6G in the EGBs. EGBs contained a huge number of tumor associated macrophages (TAMs) and TAMs were located around CD90+ TECs. These results suggest that not many but some CD90+ TECs was from bone marrow derived circulating monocytes. CD90 on activated endothelial cells has been recognized as a counterreceptor for CD11b that is a marker for monocytes and macrophages. Therefore, it is likely that CD90+ TECs may enhance influx of circulating monocytes as progenitors for TAMs.

(COI: No)

P1-337

Degradation rate of tyrosine hydroxylase by ubiquitin-proteasome pathway

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Tyrosine hydroxylase (TH) is the rate-limiting enzyme in catecholamine biosynthesis, and the portion of the enzyme controlling the intracellular stability has been assigned to the N-terminus. We have reported that TH is degraded by the ubiquitin-proteasome pathway and that the phosphorylation of Ser residues in N-terminus plays a critical role in the degradation of the enzyme. However, the mechanism by which the phosphorylation triggers this degradation pathway in the cell remains unknown. In this study, we investigated the role of the phosphorylations of TH in the degradation of this enzyme. The enhancement of proteasome activity with USP14 inhibitor decreased the quantity of TH phosphorylated at Ser19 in PC12D cells, although it did not decrease that of TH molecules and that of TH phosphorylated at Ser31 and Ser40. The inhibition of proteasome activity with MG132 increased only the quantity of TH phosphorylated at Ser19 by 5-folds for 8 hr. Moreover, we revealed that TH phosphorylated at Ser19 accounted for about 2% of all TH molecules. Therefore, we propose that the phosphorylation of Ser19 is a trigger for the proteosomal degradation and that TH molecule is degraded at a rate of about 1% per hr in PC12D cells. These results present critical information to understand the mechanism that the level of TH is maintained constant in the cells. Support contributed by: KAKENHI (25461296)

(COI: No)

P1-338

Degradation of anillin in cell cycle is mediated by proteasome

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Anillin, a scaffold protein linking actin and myosin, controls the contractile ring spatially and is required for cytokinesis. Anillin is a substrate of anaphase-promoting complex/cyclosome (APC/C) which acts as an ubiquitin ligase. Therefore, anillin level decreases in the exited cells from M phase to G1 phase. Degradation of anillin is known to be important for cell function in G1 phase beyond cytokinesis because untimely accumulation of anillin in G1 phase is toxic to the cells. Anillin is degraded through ubiquitin dependent pathway, however, it is unclear whether anillin is degraded by proteasome or lysosome.

To investigate the degradation pathway of anillin in cell cycle, HeLa S3 cells were synchronized by a double thymidine block, and treated with proteasome inhibitor MG132 or lysosome inhibitor chloroquine.

Anillin was degraded in cell cycle-dependent manner with chloroquine treatment. On the other hand, MG132 treatment induced accumulation of anillin. These findings indicate that degradation of anillin in cell cycle is mediated by proteasome.

(COI: No)

P1-339

A novel angiogenic agent COA-Cl induced PGC-1α-mediated VEGF production in cultured human fibroblasts

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COA-Cl is a recently developed pro-angiogenic agent. It induces robust tube formation in human umbilical vein endothelial cells. A direct endothelial effect, which is partly mediated by S1P receptor, contributes to angiogenesis by COA-Cl. However, the angiogenic effect of COA-Cl was augmented, when co-cultured with fibroblasts. Therefore, mechanisms by which COA-Cl promotes angiogenesis still remain elusive. Here we addressed the hypothesis that COA-Cl induces VEGF production in fibroblast. COA-Cl (100 μM, 48 h) increased basal secretion of VEGF into culture medium from 16 ± 15 to 274 ± 52 pg/mL ($p<0.05$) in human dermal fibroblasts, as evaluated by ELISA. COA-Cl also up-regulated the expression of VEGF mRNA by 2.1 fold (RT-PCR, $p<0.05$). Two transcriptional regulatory proteins, PGC-1 α and HIF-1 α, have been identified as major activators of VEGF gene. COA-Cl markedly up-regulated the mRNA expression of PGC-1 α, but not HIF-1 α, in a time- and dose-dependent manner. Conversely, siRNA-mediated silencing of PGC-1 α gene attenuated COA-Cl-induced VEGF production. The results suggest that COA-Cl induced VEGF production by up-regulating PGC-1 α in fibroblasts, which underlies the augmented angiogenic effect of COA-Cl in the presence of fibroblast.

(COI: No)

P1-340

Anti-inflammatory actions of an old hypnotic bromvalerylurea on microglia/macrophages

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We have recently noticed that an old hypnotic bromvalerylurea (BU) has an anti-inflammatory effect on microglia/macrophages, as BU suppressed expression of pro-inflammatory mediators by LPS-treated rat primary cultured microglial cells or peritoneal/alveolar macrophages. So far there are no literatures on the anti-inflammatory actions of BU, therefore, we aimed to elucidate the molecular mechanisms underlying the BU actions. When treated with interferon-gamma (IFN-gamma), phosphorylation of STAT1 was observed in macrophages that was abolished by BU. BU also suppressed IFN-gamma-induced upregulated mRNA expression for STAT1, IRF1 and iNOS. Similarly, BU suppressed LPS-induced upregulated expression of mRNA encoding IL-1beta, IL-6 and Cox2. In spite of the marked immunosuppressive effects of BU, BU did not suppress LPS-induced nuclear translocation of NFkappaB. ChIP assay revealed that BU did not prevent binding of NFkappaB to the target DNA region. BU suppressed IFN-gamma-induced STAT1 phosphorylation in microglial cells that was incubated with LPS for 3 h. When JAK1 or STAT1 expression is knocked down with siRNA, response of macrophages to LPS was almost disappeared. BU did not affect GM-CSF-induced JAK2-dependent STAT5 phosphorylation in macrophages. These results suggest that BU exert its immunosuppressive or anti-inflammatory effects at least partly through specific inhibition of JAK1.

(COI: No)

P1-341

Therapeutic effects of an old hypnotic bromvalerylurea on sepsis of rats

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Sepsis has a high mortality rate, therefore, a novel effective treatment should be developed. We recently observed marked immunosuppressive effects of an outdated hypnotic drug, bromvalerylurea (BU), on lipopolysaccharide (LPS)-activated microglial cells, resident macrophages in the brain. In this study, it was investigated whether BU treatment ameliorated cecum ligation and puncture (CLP)-induced sepsis by suppressing proinflammatory reactions of alveolar and peritoneal macrophages (AMs and PMs). BU suppressed LPS-induced production of NO, IL-1beta, IL-6, TNFalpha and chemokines by AMs and PMs in vitro. Male Wistar rats were subjected to cecum-ligation and puncture (CLP). Shortly after CLP, septic rats were subjected to subcutaneous injection of BU (twice/day) at a dose of 30 mg/kg body weight/day, which was clinically relevant. BU treatment suppressed lung edema and ameliorated blood gas test data, with decreased mortality rate of the septic rats by approximately 38% 48 hours after CLP. These effects of BU may be attributable to the suppression of accumulation of leukocytes in the lung, vascular permeability enhancement and expression of proinflammatory cytokines by AMs and PMs. Although BU is now seldom used in clinics, it is worth reevaluating as a novel agent for sepsis and related disorders.

(COI: No)

P1-342

Suppressive effects of bromvalerylurea on LPS-treated activated alveolar and peritoneal macrophages

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Recently, we have found anti-inflammatory actions of an old hypnotic/sedative bromvalerylurea (BU) on LPS-treated microglial cells. BU also suppressed LPS-induced NO release by rat alveolar and peritoneal macrophages. BU inhibited expression of mRNA encoding a wide variety of proinflammatory mediators including proinflammatory cytokines (such as interleukin-1beta (IL-1beta), IL-6, tumor necrosis factor-alpha), chemokines CCLs 2, 3, and 4, and inducible nitric oxide synthase (iNOS) by LPS-treatedalveolar macrophages. These results suggest that the inhibitory action of BU exerts at transcriptional level. However, BU did not prevent LPS-induced translocation of NFkappaB into nuclei as well as LPS-induced IkappaB degradation as observed in the microglial cells culture. BU inhibited interferon-gamma-induced and LPS-induced STAT1 phosphorylation but not GM-CSF-induced JAK2-dependent STAT5 phosphorylation. Noradrenaline suppressed LPS-induced activated reactions of microglia similarly to BU, but mainly through suppressing LPS-induced NFkappB translocation into nuclei. To determine the molecular mechanisms underlying the suppressive effects of BU, we are now conducting the studies on comparison of anti-inflammatory effects of BU and noradrenaline.

(COI: No)

P1-343

Tumor-associated macrophages; involvement of ECM proteins in induction of their M2 phenotype in experimental gliomas in the rat brain

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Dense accumulation of macrophages in gliomas has been correlated to the worse prognosis. Such macrophages in the tumor tissue (tumor-associated macrophages; TAMs) display an alternatively activated or M2 phenotype, characterized with suppressed proinflammatory nature and promoting activity for tumor growth. In this study, we aimed to elucidate the involvement of extracellular matrix (ECM) proteins in the induction of M2 phenotypes of TAMs. An experimental malignant glioma model was established by transplanting C6 glioma cells into neonatal rat forebrains. Rats developed visible tumors in the brain within 4 weeks. TAMs densely accumulated in the tissue mass. C6 glioma cells were found to expressed an ECM protein tenascin C (TNC) at a high level. TAMs in the glioma were isolated from the dissected tumor tissue and they were cultured solely or cocultured with C6 cells. TAMs were also cultured on C6 glioma cell-derived ECMs. As a result, solely cultured TAMs expressed mRNA encoding chemokines CCL2, 3, and 4 as well as IL-1beta at high levels. On the other hand, TAMs cocultured with C6 cells or those cultured on C6 cell-derived ECM displayed suppressed expression of these proinflammatory factors. Furthermore, when cocultured with C6 cells, of which TNC expression was knocked down, expression of the proinflammatory mediators were elevated. These results suggest that C6 glioma cell-derived TNC may be responsible for the polarization of TAMs to M2 phenotype.

(COI: No)

P1-344

Oct-3/4 promotes the drug-resistant phenotype of glioblastoma cells by enhancing expression of ATP binding cassette transporter G2

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Drug resistance of malignant tumor cells is a major obstacle for the efficacy of chemotherapeutic treatment. Various solid tumor cells expressed Oct-3/4, which has been implicated in the malignancy and also in poor prognosis of GBs, but little is known of its involvement in drug resistances of GB. In this study, we tried to elucidate the contribution of Oct-3/4 to drug resistance in GB cells by lactate dehydrogenase assay, poly ADP-ribose polymerase cleavage, and efflux assay of an anti-cancer drug doxorubicin. A drug efflux pump gene responsible for Oct-3/4-induced drug resistance was evaluated by quantitative PCR analysis and knockdown by shRNA. Oct-3/4 decreased the susceptibility to chemotherapeutic drugs by enhancing excretion of drugs through a drug efflux pump gene, ATP binding cassette transporter G2 (ABCG2). Moreover, expression of Oct-3/4 was well correlated to ABCG2 expression in patients with GBs. Collectively, Oct-3/4 may elevate ABCG2 expression leading to acquisition of a drug-resistant phenotype of GB cells. The present study provides evidence that a signaling pathway from Oct-3/4 to ABCG2 is a promising target to prevent GB cells turning into cells with drug-resistant phenotypes.

(COI: No)

P1-345

Olopatadine Inhibits Exocytosis in Rat Peritoneal Mast Cells by Counteracting Membrane Surface Deformation

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Besides its anti-allergic properties as a histamine receptor antagonist, olopatadine exerts mast cell stabilizing properties by inhibiting the release of chemokines. Since olopatadine bears amphiphilic features and is preferentially partitioned into the lipid bilayers of the plasma membrane, it would induce some morphological changes in mast cells and thus affect the process of exocytosis. In the present study, employing the standard patch-clamp whole-cell recording technique, we examined the effects of olopatadine and other anti-allergic drugs on the membrane capacitance (Cm) in rat peritoneal mast cells during exocytosis. Using confocal imaging of a water-soluble fluorescent dye, lucifer yellow, we also examined their effects on the deformation of the plasma membrane. Relatively lower concentrations of olopatadine (1 or 10 μM) did not significantly affect the GTP-γ-S-induced increase in the Cm. However, 100 μM and 1 mM olopatadine almost totally suppressed the increase in the Cm. Additionally, these doses completely washed out the trapping of the dye on the cell surface, indicating that olopatadine counteracted the membrane surface deformation induced by exocytosis. This study provides electrophysiological evidence for the first time that olopatadine dose-dependently inhibits the process of exocytosis in rat peritoneal mast cells. Such mast cell stabilizing properties of olopatadine may be ascribable to its counteracting effects on the plasma membrane deformation in degranulating mast cells.

(COI: No)

P1-346

Extracellular Na^+ ion dependency of hypotonic swelling of HeLa cells

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The mechanism of cell volume regulation after hypotonic swelling has been widely investigated and the role of ion channels or transporters has been reveiled. On the other hand, the swelling process just after the hypotonic challenge has been considered to be attained by the water influx driven by the difference of osmolarity between extracellular and intracellular solutions. In this study, we examine the possibility of the water influx is enhanced by the ionic influx, especially Na^+ ion. The cell volume of enzymatically suspended HeLa cells was measured by FACS. Hypotonic condition was obtained by the addition of water to the experimental solution. The cell volume was increased rapidly after hypotonic challenge as reported before. The replacement of Na^+ ion by choline suppressed the cell swelling. NKCC inhibitor, bumetanide, inhibited cell swelling by 48% and ENAC inhibitor, amiloride, inhibited cell swelling by 18%. These data suggest that water influx after hypotonic challenge is partially driven by Na^+ ion influx via NKCC or ENAC.

(COI: No)

P1-347

Dysregulation of mitochondrial formyltransferase MTFMT in gastric cancer

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Mitochondrial protein synthesis is responsible for biosynthesis of 13 proteins, which are essential for constitution of mature respiratory chains. Dysregulation of mitochondrial protein synthesis impairs oxidative phosphorylation, which shifts aerobic respiration to anaerobic glycolysis. The enhanced glycolysis, namely Warburg effect, is a hallmark of cancer. However, the molecular mechanism underlying mitochondrial dysfunction in cancer remains unclear. To investigate the regulatory mechanism of mitochondrial protein synthesis in cancer cells, we performed a systemic investigation of the expression level of genes involved in mitochondrial protein synthesis in a large clinical samples derived from patients having gastric cancer. Interesting, MTFMT, a mitochondrial tRNAMet-specific formyltransferase gene, is significantly upregulated in cancer tissues. Because the formyl-tRNAMet is specifically used for start codon ATG dysregulation of MTFMT might have profound effect on mitochondrial protein synthesis. As expected, overexpression of MTFMT in gastric cancer cells decreased mtDNA-derived mitochondrial proteins. Furthermore, the expression of MTFMT was under control of oncogene c-Myc. These results demonstrate that the activation of c-Myc-MTFMT pathway might be responsible for the metabolic changes in gastric cancer.

(COI: No)

P1-348

Identification of the major asynapsis-induced phosphorylation site of mouse HORMAD1

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HORMAD1 is a mammalian homolog of yeast Hop1, which is necessary for meiotic recombination and surveillance mechanisms. We have made Hormad1 knockout mice, and found that HORMAD1 is necessary for synapsis and synapsis checkpoint in mammalian meiosis. In yeast, DNA double strand break (DSB)-induced phosphorylation of Hop1 is necessary for its function. Mouse HORMAD1 has multiple putative phosphorylation sites, and is intensively phosphorylated in SPO11-deficient meiocytes, where extensive asynapsis occurs due to the absence of DSBs. Despite the expected importance of this asynapsis-induced phosphorylation of HORMAD1, the phosphorylation site has not yet been identified. In this study, we made phospho-specific antibodies against two candidate phosphorylation sites, Ser-307 and Ser-378, and examined their localization on meiotic chromosomes by immunostaining of spermatocyte spreads. As a result, both Ser-307 and of Ser-378 were phospholylated on unsynapsed chromosomal axes at zygotene stage, and on the XY chromosome axes at pachytene stage in wild-type spermatocytes. In addition, interestingly, Ser-307, but not Ser-378, was phosphorylated on entire unsynapsed axes in SPO11-deficient spermatocytes. We further confirmed by western blotting that the band of phosphorylated HORMAD1 was positive for the Ser-307 phosphorylation in SPO11-deficient testes. These results for the first time demonstrate that Ser-307 is the major phosphorylation site of mouse HORMAD1 on unsynapsed axes, providing a clue to reveal the molecular basis of asynapsis surveillance mechanism.

(COI: No)

P1-349

The progeny of bone marrow stem cells with metabolic memory perturbs skin homeostasis

Okamoto, Naoki[1]; Okano, Junko[1]; Kojima, Hideto[2]; Katagi, Miwako[2]; Nakae, Yuki[2]; Terashima, Tomoya[2]; Udagawa, Jun[1] (*[1]Shiga. Univ. Med. Sci., Shiga, Japan; [2]Shiga. Univ. Med. Sci., Shiga, Japan*)

The major cell population in epidermis is keratinocytes with more than 95%, while other cells such as melanocytes and Langerhans cells (LCs) are observed as minor cell population. Although it has been well known that bone marrow-derived cells (BMDCs) are mobilized to replenish the LC population upon severe inflammation, the dynamic state of BMDCs in skin remains unknown at either steady state or mild alteration of microenvironment. Using ionizing radiation at a relatively low dose for skin (10 Gy), we investigated the role of BMDCs in skin under mild alteration of microenvironment. We transplanted KSL (c-kit+Sca1+Lin-) cells, an early form of hematopoietic stem cells, from GFP reporter mice to irradiated wild-type mice in order to chase the progeny of BMDCs as GFP+ cells. The descendants of KSL cells migrate into the epidermis one month after transplantation. The population was heterogeneous and some of them are ramified with MHCII+EpCAM+Langerin+, indicating Langerhans cells. To pursue the metabolic memory on the progeny of KSL cells migrating into epidermis, we transplanted KSL cells from GFP reporter mice with diabetes mellitus to irradiated wild-type mice. The descendants from KSL cells exposed to hyperglycemia affected homeostasis of skin, indicating that dynamic cells (e.g. BMDCs) as well as static cells (e.g. keratinocytes) play an important role on the maintenance of skin microenvironment.

(COI: No)

P1-350

Species difference in expression and localization of androgen receptor in the suprachiasmatic nuclei of normal and hormone-manipulated adult rats and mice

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The suprachiasmatic nucleus (SCN) is a master pacemaker of the CNS, which regulates a wide variety of neural and bodily rhythms. Hormones are considered as critical intrinsic modifiers to the SCN clock directly through regulating expression of numerous SCN genes via their receptors. Although we have recently reported that androgen receptor (AR) expression was higher in males than females and critically more prominent in mice than in rats, detailed localization of AR expression and its hormonal regulation in the SCN remain to be clarified in the two rodents. In the present study, detailed AR distribution was immunohistochemically examined in serial paraformaldehyde-fixed sections of the SCN and compared between hormonally manipulated rats and mice. The current results demonstrated that AR expression in the SCN is upregulated by dihydrotestosterone treatment in both castrated rodents and clarified that enhanced AR expression in the rat SCN is localized to the shell part (output part), making a sharp contrast with prominent AR expression in the core part (input part) of the mouse SCN. Our observation strongly suggests that the species difference in effects of clock-controlled androgen secretion on circadian rhythmicity is attributable to the species difference in expression and localization of AR in the SCN. Androgen might be regarded as a species-dependent "clock-controlled clock modifier".

(COI: No)

P1-351

Circadian Rhythm in Skin

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Animals evolved endogenous timing clocks called circadian rhythms (~24 hours) to adapt external environment. Circadian rhythm is controlled by core clock genes (Bmal1, Clock, Period1/2/3, and Cryptochrome1/2), among which Bmal1 and Clock act as master regulators. Although biological functions of circadian rhythm are well-studied in the central nervous system as well as various peripheral tissues, its role in skin remains unclear. Here, we report epidermal cells isolated from mice show circadian changes in the gene expression of the core clock genes, and this endogenous rhythm in skin is independent of one in the central nervous system. In addition, we show that transepidermal water loss (TEWL), a reflection of the skin barrier function, rhythmically changes throughout a day. Taken together, these results indicated that circadian rhythm in skin regulated the physiological function of skin.

(COI: No)

P1-352

Effects of oxidative stress on circadian rhythm in vitro MEF from PER2::LUC mouse

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Oxidative stress is produced by reactive oxygen species (ROS), which can affect the physiological function of cellular proteins, lipids, nucleic acids and other macromolecular substances. Recent studies have focused on circadian-regulated energy metabolism, redox state, and intracellular ROS in living system and disease such as cancer and metabolic syndrome. However, how the circadian system responds to oxidative stress has not understood completely, and we have to reveal it. To clarify this question, we investigated the relationships between circadian rhythm and oxidative stress (H2O2 0.2 mM) produced at various points (CT 4, 8, 16, 20 and 22) in mouse embryonic fibroblast (MEF) cells from PER2::LUC mouse. We demonstrated that each exposure time point to oxidative stress droved the phase shifts (advance and delay), and shift direction were dependent on exposed timings. In addition, oxidative stress led to cell death in a time-dependent manner, which was consistent with the time-dependent phase shift. Furthermore, we found that H2O2 application caused amplitude reduction at a certain time point. Some kinase inhibitors (PKA inhibitor and CaMKII inhibitor) protected stress-induced reduction of amplitude. It is suggested that oxidative stress may be involved in CaMKII stress response pathway. Thus, the relationship between circadian clock and oxidative stress was strongly suggested, and it is necessary to continue to search for a signaling cascade on the phase shift and amplitude reduction due to oxidative stress.

(COI: No)

P1-353

Effects of aging on the peripheral clock gene expression rhythms

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In mammals, aging causes the disruption in sleep-wake cycles and the decline in neural activity rhythms in the SCN, suggesting that these changes are provided by age-related decline in clock gene expression rhythms. In this study, we reported age-related changes in clock gene expression rhythms in peripheral tissues using in vivo imaging system. We observed expression rhythms of PER2, one of the clock genes, in the kidney, liver, and submandibular glands of young and aged mice carrying with PER2::LUCIFERASE knock-in. We focused on the character and speed of the response to the entraining stimuli including light, food, and restraint stress. Regarding to light entrainment, the results demonstrated no differences in amplitude and phase of PER2 in peripheral tissues between young and aged mice, although the decline in amplitude of the behavioral rhythms was observed in aged mice. For the entrainment to restraint stress, there were no differences similarly. However, regarding to food entrainment, the phase of aged mice advanced more quickly than that of young mice only in the submandibular glands. In addition, expression levels of adrenergic receptors in the submandibular glands were reduced in aged mice compared to young mice. Thus, the present findings suggested that, in the submandibular glands of aged mice, food entraining signals became dominant signal corresponded with the reduction in adrenal signal.

(COI: No)

P1-354

A Fluorescence Resonance Energy Transfer Biosensor for TAK1 activity

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The stress-activated protein kinase (SAPK) signaling cascade evokes various cellular responses, such as apoptosis, differentiation and inflammation, under various cellular stresses. Here, we report a fluorescence resonance energy transfer (FRET)-based biosensor that responds to various stresses. A FRET biosensor "3592NES" was found to respond to stress-inducing reagents such as anisomycin, tumor necrosis factor-α, and interloikin1-β. Among various inhibitors for protein kinases, we found that an inhibitor against TGF-β activated kinase 1 (TAK1), 5z-7-oxozeaenol, markedly suppressed the stress-induced increase of FRET ratio in 3592NES-expressing cells. Furthermore, siRNAs against TAK1 also abrogated the stress-induced response of the 3592NES-expressing cells, indicating that 3592NES monitors the TAK1 activity. To examine whether 3592NES can monitor TAK1 activity in physiological contexts, we established Lewis lung carcinoma 3LL cells that stably express 3592NES. When the 3592NES-expressing 3LL cells were co-cultured with macrophages and stimulated with polyinosinic:polycytidylic acid (poly I:C), we observed increase in FRET ratio and induction of apoptosis. We also live-imaged 3592NES-expressing 3LL cells that were implanted subcutaneously into mice by two-photon excitation microscopy. High FRET signal was observed in tumor cells locating the periphery of tumor mass. These observations suggest that 3592NES is a versatile biosensor that monitors stress-induced TAK1 activation during inflammation and tumor development both in vitro and in vivo.

(COI: No)

P1-355

Sex-related difference in exponents of Stevens' power law

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Background: The magnitude of sensation is a power function of the intensity of the stimulus, known as the Stevens' power law. We have reported that the inverse process, "making stimulus intensity matching to a designated magnitude of sensation", is not simply an inverse process with the reciprocal exponent (Nishi et al., 2014). The purpose of this study is to examine possible sex-related differences in exponents of Stevens' power law in those two matching tasks.

Methods: Subjects were instructed to make judgments of the apparent magnitude of sensation of loudness, tone, brightness and angle of the elbow joint (M/F = 30/30). The magnitude of sensation was quantified by matching numbers to sensory stimuli ("matching task"). Inversely, intensity of stimuli was matched to natural numbers (0-100) given ("inverse matching task"). Data were analyzed by a linear mixed-effects model.

Results: For all four modalities, matching tasks and inverse matching tasks obeyed power functions with characteristic exponents ($p<0.001$). Exponents of inverse matching tasks were not reciprocal to those of matching tasks for all modalities. Exponents in males were significantly larger than those in females for brightness both in matching and inverse matching tasks ($p=0.02$).

Conclusions: It is suggested that inverse matching task is not a reciprocal function of matching task. There were significant sex-related differences in exponents in brightness.

(COI: No)