Regulation of a synaptic pathway in Autism spectrum disorders

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Neurexin-LRRTM is a key synapse organizing complex, which controls the molecular composition and functional properties of excitatory synapses. Both neurexins and LRRTMs are implicated in cognitive disorders such as autism spectrum disorders (ASD) and schizophrenia. Lack of LRRTM4 in mice reduces excitatory synapse number and function by up to 35% across brain regions and compromises several forms of synaptic plasticity. Here, we investigated the functional significance of neurexin interaction with LRRTMs. We found that LRRTMs have a differential requirement for protein domains of neurexin but require the heparan sulfate (HS) modification to induce presynaptic differentiation. Also, we generated a series of mutations in LRRTM4 substituting closely spaced stretches of positively charged residues with alanine. We found that the modified HS chains of neurexin binds to positive residues within the LRR 5-8 domains of LRRTM4. Our study reveals a novel mode of interaction between neurexins and LRRTMs which is essential for the development of excitatory synapses.

Role of dopamine receptors in modulating nicotine-induced tremor in mice

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We previously demonstrated that nicotine elicited kinetic tremor by activating the inferior olive neurons via α7 nACh receptors (*Behav. Brain Res.*, 314, 173-180, 2016). Since α7 nACh receptors are known to enhance monoamine release, we here explored the role of dopamine receptors in modulating nicotine-induced tremor. Male ddY mice were treated with nicotine (1 mg/kg, i.p.) to induce tremor. Various dopamine agonists or antagonists were injected 15 min before the nicotine injection. Brain levels of dopamine and its metabolites, DOPAC and HVA, were analyzed in mice treated with nicotine (1 mg/kg) using HPLC. Treatment of mice with the D_1 receptor antagonist SCH-23390 significantly enhanced nicotine-induced tremor whereas the D_1 agonist SKF-38393 significantly suppressed the tremor induction. The nicotine-induced tremor was inhibited the D_3 receptor antagonist U-99194, but potentiated by the D_3 agonist PD-128,907. Neither the selective D_2 antagonist L-741,626 nor D_4 antagonist L-745,870 affected nicotine-induced tremor. In addition, nicotine elevated the levels of dopamine and DOPAC in the medulla oblongata containing the inferior olive. These results suggest that D_1 and D_3 receptors exert inhibitory and facilitatory influences on nicotine-induced tremor, respectively.

Oxidative stress-mediated neural cell death induced by nanoparticles

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Nanomaterials have a variety of unique physical and chemical properties, and are being studied for biotechnological, pharmacological, and medical applications. Silica nanoparticles (SiNPs) are produced on an industrial scale and used in various fields. Despite these benefits, there is concern that exposure to nanoparticles may lead to adverse effects on certain types of cells or tissues. Because SiNPs can cross the blood–brain barrier and the blood–placental barrier, they may cause toxic effects such as hemolysis, immune responses, and developmental abnormalities in the brain and developing embryos. Although investigations of the toxicity of SiNP to neurons are essential for medicinal use, few studies have assessed the direct effects of SiNPs on cells derived from the central nervous system. In this study, we showed that treatment with SiNPs caused oxidative stress, morphological damage, and neural cell death. Furthermore, we found that these cytotoxicities was reduced by SiNP surface functionalization or protein coating, and pretreating cells with an antioxidant, suggesting that contact-induced oxidative stress may be responsible for SiNP-induced cell death. These data will be valuable for utilizing SiNPs in biomedical applications.

Development and phenotypic characterization of GAD67 knockdown mice by using a Tet-off system

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GABA is a major inhibitory neurotransmitter in the mammalian brain and regulates emotional behaviors. GABA is synthesized from glutamate by glutamate decarboxylase (GAD) which exists as two isoforms, GAD65 and GAD67, encoded by separate genes. The function of GAD65 is well investigated by using global GAD65 knockout ($GAD65^{-/-}$) mice. However, the function of GAD67, particularly in the adult brain, is not well investigated because $GAD67^{-/-}$ mice died on the day of the birth. In order to resolve the function of GAD67, we developed novel GAD67 knockdown ($GAD67^{tTA/STOP\text{-}tetO}$) mice by using a Tet-off system and investigated the biological phenotypes in those mice. Approximately 30% of $GAD67^{tTA/STOP\text{-}tetO}$ mice survived to adulthood. Treatment with doxycycline (Dox) for 3 weeks in adulthood markedly decreased the protein levels of GAD67 in the frontal cortex, hippocampus and cerebellum of $GAD67^{tTA/STOP\text{-}tetO}$ mice. Metabolome analysis demonstrated that the GABA contents were significantly decreased in the frontal cortex, hippocampus and cerebellum of Dox-treated $GAD67^{tTA/STOP\text{-}tetO}$ mice compared to Dox-treated $GAD67^{+/+}$ mice. In addition, several other metabolites were significantly decreased in the frontal cortex, but not in the hippocampus or cerebellum, of Dox-treated $GAD67^{tTA/STOP\text{-}tetO}$ mice. On the other hand, no significant difference was observed in the metabolite levels, including GABA content, in the frontal cortex between $GAD65^{-/-}$ and $GAD65^{+/+}$ mice. These results suggest that GAD67 is the major enzyme of GABA synthesis and regulates metabolite levels in a brain region-dependent manner.

Development of new fluorescent probes for imaging of prosocial signaling in the brain

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Most species in animal kingdom live in the social groups. To form social groups, one has to recognize and learn the characters of the colleagues. Furthermore, to make the bond within the social groups stronger, one has to make good communications with the others. Accumulating evidences suggested that "prosocial" signaling in brain is likely to be involved in such social activities. Oxytocin and vasopressin, neuropeptides mainly generated in hypothalamus, have emerged as key molecules in prosocial signaling. However, how oxytocin and vasopressin work in the brain during the prosocial activities remains elusive, due to the lack of the method that allows the real-time monitoring of oxytocin and vasopressin in behaving animals. Because defects in prosocial activity is likely to underlie the social isolation in human, understanding how prosocial activities are processed in the brain may lead to the development of the therapeutics for the patients. In this context, there is a pressing need for the technologies that allow the measurement of the dynamics of oxytocin and vasopressin in brain. Therefore, I aimed to develop fluorescent sensors for oxytocin and vasopressin. I engineered and screened more than a hundred of mutant fluorescent sensors, and obtained a potential oxytocin sensor and a potential vasopressin sensor, which shows a large response in the fluorescence intensity upon stimulation with the ligands. In this poster, I will present the characterizations of these new probes and discuss their potential applications.

NMDA receptor-dependent molecular plasticity in dendritic spines of the cerebral cortex after somatosensory stimulation

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The F-actin capping protein CapZ accumulates more in dendritic spines within regions where a long-term potentiation (LTP)-inducing stimulus has been applied. With the goal of developing an in vivo synaptic plasticity marker, we produced a transgenic mouse line, called AiCE-Tg, in which CapZ tagged with enhanced green fluorescence protein (EGFP-CapZ) is expressed in some spines. Twenty minutes after somatosensory stimulation under inactivation of the unilateral sciatic nerve in the AiCE-Tg mice, EGFP-CapZ signals were brighter in a subset of dendritic spines in the sensory cortex that receive preserved projections than those in the other hemisphere. That difference was abolished by an NMDA receptor blocker, MK801. Immunolabeling of α-actinin, a PSD-95 binding protein that can recruit AMPA receptors to postsynaptic sites, showed that α-actinin localization was more frequent/more accumulated in the brightest EGFP-CapZ spines (top 100) than in less bright spines (top 1000). This input-dependent redistribution of EGFP-CapZ may reflect LTP-like changes in vivo and thus may provide a useful tool for synaptic plasticity research.

Differential regulation of dopamine D1 receptor signaling in subregions of the striatum

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Recent studies demonstrated that corticostriatal projections and a histochemically defined organization of the striatum are different among subregions of the striatum. Therefore, we investigated dopamine signaling in each subregion of the striatum. Mouse striatal slices were divided into seven subregions: (1) rostral part, (2-1) intermediate medial part, (2-2) intermediate lateral part, (2-3) intermediate most lateral part, (3) caudal part, (4) most caudal part, (5) nucleus accumbens. Slices of seven subregions were treated with a D1 receptor agonist, SKF81297, and the activity of cAMP/PKA signaling was evaluated with the phosphorylation of DARPP-32 and GluA1. The effects of SKF81297 on the phosphorylation were the lowest in the subregion (3) in the rostrocaudal axis and in the subregion (2-3) in the mediolateral axis. Treatment of slices with a PDE10A inhibitor, papaverine, or SKF81297 plus a muscarinic receptor antagonist, atropine or MT3, increased the phosphorylation in subregions where the effects of SKF81297 was low. In a 6-OHDA parkinsonism model, the 6-OHDA lesion of dopaminergic innervation enhanced dopamine D1 signaling in most of subregions except subregion (3). Thus, differential regulation of dopamine D1 signaling in subregions of the striatum are mediated through activities of PDE10 and/or muscarinic receptors. Moreover, in the 6-OHDA parkinsonism model, dopamine D1 signaling is upregulated in subregions with high and low dopamine D1 signaling.

Phosphorylation of Collapsin Response Mediator Protein 1 by Semaphorin 3A-Fyn signaling regulates basal dendritic growth and arborization

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Collapsin Response Mediator Protein 1 (CRMP1) is an intracellular phoshoprotein that mediates Semaphorin3A (Sema3A) intracellular signaling. Upon Sema3A stimulation, Fyn, a Src-type tyrosine kinase, phosphorylates and activates Cycline-dependent kinase 5 (Cdk5), which subsequently phosphorylates serine 522 of CRMP1. In addition, it has been shown that Fyn directly phosphorylates tyrosine 504 (Y504) of CRMP1 (Buel et al., 2010). Then, we investigated the functional role of this phosphorylation in Sema3A signaling. We found that Fyn phosphorylated Y504 but not other tyrosine residues of CRMP1. A dominant negative mutant of CRMP1 Y504F, substitution of tyrosine 504 to phenylalanine (F), suppressed Sema3A-induced growth cone collapse of chick E8 DRG neurons. We next tested the role of Fyn and CRMP1 in Sema3A-mediated dendritic growth *in vivo. CRMP1-/-* or *Fyn -/-* single-homozygous mice as well as $Fyn^{+/-}$; $Crmp1^{+/-}$ double-heterozygous mice exhibited aberrant development of cortical layer V basal dendrites. Finally, we examined the dominant negative effect of CRMP1 Y504F on cortical dendritic morphogenesis. CRMP1 Y504F or CRMP1 WT with tdTomato was transfected in the mice cortical layer V neurons at E15 by *in utero* electroporation. CRMP1 Y504F-expressed layer V neurons showed poor development of basal dendrites compared with CRMP1 WT-expressed neurons at 5-weeks old mice. These results suggest that Fyn-induced phosphorylation of CRMP1 Y504 may participate in Sema3A-regulated axon pathfinding and cortical dendritic development.

Inactivation of the A-type current is inhibited by ERK5 phosphorylation of Kv4.2 in PC12 cells.

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Extracellular signal-regulated kinase (ERK) 5, a member of mitogen-activated protein kinase, plays important roles in the neuronal development. In our previous studies, we demonstrated that ERK5 mediates neurite/axon outgrowth and catecholamine biosynthesis in PC12 cells and sympathetic neurons. However, the regulation of membrane excitability by ERK5 remains unclear. Thus, we examined the effect of ERK5 on Ca^{2+} and K^+ channels in PC12 cells. In order to activate ERK5 signaling selectively, ERK5 and the constitutively active MEK5 mutant were overexpressed in PC12 cells. In these cells, the gene expression of L-, P/Q- and N-type Ca^{2+} channels was not increased. In contrast, those of Kv4.2 and Kv4.3 were enhanced by ERK5 signaling. Although the protein levels of Kv4.2 were not correlated to mRNA levels, phosphorylation levels of Kv4.2 were increased by ERK5 activation. Because Kv4.2 is a pore-forming subunit of A-type K^+ channels, which play essential roles in membrane excitability, we measured the A-type K^+ current by a whole-cell patch clamp method. The electrophysiological data showed that ERK5 inhibits inactivation of the A-type current, which may be involved in the neural differentiation process by affecting membrane excitability.

M_2 muscarinic receptors possibly facilitate oxytocin synthesis in the mouse supraoptic nuclei

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In the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus, oxytocin and arginine-vasopressin (AVP) are synthesized to cause the lactation and reabsorption of water in the kidney, respectively. We have previously reported that M_2 muscarinic receptors in the SON, but not PVN, promote AVP synthesis. The present study was carried out to examine whether M_2 muscarinic receptors also regulate oxytocin synthesis in the hypothalamus. M_2 receptor knockout (M_2KO) mice and wild-type (WT) mice (3-4 months old) were used in the following experiments. The oxytocin neuron, AVP neuron and M_2 muscarinic receptor were identified by immunohistochemistry. c-Fos immunoreactivity was used as a marker for neuronal activity in the hypothalamus. In M_2KO mice, the number of oxytocin neurons was significantly decreased in the SON, but not in the PVN, compared with WT mice. The muscarinic agonist pilocarpine increased the number of c-fos positive cells in SON of WT mice. However, the increase of c-fos positive cells was significantly decreased in SON of M_2KO mice. Immunoreactivity of M_2 receptor was detected in the SON region, although it seemed to be not expressed in the cell body of oxytocin or AVP neurons. These results suggest that M_2 receptors may stimulate oxytocin synthesis in SON neurons as is the case of AVP, possibly through an unidentified, indirect pathway.

Molecular Mechanism of KCNQ Channels For Reward Behavior

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Dopamine plays a key role in the modulation of the circuit activity in striatum for reward behavior. We have reported that dopamine type 1 receptor (D1R) signaling in the striatum presumably regulates neuronal excitability and reward-related behaviors through PKA/Rap1/MAPK pathway. However, how D1Rs and its downstream signaling regulate neuronal excitability and behavior remain largely unknown. We focus on the post-modification of ion channels for neuronal excitability and reward behavior because protein phosphorylation of ion channels is vital for neuronal function. In this study, we identified a voltage-gated potassium channel, KCNQ2, as a phospho-candidate that is regulated by D1R signaling. Phosphorylation of KCNQ2 by MAPK cascade altered the open probability of KCNQ2/3 channels in Xenopus oocyte. The expression of phospho-defective mutants of KCNQ2 suppressed the functional modulation of KCNQ channel by MAPK. D1R agonist, SKF38393 caused a decrease in KCNQ-sensitive current in striatal slices, whereas D2R agonist, Quinpirole did not cause the effect. These results suggest that D1Rsignaling controls the channel activity of KCNQ via its phosphorylation for neuronal excitability and reward behavior.

1-P-012 Poster Sessions

Neuronal activity backpropagating in dentate circuit

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Hippocampal sharp waves / ripples (SPW-Rs) are high-frequency oscillations emitted mainly during slow-wave sleep or quiet rest states and play a key role in memory consolidation. While SPW-Rs are initiated in the CA3 subregion and propagate to the downstream CA1 subregion, we observed that they also propagate back to the dentate gyrus. However, neither the role of CA3-to-DG SPW-Rs backpropagation nor its propagation mechanism has been fully understood. We previously demonstrated that the subthreshold membrane potentials of hilar mossy cells reflect the activity of SPW-Rs initiated in acute brain slice preparations. We thus hypothesize that mossy cells relay CA3 SPW-Rs backward to the dentate gyrus. Using *in vitro* whole-cell current-clamp technique, we simultaneously recorded the membrane potentials of up to five mossy cells in combination with recordings of local field potentials from the CA3 *stratum pyramidale*. Information theoretical analysis revealed that the activity patterns of SPW-Rs predict the combinatorial dynamics of the membrane potentials of multiple hilar mossy cells. For further confirmation, we conducted *in vivo* whole-cell recordings from mossy cells together with recordings of local field potential of the CA1 subregion in urethane anesthetized mice. We thus concluded that mossy cells are responsive to specific patterns of ripple information at the subthreshold level. Our research approaches further elucidation of brain information dynamics and will provide a new perspective to an information processing mechanism.

Novel PAC1 receptor antagonists alleviate paclitaxel-induced mechanical allodynia by inhibiting astrocytic activation

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Paclitaxel (PTX) is an anticancer agent mainly used as the primary therapy for malignancies such as ovarian, breast, and stomach cancers. However, it frequently induces severe peripheral neuropathy including mechanical allodynia, numbness in a stocking-glove distribution. Recently, we developed novel and small-molecule PACAP type 1 (PAC1) receptor antagonists named PA-8 and PA-81004. In the present study, we examined the effects of novel PAC1 antagonists on PTX-induced mechanical allodynia in mice.

Repeated administration of PTX (2 mg/kg, once a day for 5 days, i.p.) induced mechanical allodynia of the hind paw and activation of spinal astrocyte. Single intrathecal injection of PA-8 (1 nmol) suppressed both PTX-induced mechanical allodynia and astrocytic activation, suggesting that spinal astrocytes activated by PACAP/PAC1 receptor signaling are involved PTX-induced mechanical allodynia.

Next, we examined the systemic administration of the PAC1 receptor antagonists. Single oral administration of PA-8 or PA-81004 (3-30 mg/kg) dose-dependently alleviated PTX-induced mechanical allodynia. The effects of PA-81004 were more potent than PA-8. Repetitive treatment with PA-8 (30 mg/kg, p.o.) 30 minutes before PTX administration almost completely inhibited the induction of mechanical allodynia. These results suggest that PA-8 exerts both therapeutic and preventive effects. Our novel PAC1 receptor antagonists may become orally available analgesics against PTX-induced peripheral neuropathy.

Effects of newly developed small-molecule PACAP type 1 receptor antagonists on itch-like behaviors in mice.

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The role of pituitary adenylate cyclase-activating polypeptide (PACAP) in pain transmission has been well documented, but its involvement in itch transmission is entirely unclear. We recently developed novel small-molecule antagonists of PACAP type 1 (PAC1) receptor including PA-8 by *in silico* screening. In this study, using PA-8, we investigated the possible involvement of PACAP/PAC1 receptor signaling in itch.

Both intradermal (i.d.) and intrathecal (i.t.) injection of PACAP (1 pmol–1 nmol) dose-dependently elicited scratching/biting behaviors, and these behaviors were inhibited by subcutaneous pretreatment with the μ-opioid receptor antagonist naltrexone (1 mg/kg). The scratching/biting behaviors induced by i.d. and i.t. PACAP were inhibited by i.d. and i.t. co-injection of PA-8 (0.1–10 nmol), respectively. The application of 5-HT (200 nmol in EtOH) to the skin elicited scratching behaviors, and they were suppressed by i.t., but not i.d., pretreatment of PA-8 (0.1–10 nmol). Next, we examine the effects of PA-8 on pruritic models. In the itch model induced by cutaneous application of acetone/ether and water (AEW, a dry skin model) or 2,4-dinitrofluorobenzene (DNFB, an atopic dermatitis model), single oral administration of PA-8 (3 – 30 mg/kg) dose-dependently suppressed the itch-associated behaviors.

These results suggest that PACAP/PAC1 receptor signaling in the skin and/or spinal cord is involved in an itch sensation. The small-molecule PAC1 receptor antagonist may become an orally available antipruritic drug in the treatment of acute and chronic itch.

Inhibitory action of endogenous sulfur on oxidative stress-induced TRPA1 activation

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An endogenous sulfur, polysulfide (PS) is generated by oxidation of hydrogen sulfide. We previously reported that PS stimulated nociceptive transient receptor potential A1 (TRPA1) channel in sensory neurons. TRPA1 is also activated by reactive oxygen species (ROS). Here, we examined the effect of PS on responses to hydrogen peroxide (H_2O_2), one of ROS, using mouse sensory neurons and heterologously expressed mouse TRPA1 in HEK293 cells (mTRPA1-HEK). In mouse sensory neurons, H_2O_2 evoked two types of $[Ca^{2+}]_i$ responses, an early TRPA1-dependent and a late TRPA1-independent ones. Pretreatment with PS inhibited the H_2O_2-induced early responses in a dose-dependent manner. PS also suppressed $[Ca^{2+}]_i$ responses to PGJ_2, another endogenous TRPA1 agonist in mouse sensory neurons. In mTRPA1-HEK, PS inhibited $[Ca^{2+}]_i$ responses to not only H_2O_2 but also PS itself and PGJ_2. Simultaneous measurement of $[Ca^{2+}]_i$ and $[PS]_i$ showed that PS did not present in the period of the inhibiting effect of PS. The removal of extracellular Ca^{2+} and calmodulin inhibitor diminished the PS-induced suppression of $[Ca^{2+}]_i$ responses to H_2O_2. When PS was administrated intraplantary prior to H_2O_2, pain-related behaviors induced by H_2O_2 significantly decreased in mouse. The present data suggest that an endogenous sulfur desensitizes TRPA1 resulting in an inhibition of subsequent activation induced by oxidative stresses via Ca^{2+} influx through TRPA1. Calmodulin signaling may be involved in PS-induced TRPA1 desensitization.

Inhibitory effect of gabapentin for interstitial cystitis/bladder pain syndrome in rats

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Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic bladder inflammation characterized by pelvic pain and urinary symptoms, such as urinary frequency and urgency. The etiology of IC/BPS is still not completely understood, and effective drug treatments have not been established. Therefore, the present study confirmed whether repeated intravesical injection of lipopolysaccharide (LPS) causes long-lasting painful and overactive bladder in rats. We further tested the effect of gabapentin on those symptoms in a rat model of LPS-induced chronic cystitis. In the histological examination, LPS-treated showed a greater inflammatory response, severe fibrosis and abnormally thick re-epithelialization. LPS revealed hyperalgesia in the region between the anus and urethral opening at 1day post-administration compared with controls, with no recovery over 21 days. In the cystometry, LPS-treated showed bladder hyperactivity at any times tested. Gabapentin showed significant analgesic effects, and significantly prevented the increased frequency of the voiding observed in the bladders of the LPS-treated. These results suggest that LPS-induced cystitis model shows long-lasting painful and overactive bladder in pathological condition, and gabapentin is effective on both symptoms in this chronic cystitis model.

Perineural treatment with anti-HMGB1 antibody alleviates nociceptive-like behaviors in mice with chronic constriction injury of distal infraorbital nerve

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Trigeminal neuropathy, caused by injury to trigeminal nerve, manifests as orofacial numbness, paresthesias, or/and pain and are refractory to treatment with commonly used analgesics. In this study, we employed distal infraorbital nerve chronic constriction injury (dIoN-CCI) model, which mimic pathology of trigeminal neuropathy, to investigate whether high mobility group box 1 (HMGB1), a kind of damage-associated molecular patterns, is involved in trigeminal neuropathy.

Under anesthesia, silk sutures were tied loosely around the dIoN of ddY male mice. Nociceptive-like behaviors were evaluated by measurement of face grooming episodes and conditioned place preference test. Microglial activity in spinal trigeminal nucleus caudalis (Sp5c) was determined by immunohistochemistry. Anti-HMGB1 neutralizing antibody (nAb) was perineurally injected right after surgery.

In dIoN-CCI mice, the mouse face grooming time was increased compared with sham mice. In addition, dIoN-CCI evoked activation of microglia in Sp5c and preference to mirogabalin-paired chamber. Moreover, the perineural treatment with anti-HMGB1 nAb blocked the dIoN-CCI-induced face grooming, microglia activation, and preference to mirogabalin. The anti-HMGB1 nAb could be a novel therapeutic reagent for inhibiting the induction of trigeminal neuropathy.

Platelet-activating factor (PAF) is increased in neuropathic pain mice: different regulation of PAF levels between the spinal cord and the dorsal root ganglia

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Platelet-activating factor (PAF) is a potent phospholipid mediator, which is involved in the pathology of neuropathic pain after peripheral nerve injury (PNI). In PAF receptor- and its biosynthetic enzyme lysophosphatidylcholine acyltransferase 2 (LPCAT2)- deficient mice, neuropathic hypersensitivity was significantly attenuated. However, regulation of PAF level after PNI remains to be elucidated. Here, we show that PNI increases PAF levels in the spinal cord and in the dorsal root ganglia (DRG). While PAF biosynthetic activity and mRNA expression of LPCAT2 were increased in both tissues, enzymatic activity of PAF degradation and mRNA expression of plasma type of PAF-acetylhydrolase (PAF-AH), which is one of PAF degradation enzymes, were decreased only in the DRG. These results suggest that distinct mechanisms exist between the spinal cord and the DRG to regulate PAF levels after PNI, and then increased PAF levels may contribute to neuropathic pain.

Discovery of a new MrgprA3 agonist and evaluation of its effect for itch sensation

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The G-protein-coupled receptor MrgprA3 (MAS-related GPR family member A3) is expressed specifically in a subpopulation of dorsal root ganglion (DRG) sensory neurons. Recently, MrgprA3^{+} DRG neurons are identified as itch-selective neurons. While MrgprA3 responds to the anti-malaria drug chloroquine and causes strong itch, chloroquine requires high concentrations to activate MrgprA3 and also displays non-selective effects. Therefore, it is necessary to accurately evaluate the ability of MrgprA3 to cause itch sensation. In this study, we screened a series of small molecule compounds to search for agonists that activate MrgprA3 by high-throughput Ca^{2+} imaging. We identified papaverine, an opium alkaloid, that specifically evoked Ca^{2+} responses in cells expressing MrgprA3. Papaverine also increased Ca^{2+} level in primary cultured DRG neurons that were responded to chloroquine. Furthermore, we found that intradermal injection of papaverine to the cheek produced scratching behavior but not wiping behavior in mice. Papaverine-evoked scratching was resistant to the histamine H1 receptor antagonist chlorpheniramine. We further found that intradermal papaverine caused phosphorylation of extracellular signal-regulated kinases (ERK) in the superficial dorsal horn. Finally, we showed that mice lacking gastrin-releasing peptide receptors (GRPR) that are required for itch transmission in the spinal cord exhibited reduction of the papaverine-evoked scratching compared with wild-type mice. In this study, we found that papaverine potently activates MrgprA3 and may cause itch sensation via activation of MrgprA3.

Estrogen deficiency aggravates paclitaxel-induced peripheral neuropathy: involvement of HMGB1

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High mobility group box 1 (HMGB1), one of damage-associated molecular patterns (DAMPs), once released to the extracellular space, aggravates inflammation and pain. We have reported that macrophage (Mϕ)-derived HMGB1 is involved in chemotherapy-induced peripheral neuropathy (CIPN) following paclitaxel (PCT) treatment. Given our recent clinical evidence for a higher risk for CIPN in PCT-treated breast cancer women over menopause age than younger women, we examined the effect of ovariectomy (OVX) on the development of CIPN in mice treated with PCT. In naïve mice, repeated i.p. administration of PCT at 2 and 4 mg/kg, but not 1 mg/kg, developed CIPN. In contrast, treatment with PCT even at 1 mg/kg caused CIPN on OVX mice. The CIPN in OVX mice treated with PCT at 1 mg/kg was prevented by an anti-HMGB1-neutralizing antibody, recombinant human soluble thrombomodulin capable of promoting HMGB1 degradation, or β-estradiol. Together, our data suggest that estrogen deficiency aggravates CIPN after PCT treatment, and the underlying mechanisms involve HMGB1.

Novel small-molecule antagonist of PAC1 receptor ameliorates nitroglycerin-induced migraine-related behaviors in mice

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Migraine is neurological disorder that includes unilateral headache. Although migraine is highly prevalent, its pathophysiology is unclear. In humans, intravenous administration of pituitary adenylate cyclase-activating polypeptide (PACAP), but not vasoactive intestinal polypeptide (VIP), induces migraine-like attacks, suggesting that selective PACAP type 1 (PAC1) receptor antagonist could be a new anti-migraine drug. Previously, we have developed novel small-molecule antagonists of the PAC1 receptor. In the present study, we investigated the effect of PA-8, one of novel PAC1 receptor antagonist, on pain-related behaviors induced by nitroglycerin (NTG) which is widely studied and accepted as an animal model of migraine.

Single or repeated administration of NTG (5 mg/kg, i.p.) induced transient and long-lasting mechanical allodynia of the hind paw, respectively. NTG also induced the increase in the number of c-fos-positive cells in the trigeminal nucleus caudalis (TNC), light-aversive behavior, and anxiety-like behavior in mice. Single or repeated administration of PA-8 (10-30 mg/kg, i.p.) reduced NTG-induced mechanical allodynia, the number of c-fos-positive cells, and anxiety-like behavior. Single administration of PA-8 (30 mg/kg, i.p.) had a tendency to reduce the light-aversive behavior induced by NTG.

The present results suggest that PAC1 receptor is deeply involved in the NTG-induced migraine-related aversive responses and that PAC1 receptor antagonists may become novel anti-migraine drug.

Middle molecular weight heparinylphenylalanine, an RAGE blocker, prevents oxaliplatin-induced peripheral neuropathy and butyrate-induced colonic pain in mice

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We have shown that activation of the receptor for advanced glycation end-products (RAGE) by all-thiol (at)-HMGB1 participates in pathological pain. Interestingly, low molecular weight heparin (LMWH) blocks RAGE and reduces the HMGB1-dependent pain, although it has potent anti-Xa and moderate anti-IIa activities. In the present study, we assessed the anti-RAGE, anti-Xa and anti-IIa activity of middle molecular weight heparinylphenylalanine (MMWH-F), in comparison with LMWH, MMWH and heparin (HP), and tested whether it prevented the at-HMGB1-induced allodynia, oxaliplatin-induced peripheral neuropathy (OIPN) and butyrate (Bu)-induced colonic pain/hypersensitivity in mice. The RAGE-binding affinity of heparinoids was LMWH ≈ MMWH ≈ HP << MMWH-F. The potency of anti-Xa and anti-IIa activities was MMWH-F << LMWH ≈ MMWH < HP and MMWH-F (not detectable) << LMWH < MMWH < HP, respectively. LMWH, MMWH or MMWH-F, preadministered i.p. at 2.5 mg/kg, partially blocked the intraplantar at-HMGB1-induced allodynia in mice. MMWH-F as well as LMWH, but not MMWH, at 2.5 mg/kg, almost completely prevented OIPN. LMWH, MMWH or MMWH-F at 2.5 mg/kg largely prevented or reversed the Bu-induced colonic pain and hypersensitivity. Together, MMWH-F is considered a potent RAGE blocker, and useful to prevent or treat neuropathic and visceral pain without causing hemorrhage.

Establishment of an assessment method by oral administration and proposal of a new data analysis method using conditioned place preference (CPP) in rats

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Ina Research Inc.

Conditioned Place Preference (CPP) is a test to evaluate the rewarding effects related to psychological dependence induced by drugs, and is used to clarify the mechanism of drug dependence and to search for the effects of dependence of newly developing drugs with central nervous system effects. In the conditioning of CPP in rats, intraperitoneal or subcutaneous administration is generally used, but oral administration is also required to test article that cannot be dissolved. However, very few reports exist on CPP using oral administration. Therefore, we performed CPP evaluation following oral administration. In our tests, rewarding effects were noted using morphine. Therefore, etizolam, which have been reported to cause dependence in humans, but for which few reports on experiments in animals are available, was evaluated using similarly method and rewarding effects were noted. In this presentation, we report the results of our experiment and propose a new method to analysis data, taking into consideration the effects of individual differences, based on the results obtained so far.

Distinct regulation of morphine-induced conditioned place preference and withdrawal in MDGA1 knockout mice

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MAM domain glycosylphosphatidylinositol anchor 1 (MDGA1) is one of the extracellular anchor proteins which belong to immunoglobulin superfamily. MDGA1 negatively regulates inhibitory synapse via selective interaction with Neuroligin-2. Neuroligin-2 is expressed in inhibitory GABAergic neurons, and contacts synaptic terminals to form synapses including dopaminergic synapses which play a key role in rewarding systems. We previously reported that MDGA1 knockout mice actually show enhancement of inhibitory perisomatic synaptogenesis in hippocampus and impairment of cognitive functions. In this study, we investigated the characteristics of morphine-induced dependence in MDGA1 knockout mice. Acquisition of morphine-induced conditioned place preference (CPP) in MDGA1 knockout mice was slightly increased while extinction of morphine CPP was strongly impaired. In contrast, naloxone-precipitated morphine withdrawal signs were decreased in MDGA1 knockout mice. These results demonstrate that MDGA1 can help the extinction of morphine-induced reward but make the expression of somatic withdrawal signs worse.

In vivo evaluation of effects of various histamine H_3 receptor inverse agonists on methamphetamine-induced hyperlocomotion and stereotyped behavior in mice

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Pretreatment of mice with pitolisant, a histamine H_3 receptor antagonist, showed a significant reduction of the hyperlocomotion induced by METH, as compared with vehicle (saline)-pretreated subjects. The pitolisant action on METH-induced hyperlocomotion was completely abolished by a histamine H_1 receptor antagonist pyrilamine resulting in hyperlocomotion, but not by a peripherally acting histamine H_1 receptor antagonist fexofenadine, a brain-penetrating histamine H_2 receptor antagonist zolantidine, or a brain-penetrating histamine H_4 receptor antagonist JNJ -7777120. Pretreatment with a histamine H_3 receptor agonist immepip rather augmented METH (3 mg/kg)-induced behavioral abnormalities from hyperlocomotion to stereotyped biting, and, combined pretreatment of pitolisant with immepip significantly attenuated the stereotyped behaviors. Pretreatment with JNJ-10181457 or conessine, other histamine H_3 receptor antagonists, showed inhibitory effects on METH-induced hyperlocomotion similar to that of pitolisant. No significant change in locomotion was observed in mice pretreated with pitolisant, JNJ-10181457, or conessine alone. Pretreatment with pitolisant prior to a high-dose METH (10 mg/kg) significantly decreased the intensity of stereotyped behaviors and increased its latency to onset in a dose-dependent manner. JNJ-1018145, but not conessine, mimicked the inhibitory action on METH-induced stereotyped behavior.

Inhibitory effect of tetrabenazine, a VMAT2 inhibitor, on morphine-induced hyperlocomotion in mice without affecting expression levels of dopamine transporter

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A single administration with morphine induced a long-lasting hyperlocomotion in mice. Pretreatment of mice with tetrabenazine (TBZ; a reversible vesicular monoamine transporter-2 inhibitor) significantly attenuated the hyperlocomotion induced by morphine, as compared with vehicle-pretreated mice. No significant change in locomotion was observed in mice pretreated with TBZ alone. Mice treated with TBZ showed an increase in immobility time in a tail suspension test, as compared with saline-treated mice. Pretreatment with TBZ had no effect on morphine-induced alterations in expression levels of dopamine transporter in brain. TBZ inhibited dopamine turnover (the ratio of DOPAC/dopamine) and 5-HT turnover (the ratio of 5-HIAA/5-HT) in the cerebral cortex of mice challenged with morphine, as compared with saline-pretreated mice challenged with morphine. No stereotyped behavior was observed in mice treated with morphine in combination with TBZ, so that the reduction in observed locomotion did not result from induction of stereotypy. Moreover, TBZ pretreatment had no effect on stereotypy in methamphetamine-treated mice. These data support the potential antagonistic actions of TBZ on some opiate actions, and encourage further exploration of potential effects on morphine reinforcement.

The monoacylglycerol lipase inhibitor JZL184 attenuates methamphetamine-seeking behaviors in methamphetamine self-administered rats

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Methamphetamine (METH) is a highly addictive psychostimulant with reinforcing properties. We previously found that the cannabinoid CB_1 receptors drive reinstatement of METH-seeking behaviors. The purpose of this study was to determine whether the activation of endocannabinoids regulates the reinstatement of METH-seeking behaviors. Rats were tested for reinstatement of METH-seeking behaviors following METH self-administration and extinction. We investigated the effects of JZL184 or URB597, inhibitors of endocannabinoid hydrolysis, on the reinstatement of METH-seeking behaviors. JZL184 (40 mg/kg, i.p.), an inhibitor of monoacylglycerol lipase, significantly attenuated both the cue- and footshock-induced reinstatement of METH-seeking behaviors. URB597 (3.2 mg/kg, i.p.), an inhibitor of fatty acid amide hydrolase, attenuated only cue-induced reinstatement. On the other hands, we also investigated the effect of the inhibitors of endocannabinoid hydrolysis on cognitive function using the novel object recognition task in mice. The recognition index level in the test did not change in JZL184-treated mice. However, URB597 significantly decreased the recognition index level. These findings suggested that JZL184 might have potential as a new therapeutic agent with anti-craving effect, without amnestic effects, in METH addiction.

Can alcohol administration to female mice change their preference and attitude to unattractive male mouse?

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Some men sometimes invite an interested woman to bar for alcohol drinking. Alcohol makes them cheerful and improves their relationship occasionally. The man expects alcohol could decrease the threshold to open her mind, and, in some cases, lose accurate judgments about him as a sexual partner. In this research, we are trying to reveal a part of the neural activities and behavioral characteristics in male and female using a mouse model in such situation.

We have established the behavioral model for male preference of female mice, in which we found that attractiveness of male mice to female mice is dependent on their appearance, not voice nor smell. Additionally, we performed *in vivo* microdialysis analyses under the experimental conditions, and found that dopamine levels in the nucleus accumbens of female mice responded to attractive male mice, but not to unattractive male mice.

Using this model, we examined the effects of alcohol on the male preference and dopamine response of female mice.

Q1. Can alcohol-administered female mice recognize attractiveness of male mice?

Q2. Can dopamine levels in the nucleus accumbens of female mice increase at the timing to meet with an unattractive male mouse after alcohol administration?

Q3. When alcohol-administered female mouse was set in rectangular box with an unattractive male mouse in a transparent small box, does she stay close to him or opposite area of him?

Q4. After repeated conditioning with alcohol aministration like as question 3, does the preference of female mice to the unattractive male mouse increase or not?

We will try to answer these questions in this presentation. These results will present the hint for how alcohol affects the relationship between male and female.

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MEK1/2 inhibitor U0126 blocks the expression of paternal behavior

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Mating-inexperienced male mice show aggressive behavior toward pup. However, they show parental behavior after the social experiences such as mating and seeing pups. Previously, we found that the GABAergic projection in the medial preoptic area (MPOA) is suppressed in the father mice in gestation experience (FGE) which experienced mating and staying with late pregnant female. This synaptic change was reversed by using G protein signaling inhibitor GDPβ. However, the mechanism of plastic changes in Me → MPOA pathway synapses induced by cohabitation with females remains unclear. In this study, we carried out the electrophysiological recordings from MPOA neurons of sexually inexperienced male mice and FGE. It was newly found that the amplitude of inhibitory post-synaptic potential of MPOA was significantly increased by U0126 in FGE mice. Furthermore, in order to validate the effects of U0126 on the behavioral pattern, U0126, was microinjected into MPOA of FGE mice, and a behavioral test in response to pup exposure was tested. As a result, in the group administered with U0126, the proportion of individuals exhibiting aggressive behavior toward pups increased significantly. These results suggest that signaling through MEK1/2 is involved in GABAergic synaptic plastic changes in MPOA that stop attacks and induce parenting behavior.

Effects of alcohol on the representation of hippocampal place cells

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Alcohol exposure impairs the retention of spatial memory. Consistently, previous reports have demonstrated widespread changes in a variety of receptor functions and gene expressions in the hippocampus, a brain region involved in spatial memory. However, it remains unknown how these molecular mechanisms are integrated to alter neuronal spike patterns. Hippocampal neurons consist of place cells as they fire preferentially when an animal visits a specific area (a place field), which are considered to play a crucial role in spatial memory. Here, we recorded spatial spike patterns of hippocampal neuronal ensembles from freely moving rats running on familiar linearized tracks. The rats were tested in two 20-min sessions of running and during a 10-min inter-session interval, they were injected intraperitoneally with 1.5 g/kg ethanol, a dose comparable to those generally consumed by humans. The alcohol administration triggered the emergence of a subset of place cell populations, while abolishing place-selective firing of the other place cell populations. Moreover, a subset of place cells altered their place fields. These results demonstrate that hippocampal spatial maps are dynamically reorganized by ethanol administration. The neuronal mechanism may underlie alcohol-induced impairments in hippocampus-dependent memory.

The administration of glycine induces the dephosphorylation of AMPA receptors and attenuates fear memory of the contextual fear conditioning.

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In the central nervous system, glycine plays an excitatory role via binding to the NMDA receptors (NMDARs) and an inhibitory role via binding to the glycine receptors (GlyRs). Recently, it has been found that upon the increase of glycine concentration in the synaptic cleft, glycine binds to the GlyRs, mainly expressed in the extrasynapse, and introduces LTD. In addition, NMDARs and AMPA receptors (AMPARs) involve in this type of LTD. To elucidate the mechanisms underlying glycine-dependent LTD, we examined the phosphorylation of AMPARs under excess amount of glycine. Furthermore, we hypothesized that the administration of high-dose glycine would attenuate hippocampal- dependent fear memory, known to require the phosphorylation of AMPARs.

We performed biochemical analysis to examine the phosphorylation status of AMPARs in the hippocampus under different doses of glycine. Glycine decreased the phosphorylation of serine 845 of GluA1 subunit of AMPARs dose-dependently. Furthermore, high-dose glycine co-incubated with strychnine, AP5 and FK506 did not show the reduction in the phosphorylation of serine 845 of GluA1, suggesting that this effect of glycine was mediated by not only GlyRs but also NMDARs. Interestingly, the high-dose administration of glycine to the rats before fear conditioning reduced the freezing behavior during the test. Moreover, this effect was eliminated by co-administration of strychnine.

In this study, we revealed that LTD required the dephosphorylation of serine 845 of GluA1 and this type of LTD could attenuate contextual fear memory.

The cognitive impairment induced by prolylhydroxylase inhibitors in mice

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Background: In general, hypoxia can suppress neural activities and cause cognitive impairment. Hypoxia inducible factor-1α (HIF-1α) is known as a transcription factor expected to play pivotal roles in the response to hypoxia in various tissues. HIF-1 a is hydroxylated by prolylhydroxylase (PHD) in an oxygen-dependent manner, and then it is degraded in ubiquitin system. Preceding studies have revealed that PHD inhibitors increase HIF-1α and induce hypoxia-like responses. In this study, we investigated the effects of PHD inhibitors on cognitive performance in mice.
Methods: Male 5-weeks-old ddY mice were subjected to novel object recognition test for evaluation of long-term memory performance. Dimethyloxalylglycine (DMOG) and roxadustat, PHD inhibitors, are subcutaneously injected.
Results: Single administration of DMOG or roxadustat 30 min before the learning phase of novel object recognition test significantly lowered discrimination index. The suppressive effect on memory performance of 5 consecutive daily administration of DMOG was similar to that of a single administration.
Conclusion: The present results suggest the possibility that an increase of HIF-1α causes cognitive impairment. Since PHD inhibitors are candidates for the treatment of anemia, attention should be paid to the side effects on cognitive performance.

Serotonin 5-HT_4 receptor agonists improve facilitation of contextual fear extinction in an MPTP-induced mouse model of Parkinson's disease

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Previously, we found that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson's disease (PD) model mice (PD mice) show facilitation of hippocampal memory extinction via reduced cyclic adenosine monophosphate (cAMP)/cAMP-dependent response element-binding protein (CREB) signaling, which may cause cognitive impairment in PD. Serotonergic neurons in the median raphe nucleus (MnRN) project to the hippocampus, and functional abnormalities have been reported. In the present study, we investigated the effects of the serotonin 5-HT_4 receptor (5-HT_4R) agonists prucalopride and velusetrag on the facilitation of memory extinction in PD mice, because 5-HT_4R, which is a Gs protein-coupled receptor that activates adenylate cyclase, is highly expressed in the hippocampus. We found that 5-HT_4R agonists restored facilitation of contextual fear extinction in PD mice by stimulating the cAMP/CREB pathway in the dentate gyrus (DG) of the hippocampus. These findings suggest that 5-HT_4R agonists could be potentially useful as therapeutic drugs for treating cognitive deficits in PD by improving the cAMP/CREB signaling pathway in the hippocampal DG.

Nose-to-brain delivery of CGRP against fear memory retention in mice

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We previously reported that intracerebroventricular administration of calcitonin gene-related peptide (CGRP) effect on hippocampus-dependent fear memory in mice. Although CGRP plays an important role in central nervous system, it seems to be degraded before reaching the brain by intravenous administration. The intranasal route offers an alternative approach for drug delivery to the brain without the interference of the blood-brain-barrier. In this study, we evaluated the nose-to-brain delivery of CGRP to investigate the effects on fear memory retention by contextual learning test. 8-week-old male C57BL6J mice were examined to contextual fear learning test. Mice were given a 0.3 mA foot shock. After fear conditioning, mice were given saline or CGRP (0.5 nmol) by intranasal administration (i. n.). CGRP injections shortened the freezing time when compared to saline. Next we also evaluated *Bdnf* or *Npas4* mRNA in mice hippocampus. As same as intracerebroventricular administration, CGRP i.n. significantly increased the level of *Npas4* rather than saline treatment. *Bdnf* level were also significantly increased. These results suggest that nose-to-brain delivery of CGRP alleviate the fear memory with increases *Npas4* and *Bdnf* in mice hippocampus.

Prioritized experience replay for learning in the rat hippocampus

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Hippocampus is crucial for episodic-like memory formation. The dynamics of hippocampal neuronal ensembles during learning has not been well understood. To investigate the hippocampal circuit property during learning, we designed a new spatial learning task and performed multiunit recording from CA1 neurons. Analyses for hippocampal place cells revealed that hippocampus captured not only the structure of environments, but also the structure of behavior task. Further analyses for hippocampal neuronal replays, the neuronal ensemble reactivations followed by sharp-wave ripples, showed rate increases in the frequency of replays with dynamic changes in replay patterns, reflecting the newly learned pathways. Real-time neuro-feedback experiments confirmed that such prioritized experience replay supports efficient learning. These results indicate that hippocampal neurons represent the self-state in the environment with multiple axes, enabling the hippocampus to flexibly emit experience replays to support the reinforcement of a specific behavior pattern.

Towards understanding the role of neuroprotective effect of adenosine deaminase

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Adenosine deaminase (ADA) is a widely expressed enzyme that catabolizes adenosine and deoxyadenosine into inosine and deoxyinosine, respectively. Adenosine is known to play a protective role by interacting with adenosine receptors when its extracellular concentration is increased. Therefore, ADA has been considered toxic to the central nervous system under ischemia, hypoxia and tissue damage. However, ADA-deficiency results in neurological disorders. We were interested in determining whether ADA is protective or harmful in the striatum, which is especially vulnerable during cerebral ischemia. In order to determine the effects of ADA on transient ischemic stress of the striatum, we used acute rat corticostriatal slices. We found that ADA has substantial neuroprotective effects in the striatum. In addition, we examined whether the neuroprotective effect of ADA is due to the removal of deoxyadenosine. The deoxyadenosine administration does not show acute cytotoxicity, suggesting that the neuroprotective effect of ADA may be due to other mechanisms, such as the recently reported direct effect of ADA on the adenosine A_{2A} receptors.

Hyperlipidemia may attenuate rupture of a cerebral aneurysm in the mouse model.

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[Introduction] Subarachnoid hemorrhage (SAH) is a life-threatening type of stroke and can be frequently caused by a ruptured aneurysm of cerebrovascular blood vessels. Although one third of patients could survive with good recovery; one-third will survive with a disability; and one-third will die.

It is well accepted that lowering blood cholesterol level is mandatory in prevention of cerebral circulatory disorder. However, the relationship between cholesterol and cerebral aneurysm is still controversial.

In this study, we elucidate the above relationship by monitoring aneurysm and SAH in 1) aneurysm model of LDL receptor/ Apobec 1 double knock out (LA-/-) mice and that of control mice. 2) Reducing cholesterol intake by administering Cholestyramine, cholesterol lowering cationic resin, to LA -/- aneurysm model mice together with daily food.

[Method] Experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Hamamatsu University School of Medicine, Hamamatsu, Japan.

Hashimoto model of animal cerebral aneurysms was performed.

Briefly, left kidney was excised one week before the experiment. Elastase was administered to the subarachnoid space to damage cerebral artery and sustained-release deoxycorticosterone was placed subcutaneously. Drinking water was substituted with 1% salt solution. Three weeks later, the brain tissue was harvested for evaluation of cerebral aneurysm and subarachnoid hemorrhage.

[Results] 1) lesser amount of cerebral aneurysm and SAH were detected in aneurysm model of LA-/- mice compared to control mice.

2) Increasing trend of SAH was observed in LA-/- mice with cholestyramine administered group.

The effects of ambrisentan, a selective endothelin ET_A receptor antagonist for vasogenic edema after traumatic brain injury in mice

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Vasogenic edema is a severe condition resulted from disruption of blood-brain barrier (BBB) after traumatic brain injury (TBI). Endothelin (ET) is an aggravating factor for the BBB disruption. In this study, we confirmed the effects of ambrisentan, an ETA receptor antagonist for TBI-induced vasogenic edema. As a model of TBI, mice (male ddY, 6 to 7 weeks) were given fluid percussion injury (FPI) by hydraulic impact on dura mater. Ambrisentan (0.02, 0.1 and 0.5 mg/kg/day) were repeatedly administrated from mouse tail vein in 6 hour to 2 days after FPI. To evaluate vasogenic edema, the BBB disruption and brain edema were evaluated by Evans blue extravasation and brain water content, respectively. Expressions of vascular endothelial growth factor-A (VEGF-A) as an aggravating factor for the BBB disruption and angiopoietin-1 (ANG-1) as a protective factor were measured by Real-time PCR. Expression of ETA receptors was observed by immunohistochemistry. The i.v. administrations of ambrisentan ameliorated the BBB disruption and decreased in brain water content in 2 day after FPI. Ambrisentan decreased in VEGF-A and increased in ANG-1 after FPI. The immunochemical observations indicated that ETA receptors were distributed in brain endothelial cells in mouse cerebrum. In vitro experiments, treatment with ET-1 (100 nM) increased in VEGF-A and decreased in ANG-1 in brain endothelial cells (bEnd.3 cells). Treatment with ambrisentan (1 μM) inhibited the effects of ET-1. These results suggest that ambrisentan is expected to be a novel therapeutic drug for vasogenic edema.

Voluntary exercise after cerebral ischemia ameliorates disability and modifies dendritic spine density

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Cerebral ischemia is one of the main causes of disability. Although many studies report that exercise improves prognosis of cerebral ischemia, the mechanisms are not revealed. The aim of this study is to reveal the underlying mechanisms of exercise on functional recovery after cerebral ischemia. We occluded the middle cerebral artery in C.B-17/Icr-$^{+/+}$Jcl mice to obtain reproducible size of cerebral infarction. Mice were divided into 4 groups: Sham, Sham + exercise, Middle cerebral artery occlusion (MCAO) and MCAO + exercise. Exercise groups had free access to a running wheel. Wire hang test was performed on day 14 post-ischemia. Grid walking test was performed daily for 14 days after cerebral ischemia. Then, we visualized the neuronal processes and dendritic spines of pyramidal cells in the layer 5 by microinjection with Lucifer yellow. As a result, voluntary exercise contributed to functional recovery after cerebral ischemia and affected the number of dendritic spines. Our data suggest that functional recovery is caused by the change in dendritic spine density following voluntary exercise.

Differential glial responses to intracerebral hemorrhage between young and middle-aged mice

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Aging is one of the major risk factors to worse mortality rate and neurobehavioral deficits after intracerebral hemorrhage (ICH). Previous research has demonstrated that aged ICH rodents show exacerbated ICH pathology, and recently, mid-life cardiovascular factors are implied to be an independent predictive factor for later-life mild cognitive impairment and dementia. However, no study has ever compared ICH pathology between young vs middle-aged mice. In this study, therefore, we used 2-month and 8-month old mice to examine whether 8-month old mice show different patterns of glial responses after ICH. ICH was induced by unilateral microinjection of 0.025 U type VII collagenase (0.5 uL) into the right side of brains of male C57Bl/6J mice. One or 8 days after collagenase injection, animals were sacrificed, and the brains were subjected to immunostaining using an astrocyte maker GFAP antibody. In the peri-lesion area, the number of GFAP-positive cells in 8-month old mice was decreased compared to the one in 2-month old mice. These data may suggest that glial responses after brain injury are already changed even in the middle-aged mice. Future studies are warranted to examine how these mid-life glial changes affect later-life neurological and cognitive dysfunction after ICH.

Development and functional analysis of the *in vitro* BBB model derived from stroke-prone spontaneously hypertensive rats.

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Stroke-prone spontaneously hypertensive rat (SHRSP) is widely accepted as an animal model of hypertension and cerebrovascular disease. SHRSP occurs BBB dysfunction even if chronic hypertension and onset of stroke are not well-established. The properties of BBB are maintained by cross-talk between brain endothelial cells and surrounded adjacent cells, such as astrocytes and pericytes. To reveal the detailed mechanism underlay the BBB dysfunction of SHRSP, we constructed an *in vitro* BBB model using brain endothelial cells, pericytes, and astrocytes. Isolated brain capillary endothelial cells (BECs) from SHRSP showed leaky barrier function and altered composition of tight junction proteins (claudin-5 and occludin). The co-culture method of BECs and astrocytes indicated that SHRSP astrocytes had less ability to induce barrier function on BECs than did astrocytes derived from normotensive control rat (WKY/Izm). In comparison using the triple co-culture model, SHRSP model showed a weak barrier function than WKY model. These results suggest that BBB-relate cells in SHRSP have different properties and the defective interactions among these cells. This altered cross-talk may be related to occurrences of cerebrovascular diseases in SHRSP.

Stimulation of LXA_4 receptor alleviates motor dysfunction in intracerebral hemorrhage model mice

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Intracerebral hemorrhage (ICH), a bleeding into the brain parenchyma, is a devastating neurologic disease with the highest mortality among all stroke subtypes. In ICH brain, thrombin induces activation of microglia/macrophages followed by neuroinflammation. Furthermore, ICH leads to infiltration of numerous leukocytes. Recent report shows the arachidonic acid metabolite, leukotriene B_4 (LTB_4), participates pathological progression of ICH (Hijioka *et al.*, 2017). In this study, we focused on lipoxin A_4 (LXA_4), synthesized from arachidonic acid as same as LTB_4. Treatment of murine microglial cell line BV-2 cells with thrombin (30 U/mL) increased mRNA expression level of inducible NO synthase (iNOS) and interleukin-6 (IL-6). Pretreatment with LXA_4 (100 μM) suppressed thrombin-induced increases in iNOS and IL-6 mRNA expression. Moreover, immunocytochemical analysis revealed the translocation of nuclear factor-κB (NF-κB) into the nucleus induced by thrombin, and thrombin-induced nuclear translocation of NF-κB was suppressed by LXA_4. Finally, daily intravenous administration of LXA_4 receptor agonist, BML-111 (1 mg/kg) attenuated the motor dysfunction of mouse model of ICH. These data suggest that LXA_4 may be the novel therapeutic agent for ICH.

Alterations of lymphocyte count and platelet volume precede development of stroke-related symptoms in stroke-prone spontaneously hypertensive rats

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Stroke is a global health problem and leads to disability. Efficacy of current treatment in both acute and chronic phase of stroke is limited. To accelerate individualized approach to primary prevention for stroke, identification of biological parameter, which is able to predict the risk of stroke development, is an indispensable component. Stroke-prone spontaneously hypertensive rat (SHRSP) is genetic animal model of chronic hypertension that progresses to stroke. Most of SHRSP spontaneously develop stroke including cerebral infarction and cerebral hemorrhage. Hematological tests readily provide health condition information. In this study, we investigated the time course of hematological parameters in Wistar rats and SHRSP. SHRSP develop stroke-related symptoms including onset of neurological symptoms, decreased body weight and blood brain barrier leakage between 12 and 14 weeks of age. Lymphocyte counts were gradually decreased at 3 weeks before development of stoke-related symptoms and then were further decreased after the development of stroke-related symptoms. The platelet volume, as shown by the mean platelet volume and large platelet ratio, gradually increased at 3 weeks before the development of stoke-related symptoms. However, although SHRSP showed more microcytic red cells than Wistar rats, the trajectories of change in erythrocyte-related parameters were similar between Wistar rats and SHRSP. Our findings suggest that alterations of lymphocyte count and platelet volume may be predictive indicators for stroke development in SHRSP.

The expression and the role of progranulin in the neural stem cells

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Neurogenesis is transiently enhanced by cerebral ischemia, but the number of newly generated neurons are not enough to compensate for damage of brain tissue. Furthermore, it seems that the majority of newly generated endogenous neurons after cerebral ischemia fail to survive. Therefore, acceleration of endogenous neurogenesis in the brain has a potential to become a new therapeutic approach for stroke. Progranulin (PGRN) is a cysteine-rich protein which is implicated in cell proliferation and tumorigenesis. In this study, we investigated the effect of PGRN on proliferation and differentiation in cultured neural stem cells. The sphere diameter of neural stem cells used in this study increased with time and these cells had multipotency. Treatment with recombinant PGRN (rPGRN) enhanced the ability of neural stem cells to proliferate and differentiate into neurons. Proliferation of neural stem cells was promoted by oxygen-glucose deprivation (OGD) treatment, and PGRN was co-localized with nestin in these cells. These results suggest that PGRN contributes to the proliferation and differentiation of neural stem cells after cerebral ischemia.

Pathophysiological changes of progranulin in active microglia after cerebral ischemia

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Progranulin (PGRN) is implicated in neuronal protection and anti-inflammation. On the other hand, granulin (GRN), which is cleaved from PGRN by neutrophil elastase, has pro-inflammatory effects. However, pathophysiological roles of PGRN and GRN after cerebral ischemia have not been fully determined. In this study, we examined time-course of changes in the levels of PGRN and GRN and their cellular sources after cerebral ischemia using a rat microsphere-embolism (ME) model and rat primary cultured microglia. Protein and mRNA levels of PGRN in activated microglia were increased in the ischemic region of cerebral cortex on day 3 after ME. Elastase activity was increased on day 1 after ME. GRN was increased on days 1 and 3 after ME. Next, we examined effect of sivelestat, a selective neutrophil elastase inhibitor, on the levels of PGRN and GRN after ME. The level of PGRN was increased in the cerebral cortex, whereas elastase activity and GRN level were decreased in sivelestat-treated rats on day 1 after ME compared with those of vehicle-treated rats. Thus, the increase in PGRN level and inhibition of GRN production after ME caused by siverestat treatment would prevent ischemic brain injury.

Minoxidil suppressed cerebral ischemic injury by direct effect on neural tissues but not by reducing blood pressure.

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Setsunan. Unv. Pharm. Sci. Mol. Pharmacol.

Minoxidil opens ATP-sensitive potassium channel (KATP) which is an inwardly rectifying potassium channel, which expressed in the heart, kidney, blood vessel and brain. Minoxidil was developed as an anti-hypertension agent and is now clinically used as a hair restorer. Minoxidil is also attracting attention as a treatment aimed at protecting against heart and brain ischemic damage. However, the underlying mechanism is not clear. We have reported that administration of minoxidil suppressed the damage of nerve tissue after cerebral ischemia, while it was unclear whether this was a direct action of nerve tissue or a cause of lowering blood pressure. Here, we examined whether another drug administration that produces the same blood pressure lowering effect as minoxidil suppresses damage due to cerebral ischemia. Losartan alone and doxazosin alone showed a transient decrease in blood pressure, but the combined use of both drugs resulted in a sustained decrease in blood pressure equivalent to minoxidil. Damage caused by transient ischemia model of middle cerebral artery occlusion in C57/BL mice was suppressed by minoxidil administration, but the combination of losartan and doxazosin did not protect. These results suggested that the neuroprotective effect of minoxidil was not related to the blood pressure lowering effect.

Protection mechanism of gadolinium trichloride against hemorrhagic brain injury

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We investigated the effect of gadolinium trichloride (Gd) on microglial polarization and neuronal injury after intracerebral hemorrhage (ICH). An *in vivo* mouse ICH model was prepared by intrastriatal microinjection of collagenase type VII. One day after ICH, the mRNA level of proinflammatory M1 microglial markers, such as inducible nitric oxide synthase (iNOS), increased. Anti-inflammatory M2 microglial markers arginase1 (M2a, c), Ym1 (M2a), and transforming growth factor-beta (M2c) increased 1 day after ICH, and chemokine CCL1 (M2b) increased after 3 days. Gd administration decreased these M1 and M2 markers. Arginase1 and iNOS protein levels also increased 3 days after ICH, and Gd decreased them because of the decrease of cell number due to apoptosis. Next, we investigated whether Gd had an anti-inflammatory effect in an ICH model. Three days after ICH, brain edema was formed, and the number of NeuN-positive cells, which indicates neuronal nuclei, decreased in the peripheral region inside the hematoma. Gd improved the edema, neuron loss, and behavioral abnormality, without affecting the hematoma size. Furthermore, Gd improved the mortality rate by ICH. Overall, Gd evoked M1 and M2 microglial apoptosis and had an acute protective effect after ICH.

Cleavage of LRP1 and increase in LRP1-ICD in ischemic brain and excitotoxic neuronal injury

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Low-density lipoprotein receptor-related protein-1 (LRP1), a member of the LDL receptor family, plays important roles in endocytosis and intracellular signaling. LRP1 is processed by furin in the trans-Golgi network (TGN), becomes mature LRP1 and moves to the cell surface. Previous studies have shown that LRP1 suppresses glutamate excitotoxicity in primary cultured retinal ganglion cells. However, the pathophysiological role of LRP1 in the brain after cerebral ischemia is unclear. The purpose of this study was to investigate the pathophysiological role of LRP1 after cerebral ischemia and *N*-methyl-D-aspartate (NMDA)-induced neuronal injury. First, we demonstrated that LRP1 was significantly cleaved after cerebral ischemia and in NMDA-induced neuronal injury. The LRP1-intracellular domain (ICD) produced by neuronal injury was co-localized with TGN and furin. In addition, we found that furin inhibitors inhibited LRP1 cleavage and suppressed co-localization with TGN or furin. Theses findings suggest that furin-mediated LRP1 cleavage and LRP1-ICD localization are involved in ischemic neuronal injury.

α-Synuclein-induced production of inflammatory mediators through Toll-like receptors in brain pericytes

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The pathological hallmark of Parkinson disease (PD) is a widespread distribution of the aggregated α-synuclein (α-Syn) proteins in the inclusions known as Lewy bodies. Exogenous α-Syn secreted from neurons could induce inflammatory responses including microglial activation. This activated microglia was observed in the substantia nigra of patients with PD. We previously reported that pericytes, one of the blood-brain barrier (BBB) constituent cells released various inflammatory mediators in response to monomeric α-Syn. Here, we investigated whether Toll-like receptors (TLRs) mediated the α-Syn-induced production of inflammatory mediators in pericytes. In response to monomeric α-Syn, pericytes released the highest levels of IL-1β, IL-6 and MMP-9 than the other cell types of the BBB (brain endothelial cells and astrocytes). TAK242 (a TLR4 inhibitor, 5 μM) but not CU CPT22 (a TLR1/2 inhibitor, 5 μM) attenuated the increased mRNA levels of IL-1β, IL-6, MMP-9 and TNF-α induced by a 24-hr exposure of α-Syn (50 μg/mL). The initial uptake of α-Syn by pericytes was inhibited by CU CPT22 and TAK242. These results suggest that TLR4 may mediate α-Syn uptake and subsequent production of inflammatory mediators in pericytes.

Functional evaluation of the patient-derived iPS cells from *PDGFB*-variants in idiopathic basal ganglia calcification

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Idiopathic basal ganglia calcification (IBGC) is an intractable disease characterized by bilateral calcification in basal ganglia and other regions. The causative genes have been identified. Among them, the variant frequency of *PDGFB* in familial IBGC is about 10%. *PDGFB* encode platelet-derived growth factor B (PDGF-B). Previous studies showed PDGF-B is expressed in endothelial cells and neurons in the brain and PDGF-BB, a homodimer of PDGF-B, stimulates pericytes which are abundant in the brain and the Pi transport in the vascular smooth muscle cells. In this study, variant analyses of *PDGFB* for IBGC patients showed four novel pathogenic variants, c.160 + 2T > A, c.457 −1G > T, c.33_34delCT and c.342_343insG. The iPS cells (iPSCs) from three patients with novel *PDGFB* variant were established and endothelial cells were induced. Enzyme-linked immunoassay analysis showed that the levels of PDGF-BB in the blood sera of patients with *PDGFB* variants were decreased to 34.0% of that of the control levels. Those in the culture media of the endothelial cells derived from iPSCs of patients decreased to 58.6% of the control levels. As the endothelial cells developed from iPSCs of the patients showed a phenotype of the disease, IBGC-specific iPSCs will give us more information on the pathophysiology and the therapy of IBGC in the future.

Role of the microsomal prostaglandin E synthase-1 in cuprizone-induced demyelination and motor dysfunction.

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Multiple sclerosis (MS) is one of the most common demyelinating diseases. Microsomal prostaglandin E synthase-1 (mPGES-1) is a key enzyme that acts downstream of cyclooxygenase and plays a major role in inflammation and immune responses by converting prostaglandin (PG) H_2 to PGE_2. PGE_2 is highly produced in the cerebrospinal fluid of patients with MS. However, the role of mPGES-1 in MS has not been fully elucidated yet. In this study, we demonstrate the role of mPGES-1 in demyelination and motor dysfunction induced by cuprizone, one of the well established models of MS. Demyelination in the brain was induced in mice lacking mPGES-1 (mPGES-$1^{-/-}$ mice) and wild-type (WT) mice by feeding ad libitum with a powdered diet containing 0.2% cuprizone for 6 weeks under specific pathogen free condition. The expression of mPGES-1 in the brain was determined by real-time PCR. The cuprizone-induced demyelination was assessed by a myelin staining with coronal brain sections, and motor dysfunction was evaluated by the rotarod test. Cuprizone up-regulated the expression of mPGES-1 mRNA in the brain of WT mice. Interestingly, mPGES-$1^{-/-}$ mice exhibited lower degree of demyelination compared to WT mice. In addition, mPGES-1 gene deletion or COX-2 selective inhibitor celecoxib reduced cuprizone-induced motor dysfunction. These data indicate that COX-2/mPGES-1/PGE_2 system contributes to the pathophysiology of MS and open possible novel therapeutic approaches for MS.

p-Coumaric Acid Has Protective Effects against Mutant Copper–Zinc Superoxide Dismutase 1 *via* the Activation of Autophagy

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the selective death of motor neurons. In previous our study, an ethanol extract of Brazilian green propolis (EBGP) prevented mutant copper–zinc superoxide dismutase 1 ($SOD1^{mut}$)-induced neurotoxicity. This paper aims to reveal the effects of p-coumaric acid (p-CA), an active ingredient contained in EBGP, against $SOD1^{mut}$-induced neurotoxicity. We found that p-CA reduced the accumulation of $SOD1^{mut}$ subcellular aggregation and prevented $SOD1^{mut}$-associated neurotoxicity. Moreover, p-CA attenuated $SOD1^{mut}$-induced oxidative stress and endoplasmic reticulum stress, which are significant features in ALS pathology. To examine the mechanism of neuroprotective effects, we focused on autophagy, and we found that p-CA induced autophagy. Additionally, the neuroprotective effects of p-CA were inhibited by chloroquine, an autophagy inhibiter. Therefore, these results obtained in this paper suggest that p-CA prevents $SOD1^{mut}$-induced neurotoxicity through the activation of autophagy and provides a potential therapeutic approach for ALS.

ALS-associated mutant ubiquilin 2 impairs protein degradation via autophagy-lysosome system

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Ubiquilin 2 (UBQLN2) has ubiquitin-like and ubiquitin-associated domains and regulates protein degradation systems. Missense mutations of UBQLN2 have been recently identified as causal genes of familial amyotrophic lateral sclerosis (ALS). ALS-associated mutant UBQLN2 has been reported to impair protein degradation via ubiquitin-proteasome system. In the present study, we investigated how mutant UBQLN2 affects protein degradation via autophagy-lysosome system, which is classified into three pathways, macroautophagy (MA), microautophagy (mA) and chaperone-mediated autophagy (CMA). We first assessed mA/CMA activity using AD293 cells stably expressing GAPDH-HT, a marker of mA/CMA activity. Overexpressed wild-type UBQLN2 decreased mA/CMA activity, and the overexpression of mutant UBQLN2 exacerbated the decrease of mA/CMA activity. Experiments using rapamycin and mycophenolic acid, activators of mA and CMA, respectively, revealed that CMA was mainly impaired by wild-type and mutant UBQLN2. As for MA, autophagic flux assay using bafilomycin A1, a lysosome inhibitor, revealed that mutant UBQLN2 decreased MA activity in AD293 cells. These findings suggest that ALS-associated mutant UBQLN2 impairs MA and CMA and disturbs protein quality control. This disturbance would be related to the pathogenesis of ALS caused by UBQLN2 mutation.

Accumulation of sphingomyelin in Niemann-Pick disease type C cells disrupts Rab9-dependent vesicular trafficking of cholesterol

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Niemann-Pick disease type C (NPC) is a genetic disorder in which patient cells have endosomal/lysosomal accumulation of cholesterol and sphingolipids. However, the relationship between sphingolipids and cholesterol accumulation in NPC cells has not been established. Here, we investigated the role of sphingomyelin (SM) on the accumulation of cholesterol in NPC cells. Reduction of SM by inhibition of the ceramide transfer protein CERT decreased the cholesterol accumulation in NPC cells. The accumulation of SM in NPC cells inhibited the transport of cholesterol to the endoplasmic reticulum. Overexpression of Rab9 in NPC cells reduced the cholesterol accumulation, which was recovered by treatment with SM. In NPC cells that overexpressed a Rab9 constitutively active mutant, SM treatment did not lead to the cholesterol accumulation. These results indicate that SM negatively regulates the Rab9-dependent vesicular trafficking of cholesterol, and a reduction in SM levels in NPC cells recovers the Rab9-dependent vesicular trafficking defect.

Lansoprazole promotes peritoneal fibrosis by bleomycin

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Bleomycin is used as an anticancer agent in the treatment of squamous cell carcinoma. Bleomycin is also used to prepare a pulmonary fibrosis model in experimental medicine due because, it has pulmonary fibrosis as a side effect. We investigated whether the gastric ulcer drug lansoprazole could also be used to suppress pulmonary fibrosis because it suppressed liver fibrosis through suppression of Tgf-β activation in a dietary nonalcoholic steatohepatitis model. Rats were subcutaneously treated with lansoprazole (LAP, 30 mg / kg / day), bleomycin (BLM, 1 mg / kg / day) or bleomycin and lansoprazole (LAP + BLM) for 28 days. The hypertrophic visceral peritoneum was strongly induced in livers of LAP+BLM group, which could be macroscopically confirmed. Histologically analysis indicated that a strong thickening of the visceral peritoneum was observed in LAP+BLM group but neither LAP or BLM group. Furthermore, in the LAP + BLM group, immunohistochemistry indicated M2 macrophages in the visceral peritoneum thickened, and the expression of Tgfβ and Col1a1 gene were increased using real-time PCR. The bleomycin interview form reported that the hypertrophic visceral peritoneum was observed in the rats treated with bleomycin continuously. Thus, lansoprazole may have promoted the hypertrophic visceral peritoneum in the liver of rats treated with bleomycin. Both bleomycin and lansoprazole are clinically used drugs, and we plan to conduct a retrospective study to investigate whether this phenomenon is confirmed.

Study on measurement of internal anal sphincter movement in dogs (application as evaluation method on defecation disorder)-2nd report

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It is estimated that the number of potential fecal incontinence patient in Japan is about 5 millions, but no fundamental treatment has been developed. Therefore, new treatments and novel drugs have being desired to be developed at present. Screening with model animals is important for developing new therapeutic agents. Therefore, we developed a measurement method of internal anal sphincter (IAS) movement in un-anesthetized and unrestrained dogs by placing the force transducers used for gastrointestinal motility measurement in the IAS and each gastrointestinal tract, and measuring the defecation movement using a telemetry method and reported the results of the research at the 92nd Annual Meeting of the Japanese Pharmacological Society. In the previous experiments, INS was observed to contract after dosing noradrenaline and the contraction was suppressed or enhanced by α-blocker or β-blocker, respectively, but it was different under anesthesia from that under awakening. In the present study, a cholinesterase inhibitor was administered and the INS movement was determined to confirm whether the cholinergic mechanism is involved in INS; and the effects of comparative control substances were also examined to evaluate the action of drugs on INS movement.

Effect on contractile response due to carbachol and bradykinin stimulation in the rat inflammatory colon.

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Inflammatory bowel disease is a group of chronic disorders characterized by damaged smooth muscle tissues in the digestive tract. We investigated the alteration of carbachol (CCh) and bradykinin (BK) -induced contractile responses in rat colon under inflammatory conditions. Inflammation was induced by an infusion to colonic lumen of the TNBS and the preparations for ring specimen of the colon which we resected were done three days after an injection. The colon tissues were isolated from saline-treated control and TNBS-treated rats. These tissues were placed in physiological organ bath system and then, isometric force were assessed. Treatment of CCh induced dose-dependent force responses in both types of tissue, but the responses were attenuated in the TNBS-treated rat. Maximal response in TNBS-treated rat was about 60 % of control. The presence of nifedipine markedly inhibited the CCh-induced contraction. This the results suggest that the contractile response to CCh of the rat colonis muscle is caused primarily the increase in the cellular Ca^{2+} concentration. On the other hand, treatment of BK responses were significantly increased in the TNBS-treated rat. These contractile response were antagonized by BK_1 receptor antagonists. These results that BK suggests that the susceptibility to BK_1 receptor increases by inflammation.

Carbon tetrachloride mediated liver fibrosis is alleviated in α7 nicotinic acetylcholine receptor knockout mice

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Background: Cirrhosis is a condition come from excessive liver fibrosis and followed by serious secondary diseases, but there is no effective therapeutic medicine. α7 nicotinic acetylcholine receptor (α7nAChR), initially found as a receptor related to neurotransmission on neural cells. This receptor also expresses on immune cells to do anti-inflammatory action. However, there is few reports showing the relationship between α7nAChR and fibrosis.
Aim: We investigated whether α7nAChR has any effects on liver fibrosis and what is the mechanism.
Methods: Liver fibrosis model mice were established with CCl_4. The pro-fibrotic mRNA expressions and collagen content in livers were measured at 1.5 and 4 weeks. Moreover, we performed immunohistochemical staining and RT-PCR to determine which cells were involved in the mechanism.
Results: α7nAChR KO mice treated with CCl_4 showed significant decrease in pro-fibrotic mRNA expressions at 1.5 weeks and liver fibrosis at 4 weeks compared to WT mice. Furthermore, hepatocytes around fibrosis area expressed ACh transferase and activated hepatic stellate cells expressed α7nAChR.
Conclusion: The severity of fibrosis was significantly decreased in α7nAChR KO mice. Moreover, it is suggested that ACh produced by hepatocytes might stimulate hepatic stellate cells to promote collagen production.

Effects of bezafibrate to high trans-fat diet (HTD) -induced nonalcoholic steatohepatitis (NASH) model in mice

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We previously presented that bezafibrate (BF), a PPAR-pan agonist, has preventing and therapeutic effects on high fat and high cholesterol (HFHC) diet-induced nonalcoholic steatohepatitis (NASH) model in mice. In this study, we examined the effects of BF to high trans-fat diet (HTD) -induced NASH model in mice. HTD was fed to 7-week-old ob (spontaneously obese, hyperglycemia model) mice for 12 weeks to induce NASH. Half of ob mice were orally administered BF (100 mg/kg/day) for 12 weeks. Remaining ob mice were served as controls and administered the vehicle. Normal C57BL/6J mice were fed normal diet and administered the vehicle. After 12 weeks, plasma levels of AST, ALT, TC, glucose, and concentrations of hepatic TG, TC in ob mice fed HTD were markedly higher than those of normal mice. Fatty droplets, inflammation and fibrosis were observed in histopathologic examination of liver. BF significantly lowered plasma TC level, histopathologic fibrosis area and fibrosis scoring without effecting to plasma ALT levels. These results suggested that bezafibrate may suppress the progression of fibrosis in mice NASH model induced by HTD through a decrease in plasma cholesterol level or directly.

Protective effect of nitric oxide on QOL and mucosal injury induced by a constitutive administration of methotrexate to rats.

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The role of nitric oxide (NO) on quality of life (QOL) and mucosal injury induced by a single and a consecutive administration of methotrexate was investigated. Rats received methotrexate intraperitoneally either a single administration (50 mg/kg) or a consecutive administration (12.5 mg/kg/day) for 4 days. N^G-nitro-L-arginine methyl ester (L-NAME) was given subcutaneously to inhibit NO synthase (NOS). 96 h after a first administration of methotrexate, ileal tissues were collected to analyze. Both a single administration and a consecutive administration of methotrexate decreased the QOL. When methotrexate was administered consecutively, L-NAME further worsened the QOL. A consecutive, but not a single, administration of methotrexate caused a significant mucosal injury and inflammation. A consecutive, but not a single, administration of methotrexate significantly induced a constitutive NOS expression in the ileal tissue. The role of NO derived from constitutive NOS in the reduction of QOL and small intestinal by methotrexate administration may be more important in a consecutive administration than a single administration.

Role of CPI-17 on uterine smooth muscle contraction depending on pregnancy stage

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Background Smooth muscle contraction is regulated by the balance between MLC kinase and MLC phosphatase (MLCP). Phosphorylated CPI-17 can inhibit MLCP resulted in induce contraction. **Aim** This study was planned to elucidate the effect of CPI-17 on contraction in myometrium from non-pregnant and pregnant mice. Wild type C57BL6/J mice (WT), CPI-17 deficient mice (KO) and phospho-inactive mutant CPI-17 at T38 to alanine knocked-in mice (TA) were used. **Methods** Isometric force stimulated with high concentration (65.4 mM) of KCl (high K), oxytocin (Oxy; 100 nM) and Carbachol (CCh, 5-100 μM) from non-pregnant and pregnant mice. **Results** The spontaneous contraction and absolute contractile ability stimulated with CCh and high K^+ were not difference in non-pregnant and pregnant myometrium isolated from WT, KO and TA. In non-pregnant myometrium, Oxy induced spontaneous rhythmic contraction with small sustained one in WT. Oxy induced persistent weaker contraction in KO and TA than WT. On the other hand in pregnant myometrium, the Oxy-induced contractions were tended to be smaller in TA and KO than WT. **Conclusion** PKC/CPI-17 pathway induces an important role to regulate contraction by Oxy but not by CCh in both non-pregnant and pregnant myometrium. CPI-17 may be important in normal parturition induction.

Dipeptidyl peptidase-4 (DPP-4) inhibitor, linagliptin, attenuates left ventricular remodeling after myocardial infarction via DPP-4-independent pathway.

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Background: Dipeptidyl peptidase-4 (DPP-4) inhibitors not only improve impaired glucose tolerance in diabetes, but also have pleiotropic extra-pancreatic effects such as preconditioning effect for myocardial ischemia-reperfusion injury. Here, we investigated the anti-remodeling effects of linagliptin, a DPP-4 inhibitor, by use of DPP-4-deficient rats.

Methods and Results: After the induction of myocardial infarction (MI), Fischer 344 rats with inactivating mutation of DPP-4 were orally administrated with a DPP-4 inhibitor, linagliptin (5 $mg \cdot kg^{-1} \cdot day^{-1}$), or vehicle in drinking water for 4 weeks. Linagliptin did not affect hemodynamic status, body weight, and infarct size. In echocardiography, linagliptin tended to improve left ventricular (LV) systolic function, and significantly improved LV diastolic function, surprisingly. Interstitial fibrosis and macrophage infiltration were significantly lower in the linagliptin group than those in the vehicle group. Fibrosis-related gene expressions, such as collagen I and transforming growth factor-β1 (TGF-β1), and inflammation-related expressions, such as macrophage chemotactic protein 1 and matrix metalloproteinase-2 (MMP-2), were significantly suppressed in marginal area of the linagliptin-treated rats compared with the vehicle rats. The TGF-β1 and MMP-2 protein levels were attenuated by linagliptin in DPP-4-deficient cardiac fibroblasts.

Conclusions: Linagliptin can attenuate MI-induced cardiac remodeling via a DPP-4-independent pathway.

Protective role of an NAD^+-dependent deacetylase SIRT1 in doxorubicin-induced cardiotoxicity.

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Background: Doxorubicin (DOX) is an anti-cancer drug which develops heart failure. SIRT1, an NAD^+-dependent deacetylase, affords cellular protection under various stresses. Here, we investigated whether and how SIRT1 protects the heart from DOX-induced cardiotoxicity in mice.

Methods and Results: Wild type (WT) and tamoxifen-inducible cardiomyocyte-specific SIRT1 knockout (cKO) mice were treated with vehicle (Veh) or DOX (4 IP injections of 5 mg/kg/week) starting at 3 months of age. Echocardiography at 1 week after final vehicle or DOX showed that left ventricular fractional shortening (FS), an index of cardiac function, was similar in vehicle treated WT and cKO. Although DOX reduced FS in both genotypes, cKO showed lower FS after DOX than that in WT. Cardiac ANP mRNA level was also higher in cKO than WT after DOX. TUNEL-positive nuclei were unchanged in Veh-treated but were more increased by DOX in cKO than WT, indicating increased apoptosis in cKO after DOX. A long-range PCR method for analysis of mtDNA deletion, which indicates mtDNA damage, demonstrated an increase in level of mtDNA with deletion by DOX in cKO but not in WT. Immunoblotting showed that DOX significantly reduced cardiac levels of an autophagosome marker LC3-II and p62 protein which is degraded by autophagy in WT but not in cKO, suggesting activation of cardiac autophagy only in WT.

Conclusions: SIRT1 deletion worsens DOX-induced cardiotoxicity probably through enhanced mitochondrial damage via impaired autophagy.

Production of functional antibody targeting Ca^{2+}permeable channel TRPV2 ameliorating dilated cardiomyopathy in animal models

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Abnormal Ca^{2+} handling is essential in the pathophysiology of dilated cardiomyopathy (DCM). One of Ca^{2+} permeable channels, transient receptor potential cation channel, subfamily V, member 2 (TRPV2) has been suggested as a principal candidate for Ca^{2+} entry pathways and a potential therapeutic target for DCM. In this study, we produced selective antibodies recognizing TRPV2 from the outside of cell. One of antibodies inhibited the Ca^{2+} influx via TRPV2 in cultured cells and caused TRPV2 to disappear from the plasma membrane via cellular internalization. The antibody epitope existed in the turret of pore-forming outer gate of TRPV2. We tested the therapeutic efficacy of the antibody in DCM developed in the δ-sarcoglycan-deficient hamsters (J2N-k). Intraperitoneal administration of the antibody (0.5 mg/kg) for 2 weeks (once a week) prevented the progression of cardiac dysfunction as evaluated by echocardiography and improved the abnormal Ca^{2+} handling. Further, the antibody was also effective in preventing heart failure of the murine 4C30 model with end-stage DCM. Interestingly, endogenous TRPV2 accumulated in the cardiac muscle sarcolemma disappeared upon antibody administration. Thus, the produced antibody is capable of ameliorating DCM through enhanced cellular internalization, and may be a promising treatment for patients with DCM.

Development and assessment of a new *in vitro* platform of human induced pluripotent stem cell-derived cardiomyocytes for evaluating the drug-induced biological phenomena predicting clinically observed cardiac effects

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Currently available human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been known to exert a negative force-frequency relationship as one of their immature properties. In this study, we examined whether controlling the direction of contraction process and/or supplying the higher oxygen tension may overcome such limitation of the contraction movement. We prepared one layered, higher cell-density sheets of hiPSC-CMs, and simultaneously recorded the motion vectors and field potentials. In a cell sheet under spontaneous activity, a synchronous movement consisted of multiple contractions which started from various sites. During electrical stimulation, the contraction started around the pacing electrodes and we observed the positive force-frequency relationships in contraction as well as relaxation along with the frequency-dependent shortening of the field potential durations. The use of fractional analysis of motion vectors demonstrated that contraction as well as relaxation processes consisted of fast and slow phases. Increase in oxygen tension from 20 to 95% in mixed gas accelerated the fast phase of relaxation. β-Stimulation accelerated the timing of fast phase of relaxation, whereas a tyrosine kinase inhibitor dasatinib delayed it. Thus, these observations can indicate that the currently proposed procedure may become a new tool for integrating the drug-induced biological phenomena *in vitro* extrapolating to clinically observed cardiac efficacy and adverse effects.

Elucidation of expression and regulation mechanism of cell adhesion factor Gicerin/CD146 in cardiac hypertrophy model

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Gicerin/CD146 is a cell adhesion molecule which belongs to the immunoglobulin (Ig) superfamily. We have reported the existence of Gicerin/CD146 in the heart, lung and smooth muscles of blood vessels. It is expected that Gicerin/CD146 expressed in these tissues may have a function different from those in the nervous system or tumors. WE have reported that Gicerin/CD146 is involved in the hypertrophy of vessel smooth muscles. We speculate that in the heart, Gicerin/CD146 may also have some role in the hypertrophy of the cardiac muscle cells. In this study, we make a cardiac hypertrophy model rat by constricting the rat aorta (TAC, transverse aortic constriction) and examined the effect on the expression of Gicerin/CD146.We confirmed the status of hypertrophy by an expression of the β-MHC gene (β myosin heavy chain), which has been reported to be elevated during cardiac hypertrophy. Next, stretch stimulation was applied to myocardial cell line H9c2 cells. We confirmed the gene expression level of Gicerin/CD146 was influenced by TAC treatment. In cultured myocardial cells, the expression level of Gicerin/CD146 was also influenced by the stretch stimulation. Based on the above, it was suggested that the expression of Gicerin/CD146 is involved in cardiac hypertrophy as well as the stretch stimulation.

Roles of Hsp90 on cardiac remodeling in the development of chronic heart failure.

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Hsp90 is a highly conserved molecular chaperone involved stabilization of client proteins. Hsp90 clients are various signal transducers including protein kinases. It is well known that the cardiac remodeling such as cardiac hypertrophy and fibrosis is involved in the development of chronic heart failure. The cardiac remodeling is regulated by many signaling pathways. c-Raf and JNK are signal transducers involved in cardiac remodeling, and are also Hsp90 client proteins. Therefore, to clarify the roles of Hsp90 and its clients in progression to the chronic heart failure, we examined effects of Hsp90 inhibitor on the signal transducers in the development of chronic heart failure in animal models. Treatment of the animals with Hsp90 inhibitor resulted in a suppression of cardiac remodeling and a preservation of cardiac pump function. Furthermore, c-Raf/Erk signaling was attenuated by an administration of Hsp90 inhibitor. These results suggest that Hsp90 contributes to stabilization of several cardiac remodeling-associated protein kinases during the development of chronic heart failure.

Analysis of therapeutic effects of the Japanese herbal medicine ninjinyoeito on oxidative stress-induced cardiotoxicity with in vitro experimental system

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Oxidative stress is known to be involved in various pathologies such as heart failure and cancer. Mitochondria, which produce oxygen species (ROS) as a type of oxidative stress, are abundantly expressed especially in the cardiac cells. Once mitochondrial damage occurs, produced ROS is accumulating and causes mitochondria damage and subsequently lead to cardiac dysfunction. Recently, cardiac dysfunction caused by anticancer drugs and cancer itself has become a concern, and it has been suggested that oxidative stress is involved in one of these causes. The anthracycline antitumor antibiotic doxorubicin (DOX) induces severe adverse effects to non-tumor tissues including cardiac cells. DOX causes cardiomyopathy in a dose-dependent manner and consequent heart failure often limits DOX-based chemotherapy. The Japanese Kampo medicine ninjinyoeito is known to improve the quality of life (QOL) in cancer patients because of its antioxidative effects. However, molecular mechanisms of ninjinyoeito for the preventive effects of oxidative stress remain unclear. In the present study, we sought to determine the effect of ninjinyoeito on oxidative stress in the heart and DOX-induced cardiotoxicity in *in vitro* experimental system. The effects of ninjinyoeito on the oxidative toxicity in cultured cardiac cells induced by DOX will be presented.

Developmental changes in sarcoplasmic reticulum (SR) dependency of contraction and relaxation mechanisms in the mouse ventricular myocardium

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Developmental changes in contraction and relaxation mechanisms were examined in the ventricular myocardium from fetal, neonatal, and 1, 2 and 4 week-old mice. In isolated tissue, the negative inotropy by ryanodine increased with age, while that by nifedipine decreased with age. The prolonging effect on the relaxation by cyclopiazonic acid, a SR Ca^{2+} ATPase (SERCA) inhibitor, increased with age, while that by SEA0400, a Na^{+}/Ca^{2+} exchanger inhibitor, decreased with age. Carboxyeosin, a plasma membrane Ca^{2+} ATPase (PMCA) inhibitor, had no effect on the relaxation in all developmental stages. In the presence of cyclopiazonic acid and SEA0400, carboxyeosin slightly prolonged the relaxation, and its prolonging effect decreased with age. In cardiomyocytes, fluorescence imaging revealed that the SR increases with age. t-Tubules, which were absent in the cell center region until 1 week, were present throughout the cytoplasm after 2 weeks. Until 1 week, Ca^{2+} at the cell center showed slower rise than the subsarcolemmal region, but after 2 weeks, Ca^{2+} increased simultaneously across the entire width of the cell. Ca^{2+} decay time decreased with age. These results indicate that the contraction and relaxation mechanisms in the mouse ventricular myocardium convert from membrane dependent to SR dependent during the postnatal development.

Changes of thoracic and mesenteric arterial vascular function in angiotensin Ⅱ-induced hypertensive mice

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Endothelial cells play an important role in regulation of vascular function, which are affected by various bioactive substances. Angiotensin (Ang) II induces vasoconstriction and is suggested to cause vascular dysfunction. In this study, we compared the endothelium-dependent vasorelaxation response of the thoracic aorta and mesenteric artery in Ang II -induced hypertensive model mice.

In thoracic aorta, stimulation of endothelial a_2-adrenoceptor with clonidine induced a PI3-K-dependent relaxation, whereas acetylcholine (ACh) induced the relaxation via Gq-PLC-Ca^{2+} pathway. In mesenteric artery, ACh-induce relaxation was remarkable, but clonidine-induced relaxation was hardly observed. Ang II -induced hypertension significantly impaired the endothelium-dependent relaxation in aorta, but not in mesenteric artery. Experiment using NOS inhibitor revealed that vasorelaxation responses to clonidine and ACh in aorta were largely dependent on NOS activity, whereas NO played minor role in endothelial-dependent vasorelaxation in the mesenteric artery. These results suggest that NOS-dependent endothelial regulation of vascular tone is more sensitive to Ang II -induced vascular dysfunction. In addition, the difference in endothelium-dependent relaxation mechanism may be related to the susceptibility of distinct blood vessel to Ang II -induced vascular injury.

Highly neurotoxic amyloid-bassemblies from Alzheimer's disease brain, amylospheroids, inhibit endothelial Na^+, K^+-ATPase α3 activity, resulting in the inhibition of eNOS activity in human brain microvascular endothelial cells.

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Vascular deposition of amyloid-bprotein (Aβ), known as cerebrovascular amyloid angiopathy (CAA), is associated with vascular dysfunction. In the previous meeting (WCP2018), we reported amylospheroids (ASPD, 30-mers highly neurotoxic Aβ assemblies in average isolated from Alzheimer's disease brain) suppressed the vasorelaxation via endothelial eNOS inactivation through mitochondrial ROS/PKC pathway. Neuronal toxicity of ASPD was reported to be exerted by impairing the activity of Na^+, K^+-ATPase α3 (NAKα3) via the direct binding (Ohnishi et al. *PNAS*2015). Here, we sought to elucidate whether NAKα3 was involved with the eNOS inactivation by ASPD. We first detected and found the protein and mRNA of NAKα3 in cerebrovascular endothelial cells. Furthermore, we found NAKα3 was expressed in innermost endothelial layer of vascular vessels. We then examined whether the eNOS inactivation by ASPD was abolished by siRNA transfection. Remarkably the knockdown of ASPD-binding target NAKα3 by ATP1A3-siRNA transfection blocked the eNOS inactivation by ASPD. Taken together, these results suggest the endothelial toxicity of ASPD was mediated by NAKα3.

Valproic acid prevents retinal angiogenesis via a proteasome-dependent mechanism in neonatal mice

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Pathological retinal angiogenesis contributes to the development and progression of vision-threatening eye diseases, such as retinopathy of prematurity and diabetic retinopathy. Valproic acid, a widely used antiepileptic drug, exerts anti-angiogenic effects by inhibiting histone deacetylase (HDAC). We previously reported that valproic acid and vorinostat, a HDAC inhibitor, suppress pathologic retinal angiogenesis in mice with oxygen-induced retinopathy. In this study, using neonatal mouse retina, we examined the mechanisms of anti-angiogenic effects of valproic acid and vorinostat. Mice were subcutaneously injected with valproic acid, vorinostat, or vehicle once a day from postnatal day (P) 0 to P3. At P4, the delayed retinal angiogenesis was observed in mice treated with valproic acid or vorinostat. The expression level of vascular endothelial growth factor (VEGF) was reduced 2 or 6 hours after a single injection of valproic acid or vorinostat in P4 mice. Both drugs suppressed the VEGF-mediated activation of mammalian target of rapamycin pathway in proliferating endothelial cells. The proteasome inhibitor MG132 prevented valproic acid- and vorinostat-induced reduction in the VEGF expression level.These results suggest that valproic acid suppresses retinal angiogenesis by decreasing retinal VEGF levels by a proteasome-dependent mechanism.

Role of glial cells in angiogenesis in the neonatal rat retina with neurodegenerative injury

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Neuronal and glial cells play an important role in the development of vasculature in the retina. In this study, we investigated the role of glial cells in angiogenesis in retinas with neurodegenerative injuries. To induce retinal neurodegenerative injuries, *N*-methyl-D-aspartic acid (NMDA, 200 nmol) was injected into the vitreous chamber of the eye on postnatal day (P)7. Morphological changes in retinal neurons and vasculature were assessed on P14, P21, and P35. Prevention of angiogenesis and regression of some capillaries were observed on P14 in retinas of NMDA-treated eyes. However, angiogenesis started on P21, and the retinal vascular network was established by P35 in retinas with neurodegenerative injuries. The results of mechanistic analyses suggest that astrocytes activated by the injury produce and secrete fibronectin to form a scaffold for endothelial cell migration. Vascular endothelial growth factor (VEGF) released mainly from glial cells stimulates the process of angiogenesis. These results suggest that glial cells play an important role in angiogenesis in neonatal rat models of retinal neurodegeneration.

Oxidative stress induces cell death via the suppression of Orai1-mediated Ca^{2+} entry in brain capillary endothelial cells.

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Brain capillary endothelial cells (BCECs) form the blood-brain barrier (BBB) and play an essential role in maintaining BBB barrier function. Oxidative stress induces accumulation of excessive reactive oxygen species (ROS) and facilitates brain capillary cell death, leading to damage BBB. However, it remains still unclear how oxidative stress induces the cell death of BCECs. In this study, t-BBEC117 cells, an immortalized bovine brain endothelial cell line, were cultured under oxidative stress with 30 μM H_2O_2 for 24 hr. The protein expressions of Orai1 and STIM1 were not affected by oxidative stress in t-BBEC117 cells. However, the oxidative stress inhibited store-operated Ca^{2+} (SOC) entry and the suppression was rescued by the application of 10 mM N-acetyl-cycteine (NAC), a ROS scavenger. Ca^{2+} imaging study with Orai1 siRNAs revealed that SOC entry was mainly mediated by Orai1 channels under oxidative stress in t-BBEC117 cells. The application of 5 μM 2-Aminoethoxydiphenylborane (2-APB), an Orai channel activator, enhanced SOC entry under oxidative stress in t-BBEC117 cells and rescued H_2O_2-induced cell death. We show here that oxidative stress inhibits Orai1-mediated Ca^{2+} entry, and thereby facilitates the death of BCECs.

Ca^{2+}-activated Cl^- channels are involved in the proliferation and migration of brain capillary endothelial cells.

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The blood-brain barrier (BBB) contributes to the maintenance of homeostasis in the brain. Brain capillary endothelial cells (BCECs) are a major component of the BBB and, thus a delicate balance between their proliferation and death is required. Although the activity of ion channels in BCECs is involved in BBB functions, the underlying mechanisms remain unclear. In this study, the molecular components of Ca^{2+}-activated Cl^- (Cl_{Ca}) channels and their physiological role were examined using the cell line derived from bovine BCECs, t-BBEC117. Expression analyses revealed that TMEM16A was predominantly expressed in t-BBEC117 cells. Whole-cell Cl^- currents were sensitive to Cl_{Ca} channel blockers, niflumic acid and $T16A_{inh}$-A01, and markedly reduced by the siRNA knockdown of TMEM16A. The blockade of Cl_{Ca} channel activity with Cl_{Ca} channel blockers or TMEM16A siRNA induced a significant membrane hyperpolarization. The treatment with TMEM16A siRNA caused an increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) at the resting level. $T16A_{inh}$-A01 inhibited cell viability in a dose-dependent manner, and Cl_{Ca} channel blockers and TMEM16A siRNA also blocked cell proliferation. In addition, Cl_{Ca} channel blockers and TMEM16A siRNA clearly attenuated cell migration. These results indicate that TMEM16A contributes to Cl_{Ca} channel conductance and its activity regulates the resting membrane potential of and $[Ca^{2+}]_{cyt}$ in BCECs, TMEM16A Cl_{Ca} channel are involved in the maintenance of BBB functions, including the proliferation and migration of BCECs.

Development of tissue-specific histamine-responsive vascular endothelial models using a collagen vitrigel membrane

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The tightness of the endothelial barrier is tissue-dependent. We expect that tissue-specific barrier function can be induced in immature vascular endothelial cells by co-culturing with tissue-specific cells. The purpose of this study is to construct tissue-specific vascular endothelial models on a collagen vitrigel membrane (CVM) composed of high-density collagen fibrils equivalent to connective tissues *in vivo* and compare the responsivity to histamine. We used human microvascular endothelial cells (HMVECs) derived from a newborn foreskin. HMVECs were cultured in a CVM chamber with or without human dermal fibroblasts (HDFs), C6 cells (a rat glioma cell line) or HepG2-NIAS cells (a human hepatocellular carcinoma cell line) cultured on the reverse-side of CVM for up to 6 days. The endothelial barrier function was evaluated by transendothelial electric resistance (TEER). TEER values of a HMVEC monolayer were 15-20 Ω/cm^2 during culture periods. It significantly increased up to 40-60 Ω/cm^2 by co-culturing with HDFs, C6 cells and HepG2-NIAS cells. Also, TEER value was clearly decreased in the co-culture model composed of HMVECs and HDFs treated with 1 μM histamine while HMVEC monolayer model showed slight response to 100 μM histamine. Now we are investigating tissue-specific responses to histamine among the models.

Elucidation of the pathogenesis of muscle sarcopenia caused by liver fibrosis

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In patients with liver fibrosis, muscle mass and muscle strength tend to decline, which affects their prognosis. In 2016, the *Sarcopenia Criteria for Liver Disease (First Edition)* was established. However, it is unclear why hepatic fibrosis leads to muscle weakness. We induced hepatic fibrosis by performing bile duct ligation (BDL) in mice and investigated the pathogenesis of muscle atrophy caused by hepatic fibrosis. In BDL mice, the weight and cross-sectional area of the tibialis anterior muscle decreased from the first week after surgery in the early stage of fibrosis. We performed forelimb grip tests to confirm a significant decrease in muscle strength. From these results, we considered that hepatic fibrosis-dependent muscle sarcopenia model had been established.

Using this model, we investigated the cause of hepatic fibrosis-dependent muscle atrophy. We applied BDL mouse serum to cultured myotube cells in vitro, and myotube atrophy was induced. This suggested the possibility of multi-organ linkage in which atrophy-inducing factor was transmitted via blood as a mechanism of muscle atrophy dependent on liver fibrosis. Currently, in order to search for atrophy-inducing factors present in the blood, we are analyzing several cytokines that have been reported to be involved in muscle atrophy.

Effects of saturated fatty acids on skeletal muscle differentiation in an intrauterine hyperglycemic environment

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INTRODUCTION: The effect of unsaturated fatty acids on skeletal muscle differentiation in an intrauterine hyperglycemic environment has not been clarified. We explored the effect of maternal nutrition on myotubes formation using the L6 rat myoblast cell model exhibiting hyperglycemia during pregnancy.

METHODS: Rat L6 skeletal myoblasts were grown in DMEM medium containing 100 mg/dL glucose (control), and subsequently it examined the effect of palmitate against the differentiation into myotubes in differentiation medium containing control or 450 mg/L (high glucose). Phospho-Akt was detected by western blotting and the expressions of muscle differentiation markers (*myf5* and *myoD*) were evaluated by real-time PCR.

RESULTS: The gene expression of *myf5* and *myoD* and the level of the Phospho-Akt were significantly higher in high glucose than in control with myogenic differentiation. Palmitate decreased the expression level of *myf5* and *myoD* in 24 hours, however, their expression level increased again after 48 hours. Palmitate also was decreased the Phospho-Akt with differentiation.

CONCLUSION: We showed that palmitate suppressed myogenic differentiation in hyperglycemic condition. We would like to explore the influence of other unsaturated fatty acid against myogenic differentiation.

The role of calcineurin B homologous protein 3 (CHP3) in C2C12 myoblasts.

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Calcineurin B homologous protein3 (CHP3) is an EF-hand calcium-binding protein. In our previous study, we found that the expression level of endogenous CHP3 protein was increased in an early differentiation step in C2C12 mouse myoblasts. In this study, we aimed to elucidate the role of CHP3 in skeletal muscle differentiation. Live-cell time-lapse imaging revealed that CHP3 over-expression inhibited cell division of undifferentiated C2C12. Therefore, CHP3 may negatively regulate the proliferation of C2C12.

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Anti-apoptotic function of PDZRN3 protein in myoblasts

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We previously demonstrated that PDZRN3 is an important protein for myogenic differentiation from myoblasts to myotubes. In regeneration of injured skeletal muscle *in vivo*, stem cells induce MyoD expression and differentiate into myoblasts, which expand through proliferation. We reported that PDZRN3 is upregulated along with MyoD during regeneration of injured muscle. In this study, we aimed to clarify a role of PDZRN3 in proliferation of myoblasts. When exposed to serum deprivation stress, PDZRN3-depleted C2C12 myoblasts by RNAi showed higher levels of apoptotic markers as compared with those of control cells. PDZRN3-depletion also suppressed the activation of anti-apoptotic Akt, indicating the involvement of PDZRN3 in apoptotic regulation. We found that the abundance of cyclin A2 was reduced in PDZRN3-depleted C2C12 myoblasts, as was that of Mre11, which plays an important role in the repair of DNA damage. The activation of p53 was enhanced in PDZRN3-depleted cells due to the DNA damage accumulation. Overexpression of cyclin A2 restored the expression of Mre11 and attenuated caspase-3 cleavage in PDZRN3-depleted C2C12 cells subjected to serum deprivation. These results thus indicate that PDZRN3 restrains apoptosis in myoblasts through maintenance of cyclin A2 expression.

Effect of bone matrix protein osteocalcin on proliferation and neuronal differentiation of PC12 cells

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Bone matrix protein osteocalcin (OC) was recently reported to support the development of learning and memory and also prevent anxiety-like behaviors in mice. Although the mechanism through which OC affects systemic energy expenditure and glucose homeostasis has been relatively well studied, the direct actions of OC on neurons in detail are still uncovered. Therefore, we here investigated the effect of OC on neurons using rat pheochromocytoma cell line PC12, with special reference to the neurite outgrowth, cell proliferation and survival, as well as intracellular signaling. The number of PC12 cells cultured for four days in the presence of 5 to 50 ng/mL of OC was increased compared to the cells cultured in the absence of OC. The length, but not the number of NGF-induced neurite outgrowth was enhanced by OC. NGF-induced phosphorylation of Akt and ERK was both affected by pretreatment of the cells with OC. RT-PCR analysis for candidates of OC receptor revealed that mRNA expression of Gpr158, but not Gprc6a, was detected in PC12 cells. These results suggested that OC may exerts direct effect on cell growth and differentiation by binding to Gpr158 and modulation of downstream intracellular signaling.

Effect of phenytoin on PI3K/Akt signaling pathway in human gingival fibroblasts

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Gingival overgrowth is caused in response to the antiepileptic drug, phenytoin (PHT). PHT-induced gingival overgrowth is characterized by the proliferation of fibroblasts and increased collagen formation in gingiva. Interleukin -1α (IL-1α) increases basic fibroblast growth factor (bFGF) production and influences the release of bFGF in human gingival fibroblasts (hGFs). We have previously reported that PHT induced gingival overgrowth to promote cell proliferation by ETS-1 expression and reduced apoptosis through SAPK/JNK pathway, since PHT enhanced Bcl -2 mRNA and protein expression in hGFs. The present study investigated the effect of PHT on PI3K/Akt signaling pathway in hGFs to clarify the mechanism of PHT-induced gingival overgrowth. Cultured hGFs were purchased from ScienCell Research Laboratories. hGFs were cultured to semi-confluence and treated with PHT and/or IL-1α for 1, 6, 24, and 96 hours. The expression and phosphorylation of Akt, GSK-3β, PTEN, PDK1, p21Waf1/Cip1, and p27 Kip1 were measured by Western blot analysis. IL-1α increased the phosphorylation of Akt (Ser473), however, PHT did not affect that of Akt (Ser473) and GSK-3β. On the other hand, PHT decreased the expression of p21Waf1/Cip1 and p27 Kip1. These results suggest that PHT may affect the expression of p21Waf1/Cip1 and p27 Kip1 through another pathway except Akt and GSK-3β signaling.

Participation of TRPV1 in tooth movement-related pain

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The many patients complain about orthodontic force-induced pain. It has reported that jaw-opening reflex (JOR) excitability is increased in 1 (D1) day and is decreased in 7 days (D7) after orthodontic force application in rats. In this model, potential analgesic role of Receptor Potential Vanilloid 1 (TRPV1) antagonism in orthodontic force-induced pain and related features were investigated.

Rats were applied continuous orthodontic force to right maxillary first molar. TRPV1 antagonists (A-889425: 5-10 mmol/kg, AMG9810: 10-15 mmol/kg) or aspirin (560 mmol/kg) was applied to D1-D7. Inflammatory cytokines were measured by antibody arrays. Excitation of trigeminal ganglia (TG) was evaluated by expression of Glial fibrillary acidic protein (GFAP) in satellite glial cells. And, expression of mature osteoclasts was measured by TRAP staining.

All chemicals significantly reduced JOR excitability at D1. Temporal alteration of JOR excitability was associated with GFAP expression and that was significantly reduced by TRPV1 antagonists and aspirin. Although these chemicals reduced expression of mature osteoclasts at D7 significantly, distance of tooth movement was not altered. Significant increase of CINC2 and IL-6 was induced by orthodontic force application. Both TRPV1 antagonists significantly reduced CINC2 and IL-6, however, aspirin failed to reduce CINC2.

Taken together, TRPV1 antagonism, in both peripheral and central, induced broad effects on orthodontic force-induced physiological and morphological alterations.

Analysis of compensatory hypertrophy-associated enhancement of salivary secretionusing the intravital Ca^{2+}imaging and comprehensive gene analysis.

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Dysfunction of unilateral salivary glands causes compensatoryhypertrophy of the contralateral salivary gland. To examine a functional change of the hypertrophiedsubmandibular gland (SMG) after the ligation of main excretory duct (MED) of unilateral SMGs, we monitored Ca^{2+}responses and salivary secretion in rat SMG using the intravital Ca^{2+}imaging system and the fibre-optic pressure sensor, simultaneously. Submaximal dose of ACh (<120 nmol/min) induced 2 times larger increase in intracellular Ca^{2+}concentration and salivary secretion in hypertrophied SMGs than in controlSMGs, whereas the maximal dose of ACh (360 nmol/min) induced comparable responses in these SMGs. These results indicate that the ligation of MED of unilateral SMGs increased the sensitivity of contralateral SMGs to ACh. To clarify the molecular mechanism for inducing "the compensatory hyperfunction", we examined gene expression of the hypertrophiedSMG by a comprehensive analysis using the next-generation sequencing and a quantitative RT-PCR analysis. Currently, we identified 57 candidate genes of which 6 genes showed significant up- or down-regulation after MED ligation in the contralateralSMGs. Our data suggest the increase in acinar cell by the proliferation and transition from ductal cells, and the involvement of some growth factors and clock genes.

Globo-series glycosphingolipids deficiency in mice resulted in the attenuation of bone formation through decrease of osteoblasts

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Purpose: Globo-series glycosphingolipids have not only been used as markers for stem cells and tumors but are also considered to regulate maintain stemness and immune system. However, there have no reports on the involvement of globo-series glycosphingolipids in bone metabolism. In this study, we investigated the effects of genetic deletion of Gb3 synthase, which initiates the synthesis of globo-seires glycosphingolipids on bone metabolism.

Material & Methods: We examined expression levels of globo-series glycosphingolipids (Gb3, Gb4, and Gb5) in MC3T3 E1 mouse osteoblast-like cells and SaM-1 human osteoblast cells using flow cytometry. To determine whether globo-series glycosphingolipids are involved in bone metabolism, we analyzed bone phenotype of Gb3 shynthase-knockout (Gb3S KO) mice using μCT. Furthermore, we conducted a calcein double labeling method to evaluate bone formation.

Results & Conclusion: Among Gb3, Gb4, and Gb5, only Gb4 was expressed in osteoblasts. μCT analysis revealed that femoral cancellous bone mass in Gb3S KO mice was lower than that in wild type (WT) mice. Calcein double labeling also revealed that bone formation in Gb3S KO mice was lower that that in WT mice. We demonstrated that Gb4 is expressed in osteoblasts, and globo-series glycosphingolipids regulate bone mass through bone formation.

S1P/S1PR2 signaling pathway promotes bone formation on rat apicoectomy model

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Sphingosine-1-phosphate (S1P) is known as a signaling sphingolipid that regulates many cellular responses, including cellular differentiation. Signaling through the specific cell surface G-protein-coupled receptors (subtypes S1PR1 to S1PR5) mediates most of the biological action of S1P. In this study, we investigated the roles of S1PR2 signaling for bone formation on the rat apicoectomy model.

We used 10 week-old male Wistar rat, and created the apicoectomy model with bone cavity (diameter 2 mm, depth 1 mm) on a mesial root of mandibular left first molar. Then we injected S1PR2 agonist (CYM-5520) mixed with scaffold (atelocollagen). Rat sacrificed after 3 weeks, and then subjected to micro-computed tomography analysis. The handling rats and all procedures were approved by the Animal Committee of Fukuoka Dental College (No. 18014).

Injection of S1PR2 agonist promoted bone properties such as bone volume density, and trabecular number compared with control. Interestingly, we found that S1PR2 agonist showed the osteoinductive effect.

We conclude that S1PR2 signaling accelerate bone formation and induce osteoblast differentiation.

Petunidin, one of the major anthocyanins which possess potent antioxidative activity, prevents bone loss in sRANKL-induced osteopenic mice.

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Osteoporosis characterized by a decrease in bone mass, is thought to be one of the chronic and lifestyle-related diseases. We aimed to elucidate the bone protective effects of petunidin, considering its potent antioxidative activity. Seven-week-old female C57BL/6J mice were divided into three groups: control, sRANKL-induced osteopenic mice (vehicle) and 7.5 mg/kg/day petunidin-treated osteopenic mice (petunidin). Bone morphometric parameters and microarchitectural properties of the femur were determined using a micro-CT system. The vacant area observed in the marrow cavity of vehicle group reduced in size and filled with trabeculae by petunidin administration. Quantitative analyses showed that petunidin significantly increased BV/TV, Tb.Th, Tb.N, reflecting the increase in trabecular bone mass. Furthermore, bone histomorphometry analyses showed that petunidin administration significantly increased OV/TV, O.Th, OS/BS, Ob.S/BS and N.Ob/BS, suggesting that bone formation was accelerated by petunidin. In contrast, major resorption-parameters (ES/BS, Oc.S/BS and N. c/BS) were decreased in the petunidin-treated group. Histological sections of the distal femurs demonstrated that both of osteoid thickness and height of the osteoblasts were increased by petunidin administration. In conclusion, the present study showed that oral administration of petunidin improved sRANKL-induced osteopenia in mice through increased osteoid formation, reflecting accelerated osteoblastogenesis, concomitant with suppressed bone resorption.

Effects of the vigabatrin, a newer antiepileptic drug, on bone metabolism in rat

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First-generation antiepileptic drugs (AEDs) increase the risk of fracture in patients with epilepsy. Although phenytoin has been reported to adversely influence bone metabolism, little is known about the effects of recent AEDs. In this study, we examined the effects of newer AED, vigabatrin on bone metabolism in rats. Male Wistar rats were treated orally with phenytoin (20 mg/kg) or vigabatrin (50 or 200 mg/kg) daily for 6 weeks. Bone histomorphometric analysis was performed, and bone strength was evaluated using a three-point bending method. Bone mineral density (BMD) was measured using quantitative computed tomography. Administration of phenytoin significantly decreased BMD. In contrast, vigabatrin treatment did not affect BMD. However, a significant decrease in bone microstructure parameters were observed in the vigabatrin 200 mg/kg treated group. The bone formation parameters decreased after vigabatrin 200 mg/kg treatment, whereas the bone resorption parameters increased. Our data suggest that vigabatrin-induced trabecular bone rarefaction, which is associated with decreased bone formation and enhanced bone resorption may affect bone strength after chronic exposure.

Possible involvement of VEGF in sepsis-associated lung vascular hyperpermeability

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Acute lung injury is one of the common lethal complications in sepsis. It is clinically characterized by refractory hypoxemia, diffuse pulmonary infiltrates, and high permeability pulmonary edema. Several molecules could contribute to increased vascular permeability during sepsis. In this study, we investigated whether VEGF, originally known as a vascular permeability factor, plays a possible role in sepsis-associated lung vascular hyperpermeability. Initially, we examined time-dependent expression of VEGF and its receptors, Flt1 and KDR, in human pulmonary endothelial cells (HPMEC-ST1.6R) when stimulated with LPS + INFγ. Following stimulation, VEGF expression was significantly increased, but Flt1 and KDR remained unchanged. VEGF release by HPMEC-ST1.6R after stimulation was significantly increased, and it was suppressed by JNK, MEK, and p38 MAPK inhibitors. Next, when experimental mouse models of sepsis were used, septic conditions resulted in enhanced lung vascular permeability, as assessed by IgM concentrations in bronchoalveolar lavage fluid, and led to a significant increase in blood VEGF concentrations. Bevacizumab, an anti-VEGF antibody, significantly suppressed pulmonary hyperpermeability in sepsis. These results suggest that VEGF is involved as one of the factors that increase lung vascular permeability in sepsis.

IL-5/eosinophils are involved in acquisition of steroid resistance in severe asthma of mice

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It has been known that 5-10% of asthma patients are resistant to the steroid therapy. However, mechanisms underlying the acquisition of steroid resistance remain unclear. Objective of this study is to elucidate whether IL-5/eosinophils are involved in the acquisition of steroid resistance. Ovalbumin (OVA)-sensitized BALB/c mice were intratracheally challenged with OVA at 5 or 500 μg/animal 4 times. Infiltration of eosinophils into the lung, and development of airway remodeling and airway hyperresponsiveness (AHR) were evaluated 1 day after the 4th challenge. Dexamethasone and/or anti-IL-5 mAb was i.p. administered during the multiple challenges. AHR was evaluated by forced oscillation technique using FlexiVent. Infiltration of eosinophils into the lung, and the development of airway remodeling and AHR in the 5 μg OVA-induced model were significantly suppressed by dexamethasone, whereas those asthmatic responses to 500 μg OVA were not inhibited by dexamethasone. Under treatment with anti-IL-5 mAb, in which the eosinophil infiltration was strongly reduced, dexamethasone showed significant inhibition on the development of airway remodeling. However, the steroid resistance in AHR was not restored by the anti-IL-5 mAb. It was suggested that IL-5/eosinophils are involved in the acquisition of steroid resistance in the severe asthma.

Effects of LPS and TNFα on the histamine responsiveness of vascular endothelial cells

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Patients with chronic spontaneous urticarial (CSU) are often resistant to treatment with H1 antihistamines and have an increased response to intradermal injection of histamine. In this study, we evaluated the changes of responsiveness of vascular endothelial cells treated with Lipopolysaccharide (LPS) and Tumor necrosis factor (TNF)-α, known as exacerbating factors of CSU, to histamine using the impedance sensor, which can analyze the gap formation of cells in real time. Umbilical cord blood-derived vascular endothelial cells (HUVECs) were used as human vascular endothelial cells. HUVECs were cultured on the electrodes of sensor and the effects of LPS and TNFα treatment on the gap formation of cells in response to histamine were monitored. Moreover, the effects of H1 antihistamines on the gap formation of LPS- or TNF- treated HUVECs were examined. When LPS and TNFα were added simultaneously with histamine, the response of HUVECs to histamine did not increase. On the other hand, when LPS and TNFα were added a day before measurement of impedance, the responsiveness to histamine was increased and the response became difficult to be suppressed by H1 antihistamines. The increase of histamine responsiveness in vascular endothelial cells by LPS and TNFα may contribute to resistance to antihistamines in patients with CSU.

Effect of STAT inhibitor on the murine chronic graft-versus-host disease (GVHD) scleroderma model using an X-ray irradiation device

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Systemic sclerosis (SSc: scleroderma) is an autoimmune disorder characterized by progressive dermal fibrosis with diffusion to multiple organs which could be fatal. SSc could be initiated by anti-cancer therapy or graft-versus-host disease (GVHD) after bone marrow transplantation. However, the current treatment methods for SSc are only conservative. Therefore the novel agents which make fundamental treatment possible are waited. The murine chronic GVHD model induced by allogenic cell transplantation from B10.D2 mice (donner) to BALB/c mice (recipient) is known as one of the experimental scleroderma models. The aim of this study is to confirm the pathologic state of the chronic GVHD scleroderma model and evaluate the effects of STAT inhibitor. We validated the model by inspection of skin score, skin hydroxyproline (HYP) content and histopathologic examinations. The skin score and skin HYP content increased in all cell transplanted groups compared to control group. Thus, it was indicated that the SSc-like symptoms with dermal fibrosis developed on model animals. Effects of STAT inhibitor were evaluated on this model. The results indicate usefulness of the chronic GVHD scleroderma model in evaluating the anti-fibrosis effect of therapeutic agents, which leads to fundamental treatment of SSc.

Development of Retrievable Molecular Target Drug using Intelectin

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Intelectin-1 (ITLN) is a secretory protein that exists in blood, thoracis, and gut lumen. ITLN doesn't induce obvious physiologic and immunological reaction. Therefore, ITLN would induce no harmful effect when it injects into the body. In previous study, we found that ITLN specifically bound to 1,2-diol residues. The 1,2-diol residue is chemically stable, low-toxic, and is not bound with almost other proteins. Accordingly, we hoped that we could develop a retrievable molecular target drug by combining an ITLN-fused antibody and a blood purification device with a diol-coated resin.

In this study, we used ITLN-fused TNF receptor (TNFR-ITLN) instead of antibody because it doesn't need humanization. TNFR-ITLN is a soluble TNF receptor replacing Fc of etanercept with ITLN. TNFR-ITLN bound and neutralized to TNF-α as well as etanercept. In addition, TNFR-ITLN was specifically eliminated from the blood with a diol-silica gel column. On an apheresis model using LPS-treated rats (sepsis model), we measured the concentration of plasma TNF-α. When the TNFR-ITLN-injected LPS-treated rats were treated by apheresis using diol column, the transient increase of TNF-α was clearly suppressed in the blood. Furthermore, these rats were prevented from death by endotoxin shock.

According to these results, we considered that the apheresis using TNFR-ITLN plus diol column could selectively remove plasma TNF-α. A Fab-fused ITLN may specifically eliminate any pathogenic antigen from the body. This medical technology can applicable to various autoimmune diseases and it is expected to clinical application for a new blood purification method.

Effects of increased or decreased red blood cells on exercise performance in mice

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An increase in red blood cells (RBCs) is believed to improve exercise performance, because RBCs transport O_2 from the lungs to the tissues and deliver metabolically produced CO_2 to the lungs for expiration. In this study, we examined the effects of increased or decreased RBCs on exercise performance in mice. In order to vary the volume percentage of RBCs in blood (hematocrit levels), trained FVB mice were administered darbepoetin alfa (DPA), a long-acting erythropoiesis-stimulating agent, or phenylhydrazine (PHZ), a reagent inducing hemolytic anemia. The exercise performance was evaluated using a forced swimming pool. The administration of DPA or PHZ caused a significant increase or decrease in hematoctit levels, respectively. However, the partial improvement in exercise performance due to increased RBCs was observed only when higher intensity exercise was applied to mice whose hematocrit levels exceeded 70%. In addition, the decrease in exercise performance due to decreased RBCs was limited, even when the hematocrit levels was about 35%. These results suggested that the increase or decrease in RBCs had little effect on exercise performance in mice.

Change in expression of ubiquitin ligase in the hippocampus of stress-maladaptive mice

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Stress is thought to be a risk factor for psychiatric disorders, such as major depression. Previously, we reported that mice exposed to repeat excessive restraint stress showed emotional abnormality. Corticosterone (CT) elevated by repeated stress have been reported to increases the ubiquitination in the brain. The present study was designed to investigate the levels of expression of ubiquitin ligase proteins in the prefrontal cortex and hippocampus of stress-maladaptive mice and to examine whether CT alters the proteins expression levels in a mouse hippocampal HT22 cells.

Male ICR mice were chronically exposed to inadaptable stress, i.e. repeated restraint stress for 240 min/day for 14 days. After the final exposure to stress, brains of mice were rapidly removed and the hippocampus was dissected. HT22 cells were cultured in DMEM media supplemented with 10% FBS. Cell viability was measured by using MTT Assay.

Western blot analysis revealed that no change in the level of expression of E3 ubiquitin ligase Nedd4-2 was observed in among all mice. In contrast, a significant decrease in the expression level of phosphorylated Nedd4-2 (p-Nedd4-2) was observed in the hippocampus, but not the prefrontal cortex, of stressed mice. Although CT had no effect on cell survival at concentrations of less than 3 μM, pretreatment with 3 μM CT down-regulated p-Nedd4-2 in HT22 cells. These results suggested that CT is an important effector for activation of Nedd4-2 in hippocampal neurons, and Nedd4-2 plays a pivotal role in the emotional abnormality in stress-maladaptive mice.

SIRT1 activitors ameliorate the ability of cell membrane repair.

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Background: It is known that there is a function of cell survival such as oxidant stress resistance and NAD + dependent histone deacetylation by SIRT1 activitots. We previously reported that administration of activator of SIRT1, resveratorol (Rsv) ,ameliorates muscular pathophysiology of dystrophin-deficient mdx mice, and inhibition of SIRT1 suppresses membrane repair process of C2C12 myoblasts and myotubes. However, it remains uncrear whether cell membrane repair in muscle cells is accelerated by SIRT1 activators. The purpose of this study is to prove that SIRT1 activators improve cell membrane repair ability as a factor in cell survival.

Method: In the present study, we used C2C12 myoblasts and myotubes. After treating these with Rsv (50 μM) and nicotinamide mononucleotide (NMN) (5mM) (24h), we damaged the cell surface by local laser irradiation using a confocal microscope (Nikon A1). And membrane resealing process was monitored by an influx of fluorescent dye FM1-43 in cells surface after laser irradiation.

Result: We found that levels of fluorescence FM1-43 dye intake after laser-induced membrane destruction in C2C12 cells were significantly decreased in NMN (54.9 ± 18.9%) and Rsv (48.0 ± 20.4%) respectively.

Conclusion: Our data indicate a possibility that SIRT1 activators ameliorate muscular pathophysiology of muscular dystrophies through promoting plasma membrane repair.

1-P-099 Poster Sessions

The influence of sarcopenia on immune homeostasis

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Sarcopenia is defined as a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life and death, as a common negative consequence of aging. The present study is undertaken to investigate the correlation between muscle atrophy and aging-related immune deterioration by using a sciatic nerve dissected sarcopenic mouse model. The fraction of immunological cells, including T cells, B cells, macrophages, natural killer cells, and neutrophils, was observed. The systemic inflammatory responses were evaluated in lipopolysaccharide-induced sepsis animals. The T and B cell lineages were significantly suppressed in the sarcopenic model compared with control mice, and the fraction of macrophages and natural killer cells also tended to increase. The survival proportions in sarcopenic mice were significantly decreased while comparing to control mice. In conclusion, sarcopenia induces a functional impairment in immune cells. The mechanism of impaired immunological system would be assessed in our future planned study.

Antioxidative activity of dexmedetomidine as a direct free radical scavenger

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Purpose

Dexmedetomidine (DXM) is clinically used for sedation in perioperative patients. Previous studies reported that DXM is preventive against oxidative stress. Thus we hypothesized that DXM directly scavenges free radicals thereby acting as antioxidant.

Methods

Direct scavenging activity of DXM was evaluated against nine species of free radicals by electron spin resonance spectroscopy with the spin-trapping method. Fluorescence-based assays of cellular viability and intracellular free radical production were conducted using Alamar Blue and MitoROS 580.

Results

DXM significantly scavenged the following free radicals in dose-dependent manners; hydroxyl radical, superoxide anion, *t*-butoxyl radical, singlet oxygen and ascorbyl free radical. However, no scavenging activity was observed against *t*-butyl peroxyl radical, nitric oxide, DPPH and tyrosyl radical. Cellular viability of MRC-5 cells exposed to hydrogen peroxide was significantly improved in the presence of 0.1 μM DXM. 10 μM DXM significantly inhibited mitochondrial superoxide anion by Antimycin A. DXM showed no cytotoxicity up to 100 μM.

Conclusions

Although no effect was observed on nitrogen-centered radicals including nitric oxide, it was confirmed that DXM directly scavenges multiple oxygen-centered free radicals including hydroxyl radical (one of the strongest free radicals in living body) and superoxide anion (the most upstream of ischemia-reperfusion injury), which probably contributes to its antioxidative activity.

Activation of ribosomal p70 S6 kinase by selective serotonin $(5\text{-HT})_{2B}$ receptor agonist BW723C86 is mediated by epidermal growth factor/transforming growth factor-α receptor tyrosine kinase in primary cultures of adult rat hepatocytes.

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Serotonin (5-HT) can induce hepatocyte DNA synthesis and proliferation by autocrine secretion of transforming growth factor (TGF)-α through 5-HT_{2B} receptor/phospholipase C (PLC)/Ca^{2+}. In the present study, we investigated whether 5-HT or a selective 5-HT_{2B} receptor agonist BW723C86, would stimulate phosphorylation of TGF-α receptor tyrosine kinase (RTK) and ribosomal p70 S6 kinase (p70 S6K) in primary cultures of adult rat hepatocytes by using Western blotting analysis. Our results showed that 5-HT- or BW723C86-induced phosphorylation of EGF/TGF-α RTK peaked at between 5 and 10 min. On the other hand, 5-HT- or BW723C86 -induced phosphorylation of p70 S6K peaked at about 30 min. Furthermore, a selective 5-HT_{2B} receptor antagonist LY272015, a specific PLC inhibitor U-73122, a membrane-permeable Ca^{2+} chelator BAPTA/AM, an L-type Ca^{2+} channel blocker verapamil, somatostatin, and a specific p70 S6K inhibitor LY2584702 completely abolished the phosphorylation of p70 S6K induced by both 5-HT and BW723C86. These results indicate that phosphorylation of p70 S6K is dependent on the 5-HT_{2B}-receptor-mediated autocrine secretion of TGF-α. In addition, these results demonstrate that the hepatocyte proliferating action of 5-HT and BW723C86 are mediated by phosphorylation of p70 S6K, a downstream element of the EGF/TGF-α RTK signaling pathway.

Up-regulation of melatonin synthesizing enzymes in mast cells

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Recent investigations of human immunological regulation have suggested that mast cells may play an important role in maintaining homeostasis. Some of these studies have suggested that mast cell cytokines may play key roles in prevention of both viral and bacterial infections, and the development of tumors. The present study focused on the enzymes for melatonin synthesis in mast cells because of their rolls in immune response. mRNA expression from LAD2 cells, a human mast cell-derived cell line, was examined for aralkylamine N-acetyltransferase (AANAT) and hydroxyindole O-methyltransfase (HIOMT), key enzymes in melatonin synthesis. LAD2 were positive for mRNA expression of both enzymes. The mRNA levels were enhanced by stimulation with db-cAMP (500 μM) with no β-hexosaminidase (β-Hex) release; in contrast, A23187 (2 μM) did not enhanced mRNA levels but did induce β-Hex release. These results suggest that melatonin release from mast cells is involved in maintaining homeostasis, and is not involved in allergic responses.

The dual role of STIM1 *O*-GlcNAcylation on SOCE activity

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O-GlcNAcylation of the stromal interaction molecule 1 (STIM1) is known to impair store-operated Ca^{2+} entry (SOCE). Because it has not been identified the *O*-GlcNAcylation sites of STIM1, we examined whether the Serine/Threonine (Ser/Thr) residues (Ser-575, Ser-608, Ser-621, and Thr-626) of STIM1 S/P-rich domain and the adjacent region were *O*-GlcNAcylated or not using co-immunoprecipitation. The result showed that Ser-621 and Thr -626 residues were *O*-GlcNAcylated. To examine the role of the *O*-GlcNAcylation on SOCE activity, we established the STIM1 knockout HEK293 cells by the CRISPR/Cas9 system, and then, transfected T626A (Thr-626 substituted to Ala) to the cells. Surprisingly, the SOCE activity was reduced via reduced phosphorylation at Ser-621 residue in T626A transfected cells. It was also shown that the SOCE activity was reduced with the treatment of *O*-GlcNAcase inhibitor Thiamet G via reduced phosphorylation at Ser-621 residue in STIM1 wild-type transfected cells. These data may indicate that both decreased *O*-GlcNAcylation of STIM1 at Thr-626 and/or increased STIM1 *O*-GlcNAcylation at Ser-621 results in impaired SOCE activity. This is the first report showing the dual role of *O*-GlcNAcylation in regulating the SOCE activity of STIM1.

Regulation of epidermal growth factor receptor of cultured lung epithelial cells by interleukin-1β

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A549 cells are an immortalized alveolar epithelial cell line, which has been employed to investigate alveolar epithelial cell responses to several treatments. In the present study, we found that treatment of A549 cells with interleukin 1 beta (IL-1β) induced the activation of p38 mitogen-activated protein kinase (MAP kinase) and MAP kinase-activated protein kinase-2 (MAPKAPK-2), and the phosphorylation of epidermal growth factor receptor (EGFR) at serine 1047. The activation of MAPKAPK-2 and phosphorylation of EGFR were inhibited by SB203580, an inhibitor of p38 MAP kinase. In addition, MK2a inhibitor, an inhibitor of MAPKAPK-2, inhibited the phosphorylation of EGFR. Biotinylation of cell surface proteins indicated that IL-1β treatment induced the internalization of EGFR. Furthermore, the long-term treatment of A549 cells with IL-1β changed the cell morphology, with the loss of cell-cell contacts. In addition, IL-1β augmented the effects of transforming growth factor beta 1 on changes in the epithelial-mesenchymal transition. These results suggested that IL-1β regulates the functions of EGFR and induces morphological changes in alveolar epithelial cells.

PTH receptor-mediated G_s signaling is suppressed by direct binding of the subcortical cytoskeletal protein 4.1G to adenylyl cyclase type 6

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The G protein-coupled receptors (GPCRs) transduce their signaling through the activation of trimeric G proteins, but their associated mechanisms have remained unclear. It has been shown that the proteins that interact with carboxyl (C)-termini of GPCRs regulate the GPCRs-mediated signal transduction by modulating intracellular localization of the receptors. Parathyroid hormone (PTH)/PTH-related protein receptor (PTHR) is a G_s- and G_q-coupled GPCR. We previously showed that the C-terminus of PTHR directly binds to a subcortical cytoskeletal protein 4.1G. Cell surface expression of PTHR and its G_q/$[Ca^{2+}]_i$ signaling were increased by 4.1G, whereas its G_s/adenylyl cyclase (AC)/cyclic AMP (cAMP) signaling was reduced by 4.1G through unknown mechanisms. In the present study, we first found that AC type 6 (AC6) interacted with 4.1G in HEK293 cells and the N-terminus of AC6 (AC6-N) directly and selectively bound to the 4.1/ezrin/radixin/moesin (FERM) domain of 4.1G (4.1G-FERM) *in vitro*. Association of AC6-N with the plasma membrane was disturbed by the knockdown of 4.1G. Next, AC6-N was overexpressed to competitively inhibit the interaction of endogenous AC6 and 4.1G in the cells. Overexpression of AC6-N, as well as 4.1G-knockdown, augmented the cAMP production induced by forskolin, a direct AC activator, and PTH-(1-34). Taken together, our results demonstrate a model in which AC6-N associates with the plasma membrane through binding to 4.1G-FERM, resulting in low AC6 activity. The mechanism is responsible for the attenuation of PTHR-mediated G_s/AC6/cAMP signaling.

Role of NAD metabolism in maintaining intestinal homeostasis

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Nicotinamide adenine dinucleotide (NAD) is an essential cofactor associated with numerous redox reactions including energy production. NAD also serves as a substrate for ADP-ribosylation by poly(ADP-ribose) polymerase and protein deacetylation by sirtuins. Interventions using NAD precursors have been reported to have various beneficial effects on aging-associated diseases, such as obesity, diabetes, and Alzheimer disease. In mammals, NAD is synthesized from niacin (nicotinamide and nicotinic acid) and tryptophan. It is known that deficiency of niacin causes Pellagra featured by diarrhea, inflamed skin, and dementia. However, it is not fully understood why niacin deficiency causes Pellagra. Here we found that deficiency of NAD synthetase (NASD) caused the shortening of villi length in small intestine in mice. This photocopies the colitis observed in Pellagra patients. Furthermore, these mice are more sensitive to colitis induced by 5-fluorouracil treatment. These results suggest that NADS is involved in mechanism of Pellagra onset and is important for maintaining intestinal homeostasis.

Low body weight at weaning in V1a vasopressin receptor knockout mice

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Three receptor subtypes are known for neurohypophyseal hormone, Arginine vasopressin (V1aR, V1bR, and V2R). We previously reported the decrease of litter size and the delayed labor in V1aR knockout (KO) mice. In order to further elucidate the mechanism of the decreased litter size in V1aR KO mice, we counted the number of implantation sites at embryonic day 5.5. Unexpectedly, there was no difference in the number of implantation sites between WT and V1aR KO mice. This observation suggests that the pups' number decreases after the implantation. We also measured the body weight of pregnant mothers and newborns. Both WT and V1aR KO mothers showed the similar weight gain during pregnancy. The body weight of newborns at the parturition also showed no difference between WT and V1aR KO mice. However, at 3 weeks old, when pups were weaned from their mothers, the body weights of V1aR KO litters were significantly lower than those of WT. These results suggest that the pups of the V1aR KO mice show growth disorder at the time of the transition from juvenile to adult. Therefore, V1aR should be requisite for the normal growth.

Regulation of nutritional environmental responses and disease predisposition by osteocalcin in utero

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The Developmental Origin of Health and Disease (DOHaD) hypothesis, advocating long-term effect of fetal origins on adult disease, suggests that adverse environmental exposure during fetal and neonatal development might increase the susceptibility for developing a wide range of lifestyle-related diseases in later life. Indeed, our previous study demonstrated that maternal high-fat and high-sucrose feeding during gestational period deteriorated offspring's glucose and lipid parameters and triggered obesity in mice.

We recently clarified that osteocalcin, one of bone matrix proteins with placental transportability, is a mediator between glycolipid metabolism and bone metabolism through multiple mechanisms improving glycolipid metabolism. In this study, we aimed to investigate whether maternal osteocalcin administration during gestational period may ameliorate the offspring's metabolic statusthrough the function of changing the nutritional response *in utero* in mice, from an epigenetic perspective, because the biological mechanism underlying the DOHaD hypothesis is supposed to be mainly related to alteration in epigenetically regulated gene expression.

As a result, we revealed that maternal osteocalcin intake could avoid the ameliorable effects of gestational overnutrition on pups by regulating epigenomic nutritional responses.

The role of the novel sex steroid membrane receptor mPRε on metabolic homeostasis.

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Progesterone is a sex steroid hormone synthesized by the ovary, and plays a pivotal role for reproductive functions such as ovulation and the maintenance of pregnancy. Although these effects of progesterone have been implicated in nuclear progesterone receptors (PRs)-mediated classical signaling pathway, key pathways involved in non-classical progesterone signaling has been provided by the identification of membrane progesterone receptors (mPRα, mPRβ, mPRγ, mPRδ, and mPRε). Interestingly, mPRs may have been related to progesterone-mediated unknown rapid non-genomic action that cannot be currently explained by their genomic action through PRs. However, the structure, intracellular signaling, and physiological functions of these progesterone receptors are still unclear. In this study, we confirmed that mPRε, among mPRs receptors, is specifically expressed in the white adipose tissue (WAT), liver, and kidney of adult male and female mice. Furthermore, progesterone- mPRε signaling may contribute to suppression of glucose uptake and impair glucose tolerance in WAT. These findings provide new insights of regarding the non-genomic action of progesterone in metabolic homeostasis and novel therapeutic targets and strategies for metabolic disorder such as obesity and type 2 diabetes mellitus.

Novel Epac2 isoforms and their roles in pancreatic β-cell functions

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We have previously clarified the physiological roles of Epac2 (exchange protein directly activated by cAMP 2)-mediated signaling in insulin secretion induced by incretin and sulfonylureas. There have been three Epac2 isoforms identified to date: a full length Epac2A, Epac2B lacking N-terminus cAMP binding domain, and Epac2C, the shortest isoform predominantly expressed in the liver. To further investigate the cellular functions of Epac2 in pancreatic β-cell, we first intended to ablate both Epac2A and Epac2B in insulin-secreting MIN6-K8 cells by the CRISPR/Cas9 system (KO-1 cells). In the KO-1 cells, although protein expression of Epac2A was ablated, the expressions of multiple Epac2B-like isoforms were still detected. We then ablated all these isoforms and established the Epac2-null cell lines (KO-2 cells). While relatively weak activation of Rap1, a downstream molecule of Epac2, by cAMP was found in the KO-1 cells, no Rap1 activation was observed upon cAMP stimulation in the KO-2 cells. Interestingly, insulin secretion in response to Epac-selective cAMP analog and GLP-1, an incretin, was reduced in both KO-1 and KO-2 cells to the same extent, compared to the parental MIN6-K8 cells. These results indicate that Rap1 activation through newly identified Epac2B-like isoforms is not involved in insulin secretion, suggesting their roles mediating the cellular functions different from insulin secretion in pancreatic β-cells.

Tight regulation of the mitochondrial Ca^{2+} signal during glucose stimulation in pancreatic β-cells.

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Increases in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]c$) in pancreatic β-cells mediate glucose-stimulated insulin secretion. It has been suggested that increases in the mitochondrial Ca^{2+} concentration ($[Ca^{2+}]m$) are also involved in insulin secretion promoting mitochondrial ATP production. However, $[Ca^{2+}]m$ dynamics during glucose stimulation require further clarification. Using mitochondrial Ca^{2+} indicator CEPIA2*mt*, we here analyzed glucose-stimulated $[Ca^{2+}]m$ dynamics in a pancreatic β-cell line MIN6. During glucose stimulation, $[Ca^{2+}]c$ showed oscillatory changes with intervals of 2–3 min. $[Ca^{2+}]m$, on the other hand, showed very subtle and unexpected changes: it decreased with an increase in $[Ca^{2+}]c$ and increased with a decrease in $[Ca^{2+}]c$. However, upon shRNA-mediated knockdown of MICU1, a gatekeeper protein of mitochondrial calcium uniporter (MCU), $[Ca^{2+}]m$ increased in phase with $[Ca^{2+}]c$ oscillations having much greater amplitudes than those in control cells. Despite the remarkable increase in $[Ca^{2+}]m$ dynamics, glucose-stimulated $[Ca^{2+}]c$ dynamics remained almost the same. These results indicate that $[Ca^{2+}]m$ is tightly regulated by MICU1 during glucose stimulation, and that increases in $[Ca^{2+}]m$ above the baseline level seem not to be necessary for the generation of glucose-stimulated $[Ca^{2+}]c$ oscillations.

Functional effect of laminin α chains on endocrine cells in rat anterior pituitary gland

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Basement membrane proteins play important roles for cells as adherent extracellular scaffolds and signal transduction factors via integrins. Laminin, a major component of the basement membrane, is comprised of three subunits, α, β, and γ chains. Among these chains, only laminin α chain is capable of signaling via integrins. Previously, we studied components of the basement membrane in rat anterior pituitary gland, and found that laminin $\alpha 1$, $\alpha 3$, and $\alpha 5$ chains are located in the basement membrane on the parenchymal cell side, but not in the endothelial cell side. However, the effect of laminin on endocrine cells in the gland is not clear. In order to elucidate this issue, dispersed rat anterior pituitary cells were cultured on laminins containing $\alpha 1$, $\alpha 3$, or $\alpha 5$ chains as ligands. Cultured cells adhered to laminin containing $\alpha 3$ or $\alpha 5$, and the morphology of these cells changed to flat shape. Double-immunostaining showed that endocrine cells express integrin $\beta 1$ and $\alpha 3$. The antibody against integrin $\beta 1$ blocked the morphological change of the cells. Furthermore, we found that adhesion to laminins containing $\alpha 3$ or $\alpha 5$ induced growth hormone release from rat anterior pituitary cells. These findings show that laminin $\alpha 3$ and $\alpha 5$ play a functional role as a signaling molecule to regulate endocrine cells in rat anterior pituitary gland.

Anti-obesity effects of cuprizone on high fat diet-induced obese mice.

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It has been known that the increase of visceral fat is closely involved in abnormal glucose tolerance, hypertension, and hyperlipidemia, leading to the other diseases, such as atherosclerosis and type 2 diabetes. Recent reports have shown that serum copper concentration was increased in obese and/or diabetic patients. Here, we investigated the effects of copper chelator, cuprizone on high fat diet (HFD)-induced obesity in mice. We administered cuprizone (0.2%w) mixed in food pellets. Mice were divided in 4 groups, fed with (1) normal chow (NCD), (2) NCD with cuprizone (NCD+C), (3) HFD or (4) HFD with cuprizone (HFD+C) for 4 weeks, and then metabolic parameters were obtained. Serum copper levels were decreased in both cuprizone groups. Cuprizone significantly decreased the body weight in HFD+C without changes of food intake. Specifically in HFD+C, cuprizone decreased 60% of epididymal and inguinal fat weights, but did not change the weight of skeletal muscle, both soleus and gastrocnemius. Furthermore, we found that cuprizone ameliorated HFD-induced insulin resistance (ipGTT and ITT) with the modifications of gene expression pattern in liver, based on the comparison between NCD, HFD and HFD+C. These results suggest that cuprizone would be a candidate of leading compounds for anti-obesity agent.

The target of canola oil toxicity in SHRSP and mechanisms underlying the toxicity

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[Aim of the study] Canola oil (CAN) ingestion is known to shorten the life-span of male SHRSP and the life-shortening is preceded by a decrease in plasma testosterone and an increase in aldosterone. The present study was conducted to clarify the target of the toxicity. [Methods] Male SHRSP were given an AIN-93G diet containing 10w/w % soybean oil (Control) or CAN and tap water *ad libitum* for 8 weeks. At the 8th week the animals were sacrificed and plasma concentrations of testosterone, aldosterone, LH and FSH were measured using ELISA kits. Testes were isolated and histopathologically examined. Gene expressions for ACE and ACE2 in the lung, kidney and testis were measured. [Results and discussion] CAN-induced decrease in plasma testosterone and increase in aldosterone were confirmed. Both, plasma LH and FSH were similar between the Control and CAN groups. Leydig cell counts in the testis were comparable between the two groups. The ratios of gene expression for ACEs to that for ACE (ACE2/ACE) in both, the testis and the kidney of the CAN group were significantly lower than those in the Control group, while ACE2/ACE in the lung of the two groups were similar.These results demonstrate that CAN ingestion does not affect gonadotropin secretion from the pituitary while suppresses the testicular function without pathomorphological changes nor Leydig cell count. The decreased ACE2/ACE in the testis and the kidney of the CAN group may affect the local renin-angiotensin-aldosterone system (RAAS). In this presentation the relationship among testosterone, aldosterone and RAAS in the CAN toxicity is discussed.

Risk assessment of green tea EGCG for pharmacokinetic interaction with prescription drugs

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The pharmacokinetic and pharmacodynamic interactions between prescription drugs and epigallocatechin gallate (EGCG), a polyphenol and the main catechin in green tea. Many Japanese people consume green tea in beverages and foods. Functional foods that contain green tea or EGCG are claimed to reduce allergies and have inhibitory effects on lipid absorption. There are 5 prescription drugs with descriptions of possible interactions with green tea on the package inserts; however, none of them described specific interactions with EGCG. Studies have suggested the effects of EGCG on cytochrome P450 (CYP), organic anion transporting polypeptide (OATP), and biomolecules that affect the pharmacokinetics of various prescription drugs. In multiple databases in mid 2019, there are 34 drugs which showing OATP-mediated interactions with EGCG, and investigated interactions between some of these drugs and EGCG that caused inhibition of intracellular drug uptake in the literature mainly involving *in vitro* experiments, which increases the drug blood concentration and risk of associated side effects. The literature shows that the blood concentration of EGCG following green tea intake is at most 1 μM, and I could not find a manuscript on specific accumulation in tissues or organs. Based on the literature, since the pharmacokinetics of bortezomib, nadolol, warfarin, and many statins is affected by EGCG at concentrations close to the blood drug concentration range, there is a possibility that green tea consumption during drug administration could be restricted depending on evidence accumulation in the future.

Examination of left-right asymmetry in gustatory stimulus-induced brain activity in rat brain using fMRI

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The asymmetry between the hemispheres of the brain is particularly well known in humans. Language centers are unevenly distributed in the left hemisphere, and the right hemisphere is known to function primarily in spatial recognition. In addition, in animal species other than humans, reports on the asymmetry of neuronal structure due to changes in the expression level of glutamate receptor-expressing neurons in the mouse hippocampus, and the difference in symptom expression by behavioral experiments using unilateral hippocampal rats there is. However, since the dominant brain has traditionally been negative in rodents, it is not clear that there is a left-right difference in brain activity in rodents. In this study, we analyzed changes in rat brain activity during taste stimulation by sweeteners using functional MRI. However, the experimental results were asymmetric. Imaging device magnetic field inhomogeneities may be related to this asymmetry. Therefore, we tried to detect changes in brain activity in the same individual by changing the direction of the rat by 180 degrees during the MRI imaging experiment. As a result, in this experiment, it was shown that even if the direction of the rat is changed, there is no effect on the brain activity detection site, and there is a difference in the brain activity of the rat.

Coriandrum sativum leaf extract attenuates cytotoxicity induced by oxidative stress in PC12 cells

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Coriandrum sativum (CS) has been used as folk remedies for Ebers papyrus, an ancient medical record in Egypt over 3000 years. It has been reported that CS shows the effects of the antioxidant and anti-inflammatory. In this study, we examined the protective role of CS leaf extract (CSLE) against the cytotoxicity induced by hydrogen peroxide (H_2O_2) on neurite outgrowth of PC12 cells.

PC12 cells seeded onto 12-well plate (2×10^4cells/well) were cultured in DMEM medium containing FBS (-), which NGF (12.5 ng/mL) was also added at this time. After 24h, the cells were incubated for 3 days in serum free DMEM containing either CSLE (0.01 μg/mL, 0.1 μg/mL, 1 μg/mL, 10 μg/mL) or ascorbic acid (AA: 50 μg/mL) with H_2O_2. On day1 and 3, morphometric analysis of the neurites and length was performed by Neurocyte Image Analyzer software. In addition, the expression levels of neurofilament-L (NF-L) were measured by real-time RT-PCR.

NGF-induced neurite outgrowth action was significantly suppressed by H_2O_2, and significant improvement was observed in the CSLE (0.01 μg/mL, 0.1 μg/mL), dose-dependently, and AA. The result of real-time RT-PCR, NF-L level was significantly increased by adding of CSLE and AA compared to H_2O_2 group.

These results demonstrate that CSLE has cytoprotective action against hydrogen peroxide-induced cell damage as well as AA.

Japanese Nuru-Neba Diet extends Healthy Life Longevity (1): Reduction of Abdominal Fat in High Calorie-Diet Mice

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Recently, we proposed the Japanese traditional foods as the potential anti-obesity food materials based on our animal and human studies. In this study, we evaluated effects of the commercially available Japanese traditional Nuru-Neba diet (JNN): contained with root kelp, wakame, agar, white cloud ear, shiitake, nameko, okra, mekabu, cut tororo, shimeji, on the abdominal fat in the high fat mice.

5-week-old male ICR mice were divided as follows: high-fat diet group (HFD group), high-fat diet and JNN group (HFD + JNN 60 mg, HFD + JNN 180 mg and HDF + JNN 300 mg). Each mouse was reared individually and then allowed to free access to diet of 2.7 g per day for six weeks. At the end of the treatment period, the visceral fat was collected. The cholesterol concentrations were determined from plasma and the expression levels of leptin in visceral fat were measured by real-time RT-PCR.

The visceral fat was significantly and dose-dependently decreased in the HFD + JNN group compared to the HFD group. The cholesterol in plasma was significantly decreased in the HFD + JNN 300 mg group compared to the HFD group. The expression level of leptin was significantly suppressed in the HFD + JNN. These results indicate that JNN can be helpful in weight control along with maintaining high-mucopolysaccharide of intestinal mucosa.

Japanese Nuru-Neba Diet extends healthy life longevity (2): Evaluations of Mechanism of Anti-obesity effects using normal and High Calorie-Diet Mice

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In this study, we cleared the different effects of commercially available Japanese Nuru-Neba diet (JNN) on the visceral fat in normal and high fat diet mice considering about changes in adiponectin and leptin levels.
5-week-old male ICR strain mice were divided as follows: normal diet group (ND group), ND + JNN (0.35g) group, high-fat diet group (HFD group), HFD + JNN group. Each mouse was reared individually and then allowed to free access to diet of 3.5 g per day for eight weeks. At the end of the treatment period, the visceral fat was collected. The expression levels of adiponectin and leptin in visceral fat were measured by real-time RT-PCR.

In ND+JNN group not HFD+JNN group, the significant increases of adiponectin levels were seen. The changes in body weight in ND+JNN group were same as seen in those of ND group. In visceral fat leptin levels, any changes could not be seen in ND+JNN group. However, in HFD group, more than 500 times of leptin levels were detected compared to those of ND group. In HFD+JNN group, the abnormal leptin levels induced by high fat diet was significantly suppressed.
These results suggest that daily intake of NJJ activates adiponectin secretion under normal conditions and has an obesity preventive effect and that obesity is prevented by suppressing leptin resistance in the obese state.

Coriandrum sativum seed extract improves aging-induced memory impairment in SAMP8 mice

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The aging society leads to increase in diseases such as dementia. Alzheimer's disease (AD) is the most common form of dementia. It is known Coriandrum sativum (CS) impart an antioxidative effect. Therefore, it is hypothesized that CS can ameliorate the degenerative brain diseases by decreasing the oxidative stress caused by ageing. In this study, we examined whether CS seed extract (CSSE) could improve the memory impairment in SAMP8 mice or not.

10-week-old male SAMP8 mice were divided into two groups orally administrated with water (SAMP8(-)) or CSSE (SAMP8(+); 200mg/kg body weight/day). 10-week-old male ICR was used as normal control group also orally administrated with water.

The mean escape time of SAMP8(-) mice was significantly longer than that of ICR mice in Barnes maze test. However, SAMP8(+) mice showed the shorter mean escape time when compared with SAMP8(-) mice. The mRNA levels of neurofilament was significantly decreased in frontal lobe of SAMP8(-) mice, but significantly increased in SAMP8(+) mice. In addition, the mRNA levels of nNOS was significantly increased in in frontal lobe of SAMP8(-) mice, but significantly reduced in SAMP8(+) mice. It was indicated that continuous oral administration of CSSE for 12 weeks could ameliorate the aging-induced memory decline in the senescence-accelerated SAMP8 mice.

Deletion of PHD finger protein 24 (Phf24) causes elevated seizure sensitivity, emotional defects and cognitive impairment in rats.

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PHD finger protein 24 (Phf24), also known as Gαi-interacting protein (GINIP), was found to be absent in Noda epileptic rat (NER) (Behav. Genet., 47, 609, 2017). In this study, to explore the role of Phf24 in modulating CNS functions, we analyzed behavioral characteristics of Phf24-knockout (KO) rats, especially changes in seizure sensitivity, emotional responses and cognitive functions. Phf24-KO rats showed higher intensity and incidence of seizures induced by pentylenetetrazole (PTZ) and pilocarpine than F344 rats (control). Furthermore, PTZ-induced kindling was significantly facilitated in Phf24-KO rats. Anxiety-like behavior and cognitive function were analyzed by elevated plus maze and Morris water maze tests, respectively. Phf24-KO rats at old age exhibited reduced anxiety (impulsive) behaviors in the elevated plus-maze test compared with F344 (control) rats. In addition, Phf24-KO rats showed impaired learning behaviors in Morris water maze test. The memory retention ability was also disrupted in Phf24-KO rats. These results suggest that Phf24 negatively regulates epileptogenesis (seizure induction and development) and plays important roles in controlling emotion and cognition.

Molecular analyses of heart tissue in mice lacking mitochondrial protein p13

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p13 is mitochondrial protein highly expressed in heart tissue. Recently, our unbiased compound screen has shown that some cardiotonic drugs change p13 mRNA expression in vitro, suggesting p13 may play a role in cardiac function. To reveal the role of endogenous p13 in cardiac function, here, we investigated histological changes, mitochondrial complex 1 activity, and mRNA expression levels of mitochondria-related genes in the heart of p13 knockout (p13-KO) mice. Although no apparent abnormalities were observed in the weight and histology, complex 1 activity was significantly reduced in the p13-KO heart. In addition, mRNA expression levels of apoptosis-related genes, such as Bcl-xL, were significantly reduced in the p13-KO heart. These results suggest that endogenous p13 may be involved in energy metabolism and apoptosis in heart tissue.

Bleomycin-induced pulmonary fibrosis in cynomolgus monkeys

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【Purpose】

Idiopathic pulmonary fibrosis (IPF) is a progressive and intractable lung disease characterized by the proliferation of fibroblasts and loss of pulmonary function. Although many bleomycin-induced pulmonary fibrosis has been studied as IPF model in rodent, IPF model in nonhuman primates has not been reported. In this study, we investigate a cynomolgus monkey model of bleomycin-induced pulmonary fibrosis by IPF related biomarker and pathology.

【Methods】

Two cynomolgus monkeys were injected transtrachealy with bleomycin (2mg/kg) once a week for the first 2 weeks. The blood and bronchoalveolar lavage fluid (BALF) were collected at 0, 1, 4, 7, 9, 14, 21, 28 days after the first bleomycin injection, and cytokine levels were measured. On day 29, lung hydroxyproline content was measured. The formalin fixed lungs were stained with HE or Masson's trichrome for microscopic observation.

【Result and Discussion】

After bleomycin injection, BALF IL-1β levels were significantly increased on day 1 and returned almost normal level on day 4. The BALF MCP-1 levels were gradually increased and reached peak from day 4 to day 9. The BALF TGF-beta1 levels reached the maximum on days 7 or 9. The serum TGF-beta1 levels showed almost the same tendency as the BALF levels. The lung hydroxyproline contents of bleomycin injected monkeys were increased about 1.4 times more than normal. Histological examination showed a significant interstitial fibrosis with destruction of the alveolar architecture and was similar to IPF of human. This study provided new model using nonhuman primate for drug development in IPF.

Decrease the inhibitory synapse-related gene expression caused autism-like behavior in rats treated prenatal hypoxia condition

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Object : Prenatal hypoxic stress (e.g., threatened abortion) is thought to be a risk factor of neurodevelopmental disease, such as autism spectrum disorder. However, a critical target molecular which was altered by hypoxic stress in the brain and its detail molecular mechanism is still unclear. To elucidate these problems, we firstly addressed by generating hypoxia rat model.

Method: Pregnant F344 rats were exposed low O_2 condition by using hypoxic chamber for 24 hours. After postnatal day 50, autism-like behaviors in rats were carried out by using social interaction test and novel objective recognition (NOR) test. In gene expression analysis, neuro2a cells were exposed 1% O_2 condition and collected for RT-PCR.

Result : Hypoxia rats didn't show any abnormalities in general behaviors. In behavioral test, hypoxia rats showed decrease social interaction time compared to F344 rats reared at normal condition (control rats). In addition, hypoxia rats showed impairment of memory function in NOR test. Furthermore, RT-PCR results showed decrease the autism-related protein Mecp gene, inhibitory postsynaptic protein gephyrin gene and $GABA_A$ receptor $\beta 3$ subunit gene in neuro2a cells treated hypoxic condition.

Conclusion: these results indicated that the prenatal hypoxic stress might cause decrease inhibitory synapse-related genes, which destroy the excitatory/inhibitory synaptic balance and showed autism-like behavior in rats.

Fast and easy construction method of ryanodine receptor mutants aiming at genetic screening of malignant hyperthermia diagnosis

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Malignant hyperthermia (MH) is a potentially fatal pharmacogenetic disorder that manifests clinically as a hypermetabolic with skeletal muscle rigidity when MH-susceptible (MHS) individual is exposed to commonly used volatile anesthetics. At present, definitive diagnosis of MH before anesthesia requires the detection of the functional abnormality using biopsied muscle samples, a painful test for patients and requiring skillful diagnosticians. Recent reports showed that the frequency of MH episodes has increased though the MH crises are very rare case. The exact percentage of MHS is difficult to determine, but the prevalence of MH can be estimated up to 1:2750 due to the autosomal -dominant inheritance in humans. The noninvasive diagnosis such as the genetic screening of the MHS is asked for, because safer anesthesia is performed. Approximately 50 % of known cases of MH are caused by mutations in the gene locus of the ryanodine receptor type 1 (RyR1, calcium release channel) and the numbers of RyR1 mutation sites reported in the patients have been increased up to about 200, but only 48 have been formally shown to be causative; the remainder await confirmatory studies. So we have employed the fast and easy making procedures for these mutants to be expressed in stable cultured cells to be discussed in this paper.

Imaging of extracellular Ca^{2+} in the hippocampus by a novel CMOS ion image sensor

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Intracellular calcium ion (Ca^{2+}i) is one of the most important cation that controls several cellular functions, and thus there were already huge number of literature about Ca^{2+}i imaging in various cells. Although it is believed that Ca^{2+}i increase is accompanied by extracellular Ca^{2+}(Ca^{2+}o) decrease, the kinetics of Ca^{2+}o decrease remain unknown because of the limited imaging options. To overcome this limitation, we developed a Ca^{2+} image sensor (CIS) that is highly selective to Ca^{2+} but not to other cations within wide dynamic range (from 100 mM to 100 mM). We used CIS for the imaging of Ca^{2+}o in acute hippocampal slices. Stimulation with glutamate (Glu) decreased Ca^{2+}o to around 200 nM within 3 s, which returned to the baseline level (2 mM) with slow kinetics (5 min). Glu stimulates both neurons and glial cells to evoke Ca^{2+}i elevation, thereby reducing Ca^{2+}o. Thus, we tested NMDA to selectively stimulate NMDA receptor in neurons. NMDA mimicked Glu-evoked Ca^{2+}o decrease. Interestingly, Ca^{2+}o decrease was initiated at hippocampal CA1-2, before spreading along the pyramidal layers. Glutamate- and NMDA-evoked Ca^{2+}o decreases were inhibited by a NMDA receptor antagonist D-APV, suggesting involvement of neuronal NMDA receptors in decrease in Ca^{2+}o. So far, many scientists neglected Ca^{2+}o, but the CIS would tell us its importance for understanding brain functions.

Development of carbon nanotube MEA system enabling simultaneous measurement of neurotransmitter release and field potential

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Multi-electrode (MEA) assays using human induced pluripotent stem cell (hiPSC)-derived neurons are expected to predict the toxicity and the pharmacological effects. If we can measure the release of neurotransmitter using this MEA, it is possible to evaluate the drug related to release of neurotransmitters, and it is expected to improve the accuracy of medicinal effects. In this study, we aimed to develop the carbon nanotube (CNT) MEA chip, which enables in both electrochemical measurement of DA release and conventional field potential measurement.
The CNT-MEA chip was fabricated by electro-plating method. Detection sensitivity to dopamine (DA) in the fabricated CNT-MEA chip was examined by electrochemical measurement method. The change of DA release to methamphetamine (MTH) were measured using cultured human iPSC-derived dopamine neurons on CNT-MEA.
As a result of the electrochemical measurement, an oxidation peak current was observed at 0.25 V, and the detection limit and linearity of DA was less than 5 nM. We have succeeded in real time detection of DA release using human iPS cell derived DA neurons, and detected the changes in the amount of DA release depending on MTH dose. Furthermore, in the human iPSC-derived DA neuron, a change of spike pattern at MTH administration was detected by conventional field potential measurement. CNT-MEA is expected as a new MEA measurement method that improves the accuracy of toxicity prediction and the pharmacological effects.

Analysis of oxidative stress in immune cells

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Reactive oxygen species (ROS) is produced in immune cells during immune responses and is necessary for host defense and inflammation. Furthermore, ROS acts as signals for gene expression and is required for T cell proliferation and activation. While low levels of ROS play important roles in cell activation, high levels of ROS induce significant damage to cells. To monitor redox state in living cells we generated transgenic mice expressing a green fluorescent protein (roGFP) whose fluorescence varies with redox state (J Invest Dermatol. 34, 1701-1709, 2014). Since the redox state may change during *in vitro* analysis it should be necessary to fix the cells. Here we evaluate the fixation methods to analyze redox state *in vitro*. We compared aldehyde and organic solvent-based fixation methods. To fix redox state of roGFP protein N-ethylmaleimide which react thiol and modify cysteine residues in protein was used. Splenocytes were isolated from roGFP mice and treated with hydrogen peroxide (oxidized state) or DTT (reduced state) to induce the maximum oxidation and reduction status. Oxidized or reduced cells fixed with various fixation methods were accessed by flow cytometry. Organic solvents lead to a severe loss of fluorescence of roGFP protein. On the other hand, fixation with aldehyde and N-ethylmaleimide was useful to maintain fluorescence and redox status of roGFP. This system should be a powerful and convenient tool for analyzing redox state in various types of immune cells *in vitro*.

Novel ionic current measurement method and system for drug screening

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Ion channel proteins play important roles in controlling various cell functions by regulating the ion permeability of cell membranes in response to stimuli. Dysfunctions of ion channels cause severe diseases; therefore, ion channels are important drug targets. However, it is difficult to measure the detailed effects of drugs on ion channels efficiently, and drug discovery affecting ion channel proteins has been lacking as compared to that affecting other proteins such as enzymes. This study describes the development of a novel electrophysiological method that significantly increases the measurement efficiency for the ion channels. The method is based on the artificial lipid bilayer method. By contacting a gold electrode containing channels with a lipid-solution interface, the channels are incorporated into the membrane simultaneously with the formation of lipid bilayers. Using this method, ionic currents were detected in less than 1 minute; moreover, some channel properties could be measured at the single channel level. In addition, we developed an automated system based on this novel method. In this system, a driving device automatically moves the gold electrode, depending on the current detected. This automation could be the basis of a system that makes multiple measurements.

Development of photoswitchable protein that inhibits actin filament polymerization by near-infrared light irradiation

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Actin filaments in cells are essential for many cell functions such as cell migration, proliferation, and contraction. We wondered if we could regulate actin polymerization non-invasively, we could manipulate the mechanical force required for cell growth, motility, and cytoskeletal rearrangements. To this end, we noted the Rap1GAP protein. The Rap1GAP is known as GTPase-activating protein specific for Raps that are small monomeric GTP-binding proteins. The purpose of this study was to develop the genetically-encoded photoswitchable protein, which inhibited actin polymerization.

We used the near-infrared-responsive BphP1-QPAS1 optogenetic pair (1). The BphP1 was combined with plasma membrane translocating peptide, and the enzymatic domain of Rap1GAP was fused to QPAS1 and fluorescent protein (Rap1GAP-QPAS1). Then they were co-transfected to the HeLa cells. Firstly, the Rap1GAP-QPAS1 was localized at cytoplasm, whereas irradiation of 740 nm allowed Rap1GAP-QPAS1 to translocate to the plasma membrane. Moreover, the area of cells was becoming smaller than that of before irradiation. These results indicated that the enzymatic domain of Rap1GAP is translocated to the plasma membrane by irradiation of infrared, and inhibited the actin polymerization by suppressing the Rap1.

(1) Redchuk TA et al., Nat. Chem. Biol. 13, 633-639 (2017)

Less-invasive measurements using the small device, nano tag®, for locomotor activity, body temperature and gastrointestinal motility in cynomolgus monkeys or beagle dogs

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Evaluations of locomotor activity, body temperature and gastrointestinal motility in monkeys or dogs are useful to understand effects of candidate drugs on the central nervous and gastrointestinal systems. Here we describe less-invasive evaluation methods using the small device, nano tag® (15×14×7 mm).

Nano tag was subcutaneously implanted in cynomolgus monkeys. Gelatin capsule containing nano tag was orally administered in beagle dogs. Then body temperature and the amount of locomotor activity were simultaneously and continuously measured by nano tag and a telemetry system (PONEMAH system). The measured profiles obtained by nano tag approximately corresponded with those by the telemetry system, suggesting data obtained by nano tag are comparable to telemetry data. Moreover, nano tag could detect drug-induced changes of locomotor activity and body temperature in animals treated with caffeine, ketamine or thiopental. As to gastrointestinal motility, gastrointestinal residence time of nano tag was evaluated in dogs. The gastrointestinal residence time became shortened and extended by treatment with pilocarpine and loperamide, respectively. The proposed less-invasive methods using nano tag could help to evaluate effects of drugs on the central nervous and gastrointestinal systems in monkeys and dogs.

Machine learning-based quality control for contraction of cultured human-induced pluripotent stem cell-derived cardiomyocytes

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Human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are expected to take place of animal models for the assessment of drug-induced cardiotoxicity because of no possible prediction errors arising from species differences. However, currently, the qualities of hiPSC-CMs are inconsistent among product lots and must be controlled by well-trained experimenters. This labor-intensive process prevents high throughput screening. To tackle this problem, we developed an automated method for controlling the qualities of the contraction of cultured hiPSC-CMs using machine learning.

After 5–7 days of culture of hiPSC-CMs, a total of 556 bright-field videos of the hiPSC-CMs were obtained. The contractile qualities of the hiPSC-CMs were inspected by four well-trained experimenters and were labelled as either 'normal' (n = 366 videos) or 'abnormal' (n = 190 videos). The contractile properties of hiPSC-CMs were measured using the absolute changes in the pixel intensity between video frames, and these dimensions were reduced to 2 using uniform manifold approximation and projection (UMAP). We then trained the support vector machine (SVM) algorithm to classify normal or abnormal hiPSC-CMs. We found that fast Fourier transformation and data augmentation improved the classification scores of SVM. In summary, we demonstrated that the machine learning approach is applicable to the control of contractile qualities of hiPSC-CMs.

Rapid measurement of plasma concentration of a vancomycin with diamond sensor.

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Vancomycin is a glycopeptide antibiotic that kills bacteria by blocking the construction of the cell wall and used to treat different bacterial diseases including meningitis and methicillin - resistant *Staphylococcus aureus* infections. Because this antibiotic can sometimes induce renal failure and hearing loss, the plasma concentration is monitored to adjust the dose applied to individual patients. In this study, we show a rapid and simple procedure with an electrochemical approach. The sensor we used consisted of a boron-doped diamond electrode, which elicits more stable reaction than classical materials such as carbon and gold. With this sensor we examined guinea-pig plasma containing vancomycin at different concentrations. The procedure we developed allowed us to complete a series of measurement in 35 sec. Time necessary for all the processes including a sample's pretreatment did not exceed 10 min. The sensor detected the drug concentration of 1 to 50 μM, which falls into the range of the therapeutic window. Moreover, we found that the sensor was repeatedly usable for the measurement with minimal impairment of the sensitivity. The methodology described here may contribute to not only advances in personalized medicine but also reduction of the cost for therapeutic drug monitoring.

Development of a novel chemical tag tool for calcium imaging by near-infrared fluorescence

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Ca^{2+} plays important roles as a second messenger in a wide range of biological phenomena including neurotransmission. Near infrared (NIR) fluorescence is suitable for *in vivo* Ca^{2+} imaging because of its high tissue penetration, low light scattering, and minimal autofluorescence. Many types of NIR chemical probes have been developed but they lack selectivity for labeling of particular cell types upon multi-cell bolus loading. We developed DeQODE chemical tag system as a new chemical biology tool, in which a small-molecular QODE probe can visualize Ca^{2+} signals with NIR fluorescence selectively inside the target cells expressing DeQODE tag. In an application of our DeQODE tag system to primary cultures of rat hippocampal neurons, neurons expressing DeQODE tag were selectively labeled by QODE probe. We successfully visualized Ca^{2+} signals of the target neurons in response to electrical stimulation at 10 Hz. We will perform *ex vivo* and *in vivo* application of our chemical tag system.

Influence on decoding accuracy of EEG in denoised EMG using machine learning

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Recently, decoding that predicts behavior from an electroencephalogram(EEG) has been many reported, and decoding an electromyogram(ECG) from an EEG is one of them. However, there are many waveforms in the brain such as noise from experiment environment, and the EEG signal is not only directly related to the EMG but also includes the waveform reflecting another role. Filtering of Wavelength has been mainly used as a method for classifying such as EEG, but in recent years, a denoised method using deep learning has also been used for waveform analysis. The purpose of this study is to evaluate decoding accuracy of denoised EMG using deep learning and to classify the ratio of EEG reflected in muscle activity and EEG reflected in different activity.

We recorded primary motor cortex(M1) EEG and 4 or 5 regions of EMG in the rat brain. Next, the denoising EMG using Latent Factor Analysis via Dynamical Systems(LFADS), a deep learning method reported using EMG analysis. After denoising, EMG and EEG were coded each other to evaluate their accuracy.

Denoised EMG using deep learning showed higher decoding accuracy than just filtering. Moreover, the reflection ratio of EEG to electromyogram was evaluated by mutual decoding of EMG and EEG. This study suggested that the usefulness of noise removal by machine learning and the EEG classification can be made more accurate.