1-B-P-001 一般演題（ポスター）

Guaiazulene, a bicyclic sesquiterpene, disrupts the TGF-β pathway and suppresses cell migration

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Guaiazulene is a plant-derived bicyclic sesquiterpene widely used as a low-toxicity anti-inflammatory agent. However, the other bioactivities and mechanisms of action of guaiazulene were not well known. Guaiazulene inhibited colony formation of the alveolar basal epithelial adenocarcinoma cell line A549 in a soft agar medium. This suggested that guaiazulene has anticancer activity. In addition, cell migration activity was evaluated using A549 and the poorly differentiated squamous cell carcinoma cell line SAS, and the results showed that guaiazulene inhibited TGF-β-induced cell migration activity. These results suggest that guaiazulene inhibits the TGF-β pathway. TGF-β induced the formation of stress fibers during cell migration, and guaiazulene inhibited stress fiber formation. Guaiazulene also inhibited phosphorylation of Focal adhesion kinase (FAK), activated during cell migration by TGF-β. These results suggest that guaiazulene inhibits cell migration by disrupting the stress fiber formation mechanism. Furthermore, TGF-β-induced changes in the expression of epithelial and mesenchymal markers were also partially inhibited by guaiazulene. These results suggest that guaiazulene may have anticancer activity and inhibit metastatic potential.

1-B-P-002 一般演題（ポスター）

Analysis of the mechanism of salivary gland self-recovery *via* oral sensory stimulation

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Parotid glands (PGs) are atrophied markedly by long-term feeding of liquid diets. These atrophied PGs recovered to normal size with a solid diet in few days. To investigate the mechanism of this PG recovery, we compared PG weight and acetylcholine-induced salivary secretion in three groups of Wistar rats: 1) a group fed a solid diet for 17 days (control group), 2) a group fed a liquid diet for 17 days (atrophic group), and 3) a group fed a liquid diet for 14 days followed by the solid diet for 3 days (recovery group). The PG weight and salivary secretion in the atrophic group were decreased to 35 and 58% of the control group, respectively, whereas in the recovery group, they recovered to 84 and 120% of the control group, respectively. We thought that oral sensory stimulation by solid diet recovers salivary gland functions *via* the autonomic nervous system, and thus we examined the effects of pilocarpine (Pilo), isoprenaline (ISO), and nicotine (Nic) on the recovery of atrophic glands. Subcutaneous administrations of these reagents during the last 3 days of liquid diet feeding recovered PG, and PG weight of Pilo-, ISO-, and Nic-treated group was 78, 121, and 78% of the control group, respectively. These results indicate the involvement of autonomic neurotransmitters in the self-recovery mechanism by oral sensory stimulation.

1-B-P-003 一般演題（ポスター）

Histidine-rich glycoprotein possesses pleiotropic functions that contribute to the maintenance of neutrophil homeostasis

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Histidine-rich glycoprotein (HRG) is a plasma glycoprotein present in blood at a concentration of approximately 1 μM. It is known to have various physiological activities related to the homeostasis of the coagulation-fibrinolytic system, the immune system, and the blood vascular system, and until now, its effects on monocytes, lymphocytes, and platelets have been reported, but its effects on neutrophils have not been reported. Therefore, we investigated the effects of HRG on neutrophils. In the presence of HRG, neutrophils maintain a regular spherical morphology and smooth cell surface without microvilli, and F-actin polymerizes just beneath the cell membrane, giving them moderate elasticity. However, in an environment with low or no levels of HRG, such as sepsis, we found that neutrophils show reduced cellular elasticity, an amoeba-like morphology, increased cell surface microvilli, and activation of adhesion molecules such as CD 11 b, CD 62 L, and CD 162, leading to increased adhesion and frictional resistance to blood vessels. Decreased HRG also affects neutrophil functions, resulting in increased the release of NETs and ROS. In addition, the reduction markedly decreased their migration to bacteria and phagocytosis. These results indicate that HRG is a critical important protein for maintaining neutrophil homeostasis.

1-B-P-004 一般演題（ポスター）

Individual resolvin E family members work distinctly and in a coordinated manner in the resolution of inflammation

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Three main E-type resolvins (RvEs): RvE1, RvE2, and RvE3, have roles in the resolution of inflammation as anti-inflammatory activities. To investigate the roles of each RvE in the resolution of inflammation, timing of interleukin (IL)-10 release and IL-10 receptor expressions, and phagocytosis evoked by each RvE in differentiated human monocytes, macrophage-like U937 cells were examined. Here, we show that RvEs enhance the expression of IL-10, and IL-10 receptor-mediated signaling pathways and IL-10-mediated-signaling-independent resolution of inflammatory effects by activating the phagocytotic function. Thus, RvE2 mainly evoked an IL-10- mediated anti-inflammatory function, whereas RvE3 principally activated phagocytotic activity of macrophages, which may be involved in tissue repair. On the other hand, RvE1 showed both functions, although not prominent but rather acting as a relief mediator that takes over the RvE2 function and passes over to the RvE3 function. Therefore, each RvE may act as an important role/stage-specific mediator in a coordinated manner with other RvEs in the processes of the resolution of inflammation.

1-B-P-006 一般演題（ポスター）

The involvement of down-regulation of CYP3A4 in the $K_{Ca}1.1$ inhibition-induced overcoming of resistance to doxorubicin in cancer spheroid models

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The large-conductance Ca^{2+}-activated K^+ channel, $K_{Ca}1.1$, plays a pivotal role in cancer progression, metastasis, and the acquisition of chemoresistance. Previous studies indicated that the pharmacological inhibition of $K_{Ca}1.1$ overcame resistance to doxorubicin (DOX) by down-regulating multidrug resistance-associated proteins in the three-dimensional spheroid models of human prostate cancer LNCaP, osteosarcoma MG-63, and chondrosarcoma SW-1353 cells. Investigations have recently focused on the critical roles of intratumoral, drug-metabolizing cytochromes P450 enzymes (CYPs) in chemoresistance. In the present study, we examined the involvement of CYPs in the acquisition of DOX resistance and its overcoming by inhibiting $K_{Ca}1.1$ in cancer spheroid models. Among the candidates of CYP isoforms involved in DOX metabolism, CYP3A4 was up-regulated by spheroid formation and significantly suppressed by the inhibition of $K_{Ca}1.1$ through the transcriptional repression of CCAAT/enhancer-binding proteins, which are downstream of the Akt-Nrf2 signaling pathway. DOX resistance was overcome by the siRNA-mediated and pharmacological inhibition of CYP3A4 in cancer spheroid models. Collectively, the present results indicate that the up-regulation of CYP3A4 is responsible for the acquisition of DOX resistance in cancer spheroid models, and the inhibition of $K_{Ca}1.1$ overcame DOX resistance by repressing CYP3A4 transcription through the Akt-Nrf2-CEBP signaling pathway.

1-B-P-007 一般演題（ポスター）

Effects of the accumulation of hypoxia-inducible factors on the expression of L-type amino acid transporter LAT1 in colorectal cancer cells

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L-type amino acid transporter 1 (LAT1; SLC7A5) is upregulated in various types of cancer and is often associated with poor prognosis of patients, indicating its pathological significance in disease progression and malignancy. However, our understanding of the mechanisms responsible for such aberrant LAT1 expression in cancer is limited. A previous study reported that LAT1 expression is regulated by hypoxia-inducible factor-2α (HIF-2α) in von-Hippel Lindau (VHL)-deficient renal cell carcinoma cells, which accumulate HIF-2α due to the lack of VHL-dependent proteasomal degradation pathway. It has been thus hypothesized that HIF-2α accumulated under the intratumoral hypoxia may generally contribute to inducing LAT1 expression. In the present study, we assessed this possibility in colorectal cancer (CRC) cells. Experimental hypoxic treatment caused the accumulation of HIF-1α and HIF-2α in CRC cell lines *in vitro*. Expression of GLUT1 (SLC2A1), a well-known HIFs target, was drastically enhanced in a HIFs-dependent manner. However, LAT1 expression was unresponsive to the accumulation of HIFs under the tested conditions. These results indicate that LAT1 expression is not controlled by HIFs in CRC cells, questioning the expected general roles of the HIFs-mediated hypoxic response in the broad pathological increase of LAT1 in cancer cells.

1-B-P-008 一般演題（ポスター）

Functional coupling with endogenous monocarboxylic acid transporter MCT1 influences on transport function of exogenously expressed organic anion transporter OAT10 in HEK293 cells: a warning on transporter assays

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Organic anion transporter 10 (OAT10, SLC22A13) is abundantly expressed in renal tubules and mediates the transport of organic anions, including nicotinate, β-hydroxybutyrate, *p*-aminohippurate, and orotate. *In vitro* transport assays using *Xenopus* oocytes and HEK293 cells revealed that the apparent substrate selectivity of OAT10 is distinct between the two expression systems, with particularly lower uptake of β-hydroxybutyrate in HEK293 cells. By means of co-immunoprecipitation followed by LC-MS/MS-based proteomic analysis, we found monocarboxylate transporter 1 (MCT1, SLC16A1) as an endogenous transporter physically interacting with OAT10 in HEK293 cells. The uptake of β-hydroxybutyrate and nicotinate, common substrates of OAT10 and MCT1, was increased by the knockdown of MCT1 in OAT10-expressing HEK293 cells, whereas the uptake of orotate, a substrate only for OAT10, was unaffected. These results suggest that MCT1 mediates the efflux of β-hydroxybutyrate and nicotinate that were taken up by adjacently located OAT10 in HEK293 cells, resulting in distinct apparent substrate selectivity of OAT10 from that in *Xenopus* oocytes. Our findings provide a general warning that unexpected interactions with endogenous transporters in specific expression systems may interfere with assessing the properties of transporters.

1-B-P-009 一般演題（ポスター）

Regulation of Cytoprotective Function by Rab protein

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PRAF3 (alias: GTRAP3-18, ARL6IP5, HSPC127, DERP11, JWA, addicsin, hp22, jmx, Yip6b), belonging to prenylated Rab acceptor 1 (PRA1) superfamily, is highly conserved amongst vertebrates. PRAF3 plays crucial roles in membrane traffic as a GDI displacement factor *via* protein interaction with a variety of Rab proteins, as well as in the modulation of antioxidant glutathione through its interaction with the amino acid transporter EAAC1. It is known that the overexpression of PRAF3 induces the toxicity of the host cell, however, the factors capable of reducing the cytotoxicity remained unknown. My findings demonstrate that Rab1a can protect from the cytotoxicity of PRAF3-overexpressed cells. Cytoprotective effects of Rab1a protein could further suggest that PRAF3 and Rab1a are closely related to each other physiologically. I hope that the findings will contribute to the future research on membrane trafficking and neurodegenerative diseases.

1-B-P-010 一般演題（ポスター）

Fibroblast growth factor 23 (FGF23) contributes to regulation of hepcidin/ferroportin axis

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Fibroblast growth factor 23 (FGF23) is a recently discovered regulator of phosphate and mineral metabolism and has been associated with both the progression of chronic kidney disease (CKD) and mortality in dialysis patients. Serum levels of FGF23 are extremely elevated, as high as > 1 ng/mL in patients with hemodialysis (HD) but the mechanisms are poorly understood. Here, to confirm the direct implication of FGF23 in iron homeostasis, we examined the mRNA expression of hepcidin, ferroportin, and hypoxia Inducible Factors (HIFs) in HepG2 cells. Serum treatment of patients with HD resulted in an up-regulation of hepcidin expression and a down-regulation of ferroportin in HepG2 cells. A significant down-regulation of ferroportin and HIFs was observed in HepG2 cells after FGF23 treatment. Conversely, hepcidin was regulated by FGF23 in a dose- and time-dependent manner. Low concentrations of FGF23 increased hepcidin expression, and high concentrations of FGF23 revealed a change-over to down-regulation of hepcidin expression. Both hepcidin and ferroportin are well known to be the main regulators in iron homeostasis. This study, thus, demonstrated that the FGF23 implicated directly in iron homeostasis as a clinical concentrate iron is compatible with serum levels in long-term patients with HD.

1-B-P-011 一般演題（ポスター）

Role of Trichoplein-mediated regulation of primary cilia dynamics in tissue regeneration

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Primary cilia are immobile structures that extend from the surface of various cells, and it is known that they are involved in signal transduction from the extracellular environment. Signal transduction through primary cilia is associated with various intracellular processes, and it has been reported to function in tissue regeneration; however, the detailed mechanism remains unclear. Trichoplein (Tchp) is localized in the basal bodies of primary cilia and functions as a suppressor of primary cilia formation through the activation of Aurora A kinase. In this study, we created Tchp knockout zebrafish to investigate the role of Tchp in tissue regeneration. We performed a fin injury regeneration experiment. We found that the Tchp-knockout zebrafish showed a higher regenerative ability than the wild type. Furthermore, we conducted a quantitative proteome analysis using regenerated zebrafish fins to identify novel genes involved in Tchp-mediated tissue regeneration. We identified 8,756 proteins, of which the expression of 186 proteins was found to be significantly altered in the Tchp knockout zebrafish. Additionally, among these variable proteins, we evaluated the functions of some genes potentially involved in tissue regeneration. We will present the results of the evaluation.

1-B-P-012 一般演題（ポスター）

Development of live-cell super-resolution imaging technique using fluorescence-renewable molecular labeling

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Super-resolution microscopy has greatly enhanced our understanding of nano-scale molecular complexes, such as in the central nervous and immune systems. However, rapid photobleaching of fluorescent dye has hampered live-cell time-lapse super-resolution imaging for long-term nano-scale observations. In this study, we developed the "De-Quenching of Organic Dye Emission" (DeQODE) system to analyze nano-scale molecular distribution dynamics precisely. DeQODE system is a renewable fluorescence labeling technique designed to overcome photobleaching. It comprises an organic probe with a quencher moiety and a fluorescence dye moiety (QODE probe), and an intracellularly expressible single-chain antibody against the quencher (DeQODE tag). The reversible binding between the QODE probe and the DeQODE tag allows fluorogenic molecular labeling to maintain fluorescence signals during imaging experiments.

Using the DeQODE system, we achieved time-lapse super-resolution imaging by stimulated emission depletion (STED) microscopy, stochastic optical reconstruction microscopy (STORM), and super-resolution radial fluctuations (SRRF) method in cultured cells which express DeQODE tag-fused proteins. Additionally, at low QODE probe concentrations, we successfully performed single-particle tracking of labeled proteins sequentially labeled with fluorescence, obtaining trajectory data for more than 10 minutes. Our results suggest that the DeQODE system is promising for analyzing cellular functions, focusing on the nano-scale molecular distribution.

1-B-P-013 一般演題（ポスター）

Effects of *S*-allyl-L-cysteine on binding to growth hormone receptors in primary cultures of adult rat hepatocytes.

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We previously reported that *S*-allyl-L-cysteine (SAC)-induced cell proliferation was involved in intracellular insulin-like growth factor (IGF)-I secretion via the Janus kinase 2 (JAK2) / phospholipase C (PLC) pathway in primary cultures of adult rat hepatocytes. Furthermore, we demonstrated that growth hormone (GH) stimulates the GH receptors in cultured hepatocytes to promote IGF-I secretion through the JAK2/PLC pathway. In this study, we investigated whether SAC binds to GH receptors by examining the affinity between the anti-GH receptor monoclonal antibody (anti-GHR mAb) and GH receptors in the presence of SAC or GH using a GH receptor immunofluorescence technique (GH receptor imaging). The GHR in hepatocytes emitted a fluorescent signal when labeled with a fluorescent dye-coupled anti-GHR mAb. Interestingly, SAC-treated fluorescent signals tended to decrease compared to the absence of SAC, and this effect was dependent on SAC doses. A similar trend was observed in GH treatment. In contrast, S-methyl-L-cysteine, which is a structural analog of SAC and has no hepatocyte proliferation capacity, did not show any decrease in fluorescence intensity. These results indicate that SAC shares the same binding site as GH for the GH receptor. In other words, SAC induces JAK2 phosphorylation by binding to GH receptors expressed on the hepatocyte membrane.

1-B-P-014 一般演題（ポスター）

Upregulation of NR4A1 counteracts cyclic mechanical stretch-induced cell death in rat aorta smooth muscle cells via p38 signaling pathway.

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Cyclic mechanical stretch (CMS) causes vascular smooth muscle cell (VSMC) proliferation, cell death, and migration, resulting in vascular remodeling and subsequent vascular failure during hypertension. However, the effect of CMS on gene induction in cardiovascular disease remains to be determined. Previously, we demonstrated that CMS caused cell death in rat aorta smooth muscle cells (RASMCs) in a JNK- and p38-dependent manner. To investigate the role of CMS in initiating cell death signaling and MAPK-related events, we used cDNA microarrays to examine the transcript profiles of CMS-induced RASMCs and discovered that the transcripts of 29 differentially expressed genes, including NR4A1, were significantly increased in response to CMS. Quantitative polymerase chain reaction (qPCR) analysis demonstrated that this increase of NR4A1 was p38-dependent. Moreover, we studied the function of NR4A1 in the early response of RASMCs to CMS. An inhibitor (CDIM8) of NR4A1 strongly increased CMS-induced cell death in vitro. We also examined NR4A1 expression in arteries using an abdominal aortic constriction (AAC) mouse model. We discovered that hypertension enhanced NR4A1 expression at both the mRNA and protein levels in arteries treated to AAC compared to sham-operated arteries using qPCR analysis and immunofluorescence staining. Taken together, our data provide the first evidence that NR4A1 protects RASMCs from CMS-stimulated cell death via the p38 signaling pathway.

1-B-P-015 一般演題（ポスター）

The characterization of *mouse* TRPM2 isoforms, and their effects on full-length *mouse* TRPM2.

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Transient receptor potential melastatin 2 (TRPM2) assembles into tetramers to function as an oxidative stress-sensitive Ca^{2+} channel at the surface membrane. Limited information is currently available on the 10 protein isoforms of *mouse* TRPM2 (*m*TRPM2) identified to date. The present study investigated whether these isoforms function as oxidative stress-sensitive Ca^{2+} channels and their effects on full-length *m*TRPM2 activity using the HEK 293 cell exogenous expression system. Only full-length *m*TRPM2, isoform 1 localized to the surface membrane and was activated by oxidative stress. Isoform 7 was clearly recognized by protein quality control systems and degraded by ER-associated degradation (ERAD) after transmembrane proteolysis. In the co-expression system, the activation and expression of full-length *m*TRPM2 were attenuated by its co-expression with isoform 7, but not the other isoforms. This decrease in the expression of full-length *m*TRPM2 was recovered by the proteasomal inhibitor, MG132. The present results suggest that isoforms other than isoform 1 did not function as oxidative stress-sensitive channels and also that only isoform 7 attenuated the activation of full-length *m*TRPM2 by targeting it to ERAD. The present study will provide important information on the functional nature of *m*TRPM2 isoforms for elucidation of their roles in physiological and patho-physiological responses *in vivo* using mouse models.

1-B-P-016 一般演題（ポスター）

15-Keto-PGE_2 acts as a biased/partial agonist to terminate PGE_2-evoked signaling

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E type prostanoid (EP) receptors are cognates for prostaglandin E_2 that have four main subtypes: EP1 to EP4. Of these, the EP2 and EP4 prostanoid receptors have been shown to couple to Gαs-protein and can activate adenylyl cyclase to form cAMP. Studies suggest that EP4 receptors are involved in colorectal homeostasis and cancer development, but further work is needed to identify the roles of EP2 receptors in these functions. After sufficient inflammation has been evoked by PGE_2, it is metabolized to 15-Keto-PGE2. Thus, 15-Keto-PGE_2 has long been considered an inactive metabolite of PGE_2. However, it may have an additional role as a biased and/or partial agonist capable of taking over the actions of PGE_2 to gradually terminate reactions. Here, using cell-based experiments and in silico simulations, we show that PGE_2-activated EP4 receptor–mediated signaling may evoke the primary initiating reaction of the cells, which would take over the 15-Keto-PGE_2–activated EP2 receptor–mediated signaling after PGE_2 is metabolized to 15-Keto-PGE_2. Additionally, we show that in the absence of phosphodiesterase inhibitor pretreatment, prostaglandin E_2 causes accumulation of cAMP in EP2 receptors, whereas markedly low levels of cAMP accumulated in EP4 receptors. By applying the Black/Leff operational model calculation, we found that EP2 receptors have a biased ability to intrinsically activate the Gαs-protein-mediated pathway, whereas EP4 receptors have strong biased activity for the Gαi-protein-mediated pathway. The present results shed light on new aspects of 15-Keto-PGE_2, which may have important roles in passing on activities to EP2 receptors from PGE_2-stimulated EP4 receptors as a “switched agonist.”

1-B-P-017 一般演題（ポスター）

Exploration of preventive drugs for cisplatin-induced hearing loss

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Cisplatin (CDDP) is a typical drug that causes drug-induced hearing loss. CDDP is taken up into the inner ear cells via the organic cation transporter (OCT)2 and is thought to be cytotoxic, but the details are unknown. In this study, we searched for preventive drugs against CDDP-induced hearing loss using medical data and evaluated the efficacy of the preventive drugs found. Imbalance analysis was performed using data from FAERS, the U.S. Food and Drug Administration (FDA) Adverse Event Reporting System, and VigiBase, the World Health Organization (WHO) adverse event reporting database, to identify candidate preventive drugs based on the reported odds ratio (ROR) and 95% confidence interval (CI) of "hearing impairment" with CDDP use. Based on the analysis of both databases, the 5-HT_3 receptor antagonist palonosetron was selected as a candidate for preventive drugs for CDDP-induced hearing loss. CDDP (15 mg/kg) was administered intraperitoneally twice to mice and the auditory brainstem response (ABR) was measured 3 days after the last dose. Palonosetron (15 mg/kg) was administered intraperitoneally 30 minutes before each dose of CDDP. CDDP administration to mice caused significant hearing loss at 8, 16, and 32 kHz frequencies. In contrast, palonosetron significantly suppressed hearing impairment at all frequencies. These results suggest that palonosetron may be a useful preventive drug against CDDP-induced hearing loss.

1-B-P-018 一般演題（ポスター）

Fasting alleviates NMDA-induced retinal ganglion cell death in mice

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Intermittent fasting has been reported to exhibit preventive and delaying effects on senescence and age-related diseases by modulating metabolic pathways. To investigate the potential preventive effects of intermittent fasting on retinal ganglion cell loss caused by age-related retinal degenerative diseases such as glaucoma and diabetic retinopathy, we examined the effect of 24-hour fasting on NMDA-induced retinal ganglion cell death in mice. Mice were subjected to 24-hour fasting followed by intravitreal injection of NMDA (10 nmol). Histological analysis revealed that fasting for 24 hours attenuated the loss of retinal ganglion cells induced by intravitreal administration of NMDA in mice. Additionally, Western blotting demonstrated that phosphorylation of AMPK α (Thr172) and FoxO3a (Ser413), which are activated by starvation and contributes to neuroprotection, was enhanced in the retinas of mice subjected to 24-hour fasting. These findings suggest that fasting alleviates NMDA-induced retinal ganglion cell death in mice and intermittent fasting may serve as a potential preventive strategy for retinal neurodegenerative diseases such as glaucoma and diabetic retinopathy.

1-B-P-019 一般演題（ポスター）

Aldehyde reductase (ALR) affects the pathogenesis of pressure ulcer

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Background

Pressure ulcer (PU) is a tissue necrosis disorder in which the cutaneous structures are affected by pressure and shear stress, leading to ischemia and circulatory impairment, etc. Increased oxidative stress caused by ischemia and reperfusion has been reported to contribute to the pathogenesis of the disease. Aldehyde reductase (ALR) is an NADPH-dependent detoxification enzyme encoded by AKR1A and involved in the synthesis of ascorbic acid (AsA). ALR deficiency results in inadequate detoxification of carbonyl compounds, which may cause organ damage AsA is extremely reactive to radical species and functions as an antioxidant. In this study, we analyzed the effect of AKR1A on pressure ulcer formation.

Methods

Ischemia-reperfusion injury (IRI) in the skin of AKR1A knockout (KO) and wild-type control (WT) mice was compared grossly and histologically. Next, the effects of AsA administration on tissue injury of IRI in KO and WT were examined. We also examined the effects of AsA administration on viable cells in hypoxia/reoxygenation load (H/R) using mouse fibroblasts.

Result and Discussion

AKR1A deficiency aggravates pressure ulcer formation, and AsA may attenuate tissue injury caused by IRI and H/R. Since AsA is a free radical scavenger, it is possible that reactive oxygen species are involved in the aggravation of pressure ulcer formation and that AsA may be beneficial in the reduction of pressure ulcer severity. We are currently investigating expression changes of tissue injury-related molecules associated with ischemia-reperfusion, and will report on these findings as well.

1-B-P-020 一般演題（ポスター）

L-DOPA prolongs duration of lidocaine local anesthetic effect via GPR143

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Clinically, lidocaine has no vasoconstrictive action, and adrenaline is added to prolong its effect. It has been reported that L-DOPA binds to GPR143 receptor and causes vasoconstriction via the α1 receptor. However, effects of L-DOPA on the duration of lidocaine anesthesia remain unclear. 0.25% lidocaine with various ligands is injected intracutaneously into the back of guinea pigs. The number of times without response to stimulus was measured. L-DOPA dose-dependently prolonged the duration of lidocaine anesthesia. Addition of caribidopa, a dopa decarboxylase inhibitor, to 1 μM L-DOPA extended the duration of lidocaine anesthesia. 1 μM DOPA CHE, an inhibitor of GPR143, inhibited the prolongation of lidocaine with 1 μM L-DOPA. 0.25% lidocaine with 1 μM L-DOPA were combined with 1 μM yohimbine, 1 μM prazosin, 1 μM JP1302, 10 μM BRL44408, 10 μM indoramin, 0.25 μM BMY7378, 5 μM cyclazosin, 1 nM silodosin respectively. After mixing, the effect of various antagosits on lidocaine with 1 μM L-DOPA were examined. As a result, antagonists other than 10 μM BRL44408 and 0.25 μM BMY7378 decreased the duration of lidocaine anesthesia with 1 μM L-DOPA. These results indicated that peripheral vasoconstrictor activity of lidocaine with 1 μM L-DOPA is mediated at least by α_{2C}, α_{1A}, α_{1B} receptors.

1-B-P-022 一般演題（ポスター）

Evaluation of oxygen-induced retinopathy in neonatal mice with fetal growth restriction

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The present study is undertaken to investigate the effect of fetal growth restriction (FGR) on retinal neovasculature in a murine premature neonatal oxygen-induced retinopathy (OIR) model. According to the results of histological analysis, a significant decrease of neovasculature, as indicated by the decreases in the number of branch junctions, the vesicular distribution, maximal vesicular radius and microaneurysm-like tufts, were observed in OIR mice with FGR while comparing to OIR neonates with normal birth weight. The results of retinal RNA-sequencing revealed a down-regulation of angiogenic factors that trigger pathologic retina neovascularization, such as MAPK pathway and relative upstream signaling pathways in OIR mice with FGR. These results suggest that FGR neonates may be equipped with a higher capacity for retinal oxygen stress, and the risk of OIR development is lower compared to mature neonates.

1-B-P-023 一般演題（ポスター）

Regulation of trigeminal ganglion neurons innervating cornea by acetylcholine receptors

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Corneal sensory nerves have crucial roles in tear secretion and corneal wound healing in addition to the induction of ocular surface sensation. However, the regulatory system of corneal nerves is not fully understood. This study aims to examine the cholinergic regulation of trigeminal neurons innervating the cornea. In guinea pigs, corneal trigeminal neurons were labeled by treating the corneal surface with the retrograde dye FM1-43. Four days later, primary cultures of trigeminal ganglion neurons were made from the animals under anesthesia. Calcium imaging with Fura-2 was performed 24-48 hours after the dissociation. Perfusion of nicotine (1-100 μM), but not pilocarpine, concentration-dependently elevated intracellular Ca^{2+} concentration in a part of trigeminal neurons; the ratio of nicotine-sensitive neurons was 61% in FM1-43 positive corneal neurons, whilst being 38% in FM1-43 negative non-corneal ones. Compared among the populations classified with responsiveness to agonists of TRPM8, a cold sensor, and TRPV1, a polymodal nociceptor, a population showing the highest ratio of nicotine-sensitive corneal neurons was characterized by TRPM8 agonist-responsive and TRPV1 agonist-unresponsive. These findings suggest that nicotinic receptors might regulate the excitabilities of corneal sensory neurons, especially cold-sensitive ones.

1-B-P-024 一般演題（ポスター）

Morphological analysis with deep learning to identify peripheral neuropathy specified on soma or axon using an *in vitro* microfluidic device

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Chemotherapy-induced peripheral neuropathy (CIPN) is a major common adverse event associated with neurological abnormalities. In the present study, we developed a microfluidic device for *in vitro* neuronal culture, and predict neurotoxicity induced by anti-cancer drugs with different mechanisms of action based on deep learning morphological analysis.

The microfluidic culture device could separate the cell body and neurites, so that the influence on soma or axon can be analyzed independently. COP (Cyclo olefin polymer), which has excellent observability and low drug adsorption, is used as the resin material, and the bottom surface is created thin and flat enough for a clear view by microscope. Next, primary DRG neurons was cultured in the device coated with Poly-L-lysine and Laminin. After culturing with a specific medium containing insulin, neurites grew sufficiently to occupy almost the whole microfluidic channel area, and the axon elongated unidirectional along the horizontal direction.

After administration of several typical anti-cancer drugs, a deep learning AI was trained with immunofluorescence image datasets of either soma or neurites. As results, AI could accurately detected toxicity positive for both soma and neurites, even at low concentrations. Furthermore, the effects of drugs on either soma or neurites could be significantly separated by principal component analysis using toxicity positive rate calculated by AI. Therefore, this method provides an effective in vitro toxicity assessment platform for peripheral neuropathies.

1-B-P-025 一般演題（ポスター）

Risk of developing Parkinson‘s disease associated with calcitonin gene-related peptide inhibition

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Parkinson‘s disease (PD) arising from the impairment of dopaminergic neurons in the substantia nigra of the midbrain is characterized by accumulation of alpha-synuclein (α-syn). Depression is a precursor of PD and is associated with increased risk of developing PD. Previous research has demonstrated that the administration of calcitonin gene-related peptide (CGRP) to the brain exerts antidepressant effects. Therefore, we hypothesized that there exists a connection between CGRP and PD. In this study, we investigated whether CGRP deficiency or CGRP antibody treatment (galcanezumab) can lead to PD-like symptoms in C57BL6J mice. Motor function was assessed using the rotarod test, pole test, adhesive test, and catalepsy test. Depression-like behavior was assessed using the forced swim test or tail suspension test. Tyrosine hydroxylase (TH) and α-syn expression levels were determined via Western blotting. CGRP-deficient mice showed a significant decrease in motor function and TH levels. An increase in α-syn expression was observed in the substantia nigra and striatum of CGRP-deficient mice. Compared with the control, administration of galcanezumab (once weekly for 4 weeks) resulted in increased depression-like behavior and impaired motor learning. Although dopamine levels in neurons remained unchanged, a notable increase in α-syn expression was observed in the substantia nigra and striatum. These findings suggest that CGRP deficiency can induce PD-like symptoms. Long-term administration of CGRP antibodies may contribute to early-stage PD-like symptoms.

1-B-P-026 一般演題（ポスター）

Prediction method for chemically-induced pain based on deep learning of typical pain-related channels response in peripheral neurons

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Drug-induced peripheral neuropathy occurs as an adverse reaction of chemotherapy. However, a highly accurate method for assessing peripheral neuropathy and pain caused by compounds has not been established. The microelectrode array (MEA) assay using human induced pluripotent stem cell (iPSC)-derived neurons is expected to be one of the in vitro assays for predicting the mechanism of action (MoA) of pharmaceuticals. However, it is difficult to detect the reactions of drugs with different modes of action with a single parameter, and the analysis method is a challenge. To address this issue, acquiring more detailed information on neuronal network activity focused on individual cells is considered effective. In this study, human iPSC-derived sensory neurons and rat DRG neurons were cultured on 236,880 electrode CMOS-MEA, and the precise electrical activity of individual neurons was acquired. The relationship between the spontaneous activity pattern of peripheral neurons and the induced response pattern to agonists of TRPV1, TRPA1, and TRPM8, respectively, was analyzed. As a result, differences in agonist types were visualized by UMAP analysis based on spike patterns. Next, we developed a pain prediction AI that learned UMAP coordinate information and agonist type. The developed model predicted agonist type and concentrations with 81% accuracy. The CMOS-MEA, which can acquire precise electrical activity of single neurons, can increase the parameters that can detect the effects of drugs, making it effective as a method for predicting the MoA of compounds.

1-B-P-027 一般演題（ポスター）

Effects of juvenile stress on membrane potential properties of mouse medial prefrontal cortex and dorsal raphe nucleus neurons.

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Severe childhood stress, such as child maltreatment, has been suggested to be a risk factor for depression and other psychiatric disorders in later life. In addition, women are statistically more likely than men to develop depression and anxiety disorders, suggesting a gender difference in stress sensitivity. However, the molecular mechanisms involved in stress-induced changes in neural activity during childhood and adulthood and the sex differences in these changes remain unclear. In the present study, we used a patch-clamp technique to analyze the effects of electric current stimulation of the foot at 3 weeks of age (3-week foot-shock, 3wFS) on membrane potential changes in pyramidal cells of the mature medial prefrontal cortex (mPFC) in mice. Analysis of female mice revealed no differences in resting membrane potential, input resistance, or action potential firing thresholds of mPFC pyramidal cells between the 3wFS and nonFS (no foot-shock) groups. The firing frequency of action potentials in mPFC pyramidal cells evoked by depolarizing current injection was increased in the 3wFS group compared to the nonFS group. Analysis of male mice showed no significant differences in any of the above parameters. Similarly, analysis of membrane potential changes in neurons of the dorsal raphe nucleus (DRN), one of the serotonergic primitive nuclei, showed no significant changes in either male or female mice. mPFC is an inhibitory regulator of the DRN, suggesting that in female mice subjected to juvenile stress, overexcitation of mPFC pyramidal cells may result in DRN inhibition. DRNs are strongly suppressed in female mice exposed to juvenile stress, which may contribute to sex differences in the onset of depression.

1-B-P-028 一般演題（ポスター）

Difference in modulation of purine metabolisms by fibroblast growth factor 2 between cultured cortical astrocytes and microglia

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Purines, including ATP and adenosine (ADO), are important neurotransmitters in the CNS. Purine release and metabolism are contributed by astrocytes and microglia. Previously, we showed that fibroblast growth factor 2 (FGF2) modulates purine metabolisms in cultured astrocytes. However, the modulation of purine metabolisms in microglia is unclear. In this study, we investigated the expressions and activities of purine metabolic enzymes in cultured astrocytes and microglia and the effects of FGF2 on them.

Cultured astrocytes and microglia from rat cortex were treated with FGF2. The expressions of enzymes were measured by real-time PCR. Enzymatic activities were measured by incubation with solution containing ATP,AMP or ADO and measurement of metabolites with HPLC.

The expressions of NTPDase1, CD73 and ADA were higher in microglia than astrocytes, while that of NTPDase2 was higher in astrocytes. The activities of NTPDases, CD73 and ADA were also higher in microglia. FGF2 increased CD73 and ADA, and decreased NTPDase2 in astrocytes, whereas did not affect any enzymes in microglia. In addition, FGF2 phosphorylated ERK and JNK, and dephosphorylated STAT3 in astrocytes, while phosphorylated only ERK in microglia.

These results indicate that microglia have higher purine metabolisms compared to astrocytes, although FGF2 modulates it only in astrocytes. These differences may be attributed to the difference in intracellular signaling pathways.

1-B-P-029 一般演題（ポスター）

Sonic hedgehog signaling reduces vasogenic edema following traumatic brain injury in mice

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Traumatic brain injury (TBI) is a severe damage to the head that causes vasogenic edema resulted from disruption of the blood-brain barrier (BBB). Sonic hedgehog (Shh) is a hedgehog family protein, which exerts protective effects for cerebrovascular and neuronal cells after brain damages via the patched-1-smoothened-Gli signaling pathway. Thus, we investigated effects of the Shh signaling for TBI-induced vasogenic edema in model mice. The TBI model was determined by inflicting a fluid percussion injury (FPI) in the mouse cerebrum. Vasogenic edema was assessed by the Evans blue extravasation into the brain tissue and the increased brain water content. Evans blue extravasation and brain water content were increased by FPI, whereas repeated intracerebroventricular administration of recombinant Shh (0.1, 1, 10 μg/day) from 3 hours to 3 days after FPI reduced Evans blue extravasation and the brain water content in the injured cerebrum. On the other hands, Jervine, a smoothened antagonist aggravated these conditions. Administration of Shh increased expression levels of tight junction proteins and angiopoietin-1, a vascular protective factor in the injured cerebrum after FPI. These results suggest that Shh signaling alleviates TBI-induced vasogenic edema by increasing tight junction proteins and angiopoietin-1.

1-B-P-030 一般演題（ポスター）

Effects of rolipram on abnormalities of emotional behavior induced by chronic restraint stress in mice.

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Chronic stress is associated with the development of anxiety and depression. Moreover, phosphodiesterase-4 (PDE-4) has been implicated in the pharmacological effects of antidepressants. In this study, we investigated the effects of the PDE-4 inhibitor rolipram on abnormalities of emotional behavior induced by chronic restraint stress (CRS) in mice.
For exposing CRS, mice were restrained in a 50mL syringe with numerous small breathing holes for 3 hours a day for 10 consecutive days. Rolipram (1.25 mg/kg, i.p.), fluvoxamine (a SSRI, 30 mg/kg, p.o.) to evaluate the predictive validity of an animal model for depression, or vehicle were administered one hour before the restraint stress.
In mice subjected to CRS, time spent in the open arm of the elevated plus-maze, as well as the time spent in the central area of the open field apparatus decreased, indicating anxiogenic behavior. Furthermore, in the forced swim test, immobility time increased in mice exposed to CRS, implying depressive-like behavior. These behavioral abnormalities in CRS mice were ameliorated by both fluvoxamine and rolipram administered prior to restraint stress.
These results suggest that mice exposed to CRS have validity as an animal model for depression and PDE-4 may be involved in the development of anxiety-like and depression-like behaviors induced by CRS.

1-B-P-031 一般演題（ポスター）

Facial expression changes in mice during social interaction under head fixation

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Autism spectrum disorder (ASD) is a developmental disorder characterized by abnormal social communication and repetitive behaviors. To study the neural circuit abnormalities underlying these manifestations using ASD model mice, it is necessary to establish an experimental paradigm that can quantitatively evaluate the changes in the subject 's emotions toward conspecifics when it interacts with them. To this end, we conducted facial videography and machine-vision-based image analysis to examine the facial expressions of mice interacting with conspecifics under head fixation. A transparent acrylic box with holes in the front, in which an inanimate object, a male conspecific, or a female conspecific was presented, was placed in front of a male C57BL/6 subject mouse head-fixed over a freely-rotating cylindrical treadmill. Images of the face of the subject mouse were recorded by a CCD camera under infrared illumination. Comparisons of each movie with prototypical faces obtained with each stimulus indicated that the facial expression changes elicited by each stimulus were unique. Overall, this paradigm can be combined with two-photon calcium imaging to reveal abnormalities in social and emotional brain circuits in ASD model mice and will also be helpful to evaluate the effects of drugs that aim to improve possible abnormal social emotions in mouse models of neurodevelopmental and neuropsychiatric disorders.

1-B-P-032 一般演題（ポスター）

Astrocyte-mediated neuroprotective effect of noradrenaline on chemical ischemia-induced delayed neuronal cell death.

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In this study, we examined the effect of noradrenaline on delayed neuronal death induced by chemical ischemia, a model of ischemia-reperfusion injury in vivo, in the mixed culture of human astrocytoma U-251 MG cells and human neuroblastoma SH-SY5Y cells. Chemical ischemia was induced by incubating the cultures in buffered salt solution containing 0.5 mM 2-deoxy-D-glucose and 5 mM sodium azide for 3 hours. After the induction of chemical ischemia, the medium was replaced with DMEM and cultured for an additional 45 hours. Delayed neuronal cell death of SH-SY5Y cells after chemical ischemia was significantly attenuated by noradrenaline (3 μM) in the mixed culture, but not in single culture of SH-SY5Y cells. The neuroprotective effect of noradrenaline was inhibited by a nonselective α-adrenoceptor antagonist phenoxybenzamine (3 μM), but not by a nonselective β-adrenoceptor antagonist propranolol (10 μM), whereas a selective α_1-adrenoceptor agonist phenylephrine (1-30 μM) attenuated chemical ischemia-induced cell death of SH-SY5Y cells in the mixed culture. These results suggest that noradrenaline attenuates chemical ischemia-induced delayed cell death of SH-SY5Y cells by stimulating the α_1 receptor of U-251 MG cells in the mixed culture.

1-B-P-033 一般演題（ポスター）

3‘,4‘-Dihydroxyflavonol inhibits LPS-induced neuroinflammatory responses of microglia by suppressing AKT–mTOR pathway

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We investigated the effects of the synthetic flavonoid 3‘,4‘-drihydroxyflavonol on lipopolysaccharide (LPS)-induced neuroinflammatory responses in MG6 microglial cells. 3‘,4‘-Dihydroxyflavonol suppressed LPS-induced tumor necrosis factor (TNF)-α and nitric oxide (NO) production in MG6 cells. 3‘,4‘-Dihydroxyflavonol also inhibited LPS-induced phosphorylation of mammalian target of rapamycin (mTOR), which is crucial for TNF-α and NO production. LPS stimulation induced rapid phosphorylation of protein kinase B (AKT) in MG6 cells. 3‘,4‘-Dihydroxyflavonol significantly inhibited the LPS-induced phosphorylation of AKT. The inhibitory effect of 3‘,4‘-dihydroxyflavonol on TNF-α and NO production was mimicked by blockade of the mTOR and AKT pathways with mTOR inhibitor, rapamycin and AKT inhibitor, LY294002. Furthermore, LY294002 significantly inhibited LPS-induced phosphorylation of mTOR in MG6 cells. These results suggest that 3‘,4‘-dihydroxyflavonol exerts anti-neuroinflammatory effects via inhibition of the AKT-mTOR pathway in microglia.

1-B-P-034 一般演題（ポスター）

Accumulation and propagation of tau impair cognitive function and decrease acetylcholine levels in the hippocampus in wild-type mice.

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【Purposes】It has been reported that there is a correlation between the spread of tau pathology in the brain and the progression of cognitive impairment in Alzheimer's disease.
Recently, a mouse model of tau propagation using wild-type mice and synthetic tau fibrils was reported (Masuda-Suzukake et al., Brain Commun., 2020), but behavioral evaluation of the mice was not conducted. In this study, cognitive function and neurotransmitter levels were assessed in this mouse model.
【Methods】We injected synthetic soluble tau or tau fibrils bilaterally into the hippocampus of 9-week-old C57BL/6J mice. The cognitive function was assessed 3 months and 6 months after injection. The levels of neurotransmitters and their metabolites in the hippocampus were measured using HPLC-ECD 6 months after the injection.
【Results・discussions】The mice injected with tau fibrils showed cognitive impairment at 3 months and 6 months after injection, while those injected with soluble tau did not show cognitive impairment. In the hippocampus, acetylcholine levels were decreased in both groups compared with the control group, and the mice injected with tau fibrils showed a significant decrease. The results suggest that the accumulation and propagation of tau in the brain impair cognitive function and decrease acetylcholine levels in the hippocampus.

1-B-P-035 一般演題（ポスター）

A novel method of inducing a torpor-like hypometabolic state by glial activation

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Hibernation or torpor are energy-saving strategy in some thermostatic animals to survive harsh environments such as extreme cold and starvation. The ability to induce such hypometabolic states has garnered considerable interest due to its potential medical benefits. However, the mechanisms regulate these hypometabolic state remain largely unknown. Here we found that chemogenetic activation of astrocytes, a type of glial cell, could induce a torpor-like hypometabolic state in mice. In this state, body temperature and levels of oxygen consumption are kept low as in torpor. Glial activation induced hypothermia inhibited by intracerebroventricular administration of receptor X antagonist. Our findings could enable the induction of hypometabolism at any given time, and thus provide a new method for studying the mechanisms of hypometabolism. Furthermore, our results suggest the importance of glia-neural interaction in the study of hibernation and torpor.

1-B-P-036 一般演題（ポスター）

Verification of the seizure liability of compounds based on their *in vitro* functional activity in cultured rat cortical neurons and cultured human iPSC-derived neurons

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Methodical screening of safe and efficient drug candidate compounds is crucial for drug development. A high-throughput and accurate compound evaluation method targeting the central nervous system can be developed using *in vitro* neural networks. In particular, an evaluation system based on a human-derived neural network that can act as an alternative to animal experiments is desirable to avoid interspecific differences. A microelectrode array (MEA) is one such evaluation system, and can measure *in vitro* neural activity. In this study, we identified the parameters that can eliminate the effects of solvents from neural activity data obtained using MEA allow for accurate compound evaluation. Additionally, we resolved the issue associated with compound evaluation criteria during MEA using principal component analysis by considering the neuronal activity exceeding standard deviation (SD) of the solvent as indicator of seizurogenic potential. Overall, 10 seizurogenic compounds and three negative controls were assessed using MEA-based co-cultured human-induced pluripotent stem cell–derived neurons and astrocytes, and primary rat cortical neurons. In addition, we determined rat cerebrospinal fluid (CSF) concentrations during tremor and convulsion in response to exposure to test compounds. To characterize the *in vitro* to *in vivo* extrapolation and species differences, we compared the concentrations at which neuronal activity exceeding the SD range of the solvent was detectable using the MEA system and rat CSF concentration.

1-B-P-037 一般演題（ポスター）

Dynamics of process addiction formation driven by predictability

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The fundamental mechanisms underlying the formation of process addictions, such as gambling addiction, gaming addiction, and social networking addiction, are not yet understood, and no effective preventive measures or treatments have been found. These addictions are generally associated with excessive activity of the basal ganglia-dopamine system, but the commonality of what constitutes an "addictive object" is not yet clear. We have developed a new experimental model using mice to address this situation. Specifically, we observed the behavior of mice that were asked to choose between two alternatives: a "predictable alternative," which foretells the presence or absence of a reward, and an "unpredictable alternative," which does not foretell anything about the reward. We observed that the predictable option was preferred when the two options offered equal reward probabilities. This preference was maintained even when only the probability of reward for the predictable option was reduced. Furthermore, we confirmed that administering L-DOPA increases the preference for the predictable option. Conversely, the lesioning of D2 receptor-expressing cells in the striatum decreased the preference for the predictable option. Based on these results, we propose a new hypothesis that process addiction is an overestimation of predictable reward signs, i.e., signs that foretell the presence or absence of reward, based on the basal ganglia dopaminergic system.

1-B-P-038 一般演題（ポスター）

コカインがオスマウスのモテ度と社会的ランキングに及ぼす影響

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The role of the prefrontal cortex (PFC), particularly the prelimbic region (PL), in determining male dominance has been previously reported (Zhou, et.al, Science, 2017). Apart from this established dominance hierarchy, our team has developed a Female-Male Preference Test (FMPT) by comparing four male mice. This test effectively differentiates between males perceived as attractive and unattractive from a female mouse's perspective. The correlation between a male's dominance and its attractiveness to females remains elusive.

Our preliminary investigations suggest that there isn't a direct proportionality between a male's social ranking within its group and its allure to females. In our previous work, we have identified cocaine as an activator of the PFC. Further, our research has elucidated the relationship between PFC dopamine activity and cocaine reactivity, revealing that cocaine regulates sensitization and its glutamate stimulation in the PFC controls dependency (Kawahara, et.al, Int J Neuropsychopharmacol, 2021).

Given these findings, we intend to administer cocaine to the less attractive male mice to observe potential shifts in their social ranking and desirability to females. This is based on the hypothesis that cocaine-induced alterations in reward-seeking behaviors might recalibrate male-male interactions, including competitions and their inherent attractiveness to females. After examining the relationship between male dominance and attractiveness, we plan to expose the less attractive males to cocaine under conditions mirroring the Cocaine Conditioned Place Preference (Cocaine CPP) to further investigate any changes in their social hierarchy and appeal.

This study aims to provide a comprehensive understanding of the interplay between cocaine, PFC activity, and male social behaviors, potentially offering novel insights into the neuropharmacological mechanisms governing social interactions in mice.

1-B-P-039 一般演題（ポスター）

Morphine induces the expression of the receptor chaperone molecule/interferon-stimulated gene RTP4 via TLR4 in microglial cells.

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Although opioid analgesics exhibit potent antinociceptive effects, various side effects including antinociceptive tolerance limit their effective clinical use. Our previous studies show that neuronal receptor transporter protein 4 (RTP4), one of the receptor chaperone proteins, contributes to the mechanism of development of morphine tolerance. Although, studies suggest that glial as well as neuronal cells contribute to antinociceptive tolerance, the role of glial RTP4 on opiate induced tolerance has not yet been elucidated. Here, we examined the changes in RTP4 levels in microglial cells after morphine exposure.

We find that morphine treatment (1 mM, 24-hr) significantly up-regulates RTP4 mRNA levels in a microglial cell line, SIM-A9 cell. This up-regulation was not reversed by naltrexone, a mu opioid receptor antagonist, while it was significantly inhibited by a neutralizing antibody targeting toll-like receptor 4 (TLR4) and by a janus kinase (JAK) inhibitor. Furthermore, interferon (IFN)-β mRNA levels were increased by morphine treatment.

These findings suggest that in microglial cells, morphine activates TLR4, leading to type I IFN production, IFN receptor and JAK activation, and finally to RTP4 gene induction.

1-B-P-040 一般演題（ポスター）

The exploration of drugs for cancer treatment inducing cognitive impairment

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Cancer-related cognitive impairment (CRCI) is cognitive symptoms, such as impairment of memory, attention, executive functions, and processing speed, elicited by some chemotherapy and hormonal treatment in many of cancer patients and survivors. Because CRCI impairs patients' quality of life during and after cancer but there is no effective prevention and treatment for this side effect, CRCI is a significant medical problem. In this study, we explored which drugs for cancer treatment is related to cognitive impairment using the FDA Adverse Event Reporting System (FAERS), the world's largest freely available self-reported adverse events database, and validated the effect of the candidate drugs conducting *in vivo* behavioral experiments. FAERS analysis revealed that the use of some hormone-modulating drugs showed strong associations with the occurrence of cognitive impairment. We further observed cognitive impairment in mice administered with one of the hormone-modulating candidates on day 1 and 7 but not on day 14 in novel object recognition test. Further experiments will be needed to elucidate the pathophysiological mechanism in hormone-modulating drugs-induced cognitive impairment and to develop effective preventive/therapeutic approach for CRCI related to hormonal treatment.

1-B-P-041 一般演題（ポスター）

Evaluation of Brain Substances in cynomolgus monkeys Using Microdialysis Methods - From Small to Large Molecules

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Backgrounds: In this study, we measured monoamines and amino acids in the brain of the cynomolgus monkey using a small molecule microdialysis method. We also evaluated the migration of antibodies into the brain using the polymer microdialysis method. Methods: In the small molecule microdialysis method, probes were inserted into the nucleus accumbens and hippocampus of the brain, and probes were perfused with Ringer solution. After completion of preperfusion, dialysates were collected at 30-minute intervals, and at the final time point, the brain was stimulated with high potassium Ringer solution for 20 minutes. In the polymer microdialysis method, the dialysate was collected using the push-pull method because the cutoff value for the molecular weight of the probe is 1000 kDa. We administered trastuzumab, an antibody drug, to cynomolgus monkeys and analyzed its pharmacokinetics in plasma and brain tissue perfusate. Results: In the small molecule microdialysis method, serotonin, norepinephrine, dopamine, acetylcholine, Glutamic acid remained stable for 2.5 hours, but increased 2 or 3 times at 3 hours by high potassium stimulation. The metabolites of these monoamines and precursors of neurotransmitters were declined by high potassium stimulation. In the polymer microdialysis method, plasma concentrations of trastuzumab peaked about 1 hour after the beginning of intravenous infusion and maintained or gradually declined. In contrast, the concentrations in brain dialysate increased gradually and reached a peak at about 5 hours after the beginning of infusion.

1-B-P-042 一般演題（ポスター）

Spatiotemporal analysis of astrocytes and microglia surrounding microvasculature along with the maturation of blood brain barrier in in vivo brain

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NIHS

Blood vessels in the central nervous system (CNS) have strong barrier functions called blood brain barrier (BBB), which enables the CNS-specific pharmacokinetics and homeostasis. Recent studies have clarified that the surrounding astrocytes and microglia actively regulate the formation and maturation of BBB. However, the precise spatiotemporal changes and the underlying mechanisms for respective cell types remain to be elucidated.
In this study, we investigated the period of cerebrovascular BBB formation in rat brains using Evans Blue, which has already been accepted not to penetrate into mature BBB. We found that rat cerebrovascular BBB was formed between day 4 and day 15 after birth. By 2D and 3D image analysis of the immunohistochemical data using IMARIS (Oxford Instruments), we clarified the cell number, three-dimensional structure, and coverage rate of astrocytes and microglia in the process of BBB maturation. Of interest, microglia directly contacted with the capillaries and the coverage rate reached the maximum at day 15 after birth, which is correlated with BBB maturation. We are currently analyzing the spatiotemporal dynamics specific to respective cell types. Our study strongly suggests that both of astrocytes and microglia directly contact with brain microvasculature and play important roles in BBB maturation.

1-B-P-043 一般演題（ポスター）

Neurotoxicity assessment of fluoxetine using multielectrode array recordings of human iPSC-derived neurons

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Seizure is one of the major causes of the cessation of development of central nervous system drugs. Multielectrode array (MEA) system has advantages in high-throughput neurotoxicity tests. Although rat behavioral tests are mainly used to evaluate neurotoxicity in drug development, it is challenging to identify human neurotoxicity by animal tests due to differences in species.

Human induced pluripotent stem cells (hiPSC)-based experiment might be valuable to evaluate human neurotoxicity. In this study, we examined the neurotoxicity of fluoxetine by MEA using hiPSC-derived neurons. We exposed the hiPSC-derived neurons XCL-1 to the drug and performed MEA recordings using MED64-Presto.

Exposure of fluoxetine to hiPSC-derived neurons reduced neural network activity, such as the number of spikes and network bursts in a dose-dependent manner. In rat cortical neurons, the network activities were also reduced by fluoxetine in a comparable dose-dependent manner to hiPSC-derived neurons.

MEA recordings in network activity of hiPSC-derived neurons could be an effective tool for neurotoxicity screening in drug development.

1-B-P-044 一般演題（ポスター）

Effect of simultaneous mother-offspring administration of the traditional herbal medicine Yokukansan on emotional abnormality induced by prenatal stress in mice

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The aim of the present study was to examine the effect of simultaneous mother-offspring administration of Yokukansan (YKS), a traditional Japanese herbal medicine, on the emotional abnormality induced by prenatal stress in mice. The dry powder extract of YKS used in the present study was supplied by Tsumura & Co. (Tokyo, Japan). YKS was mixed with powdered rodent chow at a concentration of 3% and fed to mice from birth until they reached 7 weeks of age. We then examined the effect of treatment with YKS on emotional abnormality induced by prenatal restraint stress. The open field test revealed that mice exposed to prenatal stress exhibited a significant decrease in the time spent in the central area, and this anxiety-like behavior was suppressed by YKS treatment. The expression levels of dopamine D2 receptors were significantly increased in the hippocampus and prefrontal cortex of mice exposed to prenatal stress, and these changes reduced to the same level as the control group by treatment with YKS. Additionally, similar results were obtained for the serotonin transporters in the prefrontal cortex. These results suggest that simultaneous mother-offspring administration of YKS could ameliorate increased anxiety sensitivity in offspring induced by prenatal stress by affecting the function of the monoamine system.

1-B-P-045 一般演題（ポスター）

Repeated prophylactic treatment of a curcumin derivative CUD003 prevents lipopolysaccharide-induced depressive-like behavior by inhibiting excessive inflammation in mice.

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Previously, we revealed that repeated prophylactic treatment with CUD003, a synthetic derivate of curcumin, suppressed the development of depressive-like behavior at lower doses than curcumin in a lipopolysaccharide (LPS)-induced depression model. In this study, we investigated the possible mechanism of the preventive effect of CUD003 on the LPS-induced depression-like behaviors in mice. Male BALB/c mice were orally pretreated with curcumin or CUD003 for 5 days before receiving single intraperitoneal injection of LPS (0.5 mg/kg). Plasma interleukin (IL)-1β and tumor necrosis factor (TNF)-α levels were assessed by enzyme-linked immunosorbent assay. Furthermore, reactive oxygen species (ROS) level in the hippocampal CA1 region was assessed by dihydroethidium staining. Twenty-four hours after LPS administration, IL-1β and TNF-α levels in plasma increased. In addition, enhanced oxidative stress with elevated level of ROS was detected in the hippocampus. Pretreatment with CUD003 (3 or 10 mg/kg) and 10 mg/kg curcumin but not 3 mg/kg curcumin suppressed all these changes. These results suggest that the mechanism of preventive effect of CUD003 on the LPS-induced depressive-like behavior in mice is related to suppression of inflammatory cytokines and reduction of oxidative stress.

1-B-P-046 一般演題（ポスター）

Social stress induces microglial contact with synapses, contributing to the release of heparan sulfate from synaptic proteoglycans in mice

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Chronic stress due to aversive and demanding conditions induces dendritic atrophy and synaptic loss of pyramidal neurons in the medial prefrontal cortex (mPFC), leading to depression-related behaviors. Chronic stress reportedly activates microglia via the innate immune receptors TLR2/4, leading to dendritic atrophy and synaptic loss of mPFC neurons and depression-related behaviors in mice. Here, we examined the mechanistic link between microglial activation and neuronal dysfunctions under social defeat stress in mice. Serial electron microscopy showed that social stress transiently increased the interaction between microglial processes and synapses, preferentially presynaptic sites. Proteoglycans reportedly include putative TLR2/4 ligands and are involved in synaptic development and functions. Carbohydrate LC-MS analysis showed that social stress specifically decreased heparan sulfate, known to be a TLR4 ligand, in synaptosomes but not in the whole mPFC tissue. This heparan sulfate decrease was abolished in TLR2/4 knockout mice, which lack stress-induced microglial activation. These findings suggest that social stress induces microglial contact with synapses, where TLR2/4 contributes to the release of heparan sulfate from synaptic proteoglycans, perhaps to activate microglia.

1-B-P-047 一般演題（ポスター）

Migratory and proliferative effects of arresten in rat cardiac fibroblasts

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Arresten, a cleaved fragment of type IV collagen α1 chain, is expressed in normal rat cardiac tissue. However, its function on the cardiac fibroblasts has not been fully elucidated. This study investigated the effects of arresten on migration and proliferation of cardiac fibroblasts and underlying mechanisms. Cardiac fibroblasts were isolated from ventricles of adult Wistar rats. Cell migration and proliferation were measured by Boyden chamber assay and cell counting assay, respectively. Phosphorylation of extracellular signal-regulated kinase (ERK) and Akt (Ser473) was evaluated by Western blotting. Arresten significantly promoted the migration (250 ng/ml, 24 h) and proliferation (100 ng/ml, 48 h) in cardiac fibroblasts. Arresten (30 min) enhanced phosphorylation of ERK (250 ng/ml) and Akt (Ser473) (100 ng/ml). The arresten (250 ng/ml, 24 h)-induced migration was suppressed by PD98059 (10 μM), an inhibitor of MEK/ERK. The arresten (100 ng/ml, 48 h)-induced proliferation was suppressed by LY294002 (1 μM), an inhibitor of PI3K/Akt. This study for the first time demonstrated that arresten promotes the migration via MEK/ERK signaling pathway, while it promotes the proliferation via PI3K/Akt signaling pathway.

1-B-P-048 一般演題（ポスター）

Elucidation of protective mechanism against AngII-induced cardiomyocyte injury by Moku-boi-to

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Heart failure is a rapidly growing public health problem, affecting over 30 million people worldwide. Current therapeutic approaches aim to regress cardiac hypertrophy but have limited efficacy. In this context, Japanese Kampo medicines are gaining attention as safe and potentially effective therapeutic agents. This study focuses on understanding the impact and mechanisms by which Moku-boi-to (MBT), a Japanese Kampo medicine, provides potential cardioprotective benefits against AngII-induced cardiomyocyte hypertrophy. By addressing this knowledge gap, we aim to contribute to the development of novel therapeutic strategies. Here, we found that MBT exhibited preventive effects against AngII-induced cardiomyocyte hypertrophy and cell death. One of the ways MBT exerted its benefits was by enhancing intracellular Ca^{2+} signaling regulation and improving mitochondrial function. However, it was unexpected that MBT did not provide additional effects when combined with the AT_1 receptor blocker losartan. These findings shed light on the AT_1 receptor-mediated cardioprotective potential of MBT and provide valuable insight into the underlying mechanisms responsible for alleviating AngII-induced dysfunction in cardiomyocytes. Our results suggest that MBT holds promise as a safe and effective prophylactic agent for cardiac hypertrophy.

1-B-P-049 一般演題（ポスター）

Doxorubicin leads to cardiomyocyte death by causing the accumulation of dysfunctional mitochondria through its inhibition of the autophagy fusion process.

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Doxorubicin (Dox), an anthracycline antibiotic, is a drug that inhibits the replication of DNA and metabolic processes in cancer cells that have high proliferative potential, thereby acting as an anti-cancer agent. Although Dox can cause severe side effects such as myocardial damage and heart failure, the exact mechanism by which it leads to myocardial injury remains uncertain. In this study, we examined the impact of Dox on the mitochondrial quality control system and the regulation of mitochondrial respiration and autophagy in H9c2 rat myoblast cells cultured in vitro using western blotting, immunohistochemistry, the Seahorse XF24 system, and flow cytometry. Our research sheds light on the processes that drive the influence of Dox on mitochondrial function. We showed that Dox does not hinder the start of autophagic flux or the functioning of lysosomes. However, Dox affected the mitochondrial quality control system, causing a shift towards fission and impairing the regulation of mitochondrial respiration. This led to an increase in oxidative stress and the inhibition of autophagy, especially the fusion of autophagosomes with lysosomes. The inhibition led to a substantial decline in autolysosome formation, which was attributed to the buildup of dysfunctional mitochondria and a subsequent surge in oxidative stress, resulting in heightened myocardial cell death.

1-B-P-050 一般演題（ポスター）

Effect of nicorandil on cardiac function and survival in cardiac-specific Bcl -2–associated athanogene (BAG) 3 knockout mice

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Bcl-2–associated athanogene (BAG) 3 is known as a regulator of cell death as well as autophagic protein turnover in the heart, and disruption of the *BAG3* gene in a cardiac-specific manner can lead to cardiac failure in mice. However, little is known about therapeutic approaches to the cardiac failure induced by *BAG3* disruption. We characterized mouse ablated *BAG3* gene using a Cre-recombinase-loxp site system in a cardiac-specific manner (BAG3$^{f/f}$ crossbred with Cre transgenic mice; BAG3 cKO) and examined the therapeutic effect of nicorandil in BAG3 cKO mice. Cardiac BAG3 level was markedly attenuated in BAG3 cKO mice compared with hearts of BAG3$^{f/f}$ mice. BAG3 cKO mice showed evidence of cardiac disease, including a reduction in fractional shortening detected by echocardiography, as well as cardiac fibrosis at 6 months of age. BAG3 cKO mice also showed premature death (~40%) up to 6 months of age. Mice were treated with nicorandil (81 mg/L drinking water) beginning at 1 month of age. Nicorandil treatment was able to prevent the reduction of fractional shortening, cardiac fibrosis, and premature death. Thus, although the underlying molecular mechanisms remain unclear, long-term treatment with nicorandil appears to inhibit cardiac disease induced by cardiac-specific *BAG3* ablation.

1-B-P-051 一般演題（ポスター）

Cardiac contractility assessment of BCR-ABL tyrosine kinase inhibitors using human iPS cell-derived cardiomyocytes and real-world database.

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BCR-ABL tyrosine kinase inhibitors (BCR-ABL TKIs) have improved the survival of patients with chronic myeloid leukemia. Growing evidence suggest that cancer therapeutics-related cardiac dysfunction has become an important serious adverse event. BCR-ABL TKIs has been also reported to induce left ventricular dysfunction and cardiac failure in clinical settings. We have previously developed an imaging-based contractility assay using human iPS cell-derived cardiomyocytes (hiPSC-CMs). Here we investigated the effect of BCR-ABL TKIs on contractility of hiPSC-CMs using the contractility assay. We used iCell cardiomyocytes 2.0 (Fujifilm Cellular Dynamics International). Motion analysis was performed using cell motion imaging system (SI8000, Sony). We found that nilotinib and imatinib decreased contraction velocity of hiPSC-CMs by chronic treatment. In contrast, bosutinib had little effect on contraction velocity. To confirm the *in vitro* data, we analyzed the cardiotoxicity risk of BCR-ABL TKIs by the real-world pharmacovigilance data, which were analyzed using FDA Adverse Events Reporting System (FAERS). We found that hiPSC-CMs data was correlated with FAERS signals. In conclusion, these results suggest that imaging-based contractility assessment using hiPSC-CMs is a useful platform to assess cancer therapeutics-related cardiac dysfunction in human.

1-B-P-052 一般演題（ポスター）

Role of mammalian target of rapamycin in the formation of retinopathy of prematurity-like vascular abnormalities in neonatal rats

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Retinopathy of prematurity (ROP) is the major cause of blindness in children. We examined the role of mammalian target of rapamycin (mTOR) on the formation of abnormal retinal blood vessels in a rat model of ROP. To induce ROP model, rats were treated subcutaneously with KRN633, an inhibitor of vascular endothelial growth factor (VEGF) receptor tyrosine kinase, on postnatal day 7 (P7) and P8. The ROP model rats were treated subcutaneously with the mTOR inhibitor rapamycin from P11 to P13. Changes in retinal vasculature, phosphorylated ribosomal protein S6 (pS6), a downstream indicator of mTOR activity, and proliferative status of vascular cells were evaluated on P14 using immunohistochemistry. For comparison, KRN633 was administered according to similar protocols. Rapamycin prevented increases in the arteriolar tortuosity, capillary density, and number of proliferating vascular cells as well as abolished the pS6 immunoreactivity in ROP rats. KRN633 almost completely abolished the abnormalities of retinal vasculature. These results suggest that activation of the mTOR pathway contributes to the onset of ROP-like abnormal retinal blood vessels. Inhibition of mTOR may be a promising approach to treat abnormal retinal blood vessels in ROP.

1-B-P-053 一般演題（ポスター）

Involvements of apelin derived from perivascular adipose tissue on modulation of vasorelaxation and kidney function in metabolic syndrome

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The perivascular adipose tissue (PVAT) regulates arterial tone. When comparing the effects of renal arterial PVAT on vasorelaxation response in SHRSP.Z-*Lepr*^{fa}/IzmDmcr rats (SPZF) and SHR/NDmcr-cp rats (CP), metabolic syndrome (MetS) model strains, enhanced acetylcholine-induced relaxation by PVAT was observed only in female CP. Furthermore, mRNA levels of apelin in PVAT are positively correlated with the enhancing effects of PVAT. MetS increases the risk of kidney dysfunction. Therefore, we investigated the relationship between apelin levels in renal arterial PVAT and kidney dysfunction in MetS model rats.

Male and female SPZF and CP aged 23 weeks were used. Systolic blood pressure (sBP) was measured using the tail-cuff method. Renal arterial PVAT was obtained from each rat, and mRNA transcript levels of apelin in the PVAT were examined by quantitative real-time polymerase chain reaction. Insulin, glucose, and creatinine in the serum and protein in the urine were measured using commercial kits. eGFR and HOMA-IR were calculated.

The highest apelin mRNA levels in PVAT and eGFR, while the lowest sBP, urine protein, and HOMA-IR levels were observed in female CP. Apelin mRNA levels in PVAT were not correlated with eGFR, urinary protein, sBP, and HOMA-IR levels.

This study suggests that apelin levels in PVAT are associated with PVAT-enhancing effects on vasorelaxation; however, the levels might not be directly affected by high blood pressure, insulin resistance, and kidney dysfunction in MetS.

1-B-P-054 一般演題（ポスター）

Analysis of the regulatory mechanism of expression of cell adhesion molecule Gicerin / CD146 in cardiomyocytes

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Gicerin / CD146 is a cell adhesion molecule which belongs to the immunoglobulin (Ig) superfamily. We have reported that gicerin / CD146 is involved in the hypertrophy of vessel neointima consists of smooth muscles. We speculate that gicerin / CD146 may also have some role in the hypertrophy of the cardiac muscle cells. In this study, we made a rat cardiac hypertrophy model by constricting the aorta (AAC, ascending aortic constriction) and examined the effect on the expression of gicerin / CD146. We confirmed the gene expression level of gicerin / CD146 was influenced by the AAC treatment. Next, stretch stimulation was applied to myocardial cell line H9c2 cells to confirm that gicerin / CD146 may participate in the cellular hypertrophy model. We also treated the cells with inhibitors MAP pathway enzymes. In cultured myocardial cells, the expression level of gicerin / CD146 was increased by the stretch stimulation and decreased by inhibiting the MAP pathway. Based on the above results, it was suggested that the expression of gicerin / CD146 is involved in cardiac hypertrophy, and that the MAP pathway may be involved in the expression of gicerin / CD146 RNA in the cardiomyocyte.

1-B-P-055 一般演題（ポスター）

Evaluation of usefulness of a mouse model of polymicrobial sepsis induced by intraperitoneal injection of fecal suspension.

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Mediford Corporation

Sepsis is a severe organ damage resulting from an uncontrolled immune response to infection pathogen. In treatment of sepsis in the early stage, antibiotics and other supportive agents are used. The animal model of sepsis have been created mainly by cecal ligation and puncture (CLP) or lipopolysaccharide (LPS) injection. However, these models have room for improvement. The objective of this study was to induce the mouse model of sepsis by intraperitoneal injection of fecal suspension and to evaluate validity of the model by application of vancomycin and prednisolone. Fecal contents in the cecal of mice were suspended in PBS, and the suspension was administered intraperitoneally to the same strain of mice. For evaluation of the usefulness of this model as a possible clinical application, vancomycin and prednisolone were subcutaneously administered after intraperitoneal administration of the cecal contents. As results, the survival rate of mice decreased in a dose-dependent manner of the fecal suspension. In the subsequent experiments, similar survival rate was obtained in the model group and treatment with vancomycin and prednisolone improved the survival rate. The model of sepsis induced by intraperitoneal administration of fecal suspension is reproducible and may be useful as an animal model of sepsis.

1-B-P-056 一般演題（ポスター）

Are leukotrienes involved in the secretory phospholipase A_2-induced neuronal apoptosis?

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Neurological diseases *e.g.* brain ischemia are associated with secretory phospholipase A_2 ($sPLA_2$). The group IB $sPLA_2$ ($sPLA_2$-IB) induced neuronal cell death via apoptosis, which were accompanied with chromatin condensation and DNA fragmentation. Previously, we had established the $sPLA_2$-IB-induced neuronal apoptosis as the *in vitro* model for cerebral ischemia. We reported that lipoxygenase inhibitors prevented neurons from the toxicity of $sPLA_2$-IB, suggesting an involvement of LTs to the neurotoxicity of $sPLA_2$-IB. Furthermore, leukotriene (LT) receptor blockers prevented neurons from the $sPLA_2$-IB-condensed chromatin and fragmented DNA, suggesting an involvement of LTs in the $sPLA_2$-IB-induced neuronal apoptosis. In the present study, we ascertained whether LTs were involved in the $sPLA_2$-IB-induced neuronal apoptosis. Prior to neuronal apoptosis, $sPLA_2$-IB generated LTB_4 in the primary culture of rat cortical neurons. The $sPLA_2$-IB-generated-LTB_4 was reduced vitamin E (radical scavenger) and nimodipine (L-voltage-dependent calcium channel blocker). Since reactive oxygen species (ROS) and calcium influx via L-VDCC were located at the up-streams of $sPLA_2$-IB-induced neuronal apoptosis, LTB_4 was located in its down-stream. However, the neurotoxicity of LTB_4 was not detected. In addition, we could detect no neurotoxicity of LTC_4, LTD_4 or LTE_4. Further studies are required to clarify how LT receptor blockers prevented neurons from the $sPLA_2$-IB-induced apoptosis.

1-B-P-057 一般演題（ポスター）

Development of Non-Alcoholic Steatohepatitis (NASH) Model Using a Chimeric Mouse with Humanized Livers (PXB-Mouse®) and Evaluation of Efficacy of Pioglitazone

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Non-alcoholic steatohepatitis (NASH) is characterized by accumulation of fat (steatosis), inflammation, and fibrosis in the liver without alcohol consumption. However, currently, there are no approved therapeutic agents for NASH in Japan, and it is considered there is no animal model that appropriately presents the complexity of NASH. Chimeric mice with a human hepatocyte (PXB-Mouse®) are characterized by replaced mouse liver cells with human liver cells. They show the capacity to mimic the function of the human liver. Therefore, the animal could be an appropriate animal model for examining efficacy of potential therapeutic agents for NASH. The aim of the present study is to develop a NASH model using PXB-Mouse® and evaluate the efficacy of pioglitazone. The study group of the NASH model was fed a choline-deficient L-amino acid-defined (CDAA, patented by our company) diet from Day 0 for 84 days. An efficacy study on pioglitazone was performed by oral administration from Day 0 for 84 days. Plasma AST, ALT, T-cholesterol, and liver HYP levels in the NASH model group were elevated compared with those of the control group. The pioglitazone-administered group showed decreased levels of plasma AST, ALT, T-Cholesterol, and liver HYP compared with those of the NASH group, suggesting that feeding PXB-Mouse® with the CDAA diet induce the of NASH-like symptoms, and that pioglitazone treatment may have potential efficacy for NASH-treatment. It is concluded that the NASH model using PXB-Mouse® could be considered a valid model for studying NASH.

1-B-P-058 一般演題（ポスター）

Characteristics of increased humoral immunity and drug resistance associated with Asparaginase allergy

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L-Asparaginase (ASNase), a key drug in the treatment of childhood acute lymphoblastic leukemia, often causes allergic reactions and drug resistance. In this study, we examined the effect of cyclophosphamide (CY) on the immune response to ASNase.

Male BALB/c mice were sensitized by ASNase. CY was i.p. injected prior to ASNase sensitization. Total IgE level and ASNase activity in the sera were measured. RBL-2H3 cells were sensitized by the sera and stimulated by ASNase to determine β-hexosaminidase (β-Hex) release. Mice spleen cells were cultured for 48 hrs and cytokines in the medium were measured using a Bio-Plex Th1/Th2 assay kit.

ASNase sensitization induced ear edema and increased serum IgE levels in mice. CY at 150 mg/kg augmented these responses. CY at 300 mg/kg increased serum IgE levels, but decreased ear edema and serum ASNase activity. Sera of CY 150 mg/kg-treated mice induced higher β-Hex release from RBL-2H3 cells than normal anti-ASNase sera, though those of CY 300 mg/kg-treated mice did not induce β-Hex release. After removing IgG from the sera of CY 300 mg/kg-treated mice, β-Hex release became higher than normally sensitized mice. ASNase sensitization induced a Th2-biased immune response, and the addition of CY further enhanced the Th2-bias in a dose-dependent manner.

CY administration enhanced Th2-biased immune responses and increased IgE and IgG production in the ASNase-sensitized mice. These findings suggest that CY may play a role in the development of ASNase allergy and drug resistance.

1-B-P-059 一般演題（ポスター）

Analysis of oxidative stress sensitivity of various immune cells using redox state monitoring mice

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Background: Oxidative stress is involved in many age-associated diseases, as well as in the aging process. Although reactive oxygen species (ROS) were thought to be primarily cytotoxic, recent studies indicate that ROS are important for cell function. In the immune cells, ROS signal appear to be essential for the activation of immune cells. We have generated transgenic mice expressing redox-sensitive green fluorescent proteins (roGFPs) (Biochim Biophys Acta Gen Subj. 1867, 130302, 2023). Two types of roGFP mice were constructed: CRO and MRO mice, which measure the redox state of whole cells and mitochondria, respectively. In this study, we measured the redox state of various immune cells using roGFP mice and analyzed their susceptibility to oxidative stress.

Methods: Immune cells isolated from CRO and MRO mice were stained with cell surface markers for B cells, T cells, NK cells, neutrophils, and eosinophils. The redox state of immune cells was measured by flow cytometry. In addition, the cells were treated with various concentrations of hydrogen peroxide, and the susceptibility to oxidative stress of various immune cells was analyzed.

Results: The basal redox state of each cell type differed significantly. The susceptibility to oxidative stress was also differed dramatically among cell types. Furthermore, redox state differed considerably between CRO and MRO mice. Monitoring redox state using a mouse model of the disease should be a powerful and convenient tool for the elucidation of the activation mechanism of immune cells from a new perspective.

1-B-P-060 一般演題（ポスター）

Role of ERM proteins in the regulation of actin cytoskeleton in migrating alveolar macrophage.

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The motility of macrophages is essential for the innate immunity system. The efficient movement of macrophages requires close coordination between processes at the leading and trailing edges of the cell. When macrophages sense chemotactic factors, they exhibit a polar morphology that is characterized by the formation of lamellipodia at leading-edge and uropod at the trailing-edge. In the lamellipodia formation, actin filament polymerization produces a protrusive force on the cell membrane that promotes the enlargement of the lamellipodia. In uropod formation, Rho, ROCK and actomyosin bundles, generate forces and maintain contractility posteriorly. The ERM (Ezrin/Radixin/Moesin) proteins are also accumulated to the uropod and link cortical actin-based cytoskeletons to the plasma membrane, thereby regulating morphology and motility. However, the relationship between actin-membrane linkage and actin polymerization is still remain elusive. In addition, previous findings are based on experiments with bone marrow-derived immune cells and it is not known whether similar results can be achieved with tissue-specific macrophages such as alveolar macrophages. In the present study, we prepared alveolar macrophages isolated from bronchoalveolar lavage fluids of mice and analyzed the involvement of ERM and the actin polymerization system in cell migration. Based on our present findings, we will discuss the coupling between actin-membrane linkage and actin polymerization in directed cell migration of alveolar macrophages.

1-B-P-061 一般演題（ポスター）

Production of Bleomycin-induced pulmonary fibrosis model mice by oropharyngeal aspiration and evaluation of the efficacy of Nintedanib

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Idiopathic pulmonary fibrosis (IPF) is a progressive disease that causes respiratory failure. However, treatment for IPF is limited because there are no effective therapeutic agents to date. Since it has been known that bleomycin, an anticancer drug, causes pulmonary fibrosis, IPF model animals have been produced by administration of bleomycin in the lung. In the present study, we attempted to create the IPF model by oropharyngeal aspiration (OPA) of bleomycin and the effects of Nintedanib were evaluated. C57BL/6N male mice were administered bleomycin via OPA at day 0. Nintedanib at 10 or 30 mg/kg were orally administered twice a day from days 0 to 21. We evaluated the effects of Nintedanib by means of Micro-CT lung imaging, quantification of cytokine levels in bronchoalveolar lavage fluid (BALF), hydroxyproline (HYP) level in the lung, pulmonary function test, and pathological examinations in the lung. As results, the control group showed higher levels of HYP, as well as TGF-β1, IL-12 (p40) and eotaxin in BALF than those in the normal group, showing induction of fibrosis. The Nintedanib 30 mg/kg group showed lower levels of HYP, TGF-β1 and IL-12 (p40) than those in the control group. These results indicated that Nintedanib has anti-fibriotic effects on IPF.

1-B-P-062 一般演題（ポスター）

Pharmacological studies on COPD model induced by exposure to cigarette smoke and poly(I:C) in mice

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Creating an animal model of pulmonary emphysema, a type of COPD, requires exposure to cigarette smoke (CS) for more than 3 months. The pulmonary emphysema model was induced by combination of CS exposure and polyinosinic-polycytidylic acid [poly(I:C)] administration for 4 weeks in mice, and the effects of anti-thymic stromal lymphopoietin (TSLP) antibody (Ab) and rolipram (Rol) were examined.

CS was exposed for 4 weeks (Days 1 to 26) and poly(I:C) was administered nasally for 4 days. From Days 15 to 25, the anti-TSLP Ab was nasally administered every other day, and Rol was orally administered daily. In the model, the peak expiratory flow (PEF) and forced expired volume at 0.05 sec/forced expired volume ($FEV_{0.05}$/FVC) were decreased, and the tissue damping, inflammatory cells in bronchoalveolar lavage fluid (BALF) and lung tissue, and pulmonary air space size were increased on Day 27. The anti-TSLP Ab inhibited the decrease in PEF and the increase in eosinophils in BALF, but Rol did not suppress these changes.

Based on these results, in the pulmonary emphysema model, respiratory function depression, inflammatory cells infiltration and alveolar expansion were observed, and the anti-TSLP Ab inhibited the respiratory function depression and inflammatory cells infiltration, but the effect of Rol was not clear.

1-B-P-063 一般演題（ポスター）

Endothelium-dependent and -independent vasodilator effects of propyl gallate

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It has been reported that gallic acid induced vasocontraction and vasorelaxation. However, the effects of ester compound of gallic acid in vasoconstriction remain unclear. This study investigated the mechanism of vasorelaxation induced by propyl gallate (PG) in the endothelium-intact (E (+)) and -denuded (E (-)) rat aorta. 1) PG phenylephrine (PE)-induced contraction in a dose-dependent manner on the E (+) and E (-) rat aorta. However, this inhibitory effect was stronger in E (+) aorta than that in E(-) aorta. 2) 4-aminopylidine, apamin or SQ22536 significantly recovered PG-evoked PE-induced contraction in E (-) aorta. 3) PG induced increases cAMP levels in E (-) aorta. 4) Pretreatment with L-NAME, but not indomethacin, significantly reduced PG-evoked E (+)-dependent vasorelaxation. 5) PG induced increases of eNOS phosphorylation in HUVECs. Pretreatment with Akti-1/2, a Akt inhibitor, or LY294002, PI3K inhibitor, but not PP2, Src inhibitor, significantly reduced PG-evoked eNOS phosphorylation. In conclusion, these results suggested that PG-induced endothelium-independent vasorelaxation partly related to activating K+ channel and/or cAMP pathway. Moreover, PG-induced endothelium-dependent vasorelaxation related to NO release from endothelium by activating eNOS via PI3K/Akt signal pathway.

1-B-P-064 一般演題（ポスター）

Coix seed polysaccharides alleviate type 2 diabetes mellitus via gut microbiota-derived short-chain fatty acids activation of IGF1/PI3K/AKT signaling

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ABSTRACT

Type 2 diabetes mellitus (T2DM) has become a worldwide concern in recent years. Coix seed (CS) as a homologous substance of traditional Chinese medicine and food, its polysaccharides can improve the symptoms of patients with metabolic disorders. Since most plant polysaccharides are difficult to digest and absorb, we hypothesized that Coix seed polysaccharides (CSP) exert hypoglycemic effects through the gut. In this study, the underlying mechanisms regulating hypoglycemic effects of CSP on a T2DM mouse model were investigated. After treatment with CSP, serum insulin and high-density lipoprotein cholesterol levels were increased, while total cholesterol, triglycerides and low-density lipoprotein cholesterol levels were decreased in T2DM mice. In addition, CSP treatment helped repair the intestinal barrier and modulated the gut microbial composition in T2DM mice, mainly facilitating the growth of short-chain fatty acid (SCFA)-producing bacteria, Spearman's analysis revealed these bacteria were positively related with the hypoglycemic efficacy of CSP. Colonic transcriptome analysis indicated the hypoglycemic effect of CSP was associated with the activation of the IGF1/PI3K/AKT signaling pathway. Correlative analysis revealed that this activation may result from the increase of SCFAs-producing bacteria by CSP. GC-MS detection verified that CSP treatment increased fecal SCFAs levels. Molecular docking revealed that SCFAs could bind with IGF1, PI3K, and AKT. Our findings demonstrated that CSP treatment modulates gut microbial composition, especially of the SCFAs-producing bacteria, activates the IGF1/PI3K/AKT signaling pathways, and exhibits hypoglycemic efficacy.

1-B-P-065 一般演題（ポスター）

Vasodilator effects of Soy Isoflavone Fermentation Products

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Vasodilator effects of Soy Isoflavone Fermentation Products.

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It has been reported that isoflavones induced vasorelaxation. However, the effects of hydroxylated isoflavones in vasoconstriction remain unclear. This study investigated the mechanism of vasorelaxation induced by soy IF yeast fermented product (soyF) containing hydroxylated isoflavones in rat aorta. 1) SoyF inhibited KCl and phenylephrine (PE)-induced contraction in a dose-dependent manner. However, these relaxations were not difference between endothelium-intact or -denuted aorta. The inhibitory effect was stronger in PE-induced contraction than that of KCl-induced contraction. 2) Moreover, soyF significantly inhibited U46619-induced contraction and PDBu-induced contraction in Ca-free PSS. 3) Pretreatment with SQ22536, but not ODQ, significantly reduced soyF-evoked inhibition of PE-induced contraction. 4) Forskolin inhibited PE-induced contraction, and pretreatment with soyF significantly enhanced the vasorelaxation of forskolin. 6) SoyF significantly increased cAMP levels. Moreover, higher cAMP levels were obtained with soyF plus forskolin treatment than the total cAMP levels obtained with separate soyF and forskolin treatments. In conclusion, these results suggested that soyF-induced vasorelaxation related to inhibition of contractile element by partly increasing cAMP level via phosphodiesterase inhibition.

1-B-P-066 一般演題（ポスター）

Red ginseng extract modulates cellular energy homeostasis and protects against cell death during nutrient deprivation.

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Red Ginseng Extract (RGE) is known to have various health benefits such as fatigue relief and tonicity. However, the effects of RGE on intracellular energy metabolism are not well understood. In this study, we treated HK-2 cells, a human proximal tubular cell line, with RGE and examined the effects on cell proliferation and changes in intracellular metabolites. First, RGE was found to promote cell proliferation in HK-2 cells in a concentration-dependent manner; LC-MS/MS analysis revealed that RGE significantly increased intracellular pantothenate, proline, and glutathione levels. In contrast, extracellular (culture medium) pantothenate levels decreased in a RGE concentration-dependent manner, suggesting that RGE enhances intracellular uptake of pantothenate. Pantothenate is synthesized intracellularly to coenzyme A (CoA), which is then converted to acetyl CoA and involved in various intracellular events, including energy metabolism. We found that inhibition of the synthesis of coenzyme A from pantothenate inhibited the effect of RGE on HK-2 cell proliferation. These results suggest that RGE activates cell proliferation via an increase in intracellular pantothenate levels and is involved in the activation of antioxidant effects and energy metabolism. Next, we investigated the protective effect of RGE during nutrient starvation in HK-2 cells. The results showed that RGE abrogated intracellular stress signals and inhibited cell death in HK-2 cells during acute nutrient starvation. Collectively, our results suggest that RGE acts as a regulator of intracellular energy metabolism under both nutrient and starvation conditions.

1-B-P-067 一般演題（ポスター）

Inhibitory effect of Brazilian propolis (AF-08) components on platelet aggregation

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Brazilian propolis (AF-08) is a dietary supplement containing various flavonoids and possesses many biological activities. Flavonoids and a diet of fruits and vegetables containing them have been shown to reduce the risk of cardiovascular diseases (CVDs). Most of CVDs are caused by the formation of arterial thrombi due to the close interaction of platelet aggregation and blood coagulation. We have previously shown that AF-08 inhibits platelet aggregation without affecting blood coagulation. In the present study, we compared the effects of AF-08 components on the inhibitory effect of AF-08 on platelet aggregation.

Major constituents of AF-08 (apigenin, kaempferol, chrysin, *trans*-cinnamic acid, and artepirin C) were performed quantative analysis by HPLC system. Human platelet-rich plasma (PRP) was obtained from human blood anticoagulated with sodium citrate and incubated with serial dilutions of AF-08 components for 10 min to assess its inhibitory effect on platelet aggregation caused by collagen.

Among the major constituents of AF-08, apigenin and chrysin inhibited platelet aggregation, although kaempferol, *trans*-cinnamic acid, and artepirin C did not affect platelet aggregation. The inhibitory effect of AF-08 on platelet aggregation was suggested to be partially due to apigenin and chrysin. Therefore, AF-08 containing apigenin and chrysin may be effective in suppressing platelet-based arterial thrombus formation and reducing the risk of CVDs.

1-B-P-068 一般演題（ポスター）

Orengedokuto inhibits TNF-α-induced HAVIC calcification obtained from calcific aortic valve stenosis patients

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Although calcific aortic valve stenosis (CAVS) is the most common heart valve disease in aging society, there is no effective medical treatment. Although orengedokuto (OGT), a herbal medicine, was used for treating inflammatory symptoms, the effect of OGT on aortic valve calcification (AVC) is unknown. So, we aimed to discover whether OGT inhibits AVC. Calcified AV were obtained from CAVS patients. Human aortic valve interstitial cells (HAVICs) were isolated from AV by collagenase treatment and cultured in α-MEM with 10% FBS. The medium containing TNF-α (30 ng/mL) was replenished every 3-4 days. We acknowledged that TNF-α strongly accelerated HAVICs calcification. We found that OGT (10-30 μg/mL) inhibited TNF-α-induced HAVIC calcification, significantly, which maintained cell viability. The elevation of alkaline phosphatase activity and gene expression of bone morphogenetic protein 2 induced by TNF-α was significantly inhibited by OGT (10 μg/mL). OGT also inhibited the acceleration of AVC in spontaneously hypertensive rat caused by aging and hypertension confirmed *in vivo*. These data showed that OGT inhibited AVC both *in vitro* and *in vivo*. The molecular mechanism of OGT inhibiting AVC needs to be further clarified for establishing medical therapy of CAVS.

1-B-P-069 一般演題（ポスター）

Consideration about the issue of education of the harmful effect of drugs: from the viewpoint of pharmacology of nursing

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It is understood that the safety management of drugs is important, but it is a fact that a medical accident regarding drugs still happens. In the case of pharmacotherapy, we have carried on a team medical care, namely the doctor writes a prescription, the pharmacist dispenses according to a prescription, and then the nurse medicates a patient. Therefore, we believe that the nurse needs to acquire knowledge and skill about the safety management of drugs, to prevent the occurrence of harmful effect of drugs in the current medical care. In response to the voices of the victim of harmful effect of drugs in Japan, Ministry of Education, Culture, Sports, Science and Technology have surveyed since 2010 on the education to attain the extermination of the harmful side effect induced by unsuitable using drugs in each school of medicine, dentistry, pharmacy, and nursing. According to the report of 2022, we can confirm to learn about harmful effect of drugs at all universities of medicine, dentistry, and pharmacy except Kanazawa Medical University. However, in school of nursing, only 254 schools out of 299 carried out the education of harmful effect of drugs. In this study, we analyzed status and issues of pharmacology education in the education of nursing, from the viewpoint of the education for harmful effect of drugs. In addition, we examined what is the requirement as a nurse to check and prevent the harmful side effect induced by unsuitable using drugs if we could find the suitable education method of harmful effect of drugs because a school hour was tight and limited on the school of nursing. We carried out this study with the approval of the Saitama Prefectural University Ethical Review Board (21054). As a result, 76% of students could explain or know thalidomide incident. Only 34% of students could understand the difference between harmful effect and drug abuse, until they took a pharmacology class. On the other hand, it became clear that 84% of students thought that it was possible to prevent or relieve harmful effect of drugs as a nurse.

1-B-P-070 一般演題（ポスター）

Autophagy is involved in the branching morphogenesis of the fetal mouse submandibular gland

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Autophagy is defined as a mechanism that transports cellular components to lysosomes for degradation. This mechanism also plays a role in the removal of unwanted organelles, in addition to providing nutrients as a starvation response by recycling the degradation products. The mouse submandibular gland (SMG) rudiment is very small organ such as approximately 300 μm in diameter at embryonic day 13 (E13). The epithelium of SMG rudiment then undergoes active proliferation in contact with the mesenchyme and develop the duct systems in the gland (branching morphogenesis). In this study, we investigated whether autophagy is involved during salivary gland organogenesis. The organs of E13 SMG rudiments were cultured with Bafilomycin A1 or Torin 1 which are inhibitor or promoter of autophagy reactions, respectively. Cultured SMGs were photographed, counted the number of endpieces and analyzed the area of epithelial rudiments. Moreover, the autophagy related proteins such as Atg5, LC3- Ⅰ/Ⅱ and p62 were analyzed by Western blotting. Bafilomycin A1 suppressed branching morphogenesis of E13 SMG rudiments. The suppression of branching morphogenesis was also showed in administration of Torin 1 in the rudiments. The Atg5, LC3- Ⅰ/Ⅱ and p62 proteins in cultured SMG rudiments were induced by Bafilomycin A1. These results suggest that autophagy is involved in the branching morphogenesis of developing fetal mouse SMG.

1-B-P-071 一般演題（ポスター）

Survey of a University Nursing Students‘ Self-Learning and Perceptions of Medication in Hospital Practice

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Purpose: This study investigated self-learning and perceptions of medication in university nursing students in hospital practice.

Methods: The participants were second- and third-year nursing students studying at a university and had completed their hospital nursing practice in 2022. The data were collected using web-based survey through Microsoft Forms. All items were investigated with their relationships to year of study. This study was approved by the university‘s Nursing Research Ethics Review Committee.

Results:Responses were received from 20 second-year students and 26 third-year students. It was found that a significantly higher percentage of third-year students than second-year students self-learned about medication side effects. A higher percentage of third-year students than second-year students used the Drug Information(DI) in the electronic medical records in their study. No significant differences by year were seen in the other items.The percentage of students who reported that they could collect and observe information on medication was over 80% for both second and third-year students. However, only 40% in both years reported being able to provide support for medication.

Conclusion:Educational strategies are needed to enhance nursing experience and learning about medication during training.

1-B-P-072 一般演題（ポスター）

Analysis of Selectively Enriched RNAs in Extracellular Vesicles

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Extracellular vesicles (EVs) play a crucial role in transporting functional RNAs to target recipient cells in various physiological processes, holding great potential for applications in therapy and diagnostics. The highly diverse EVs have different RNA profiles from their originating cells, indicating a selective and active loading process for specific RNAs. However, the precise molecular mechanisms governing the selective loading of mRNA into EVs remain undiscovered.

In our research, we focused on small EVs (sEVs) obtained through sucrose density gradient ultracentrifugation and found regarding RNA loading into sEVs. We identified RAB13 as one of the enriched RNAs in sEVs, and we revealed that a specific segment of the 3' untranslated region of the RAB13 gene plays a crucial role in concentrating RNA content within sEVs. This finding has the potential to enable technology for loading targeted RNAs into sEVs, which could lead to more efficient and novel targeted therapies and diagnostics.

1-B-P-073 一般演題（ポスター）

Construction of a new extracellular vesicles isolation method and its quantification system

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Extracellular vesicles (EVs) are known to play an important role in intercellular communication to deliver bioactive molecules such as proteins, nucleic acids, and lipids. Therefore, EVs are attractive research topics in the therapeutic and diagnostic areas. The foundational technology of EVs research is EVs isolation, but there are issues with reproducibility and purity. The reasons for this are,

(1) Manual operation is required for many EVs isolation methods.

(2) Low purity of isolated EVs makes stable EVs isolation difficult.

(3) It is difficult to construct a quantifiable evaluation system for isolated EVs.

Then, we have developed a fully automated method to isolate EVs by combining an immunoprecipitation-based isolation method with reagents that minimize the influence of non-EVs components. This method enables the isolation of EVs with reproducibility, purity, and stability. Furthermore, it is also possible to quantify EVs by system for EVs marker-specific detection of isolated EVs by chemiluminescence enzyme immunoassay (CLEIA). Here we report various data obtained related to biomaterial-derived EVs using our EVs isolation method and quantification system by EVs-CLEIA.

1-B-P-074 一般演題（ポスター）

Isolation of plasma extracellular vesicles excluding lipoproteins by using polyanion and a divalent cation

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Extracellular vesicles (EV), cell-derived spherical particles enclosed by a lipid-bilayer, contain various molecules and mediate cell-to-cell communication. EV in plasma have been expected as potential disease biomarkers. However, the high concentration of lipoproteins in plasma, which have similarities in size, density, and contents to EV, hampers analysis on plasma EV. To overcome this issue, we aimed to develop an effective isolation method for plasma EV excluding lipoproteins (HDL, LDL/VLDL), by using polyanion and a divalent cation. Human plasma was mixed with 1) phosphotungstic acid and $MgCl_2$, 2) heparin and $MnCl_2$, and 3) polyethylene glycol (PEG). The mixture was centrifuged to separate a supernatant and pellet. 4) Dextran sulfate-conjugated cellulose (DEC) beads were used for the additional depletion of LDL/VLDL. The protein expression of Apo A-I (HDL marker), Apo B (LDL/VLDL marker), and CD9 (EV marker) in supernatant and pellet was measured by ELISA. Negative staining was performed for the observation of particle shape by using transmission electron microscopy. 1) phosphotungstic acid and $MgCl_2$ or 2) heparin and $MnCl_2$ could not remove lipoproteins from plasma EV. In contrast, 3) PEG could remove HDL and most of LDL/VLDL from plasma EV. Unfortunately, 4) The DEC beads removed not only LDL/VLDL but EV from the pellets of plasma mixed with PEG. Exploring the higher yielding method for isolation of plasma EV with higher purity is required, and further research is necessary to compare this method with conventional EV isolation ones.

The roles of excitation-transcription coupling in vascular smooth muscle cells: Optogenetic insight

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Prolonged stress on blood vessels induces chronic inflammation, as well as the dedifferentiation and proliferation of vascular smooth muscle cells (VSMCs), leading to vascular remodeling. Pressure overload, one of the major stresses causing vascular remodeling, depolarizes VSMCs and results in Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCCs). We have hypothesized that sustained depolarization induces excitation-transcription coupling (E-T coupling) in VSMCs and upregulates proinflammatory genes mainly using *ex vivo* preparations. To examine if sustained depolarization of VSMCs induced the E-T coupling and vascular remodeling in live mice, we utilized an optogenetic approach using mice expressing ChR2 specifically in smooth muscle (SMC-ChR2-YFP). We confirmed VSMC-specific expression of ChR2 in the carotid arteries of SMC-ChR2-YFP mice. Light stimulation successfully induced an increase in cytosolic $[Ca^{2+}]$ through VDCCs in VSMCs freshly isolated from mouse carotid arteries. Similar results were obtained in tissue preparations from carotid arteries. Furthermore, light stimulation of the carotid arteries *in vivo* induced pro-inflammatory gene transcription. These results suggest that sustained depolarization specific to VSMCs can cause E-T coupling *in vivo* and trigger vascular inflammation, which potentially lead to vascular remodeling.

1-B-P-076 学生セッション（ポスター）

Inhibitory effect of CD34 on human aortic valve calcification

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While various osteogenic factors induce the ectopic calcification of human aortic valve, the cellular origin of valve calcification remains unclear. Recently, we have demonstrated that human aortic valve interstitial cells (HAVICs) are positive on both mesenchymal stem cell markers (CD73, 90, 105), and endothelial cell marker (VEGFR2), then CD34-negative cells are responsible for aortic valve calcification. While normal HAVICs are physiologically CD34-positive, the pathophysiological role of CD34 in HAVICs obtained from calcified aortic stenosis patients is yet unknown. To confirm whether the transformation from CD34-positive to -negative occurs in HAVICs, we compared the effect of two cytokines; TNF-α and TGF-β. Interestingly, TNF-α unlike TGF-β induced HAVIC calcification, decreasing the gene expression of CD34 and extracellular matrix proteins, tenascin X and MGP. Overexpression of CD34 significantly inhibited TNF-α-induced HAVIC calcification, maintaining tenascin X and MGP gene expression. Further, we confirmed that, in CD34-negative cells, lower expression of tenascin X was shown in comparison to CD34-positive cells by using a DNA microarray. These results suggest that the role of CD34 is to inhibit the HAVIC calcification by maintaining the expression extracellular matrix proteins such as Tenascin X.

1-B-P-077 学生セッション（ポスター）

Pathophysiological roles of a macromolecular complex of the cardiac KCNQ1 channel

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The cardiac I_{Ks} channels composed of both alpha subunit KCNQ1 and beta subunit KCNE1 contribute to the repolarization phase of cardiac action potential. Mutations of I_{Ks} channel genes are associated with the development of lethal arrhythmias due to congenital QT prolongation syndrome. Furthermore, these arrhythmias are affected by various intracellular regulators such as Ca^{2+}, CaM, ATP, PKA, and NO via KCNQ1 molecular complex. Our results suggest that membrane proteomics have a relationship between these complexes and sepsis-related signaling. However, this relationship during sepsis has not been elucidated.

Therefore, we test whether I_{Ks} channels play a role in sepsis-induced cardiac dysfunction by using genetically engineered (I_{Ks}-Tg) mice (13-20 weeks, genetic background: C57BL/6J) which express human I_{Ks} channels (tandem protein of KCNE1 and KCNQ1). A sepsis model, the Cecal Slurry (CS) intraperitoneal injection technique, was employed to investigate the effect of cardiac-specific I_{Ks} channel expression. We showed that the sepsis score of I_{Ks}-Tg male mice (n = 7) was significantly lower than that in age-matched wild-type male mice (n = 19). To seek the mechanisms, we test the effects of CS on action potential between I_{Ks}-Tg mice and wild type mice by path-clamp method. Action potential duration (APD) was prolonged in 1-day wild-type male mice after CS injection. However, CS did not change APD in the I_{Ks}-Tg male mice. These results suggest that the I_{Ks} channels have a protective role in CS-induced APD prolongation.

1-B-P-078 学生セッション（ポスター）

Involvement of peptidylarginine deiminase 2 and 4 in the pathogenesis of TNBS-induced colitis in mice

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Peptidylarginine deiminase (PAD) is an enzyme, which citrullinates arginine residues of proteins. PAD4 is known to be an important factor in the induction of neutrophil extracellular traps (NETs), which are implicated in the pathogenesis of various inflammatory diseases. PAD2 is also reportedly involved in the pathogenesis of inflammatory diseases, but the details are not fully understood. In this study, we investigated the pathogenic roles of PAD2 and PAD4 in inflammatory bowel disease using a trinitrobenzene sulfonic acid (TNBS)-induced murine colitis model. PAD2- and PAD4-deficient (PAD2KO and PAD4KO) mice were generated by CRISPR-Cas9-mediated genomic editing. Colitis was induced by an intrarectal injection of TNBS. TNBS injection produced severe colitis, accompanied by body weight loss, increase in myeloperoxidase activity, and inflammatory cytokine expression in wild-type (WT) mice. In contrast, the severity of colitis with these inflammatory responses was significantly reduced in either PAD2KO or PAD4KO mice. NETs formation in peritoneal neutrophils was significantly suppressed in PAD4KO but not PAD2KO mice compared with WT mice. In contrast, macrophage extracellular traps formation in peritoneal macrophages was affected neither PAD2KO nor PAD4KO mice. In conclusion, PAD2 and PAD4 contribute to the pathogenesis of TNBS-induced colitis. PAD4 is involved in NET formation while PAD2 is not involved in the NET formation, suggesting that PAD2 may contribute to the progression of colitis via a mechanism different from NETs formation and PAD4.

1-B-P-079 学生セッション（ポスター）

Investigation of factors contributing to fibrosis in chronic ulcerative colitis in mice

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Chronic inflammatory disease leads to excessive fibrosis in the intestine. Statin drugs that inhibit the Ras and Rho pathways may be useful in fibrotic diseases. Therefore, we established an experimental animal model of colorectal fibrosis and exmamined the effect of pravastatin on intestinal fibrosis. Male C57BL/6N mice were given dextran sulfate sodium (DSS) ad libitum for 7 days, followed by a 14-day rest period. This was repeated for one cycle up to a maximum of three cycles. Body weight and fecal condition were measured nd expressed as Disease Activity Index (DAI). 14-day rest period within the third cycle (Day 49-63), pravastatin was administered orally once daily for 14 days. In the DSS drinking group (control group), DAI increased and colon length shortened. Furthermore, collagen fibers and type I collagen increased in the submucosal tissue. p-SMAD3 and Rock1/RhoA expression increased. Pravastatin treatment had no effect on DAI and colon length compared to the control group. In addition, collagen fibers and type I collagen in the submucosal tissue increased as in the control group. However, the ratio of Rock1/RhoA expression was significantly decreased compared to the control group. Repeated treatment with DSS was shown to cause fibrosis with increased type I collagen in the submucosal tissue. Pravastatin inhibited ROCK1 but it failed to inhibit fibrosis. It is hypothesized that pravastatin may have contributed to the increase in fibrosis by decreasing geranylgeranyl pyrophosphate expression and exacerbating inflammation.

1-B-P-080 学生セッション（ポスター）

Deficiency of leukotriene B4 receptor type 1 (BLT1) ameliorates ovalbumin-induced allergic enteritis in mice

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Leukotriene B4 receptor type 1 (BLT1), a high-affinity receptor for leukotriene B4 (LTB4), plays an important role in inflammatory responses, including allergic airway inflammation. In this study, we examined the effect of genetic BLT1 deletion (KO) on ovalbumin (OVA)-induced allergic enteritis, a gastrointestinal form of food allergy in mice. Repeated oral OVA challenges after sensitization with OVA/alum induced allergic enteritis, characterized by systemic allergic symptoms (scratching, immobility, and swelling), diarrhoea, colonic oedema, and colonic goblet cell hyperplasia, accompanied by increased colonic peroxidase activity, colonic inflammatory cytokine expression, and serum OVA-specific IgE levels. The severity of enteritis was significantly attenuated in KO mice compared with wild-type (WT) mice, without an increase in serum OVA-specific IgE levels. The accumulation of neutrophils, eosinophils, M2-macrophages, dendritic cells, CD4+ T cells, and mast cells was observed in the colonic mucosa of allergic enteritis, and such accumulations were significantly lower in KO mice than in WT mice. BLT1 expression was upregulated and colocalized mostly in neutrophils and partly in eosinophils and dendritic cells in the colonic mucosa of allergic enteritis. These findings indicate that BLT1 deficiency ameliorates OVA-induced allergic enteritis in mice, and that LTB4/BLT1 contributes to neutrophil and eosinophil accumulations in the allergic colonic mucosa. BLT1 is therefore a promising drug target for the treatment of food allergies.

Ca^{2+}-highly permeable TRPV6 regulates colonic mucosal barrier functions to protect against dextran sulfate sodium-induced murine colitis

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Transient receptor potential vanilloid 6 (TRPV6), which is a highly Ca^{2+}-permeable cation channel, is expressed in gastrointestinal epithelia and implicated in maintaining Ca^{2+} homeostasis via transcellular Ca^{2+} transport. In this study, we investigated the local roles of TRPV6 in colonic mucosal barrier functions and pathogenesis of colitis in mice. Colitis was induced in TRPV6KO and wild-type (WT) mice by 7-days treatment with dextran sulfate sodium (DSS). Intestinal permeability was evaluated by FITC-dextran methods. The colonic mucus secretion determined histochemically, epithelial cell proliferation determined immunohistochemically, and expression of adherence and tight adherence junction proteins determined by western blotting were examined to evaluate the mucosal barrier functions. The severity of DSS-induced colitis was significantly aggravated in TRPV6KO mice compared with WT mice. Intestinal permeability was also significantly increased in TRPV6KO mice compared with WT mice even in normal conditions (without DSS). Although there is no difference in the colonic mucus secretion and epithelial cell proliferation between WT and TRPV6KO mice, the expression of adherence junction protein E-cadherin, and tight junction protein claudin-3 and occludin was significantly lower in TRPV6KO than WT mice in normal conditions. These findings suggest that TRPV6 plays a critical role in regulation of mucosal barrier functions via expression of adherence and tight junction proteins to protect against colitis.

Protective role of peptidylarginine deiminase 2 and 4 in pathogenesis of DSS-induced colitis in mice.

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Peptidylarginine deiminase (PAD) is an enzyme, which citrullinates arginine residues of proteins, and is involved in extracellular traps (ETs). ETs are phenomenon in which histones and granulocyte proteins are released from neutrophils and macrophages into the extracellular space with DNA, and are associated with the pathogenesis of various inflammatory diseases. PAD4 is expressed mainly in neutrophils and contributes to ETs in neutrophils while PAD2 is widely expressed including macrophages. We recently showed that PAD4-derived ETs plays a detrimental role in TNBS-induced colitis. In the present study, we investigated the role of ETs derived from PAD2 and PAD4 in DSS-induced colitis. Colitis was induced in male mice lacking PAD2 (PAD2KO) and PAD4 (PAD4KO) mice by DSS solution for 7 days. DSS treatment caused severe colitis accompanied by weight loss, diarrhea, and bloody stool. Further, increase in MPO activity and inflammatory cytokine expression as well as induction of ETs were observed. The severity of colitis with increase in MPO activity and cytokine expression was significantly exacerbated in either PAD2KO or PAD4KO mice compared with wild-type (WT) mice. In particular, rectal bleeding were typically observed in these KO mice. These findings suggest that PAD2 and PAD4 play the protective role in DSS-induced colitis. Thus, PAD2 and PAD4 may have a different involvement in the pathogenesis of DSS- and TNBS-induced colitis.

1-B-P-083 学生セッション（ポスター）

Exploration of glycyrrhizin derivatives exploiting bioconversion by endophyte inside licorice root.

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Almost all plants are not axenically and have commensal “endophyte” in their body. We yielded slightly modified 2 derivatives of glycyrrhizin from licorice endophyte. Glycyrrhizin has been known to inhibit high-mobility group box (HMGB)1, a proinflammatory cytokine-like protein, by direct interaction. Here we investigated the effect of the glycyrrhizin derivatives on HMGB1 with primary-cultured macrophage. Both derivatives were hydroxylated at the C -15 position and one was further ketonated at the C-3 position. These derivatives had lower toxicity than glycyrrhetinic acid; however, lost the ability to inhibit HMGB1. This was probably due to increased polarity at the C -15 position. Accordingly, taking advantage of ketone at the C-3 position, we synthesized a methyl ester at the C-30 position. This compound suppressed the interleukin-1b mRNA expression more strongly than glycyrrhizin in macrophages challenged by HMGB1, and showed neuroprotection based on edema relief in an *in vivo* murine intracerebral hemorrhage (ICH) model. These results show that this glycyrrhizin derivative is a promising compound for the ICH therapy.

1-B-P-084 学生セッション（ポスター）

Whole-brain mapping of neuronal activation by peripheral inflammation

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While inflammation is a natural process, chronic or excessive inflammation in the brain can be harmful. After administration of neuroinflammatory compounds such as lipopolysaccharide (LPS), its signals reach the brain through three main pathways: (1) humoral pathway, (2) afferent vagal pathway, and (3) spinal neuronal pathway. This study aims to gain new insights into mechanisms underlying inflammation-induced brain activation. Neuronal activity is evaluated by c-Fos immunostaining in the LPS-induced inflammation states. LPS activated neurons in the medial prefrontal cortex (mPFC), anterior cingulate cortex (ACC), insular cortex (IC), hypothalamic paraventricular nucleus (PVN), basolateral amygdala (BLA), thalamic paraventricular nucleus (PVT), hippocampus ventral CA1 (vCA1), locus coerules (LC) and nucleus tractus solitarius (NTS). The activation of the ACC, IC, PVN, and BLA was blocked by intracerebroventricular injection of alloxan, a toxic material to destroy ependymal cells around the ventricle, while the activation of the mPFC, ACC, vCA1, LC, and NTS was blocked by vagotomy. These results suggest that ACC, IC, PVN, and BLA may transmit peripheral inflammatory signals to the brain via the humoral pathway, while mPFC, ACC, vCA1, LC, and NTS transmit signals via the afferent vagal pathway.

1-B-P-085 学生セッション（ポスター）

Investigation of the transcriptional regulation of inflammation-related genes by the CCR4-NOT complex in the pathophysiology of ARDS/Acute Lung Injury.

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Acute respiratory distress syndrome (ARDS) is an acute lung injury with high mortality, linked to various factors like sepsis, aspiration, pneumonias, and SARS-CoV-2 infections. No effective drugs are currently available, necessitating further understanding of ARDS's pathogenesis. The CCR4-NOT complex, a large multimeric protein complex, contributes to mRNA regulation, including transcription, translation, and degradation. While the significance of cytokine mRNA degradation in inflammation has been postulated, the roles of CCR4-NOT complex-mediated mRNA decay remain elusive. We studied the CCR4-NOT complex's role in acute lung injury using Cnot3 heterozygous mice, which are induced by tamoxifen treatment in *Cnot3*$^{flox/+}$;*Cre-ERT*$^{Tg/+}$ mice and partly impair CCR4-NOT-mediated mRNA degradation. Cnot3 heterozygous mice showed severe lung injury following tracheal acid instillation, as evidenced by increased wet to dry ratio and lung histology, compared with wild type mice. Cytokine mRNA levels, including IL-1b, NOS2, and CCL2, were significantly elevated in the lungs of Cnot3 heterozygous mice. The mRNA decay rates remained unaffected in Cnot3 heterozygous MEFs, but transcription levels were upregulated. RNA-seq and subsequent pathway analysis revealed PU.1 is likely involved in enhanced IL-1b expression. Further studies for the transcription and mRNA degradation of inflammatory genes by the CCR4-NOT complex in severe acute lung inflammation should contribute to development of novel RNA therapeutics in ARDS.

1-B-P-086 学生セッション（ポスター）

Inhibitory effect of selective serotonin reuptake inhibitors on inflammatory cytokine production in immune cells

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Sepsis is a systemic inflammatory syndrome triggered by infections. Controlling the abnormal cytokine-producing pathology (cytokine storms) that contributes to the severity of sepsis is thought to be an effective treatment, but there is no specific drug for this as yet. Recently, there have been many reports that antidepressants, selective serotonin reuptake inhibitors (SSRIs), have anti-inflammatory effects. In fact, some SSRIs are in clinical trials for application in the treatment of some inflammatory diseases. In this study, to clarify the usefulness of SSRIs as "cytokine storm inhibitors," we aimed to elucidate their effects on the production of inflammatory cytokines (interleukin-6; IL-6) in immune cells. In murine macrophages and dendritic cells, SSRIs significantly suppressed IL-6 production induced by Toll-like receptor 3 (TLR3), TLR4 or TLR9 agonist, but not by TLR7 agonist. In murine lymphocytes, SSRIs also suppressed IL-6 production induced by T cell activator. According to a comparison of five SSRIs, fluoxetine, which is a potent inhibitor of IL-6 production and has low toxicity, was considered the most desirable SSRI as a cytokine storm inhibitor. An examination of the structural requirements indicated that the nucleophilicity of the N atom of fluoxetine has a critical role in anti-inflammatory effect. Overall, our findings suggest that SSRIs, especially fluoxetine, may represent an unprecedented cytokine storm inhibitor with a multifaceted anti-inflammatory effect.

1-B-P-087 学生セッション（ポスター）

Solubilization of insoluble glycyrrhizin derivative having anti-HMGB1 effect and its biological activity

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We have so far investigated the effect of the glycyrrhizin derivatives on high-mobility group box (HMGB)1. We found a glycyrrhizin derivative having potent anti-HMGB1 activity; however, the derivative was very insoluble. In this study, we succeeded in solubilizing the glycyrrhizin derivative. The glycyrrhizin derivative preparation can be injected intravenously and showed higher transition ratio in the brain than p.o. administration in an *in vivo* murine intracerebral hemorrhage (ICH) model. Intravenous injection of the low-dose (4 mg/kg) glycyrrhizin derivative preparation showed more potent suppression effect on interleukin-1beta mRNA expression than p.o. administration of the suspension of the high-dose (50 mg/kg) glycyrrhizin derivative. Also, intravenous injection of the glycyrrhizin derivative preparation potently suppressed the ICH-induced brain edema. This glycyrrhizin derivative preparation may be a hint for the development of novel ICH treatment medicines.

Role of microsomal prostaglandin E synthase-1 in skin inflammation and T-cell immune response of imiquimod-induced psoriasis in mice.

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Psoriasis is a chronic inflammatory disease associated with abnormalities in the immune system. Microsomal prostaglandin E synthase-1 (mPGES-1), a terminal enzyme for prostaglandin (PG) E_2 biosynthesis, highly expresses in the skin of psoriasis patients. However, the detailed role of mPGES-1 in psoriasis remains unclear. In this study, we investigated the role of mPGES-1 in psoriasis-like skin inflammation induced by imiquimod (IMQ), one of the well-established models of psoriasis. Psoriasis-like skin inflammation was induced in mice lacking mPGES-1 (mPGES-$1^{-/-}$ mice) and wild-type (WT) mice by administrating IMQ. The expressions of mPGES-1 mRNA and protein were highly induced in WT skin by IMQ, which correlated to the increase of skin PGE_2. The production of PGE_2 was abolished in mPGES-$1^{-/-}$ skin, indicating that mPGES-1 is a responsible enzyme for skin PGE_2 production. Interestingly, mPGES-$1^{-/-}$ mice exhibited severer symptoms of psoriasis compared to those of WT mice, indicating the protective role of mPGES-1 in the development of psoriasis. In addition, the skin expression of IL-17A, an aggravating factor for psoriasis, was significantly higher in mPGES-$1^{-/-}$ mice compared to WT mice in response to IMQ. Furthermore, compared with WT mice, the number of IL-17A-producing TCRβ^+ cells was significantly increased in mPGES-$1^{-/-}$ skin, suggesting an importance of mPGES-1 in the IL-17A-related T-cell immune response. Taken together, mPGES-1/PGE_2 system plays a protective role in psoriasis, partly by regulating T-cell immune response associated with IL-17A.

1-B-P-089 学生セッション（ポスター）

Topical treatment of the co-culture supernatant from Lactobacillus and Saccharomyces significantly ameliorates the inflammatory responses in a mouse model of atopic dermatitis via inhibition of cytokine production by epithelial keratinocytes.

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Approximately 10% of dogs suffer from atopic dermatitis (AD) and long-term treatment for AD impacts pet owners both physically and economically. In this study, we focused on the co-culture supernatant from Lactobacillus and Saccharomyces (LS) as new probiotics and examined the efficacy of LS on the development of AD. Anti-inflammatory effect of LS was confirmed using human epithelial keratinocytes (HaCaT). Pro-inflammatory cytokines production by stimulated HaCaT treated with LS were measured. Anti-allergic properties of topical treatment of LS were examined in a mouse model of AD. After AD symptoms were developed, daily topical treatment of LS was performed for 3 weeks. Clinical symptoms were monitored weekly, and immune responses were analyzed. Secretion of IL-6, IL-8, TARC and TNFα by stimulated-HaCaT was significantly decreased by co-cultured with LS. Whereas no significant change of clinical symptom was observed by LS treatment, allergy-related immune reactions including the number of IgE-positive B cells and activated dendritic cells in the local lymph nodes was significantly decreased in the LS treatment group. Our findings suggest that LS has supplemental therapeutic effects on the AD via direct inhibition of inflammatory cytokines released from epithelial keratinocytes.

1-B-P-090 学生セッション（ポスター）

Anti-aging effects of Mongolian Berry Extract on Human Epidermal Keratinocytes

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Mongolian Berry (MB) is a type of goji berry that grows in the highlands of China and Mongolia. Due to its diverse nutritional content, it is expected to have strong antioxidant properties and potential anti-aging effects. Actually, MB contains a significant amount of the antioxidant compound such as proanthocyanin, and our findings indicates that approximately 4 times higher superoxide dismutase activity, an antioxidant enzyme, compared to blueberries.
Oxidative stress refers to a condition in the body where the oxidative forces exceed the antioxidant defenses. Reactive Oxygen Species (ROS) are identified as factors that can induce oxidative stress. ROS are produced during normal physiological processes, but they can also be generated by factors such as inflammation, UV radiation, and radiation. When the body's ability to eliminate ROS cannot keep up with their production, an excess of ROS can lead to DNA damage, protein denaturation, enzyme inactivation, and more. These reactions are closely related to various diseases, including aging and cancer. To prevent oxidative stress, maintaining a well-functioning antioxidant defense mechanism is crucial.
So far, there haven't been detailed studies on MB, and its application in the field of veterinary medicine remains a future challenge. The objective of this study is to comprehensively examine the antioxidant and cell-protective effects of Mongolian Berry on human epidermal keratinocytes.

1-B-P-091 学生セッション（ポスター）

Exploring of anti-colon cancer mechanisms of endocannabinoid 2-arachidonoylglycerol.

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The numbers of cancer patients and deaths by colorectal cancer (CRC) in Japan are increasing every year. Cyclooxgenase-2 (COX-2) is an enzyme and biomarker of CRC that produces prostaglandins (PG) from arachidonic acid and plays important roles in the inflammation. We have been reported that COX-2 expression is induced by PGE_2 stimulation in HCA-7, a human early colon cancer cells.

2-arachidonoylglycerol (2-AG) is an endocannabinoid that has been reported to exhibit anticancer effects against various cancers. However, the detailed mechanisms of anticancer effects of 2-AG have not been clarified. Therefore, the purpose of this study is to elucidate the effects of 2-AG on HCA-7 cells and their mechanisms.

As the results, COX-2 expression induced by PGE_2 was significantly and concentration-dependently inhibited by pretreatment with 2-AG in HCA-7 cells. This effect was not observed with anandamide, a similar endocannabinoid as 2-AG, or their metabolites. Interestingly, 2-AG did not affect the transcriptional activity of COX-2, whereas RT-PCR showed that COX-2 mRNA expression was suppressed by 2-AG.

These results suggest that 2-AG but not anandamide, suppresses COX-2 expression induced by PGE_2 in HCA-7 cells, and that this effect of 2-AG may not be due to transcriptional inhibition, but rather to decrease of mRNA stability.

Development of a Therapeutic Agent for Spinal Cord Injury Using Lipid Mediator Derived from Pig Liver Degradation Products

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Purpose: Lysophospholipids in porcine liver protease degradation products (PLDP) have shown the potential to ameliorate damage in rats with spinal cord injury (SCI) by the amputation method. In this study, we evaluated the effect of PLDP on healing in SCI mice developed using an impactor to reduce variability from different transection methods.

Methods: Mice were subjected to SCI by impact loading with an impactor. PLDP or sterile water was orally administered for 14 days from the day of treatment, and motor function was evaluated with the Basso Mouse Scale (BMS) and the Basso, Beattie, Bresnahan Locomotor Rating Scale (BBB).

Results: The PLDP group displayed a trend towards improvement in motor function over controls according to the BMS, while BBB scores showed a significant amelioration.

Discussion: The impactor method reduced inter-subject differences and demonstrated that PLDP promoted paraplegia recovery. The discrepancy between the BMS and BBB results may have been due to the latter method's more detailed multi-joint assessment of lower limb function.

Summary: Our results indicate that phospholipid components may have therapeutic effects in SCI. Future studies including immunostaining and compositional analysis will clarify the effects of individual lysophospholipids in mice with SCI using the impactor method.

1-B-P-093 学生セッション（ポスター）

Keratide®, a peptide derived from waterfowl feather keratin, contributes to stress resistance of epidermal keratinocytes.

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The skin is the largest organ in the human body and serves as a barrier that protects the body from external stimuli. These functions are gradually lost with aging. Therefore, it is important to maintain the skin functions. In recent years, there have been reports of peptides derived from natural proteins having physiological activity, but there are few reports on the physiological activity of peptides derived from keratin, a protein that constitutes the epidermis. Therefore, we investigated keratide®, a peptide derived from waterfowl feather keratin, to clarify its bioactivity.

We examined the physiological effects of keratide® using keratinocyte cell lines. We found that keratide® improves oxidative stress tolerance via induction of glutathione, and induces expression of filaggrin and involucrin, which enhance skin moisturizing and barrier function. Furthermore, experiments with inhibitors revealed that the expression of filaggrin and involucrin is mediated by the ERK MAPK pathway and the TGF-β pathway.

These results indicate that keratide®, as a bioactive peptide, induces epidermal keratinocytes to tolerate oxidative stress and induces proteins that contribute to skin moisture retention, suggesting that keratide® is pharmacologically useful.

Apelin suppresses pulmonary inflammation and ameliorates COPD pathogenesis in elastase-induced emphysematous mice.

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Chronic obstructive pulmonary disease (COPD) is a refractory respiratory disorder characterized by airway inflammation, emphysema, and mucus retention, primarily classified into two subtypes (airway- and emphysema-dominant type). In recent years, COPD has been recognized not only as a pulmonary disease but also as a systemic inflammatory condition, demanding a comprehensive therapeutic approach with various targets. Recently, the bioactive peptide apelin has gained attention as a therapeutic target for COPD-associated comorbidities. Interestingly, the expression of apelin and its receptor was found to be significantly decreased in various COPD animal models, but the exact impact of apelin on COPD pathogenesis remains unclear. In this study, intraperitoneal administration of apelin did not have any significant impact on COPD pathogenesis in airway epithelial-specific Na^+ channel (ENaC) overexpressing mice (airway-dominant type), which was established in our laboratory. In contrast, intraperitoneal administration of apelin to the elastase model (emphysema-dominant type) significantly reduced the total cell and neutrophil counts in bronchoalveolar lavage fluid (BALF). Additionally, it significantly improved emphysema and suggested that apelin could ameliorate the inflammatory state in emphysema-dominant COPD pathogenesis. This study is the first to reveal the potential of apelin in improving emphysema-dominant COPD pathogenesis, proposing apelin as a novel therapeutic target for this particular subtype of COPD.

1-B-P-095 学生セッション（ポスター）

Melatonin inhibits voltage-gated K_V4.2 channels in rat pinealocytes

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Melatonin is synthesized in and secreted from the pineal glands, which regulates the circadian rhythms. However, the effects of melatonin on pineal ion channels remains unclear. In the present study, the effects of melatonin on voltage-gated K^+ (K_V) channels in rat pinealocytes were examined by whole-cell patch clamp configuration. The application of melatonin reduced pineal K_V currents in a concentration-dependent manner. Expression analysis revealed that K_V4.2 channels were highly expressed in rat pineal glands. In HEK293 cells expressed with K_V4.2 channels, melatonin decreased outward K_V4.2 currents. This inhibition was observed even in the presence of luzindole, an antagonist of melatonin receptors. Melatonin also blocked the activity of K_V4.3, K_V1.5 and K_V1.1 channels in reconstituted HEK293 cells. In pinealocytes treated with K_V4.2 siRNA, melatonin-sensitive K_V currents were attenuated. Furthermore, the application of melatonin caused membrane depolarization in rat pinealocytes. These results strongly suggest that melatonin directly inhibits K_V4.2 channels and results in membrane depolarization in rat pinealocytes.

1-B-P-096 学生セッション（ポスター）

Examination of the biased activities of prostaglandin D_2 metabolite via CRTH2 receptors.

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Chemoattractant receptor-homologous molecule on Th2 cells (CRTH2) receptors belong to a distinct family from the family of other prostanoid receptors. CRTH2 receptors are reported to be mainly coupled to Gi protein, but also to Gq protein. Prostaglandin (PG) D_2 is considered as the major ligand of CRTH2 receptors, however, the metabolites of PGD_2 are also known to activate CRTH2 receptors although the detailed mechanisms haven't been elucidated.

In this study, we aimed to elucidate the functional differences of CRTH2 receptors-mediated signaling activate by PGJ_2, a major metabolite of PGD_2.

Using HEK293 cells stably expressing CRTH2 receptors, the effects of PGJ_2 on cAMP formations as well as intracellular Ca^{2+} concentrations were examined.

As the results, PGJ_2 showed little inhibition of cAMP formation when compared to PGD_2, possibly because of lower affinity to the receptors. However, PGJ_2 could induce similar levels of the intracellular Ca^{2+} concentration as that was induced by PGD_2. Since PGD_2 has been reported to exacerbate allergic inflammations such as asthma by acting on CRTH2 receptors, its metabolite PGJ_2 could augment allergic reaction, by sustaining Ca^{2+}-mediated construction of brouchial tubes as a biased ligand of the receptors.

1-B-P-097 学生セッション（ポスター）

Priming effects of IFN-γ on poly(I:C)-induced IL-6 production in human bronchial epithelial cells

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Interleukin (IL)-6 is recognized as a factor associated with disease severity in COVID-19. The mechanism underlying the overproduction of IL-6 by SARS-Cov-2 remains unclear. Respiratory viruses initially infect bronchial epithelial cells (BEC) that produce various mediators. We have previously shown that pretreatment of human BEC (NCl-H292) with interferon (IFN)-γ markedly increased poly(I:C)-induced IL-6 production via upregulation of toll-like receptor (TLR) 3. In this study, we further investigated the feature of poly(I:C)-induced IL-6 production in IFN-γ-primed NCl-H292. Priming effects of IFN-γ on poly(I:C)-induced IL-6 production were observed not only in NCl-H292 but also in human primary HBEC and A549, another BEC. The Janus kinase (JAK) inhibitor tofacitinib inhibited IFN-γ-primed upregulation of TLR3 and poly(I:C)-induced IL-6 production. Chromatin immunoprecipitation revealed that IFN-γ stimulated histone modifications at region associated with the IL-6 gene locus. In mouse bronchial inflammation model, IFN-γ priming significantly increased poly(I:C)-induced lung IL-6 mRNA and protein levels in the alveolar lavage fluid. Taken together, priming of bronchial epithelial cells with IFN-γ markedly increases poly(I:C)-induced IL-6 production via JAK-dependent upregulation of TLR3 and chromatin remodeling at IL-6 gene locus. These mechanisms may be involved in severe respiratory inflammation with excess production of IL-6 following infection with RNA viruses.

Treatment with resveratrol, a SIRT1 activator, attenuates aging-associated skin thinning in mice.

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[Background] Skin thinning associated with aging leads to various health disadvantages such as decubitus. Cellular senescence has been implicated in skin thinning, and previous in vitro studies have demonstrated that SIRT1, an NAD^+-dependent protein deacetylase, suppresses cellular senescence in keratinocytes and dermal fibroblasts. This study aimed to elucidate whether activation of SIRT1 attenuates age-related skin thinning in mice.
[Method and Results] DDY mice were fed a normal diet or a diet containing a SIRT1 activator resveratrol (RSV, 0.4 g/kg diet) for 37 weeks starting at 23 weeks of age. Back and ventral skin samples were obtained from 60-week-old mice (Old), resveratrol-fed 60-week-old mice (Old+RSV), and 20-week-old mice (Young). Skin thickness was examined histologically in Hematoxylin-Eosin staining. Epidermal cell proliferation was evaluated by immunohistochemistry using an anti-Ki67 antibody. In the back skin, dermal and epidermal thicknesses were significantly decreased in Old compared to Young, but these aging-related changes was attenuated in Old+RSV. In ventral skin, fat layer thickness was significantly thinner in Old than those in Young. Fat layer thickness was also maintained in Old+RSV. The percentage of Ki67-positive cells in epidermis was significantly decreased in Old compared to Young, but this was preserved in Old+RSV.
[Conclusion] These findings suggest that activation of SIRT1 by administration of resveratrol attenuates skin thinning with aging partly via maintaining epidermal cell proliferation.

FABP2 is a important molecule for the α-synuclein pathologies in enteric neurons

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[Background/Purpose] Parkinson's disease and dementia with Lewy bodies are caused by neuronal cell death induced by α-Synuclein (α-Syn) accumulation. We have previously reported that fatty acid binding protein (FABP) plays an essential role to the α-syn pathology in the brain. Recently, it has become clear that pathological α-Syn is transmitted from the gut to the brain via the vagus nerve. However, the detailed mechanism of such α-Syn propagation is still unclear. Accordingly, we focused on the process of α-Syn uptake into enteric neurons, and tried to identify the key molecules of that process. [Methods] We used primary cultured neurons from murine small intestinal myenteric plexus. We treated fluorescence labeled α-Syn PFF to the primary neurons, and observed α-Syn uptake by immunocytochemistry. [Results] Intracellular uptake of α-Syn into primary neurons was observed. Interestingly, taken up α-Syn was colocalized with intestinal-FABP (FABP2). Furthermore, the fluorescence intensity of taken up α-Syn correlated with that of the 2nd antibody against anti-FABP2 1st antibody. [Conclusion] This results indicates that FABP2 is involved in the process of the intracellular uptake and/or accumulation of α-Syn. Therefore, FABP2 is an important molecule for elucidating the mechanism of α-Syn pathology in the gut.

1-B-P-100 学生セッション（ポスター）

Regulation of peripheral organ activity by the insular cortex

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The insular cortex serves as a hub cortical region that is bidirectionally connecting to an extensive cortical and subcortical brain areas and has been shown to modulate emotional behavior both in humans and rodent models, including fear and facial expressions, anxiety, and depression. In addition, accumulating evidence demonstrates that the insular cortex regulates peripheral organs through autonomic controls. However, the detailed neurophysiological mechanisms remain to be fully unknown. To address this issue, we examined how inactivation of insular cortex with the GABAA receptor agonist muscimol affects an electrocardiogram signal and a peripheral blood glucose concentration in freely moving rats. Inhibition of the insular cortical activity reduced heart rates and increased variability of blood glucose. Power spectral analysis of heart rate variability exhibited revealed that the insular cortical inhibition decreases the low-frequency components and the low- and high-frequency power ratios, a measure of sympathetic activity, and decreases but does not alter the high-frequency components, a measure of parasympathetic control. These results indicate that the insular cortex regulates peripheral physiological signals such as heart rate and blood glucose levels through the modulation of both sympathetic and parasympathetic tone.

1-B-P-101 学生セッション（ポスター）

Functional analysis of myelin protein Zwilling in medaka fish

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Although ncRNAs are defined as RNAs that are not translated into proteins, recent studies have reported that some ncRNAs indeed coded small proteins and those proteins regulated various biological processes.
LOC105354516 is annotated as a ncRNA that is highly expressed in the adult brain of medaka (Oryzias latipes). Reanalysis of publicly available ribosome profiling data revealed that two small proteins are translated from LOC105354516. These proteins were highly homologous to zebrafish Zwilling. Zwilling has been reported to be a myelin protein, but its physiological function is unknown. Therefore, we named LOC105354516 as ozwi and continued to analyze its function.
The expression pattern of ozwi mRNA showed high homology with that of mbpa (myelin basic protein), suggesting that ozwi gene also encoded a myelin protein.
We generated ozwi mutants in which a large portion of the ozwi gene was deleted and performed an open field test using them to evaluate their anxiety-like behavior. Compared to the wild type, ozwi mutants spent more time at the edge of the tank and showed higher anxiety. Furthermore, to explore other physiological function of the ozwi gene, we are currently comparing myelin in WT and ozwi mutants by electron microscopy and searching for proteins that form a complex with the ozwi protein.

1-B-P-102 学生セッション（ポスター）

Pathophysiological role of TRPC3 in the development of neuropathic pain

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Neuropathic pain is a pathological pain condition that often caused by peripheral nerve injury. Accumulating evidence suggests that central nervous system (CNS) inflammation, which is mediated by the interaction between neurons and glial cells/peripheral immune cells, plays a pivotal role in the development of neuropathic pain. Transient receptor potential canonical (TRPC3), a Ca^{2+}-permeable nonselective cation channel, is widely expressed in the CNS, primary sensory neurons, and peripheral immune cells. In this study, we investigated the involvement of TRPC3 in neuropathic pain after peripheral nerve injury. Naïve TRPC3-knockout (KO) mice displayed normal mechanical/thermal sensitivities, but TRPC3-deficiency impaired mechanical/thermal hyperalgesia in a mouse model of partial sciatic nerve ligation (pSNL). Using bone marrow chimeric mice, we also showed that TRPC3 in CNS or primary sensory neurons, but not in peripheral immune cells, is required for the development of pSNL-induced mechanical hyperalgesia. Moreover, intrathecal administration of GSK1702934A, a TRPC3 agonist, induced acute mechanical hyperalgesia. Overall, TRPC3 plays an important role in the development of neuropathic pain after peripheral nerve injury.

Comprehensive analysis of brain-spinal cord top-down signaling for neuropathic allodynia

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Allodynia, pain caused by innocuous stimuli, is a hallmark symptom of neuropathic pain. Since this symptom is resistant to existing analgesics, it is important to elucidate its underlying mechanism that provides a clue for developing novel therapies. Pain information in the spinal dorsal horn (SDH) is strongly controlled by top-down signals from the brain. While the brainstem is a well-known region of this control, but little is known about the role of other regions. In this study, we comprehensively explored the brain regions with neurons that directly project to the SDH using a whole-brain imaging system. Among many brain regions identified, we examined the role of some regions in neuropathic allodynia and found that activation and inhibition of the rostral ventromedial medulla (RVM)-SDH and primary somatosensory cortex (S1)-SDH neural pathways, respectively, attenuated behavioral response related to allodynia after nerve injury. Furthermore, inhibition of the S1-SDH pathway also reduced the number of c-FOS-positive neurons in the superficial lamina in SDH in response to optogenetic stimulation of primary afferent Aβ fibers. These data indicate the importance of these top-down signaling pathways in neuropathic allodynia and propose that these pathways may be therapeutic targets for neuropathic pain.

1-B-P-104 学生セッション（ポスター）

Analgesic effects of Disulfiram in carcinoma model mice developing allodynia

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Disulfiram (DSF), used as a treatment for alcoholism, has been found to have various points of action. We have reported that DSF exerts its anticancer effects by directly binding to and inhibiting the intracellular protein FROUNT, which promotes chemokine receptor CCR2 and CCR5 signaling expressed on macrophages (Nature Communications. 2020: 609). In addition, it has been reported that these chemokine receptor signals are deeply involved not only in "cancer" but also in "pain". In this study, we examined the analgesic effect of DSF by FROUNT inhibition in a new mouse model of carcinoma bearing allodynia.

To create the carcinoma model, we subcutaneously transplanted a mouse lung cancer cell line, Lewis Lung Carcinoma (LLC), into the right back of mice. The mice developed a significant decrease in pain threshold in both legs about 2 weeks after transplantation. In addition, the number of CD11b-positive microglia, an activated microglial marker, were increased in the dorsal horn of the spinal cord. Next, to evaluate the analgesic effect, intraperitoneal administration of DSF significantly improved allodynia. Furthermore, this allodynia was significantly attenuated in FROUNT-deficient mice. These results suggest that DSF may be a useful therapeutic agent for cancer patients suffering from pain.

1-B-P-105 学生セッション（ポスター）

Endothelin A receptor agonist ET-1 suppresses μ-opioid receptor activities with different efficacies caused by morphine or fentanyl

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Endothelin-1 (ET-1), known as a vasoconstrictor, induces pain signals through its specific receptor ETA (ETAR). We previously reported that the novel selective ETAR antagonist compound E (provided by Eisai Co., Ltd.) restored the ET-1-induced attenuation of morphine-induced analgesia with HEK293 cells stably expressing both ETAR and μ-opioid receptor (ETAR/MOR cells) and mice model. Further, among several opioids including morphine, we found that ET-1 almost completely suppressed the MOR activity induced by fentanyl (attenuated to 4.8 %) compared to that of morphine (attenuated to 37%), indicating that analgesic effects induced by fentanyl could be more influenced by endogenous ET-1. Several studies have reported that the fentanyl binding site to MOR is different from that of morphine, and these differences may also be involved in the distinct attenuation of MOR activity by ET-1, although the precise mechanism remains unclear. In the present study, in order to analyze the mechanism by which the analgesic attenuation effect of ET-1 differs among opioids, we are in the way to investigate and compare the inhibitory effect of ET-1 on MOR activity by morphine or fentanyl.

2-B-P-001 一般演題（ポスター）

Extract of Arachis hypogaea activates transient receptor potential vanilloid 4 channel

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Transient receptor potential vanilloid 4 (TRPV4) is a Ca^{2+}-permeable non-selective cation channel and its activating stimuli include anandamide, bisandrographolide, citric acid, arachidonic acid metabolic products by epoxygenases, hypo-osmotic cell swelling, and warm temperature. TRPV4 is involved in Ca^{2+}-dependent signal transduction in several tissues. Since the activation of TRPV4 facilitates adherence-junction formation in the skin epithelium, compounds that activate TRPV4 are expected to maintain or improve the barrier function of epidermal cells. In this study, we found that the extract of *Arachis hypogaea* (*A. hypogaea*) activates human TRPV4 (hTRPV4). In the Ca^{2+}-imaging experiment, the application of *A. hypogaea* extract exhibited the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increases in HEK293T cells expressing hTRPV4. The $[Ca^{2+}]_i$ increases by application of *A. hypogaea* extract were not observed in HEK293T cells expressing TRPV1, TRPV2, TRPV3, TRPM8, or TRPA1. Moreover, the application of *A. hypogaea* extract enhanced transepithelial electrical resistance in the keratinocyte monolayer. These results suggest that *A. hypogaea* extract is useful for maintaining and improving the barrier function of epidermal cells.

2-B-P-002 一般演題（ポスター）

Inhibition of amino acid transporter suppresses activation of microglial cell lines

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Microglia are innate immune cells in the brain and play an essential role in the maintenance of brain immune homeostasis while their chronic overactivation is involved in the onset and exacerbation of various neurodegenerative diseases. Therefore, regulation of microglial activation is one of the therapeutic strategies for these diseases. The molecular targets for suppression of the microglial activation may include an amino acid sensor, the mammalian target of rapamycin complex 1 (mTORC1) signaling. In the present study, we hypothesized that inhibition of amino acid transporter LAT1/SLC7A5 may suppress the microglial activation by inhibiting mTORC1 signaling. Murine and human microglial cell lines BV2 and HMC3 showed a time-dependent uptake of [^{14}C]Leu in a Na^+ free transport buffer which was inhibited by a LAT1 inhibitor nanvuranlat. A non-competitive LAT1 inhibitor OKY-034 also suppressed the [^{14}C]Leu uptake in a concentration-dependent manner. Thus, LAT1 is functionally expressed in the microglial cell lines and inhibited by OKY-034. Lipopolysaccharide (LPS)-induced increase in gene expressions of the proinflammatory microglial marker CD86 and the proinflammatory cytokine IL-1β was significantly suppressed by OKY-034 to a level similar to that of rapamycin, an inhibitor of mTORC1 signaling, suggesting that this LAT1 inhibitor may suppress microglial inflammation possibly by inhibiting mTORC1 signaling. Thus, LAT1 inhibitors would be possible candidates for the treatment of neurodegenerative diseases accompanied by microglial activation.

2-B-P-003 一般演題（ポスター）

Activation of NMDA receptors attenuate MCAO-induced neurodegeneration in mouse brain

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Activation of N-methyl-D-aspartate (NMDA) receptors has been shown to induce either neuronal cell death or neuroprotection against excitotoxicity in cultured neurons in vitro. To elucidate in vivo neuroprotective role of NMDA receptors, we investigated the effects of pretreatment of NMDA on cerebral ischemia-reperfusion injury to the mouse brain in vivo. Middle cerebral artery occlusion (MCAO) was performed to induce cerebral ischemia-reperfusion injury in mice 24 h after the administration of NMDA (75 mig/kg, i. p.). Neurological assessment and cerebral ischemic volume were measured by Zea longa score and TTC staining at 24 h after reperfusion. MCAO induced severe damages in mouse brain, with increasing neurological deficits by Zea longa score. However, the prior administration of NMDA at 24 h before significantly prevented cerebral infraction area by MCAO. In addition, MK-801 was effective in abolishing this brain protective effect of NMDA against the brain damage induced by MCAO. These results suggested that in vivo activation of NMDA receptor is capable of protecting against MCAO-induced cerebral ischemia-reperfusion injury.

2-B-P-004 一般演題（ポスター）

Antidepressant-like activity by TrkB overexpression using brain-directed adeno-associated virus

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Tropomyosin related kinase B (TrkB) is a neurotrophic factor receptor that plays an important role in neurogenesis, generating neurons from neural stem cells. A decline in hippocampal neurogenesis has been reported to be related to the pathology of various neurodegenerative diseases such as depression. Therefore, promoting TrkB-mediated neurogenesis may be a potential therapeutic target for these diseases. In this study, we investigated the effects of TrkB overexpression on neurogenesis and depression-like behavior in a mouse model exposed to chronic social defeat stress (CSDS). For such purpose, we used the blood-brain barrier-permeable adeno-associated virus serotype PHP.eB containing a gene of Flag-tagged mouse TrkB (AAV-mTrkB). In mice intravenously (*i.v.*) administered AAV-mTrkB the area of hippocampal newborn neuron marker Dcx-positive cells was significantly higher than that in the control AAV-treated group, suggesting that AAV-mTrkB promotes neurogenesis. After CSDS exposure, stress-susceptible mice and stress-resilience mice were separated and injected with each AAV. In the forced swimming test, the immobility time in stress susceptible mice *i.v.* administrated AAV-mTrkB was significantly shorter than the control AAV-treated group, suggesting that AAV-mTrkB shows an antidepressant-like activity. Taken together, peripheral administration of AAV-mTrkBmay promote neurogenesis and show antidepressant-like activity. Further analysis of its pharmacological effects is needed for treatment of neurodegenerative diseases.

2-B-P-005 一般演題（ポスター）

Involvement of mitochondrial Na^+/Ca^{2+} exchanger in the pathogenesis of pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH) is a severe and progressive disease that leads to right heart failure. The pathogenesis of PAH is generally characterized by vasoconstriction, upregulated proliferation, migration, and pulmonary vascular remodeling in lung tissue. Recent studies using genetic analyses and experimental models have suggested that the hypercontraction of pulmonary arteries induced by Ca^{2+} signaling abnormality may be involved in the pathogenesis of PAH. We recently showed that the upregulation of mitochondrial Na^+/Ca^{2+} exchanger (NCLX) contributes to the development of hypoxia-induced PAH, using NCLX genetically engineered mice. In the present study, we investigated the pathological mechanisms of NCLX in hypoxia-induced pulmonary hypertension. Pressure-induced arterial constriction was relaxed by specific NCLX inhibitor CGP-37157. Moreover, CGP-37157 suppressed hypoxia-induced migration of pulmonary arterial smooth muscle cells. These findings suggest that NCLX contributes to the development of pulmonary hypertension by promoting vascular hypercontraction and migration of pulmonary artery cells.

2-B-P-006 一般演題（ポスター）

Myocardial TRPC6 modulates stretch-induced increase in contractility via Zn^{2+} mobilization.

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TRPC6 has been previously reported to be involved in cardiac mechanosensitive responses, e.g., the Anrep effect. However, its role in the Frank-Starling mechanism (FSM) remains unclear. This study investigated whether TRPC6 contributes to the stretch-induced increase in contractile force associated with the FSM. Here, we used isolated ventricular cardiomyocytes from wild-type (WT) and $TRPC6^{-/-}$ mice hearts. The cells were electrically stimulated at 4 Hz in normal Tyrode solution at 37 ° C. Axial stretches were applied using the carbon fibre technique to generate the end-systolic force-length relation (ESFLR) curve. The slope of the ESFLR curve, an indicator of cellular contractility, was significantly steeper in $TRPC6^{-/-}$ mouse cardiomyocytes than in WT mouse cardiomyocytes. Transcriptome and real-time polymerase chain reaction analysis revealed that the genetic deletion of TRPC6 led to an increase in metallothionein 1 and 2, which is associated with intracellular Zn^{2+} concentrations ($[Zn^{2+}]_i$), along with an increase in ZIP8, a zinc transporter. Subsequently, zinc imaging unveiled an elevation in $[Zn^{2+}]_i$ in $TRPC6^{-/-}$ mouse cardiomyocytes. Interestingly, the addition of Zn^{2+} to the normal Tyrode solution also prompted the contractility in WT mouse cardiomyocytes, while this augmentation was blocked by rac-3, a ZIP8 inhibitor. These results suggest that TRPC6 contributes to alterations in cardiac muscle contractility, associated with the FSM, by regulating $[Zn^{2+}]_i$ via ZIP8.

2-B-P-007 一般演題（ポスター）

Targeting the coiled-coil domain of TRPC6 channel with the CRISPR/Cas9 system in mouse-podocyte cell line

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Transient Receptor Potential Canonical 6 (TRPC6) is a tetrameric Ca^{2+}/Na^{+}-permeable cation channel, and is expressed in various types of cells including podocytes in kidney glomerulus. Mutations of this gene are known to be associated with the two major causes of nephrotic syndrome (NS), namely minimal change nephrotic syndrome and focal segmental glomerular sclerosis. We have previously reported that a negative feedback regulation triggered by cellular Ca^{2+} elevation (calmodulin-mediated Ca^{2+}-dependent inactivation, CDI) is impaired in NS-associated TRPC6 mutations. However, the pathophysiological significance of impaired CDI is largely unknown. In this study, we evaluated the activities of NS-associated TRPC6 channels by using the patch-clamp recording. The inactivation of inward currents in NS-associated mutations was significantly delayed compared to that of the wild-type, and their total current densities which were calculated by integrating the inward currents exhibited a strong correlation with the age at the disease presentation. To elucidate the functional importance of the channel surface expression or delayed inactivation, the CRISPR/Cas9 system was used to edit the TRPC6 gene in mouse podocyte MPC-5 cells. The established cell line expressing CDI impaired-TRPC6 exhibited excess currents, while the expression level of TRPC6 on the cell surface remained to be unchanged. These results indicated the impact of CDI on NS onset and progression, and suggested that evaluation of the CDI in TRPC6 may contribute to pathological prediction of the onset and prognosis of NS.

2-B-P-008 一般演題（ポスター）

Effect of novel selective TRPC3/6 dual inhibitor L862 to rat PAN-induced nephropathy

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Nephrotic syndrome is a kidney disorder characterized by high urinary protein and low serum albumin caused by the impairment of glomerular podocytes. It has been reported that Transient Receptor Potential Canonical 6 (TRPC6) mutations found in patients with focal segmental glomerulosclerosis (FSGS) often cause hyperactivated channel currents. Regarding this mechanism, we have previously shown that the disruption of Calmodulin-mediated Ca^{2+}-dependent inactivation in TRPC6 channel led to prolonged cation influx and disorganized cytoskeleton in the podocytes. Aside from this, it is also known that in a model animal of chronical damaged kidney, the expression of both TRPC3 and TRPC6 are enhanced, suggesting that both of these molecules could be rational therapeutic targets.
Here, we developed “L862”, a novel selective TRPC3/6 dual inhibitor. This compound has a superior pharmacokinetic property compared to previously reported compounds, which allows it to be administered orally. We investigated the effect of L862 in normal rats and puromycin aminonucleoside (PAN)-induced rat nephrotic model. This compound exerted significant improvement of proteinuria, while no apparent toxicities were observed in normal rats. These results suggest that L862 would be a promising therapeutic compound for nephrotic syndrome, as well as other TRPC3/6-related diseases.

2-B-P-009 一般演題（ポスター）

Mechanisms underlying mutant astrocyte-mediated demyelination in Alexander disease

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Alexander disease (AxD), a rare neurodegenerative disease, is caused by the mutation of *GFAP* gene whose expression is enriched in astrocytes. Thus, AxD is "a primary astrocyte disease". AxD patients mainly show severe neurological symptoms such as psychomotor developmental delay, motor deficits etc. and white matter degeneration in their brains. However, molecular pathogenesis that leads from mutant astrocytes to white matter degeneration remains largely unclear, although AxD astrocytes are thought to gain neurotoxicity. Here, we examined the structural changes in the corpus callosum (CC) of AxD model mice carrying human mutant *GFAP* (Tanaka et al., *GLIA*, 2007). We revealed followings. (1) Immunohistochemical data showed that demyelination occurred in the CC of AxD. (2) AxD astrocytes enhanced "astrocyte reactivity" and mainly occupied demyelinated areas, suggesting that AxD astrocytes should contribute to local demyelination. (3) AxD astrocytes highly expressed Galectin-3 and Lipocalin-2, both of which are thought to control astrocytic phagocytosis. (4) Electron microscopic analysis showed that AxD astrocytes engulfed myelin, but not WT. Together, all these findings suggest that AxD astrocytes may phagocytose myelin by acquiring their abnormal phagocytic ability, thereby leading to white matter degeneration.

2-B-P-010 一般演題（ポスター）

Alzheimer patients‘ amyloid β oligomer interacts with Na^+, K^+-ATPase α3 in brain pericytes, leading to the activation of amyloidogenic APP processing protease, δ-secretase, in cortical neurons

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Cerebrovascular dysfunction modifies amyloid β (Aβ) pathologies in neurons, worsening Alzheimer‘s disease (AD), and however, the detailed molecular mechanism is still mostly missing. To unrevealed the mechanism, we developed a new *in-vitro* tri-culture system, consisting of brain endothelial cells, brain pericytes, and cortical neurons. Through analyses with this culture system, we found that the dysfunction of the pericytes induced by amylospheroids (ASPD), an AD patient-derived Aβ oligomer, activates δ-secretase, one of the amyloidogenic proteases, in the neurons, and these findings were reported at the previous annual meeting. Although the activated δ-secretase has been reported to play a significant role in the onset and progression of AD, the mechanism underlying its activation remains unclear. Therefore, the binding target of ASPD in the pericytes was explored, and then, we found that the membrane Na^+, K^+-ATPase α3 (NKAα3) serves as a binding target of ASPD by the confocal three-dimensional (3D) imaging and its high-content analyzing. Consistent with the results of image analyses, Knockdown of NKAα3 in the pericytes using siRNA attenuated the activation of δ-secretase by ASPD. In summary, we here show that the pericyte dysfunction may contribute to the activation of δ-secretase in AD.

2-B-P-011 一般演題（ポスター）

Chronic administration of oxytocin affects social behavior and hippocampal astrocyte levels in a dose-specific manner in healthy female mice

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Background

Oxytocin (OT) attenuates the impairment in social behavior, a core symptom of psychiatric disorders, in a sex-dependent manner. Recently, we revealed that the therapeutic effect of OT differed dose-independently in depression model of female mice. However, it is remains unclear whether OT also exerts the unique effect on females under non-stress conditions. Thus, we investigated the effect of OT administration on the social behavior and hippocampal astrocytes expression levels, a key regulator for social behavior, in healthy female mice.

Methods

Adult female C57BL/6J mice were intraperitoneally injected with OT (0.01, 0.1, or 1.0 mg/kg) for 3 weeks. To assess the effects of OT treatment on social behavior, mice were subjected to the social interaction test (SIT). Then, hippocampal samples of mice were collected, and glial fibrillary acidic protein (GFAP) levels, an astrocyte marker, were evaluated by western blotting analysis.

Results

OT (0.01 mg/kg) group showed a significantly lower social interaction rate in the SIT and GFAP levels in the hippocampus than the vehicle group. The social interaction rate and GFAP levels of OT (0.1 and 1.0 mg/kg) groups were comparable to the vehicle group.

Discussion

Social behaviors require OT signaling in the hippocampus. Previous studies showed that astrocytic OT signaling modulated neuronal activity. These results suggest that low dose of OT (0.01 mg/kg) may have deleterious effects on social behavior and hippocampal astrocytes in health females.

2-B-P-012 一般演題（ポスター）

Analysis for Neuroleptic Malignant Syndrome Using the Japanese Adverse Drug Event Report (JADER) Database.

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Neuroleptic malignant syndrome (NMS) is a rare but serious and sometimes fatal complication in patients taking antipsychotic drugs, and its underlying mechanism still remains unclear. The pharmacotherapy for psychotic disorders is complicated and often involves a combination of two or more drugs, including drugs other than antipsychotics. We used the Japanese Adverse Drug Event Report (JADER) database to broadly investigate the drugs associated with NMS, following their related pathways, as well as the drug-drug interactions (DDIs) in NMS. Single-drug signals were evaluated using the reporting odds ratio and proportional reporting ratio, and drug pathways were investigated using the Kyoto Encyclopedia of Genes and Genomes. DDIs were evaluated using the Ω shrinkage measure and Chi-square statistics models. All drugs associated with 20 or more NMS cases in the JADER database exhibited signals for NMS. Pathways associated with the drugs included the dopaminergic or serotonergic synapses. DDIs leading to NMS were confirmed for several drug combinations exhibiting single-drug signals. Although this study confirmed the significant association of various drugs, including non-psychotics, with NMS and suggested that various pathways related to these drugs may be involved in the progression of NMS, further investigation is needed to elucidate the pharmacological mechanisms.

2-B-P-013 一般演題（ポスター）

Activity dependent reduction of synaptic molecules in hippocampal mossy fiber terminals after novel context exploration

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In the dentate gyrus (DG), the novel context (NC) exploration activates a sparse population of granule cells (GCs). Stimulated GCs reshape computations in the hippocampal neural circuitry by modifying their synaptic plasticity, particularly at mossy fiber terminals (MFTs). Nevertheless, it remains elusive what molecular changes occur at MFTs of GCs which are active during the NC exposure.

In this study, we used an artificial, activity-dependent promoter called Robust Activity Marking (RAM) to label activated neurons. In the DG, we introduced AAV expressing V5 tag fused synaptophysin (V5-SYP) via RAM promoter to label MFTs of GCs that exhibited activity during the NC exploration. The number of V5-SYP positive MFTs was threefold higher in mice exposed to the NC compared to unexposed counterparts. Next, we quantified various presynaptic molecules in V5 positive and negative MFTs, one day subsequent to exposure to the NC. We found V5-SYP positive MFTs exhibited significantly diminished levels of active zone molecules such as Munc13-1, RIM1, and CAST. Conversely, the amount of Cav2.1 showed no difference between the two types of MFTs. These findings suggest that MFTs of activated GCs attenuate their synaptic transmission by downregulating key molecules that constitute the synaptic machinery of transmitter release.

2-B-P-014 一般演題（ポスター）

Cyclophillin A is involved in the clathrin-mediated endocytosis of α-synuclein by brain pericytes

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Parkinson's disease (PD) is characterized by widespread distribution of Lewy bodies, which are mainly composed of aggregated α-synuclein(α-Syn), in the brain. We previously revealed that pericytes, one of the blood-brain barrier-constituting cells, take up α-Syn and degrade it. Therefore, an efficient uptake of a-Syn by pericytes enable to establish a novel disease modifying therapy of PD utilizing the α-Syn d egradation system in pericytes. In this study, we investigated the intracellular uptake mechanism in pericytes for α-Syn.

We used primary cultures of rat brain pericytes. Increasing concentrations of extracellular α-Syn ranging from 0.05 to 10 μg/mL resulted in the increased cellular accumulation of α-Syn in pericytes. The cell/medium ratio of α-Syn in pericytes showed a significant decrease with the increased concentration of extracellular α-Syn. Furthermore, the uptake of α-Syn by pericytes was decreased at 4°C and in the presence of chlorpromazine. Cyclosporin A(CsA), a P-glycoprotein (P-gp) inhibitor, increased the uptake of α-Syn by pericytes. However, siRNA-mediated knockdown of P-gp failed to increase the uptake of α-Syn by pericytes. Knockdown of cyclophillin A (CypA), a molecular target of CsA to inhibit calcineurin activity, decreased the uptake of α-Syn by pericytes.

These results suggest that α-Syn uptake by pericytes is mediated by saturable transport system, clathrin-mediated endocytosis, and a CypA-dependent mechanism. In addition, inhibiting calcineurin activity would contribute to the enhanced α-Syn uptake, leading to α-Syn degradation by pericytes.

2-B-P-015 一般演題（ポスター）

Developmental neurotoxicity of glutaraldehyde: studies in neuron/astrocyte model and zebrafish

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The genotoxicity, development toxicity, carcinogenicity, and acute or chronic toxic effects of glutaraldehyde (GA), particularly during occupational exposure through its use as a fixative, disinfectant, and preservative, are well-documented but its effects on neurotoxicity have not been investigated. We performed *in vitro* and *in vivo* studies to examine the developmental neurotoxicity (DNT) of GA. Neurite outgrowth was examined in an *in vitro* co-culture model consisting of SH-SY5Y human neuroblastoma cells and human astrocytes. Cell Counting Kit-8, lactate dehydrogenase assay, and high-content screening revealed that GA significantly inhibited neurite outgrowth at non-cytotoxic concentration. Further studies showed that GA upregulated the mRNA expression of the astrocyte markers *GFAP* and *S100β* and downregulated the expression of the neurodevelopmental genes *Nestin, βIII-tubulin, GAP43*, and *MAP2*. Furthermore, *in vivo* zebrafish embryo toxicity tests explored the effects of GA on neural morphogenesis. GA adversely affected the early development of zebrafish embryos, resulting in decreased survival, irregular hatching, and reduced heart rate in a time- and concentration-dependent manner. Furthermore, the width of the brain and spinal cord was reduced, and the myelination of Schwann cells and oligodendrocytes was decreased by GA in transgenic zebrafish lines. These data suggest that GAs have potential DNT *in vitro* and *in vivo*, highlighting the need for caution regarding the neurotoxicity of GA.

2-B-P-016 一般演題（ポスター）

Expression analysis of equilibrative nucleoside transporter 3 (ENT3) in cultured astrocytes

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We have found that cultured differentiated astrocytes pretreated with *N*^{6}, *2'-O*-dibutyryladenosine 3',5'-cyclic monophosphate (DBcAMP), a permeable analogue of cAMP, incorporate thymidine, but not uridine, via nucleoside transporters including equilibrative nucleoside transporters (ENTs) into TCA insoluble fraction for repair on DNA injury in the presence of hydrogen peroxide (H_2O_2) at an early time, and these phenomena are specific in differentiated astrocytes, but not undifferentiated astrocytes and neurons.

We studied expression of ENT3 and LIMPII (Lysosomal Integral Membrane Protein II) in cultured astrocytes by RT-PCR and western blot analysis and immunocytochemistry. ENT3 mRNA and protein expression were found by RT-PCR and western blot analysis.

Astrocytes were double stained by anti-GFAP antibody or anti-LIMPII antibody, and anti-ENT3 antibody. ENT3 was co-expressed with GFAP or LIMPII. We could confirm ENT3, that is assumed to be presented in lysosomes on cultured astrocytes.

These results indicate that ENT3 expressed in lysosomes in astrocytes. Lysosomes incorporate foreign matters by phagocytosis and disassemble them and decomposition products including nucleosides were transported from lysosomes to cytosol for reuse.

When H_2O_2-induced thymidine incorporation into astrocytes was increased, delayed cell death was suppressed for DNA repair involved in nucleosides supply via nucleoside transporters, including ENTs. ENT3 existed in lysosomes might transport nucleosides from lysosomes to cytosol for DNA repair in astrocytes exposed by H_2O_2.

2-B-P-017 一般演題（ポスター）

A novel genetic risk factor, Midnolin, promotes neurite outgrowth in PC12 cells.

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Parkinson's disease (PD) is the second most common neurodegenerative disease. It is generally understood that loss of dopaminergic neurons in substantia nigra projecting to striatum results in motor impairments. Although more than twenty causal genes have been identified to date, the 90% of patients with PD are sporadic, and pathophysiological mechanism of PD remains unclear. We previously identified a novel genetic factor, *Midnolin* (*MIDN*), for PD in Yamagata and British cohort studies. Here, we attempted to clarify the physiological role of Midn in PC12 cells. First, two monoclonal cell lines were created where frame-shift mutations were introduced in *Midn* by CRISPR/Cas9 method (+1/+1 insertion and -1/-1 deletion). Whereas wildtype PC12 cells promotes neurite outgrowth in response to NGF, the effect was completely blocked in both of *Midn* mutant PC12 cells, suggesting that Midn is essential for neurite outgrowth in PC12 cells. Neurofilament is often regarded as an index of neuronal differentiation. We therefore measured both the promoter activity and protein levels of neurofilament light chain (NF-L). NGF increased the activity of NF-L promoter and upregulated NF-L protein in wildtype PC12 cells, which was largely inhibited in *Midn* mutant PC12 cells. These results suggest that MIDN dysfunction may trigger the pathogenesis of PD.

2-B-P-018 一般演題（ポスター）

Midnolin regulates the transcriptional activity of early growth response 1

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Parkinson's disease (PD) is an age-associated progressive neurodegenerative disease. Previously, we identified *Midnolin* (*MIDN*) as a genetic risk factor for PD. Although *MIDN* copy number loss increases the risk of PD, the molecular function of MIDN is unknown. To investigate the role of MIDN, we generated *Midn* knockout (KO) PC-12 cells and performed RNA-Sequencing. *Midn* KO altered the expression of many genes. While MIDN mainly localizes in the nucleus, MIDN has no DNA binding domain. We, therefore, assumed that MIDN might bind to certain transcription factor(s) (TF(s)) and regulate gene expression. We focused on a TF, early growth response 1 (EGR1) because the promoter region of many genes affected by *Midn* KO have EGR1 binding regions in common. At first, we confirmed the interaction of MIDN and EGR1 by immunoprecipitation. Then, to examine whether MIDN affects the EGR1-dependent transcription, we developed a reporter plasmid that can monitor EGR1-dependent transcription by measuring luciferase activity. Using the reporter, we confirmed that *Midn* KO reduced EGR1 transcription activity. These results suggest that the interaction of MIDN and EGR1 promotes EGR1-dependent transcription.

2-B-P-019 一般演題（ポスター）

The role of microglial testosterone signaling in the sex-related differences in Alzheimer's disease

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Epidemiological studies have shown a lower prevalence (AD) prevalence in men, although the underlying mechanisms are unclear. Microglia, the primary innate immune cells in the brain, release inflammatory cytokines and degrade aggregated amyloid β (Aβ) via autophagy, implying their role in sex-related AD susceptibility. In this study, we investigated the effect of testosterone, the major sex hormone in males, on Aβ-induced autophagy in microglia via GPRC6A, a non-genomic testosterone receptor. We confirmed that GPRC6A, but not the nuclear androgen receptor, is expressed in mouse microglial MG6 cells, indicating that GPRC6A mainly mediates testosterone signaling in MG6 cells. Testosterone suppressed ERK phosphorylation, activating autophagy and enhancing Aβ degradation. Extracellular Aβ was internalized by MG6 cells and co-localized with LC3, an autophagosome marker. Furthermore, co-stimulation with Aβ and testosterone amplified autophagic vacuoles, strengthening the link between testosterone and Aβ-induced autophagy. Genetic GPRC6A knockdown as well as GPRC6A inhibition counteracted this effect, implying testosterone-GPRC6A augmentation of Aβ-induced autophagy. This mechanism may contribute to the low susceptibility to AD in men.

2-B-P-020 一般演題（ポスター）

Alteration of gen expression in the medial prefrontal cortex (mPFC) of FABP3 KO mice

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Polyunsaturated fatty acids (PUFAs) are essential for brain development and function. Increasing evidence has shown that an imbalance of PUFAs is associated with various human psychiatric disorders, including autism and schizophrenia. Fatty acid-binding proteins (FABPs), cellular chaperones of PUFAs, are involved in their intracellular trafficking, signal transduction, and gene transcription. We previously demonstrated that FABP3 is robustly expressed in the GABAergic inhibitory interneurons in the medial prefrontal cortex (mPFC) of both juvenile and adult wild-type mice. Although the expression of FABP3 becomes evident after birth, the function of FABP3 is largely unknown in postnatal brain. In particular, the effects of FABP3 deletion in the mPFC GABAergic inhibitory interneurons are unclear. In this study, we comprehensively investigated the changes in gene expression due to the presence or absence of FABP3 in the mPFC at postnatal day 24 (P24), when FABP3 expression shows its highest value. We isolated nuclei from t he mPFC of wild-type and FABP3 gene knockout mice and conducted single-cell RNA sequencing. Focusing on the GABAergic inhibitory interneurons, we identified numerous genes with differential expression in gene groups involved in brain development, adult behavior and regulation of neuron differentiation. These results suggest that FABP3 is involved in the development of inhibitory synapse in the mPFC.

2-B-P-021 一般演題（ポスター）

Neohesperidin produces antidepressant-like effects via mechanistic target of rapamycin complex 1 in the medial prefrontal cortex in male mice

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Previous studies demonstrated that the extract of the fruit of *Citrus aurantium* (bitter orange) produces antidepressant-like and anxiolytic-like effects. However, it is unclear which constituents of *C. aurantium* are involved in these beneficial effects. Here, we examined the antidepressant-like effects of neohesperidin (NH), a flavanone glycoside found in *C. aurantium*, in naïve and repeated prednisolone (PSL)-induced depression model mice. We found that oral (p.o.) administration of NH (5 and 50 mg/kg) dose-dependently produced antidepressant-like effect in naïve mice in the forced swim test (FST) 1 h after the treatment without affecting locomotor activity and anxiety-like behavior in the open field test (OFT). However, NH failed to produce antidepressant-like effect in the FST 24 h after the treatment. We also examined the effect of NH (50 mg/kg, p.o.) on depression-like behavior induced by repeated subcutaneous injections of PSL (50 mg/kg, once a day for 5–6 days). Repeated PSL injections significantly increased immobility in the FST, which was not reversed by acute intraperitoneal injection of desipramine (30 mg/kg). In contrast, a single dose of NH (50 mg/kg, p.o.) blocked repeated PSL-induced depression-like behavior in the FST 24 h after the treatment, which was blocked by intra-medial prefrontal cortex (mPFC) infusion of rapamycin (0.01 nmol/side), a mechanistic target of rapamycin complex 1 (mTORC1) inhibitor. These results suggest that NH produces antidepressant-like effect via mTORC1 activation in the mPFC.

2-B-P-022 一般演題（ポスター）

Implication of TRUSS in prefrontal cortex in the development of ethanol dependence

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It is estimated that the number of alcohol dependant is over a million in Japan. Although a lot of study suggested candidates, certain mechanism underlying the development of alcohol dependence is still unclear. Our previous findings suggested that tumor necrosis factor receptor-associated ubiquitous scaffolding and signaling protein (TRUSS) may play role in the development of ethanol dependence. In the present study, we investigated the expression of TRUSS in ethanol depencence. Mice were treated with liquid diet containing ethanol for 7 days. Using the escalating ethanol dosage schedule, the mice were fed the ethanol diet as follows: 1st day: 1 w/v%; 2nd and 3rd day: 3 w/v%; 4th to 7th day: 4 w/v% ethanol diet, respectively. The control mice were given the same volume of ethanol-free liquid diet with sucrose substituted in isocaloric quantities for ethanol. The mice chronically treated with ethanol revealed severe withdrawal signs after discontinuation of ethanol. The mice were killed by decapitation and the prefrontal cortex was dissected. The expression of TRUSS in prefrontal cortex was significantly increased in ethanol dependence. We next investigated the role of TRUSS in the development of ethanol dependence using TRUSS antibody. We observed that the treatment of TRUSS antibody, significant prevented in the development of ethanol dependence. In conclusion, our findings suggest that the TRUSS may regulate the development of ethanol dependence.

2-B-P-023 一般演題（ポスター）

Effect of oral administration of probiotic *Bifidobacterium breve* on epileptic seizures in a mouse model of kindling induced by pentylenetetrazole

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Repetitive low-dose administration of the $GABA_A$ receptor antagonist pentylenetetrazole (PTZ) to mice gradually lowers the threshold for convulsive seizures and induces tonic-clonic seizures. Therefore, PTZ has been used to create chemical-kindling mouse models for epilepsy research. Recently, it has been reported that administration of probiotics has various beneficial effects on central nervous system function. The purpose of the present study was to investigate the effects of oral administration of probiotic *Bifidobacterium breve* strain A1 (*B. breve* A1) on the tonic-clonic seizure of PTZ-induced kindling model mice (KD mice). PTZ (37 mg/kg) was administered intraperitoneally to mice every other day for 15 days. On the other hand, living or heat-killed *B. breve* A1 at a volume of 0.25 mL (3.25×10^9 cfu organisms) was administered orally every other day for 15 days starting the day before the first PTZ injection. The mean seizure score in KD mice gradually increased with repetitive injections of PTZ. Oral administration of viable but not nonviable *B. breve* A1 to KD mice resulted in a significant decrease in mean seizure score at all injections after the fourth PTZ injection. These results suggest that at least *B. breve* A1 living in the gut lumen leads to amelioration of tonic-clonic seizures in PTZ-induced KD mice via an unknown signaling pathway.

2-B-P-024 一般演題（ポスター）

Intracellular calcium dynamics induced by Propofol and Nicotine - Observations using the intracellular organelle calcium indicator CEPIA (calcium-measuring organelle-entrapped protein indicators)

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Propofol is known to cause vascular pain and hypotension as adverse effects. Prolonged use of propofol at high doses can produce fatal propofol infusion syndrome (PRIS). We have reported that propofol increased intracellular calcium ($[Ca^{2+}]_i$), which was accompanied by changes in the endoplasmic reticulum (ER) morphology, suggesting that the propofol-induced $[Ca^{2+}]_i$ elevation was due to calcium leakage from the ER. We have also found that nicotine (>100 μM) elevates $[Ca^{2+}]_i$ by a nicotinic receptor-independent mechanism, suggesting that nicotine mobilizes calcium from the ER to the cytoplasm. In this study, we aimed to further elucidate the mechanism of propofol- and nicotine-induced $[Ca^{2+}]_i$ elevation using the intracellular organelle calcium indicator CEPIA.

We used HeLa cells expressing CEPIAs and observed their fluorescence changes by fluorescence microscopy.

Propofol decreased the $[Ca^{2+}]_i$ in the ER and simultaneously increased that in the cytosol in a concentration-dependent manner. The structural changes and the decrease in calcium concentration in the ER were synchronized. Thus, it was strongly suggested that propofol leaks calcium from the ER into the cytoplasm. In contrast, nicotine unexpectedly increased calcium concentrations at all sites in the cytosol, ER, and mitochondria. Nicotine may cause complex changes in calcium dynamics through extracellular, cytosolic, and intracellular organelles.

CEPIA has proven to be useful in elucidating drug-induced calcium kinetics by visualizing detailed calcium dynamics in intracellular organelles.

2-B-P-025 一般演題（ポスター）

ヒスタミン神経の化学遺伝学的な活性化は嗅周皮質のH_2受容体活性化を介して忘却した物体記憶の想起を促進させる

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Histamine within the central nervous system is a promising target for reactivating forgotten memories. We have previously demonstrated that histamine H_3 receptor inverse agonists enhance histamine release in the perirhinal cortex (PRh) and facilitate retrieval of forgotten object memories. Nevertheless, considering that histamine H_3 receptors are expressed in non-histaminergic neurons as well, it is plausible that other neurotransmitter systems might also be involved in memory recovery. This study directly tested the contribution of central histamine signaling to retrieval recovery. We virally targeted hM3Dq, the Gq-coupled excitatory designer receptor exclusively activated by designer drugs (DREADD), to histaminergic neurons in the tuberomammillary nucleus of HDC-Cre mice. One week after the training session of the novel object recognition task, mice underwent a test session where one familiar and one novel objects were presented. The pre-test injection of clozapine-N-oxide to the mice receiving AAV-DIO-hM3Dq increased discrimination between novel and familiar objects, indicating enhanced memory retrieval. This effect was blocked by intra-PRh infusions of ranitidine, a histamine H_2 receptor antagonist. These results indicate that chemogenetic activation of histamine neurons promotes memory retrieval through PRh H_2 receptor activation.

2-B-P-026 一般演題（ポスター）

Precision drug discovery for schizophrenia

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Schizophrenia drug discovery has been a series of failures since the development of dopamine D2 receptors agents. These agents have some effect on schizophrenia, but not enough. One reason for this is estimated to be that although schizophrenia is diagnosed based on the psychological symptoms, there are multiple underlying biological dysfunctions. Recent advances in molecular biology have facilitated genome analysis, and many genes associated with schizophrenia have been reported. Thus, based on this data, we have embarked on developments of precision drug discovery for schizophrenia using model animals with the genetic mutations. The GWAS meta-analysis was shown that UGT1A1 gene mutation is a risk factor for the schizophrenia (Prata 2019). We demonstrated hyper serotoninergic transmission to the frontal cortex in the rat model, On the other hand, the dopaminergic transmission was intact (Miura 2022). Based on the findings, we developed a novel anti-psychotic agent (TRM001) for UGT1A1 gene mutations associated schizophrenia. We demonstrated that TRM001 administration rapidly ameliorates abnormal behavior in the model rat. TRM001 has been shown to be safe in healthy subjects. UGTA1 gene mutations associated schizophrenia can be easily diagnosed by genetic mutations and indirect bilirubin concentration in the blood. Since it is also possible to select patients with UGTA1 gene mutations associated schizophrenia, TRM001 could be worthy of proceeding to second clinical trials.

2-B-P-027 一般演題（ポスター）

Enzymatic activity of nardilysin plays significant roles in the regulation of central nervous system activity.

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Nardilysin (N-Arginine Dibasic Convertase: NRDC) is a metalloendopeptidase belonging to the M16 family, which enzymatically cleaves on the N-terminal side of the arginine residue at a dibasic site. Although prior studies identified Dynorphin-A, somatostatin-28, α-neoendorphin, and glucagon as *in vitro* substrates for NRDC, but its *in vivo* substrates remain elusive.

We generated and analyzed whole body knockout mouse of NRDC, which displayed impaired axonal maturation and hypomyelination in the CNS. These findings were attributed to reduced ectodomain shedding of neuregulin 1 (NRG1), a myelination promoting factor, because NRDC enhances ectodomain shedding of NRG1 via the complex formation with ADAM17 or BACE1.

To elucidate the *in vivo* role of NRDC enzymatic activity, we generated NRDC E>A knock-in mice, where the enzymatically active glutamate (E) was substituted by alanine (A). Adult NRDC E>A KI/KI mice showed growth retardation, aberrant behavior, and epileptiform vertical head movements which are reminiscent of NRDC-KO mice. However, they lacked certain traits seen in NRDC-KO mice, such as ventricular enlargement and hypomyelination. Additionally, we found no decrease in NRG1 shedding in the brains of NRDC E>A KI/KI mice, which was observed in NRDC-KO mice. These results indicated the strong link between myelination and NRDC-induced NRG1 shedding, at the same time, suggested that there are unknown underlying mechanisms by which NRDC enzymatic activity regulates behaviors.

2-B-P-028 一般演題（ポスター）

The analysis of cortical microstructure in maternal hypoxia rat model

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Maternal hypoxic stress such as threatened abortion at early pregnancy is thought to be a risk factor for children's neurodevelopmental disorder onset. We previously reported that maternal hypoxia caused neurodevelopmental disorder-like behavior in rat and both NeuN-positive cells and glial fibrillary acidic protein-positive cells were decreased in rat anterior cingulate cortex (ACC) by treating with maternal hypoxic stress, which indicated that ACC might be involved in the onset of hypoxia-related neurodevelopmental disorder. Here, we conducted the microstructural analysis in ACC by using immunohistochemical studies. First, to check which neural subtypes decrease in ACC, immunofluorescent double staining was conducted. As results, glutamatergic pyramidal neurons were decreased by maternal hypoxia. In addition, glutamatergic neural presynaptic marker VGLUT1 density was decrease around pyramidal neuron in hypoxia rats' ACC. Furthermore, microstructural analysis by using transmission electron microscopy revealed that the number of presynaptic vesicles was decrease in hypoxia rat ACC. These results suggested that maternal hypoxia might cause abnormal behaviors by hypoactivity of glutamatergic neural networks.

2-B-P-029 一般演題（ポスター）

LIT-001, a non-peptide oxytocin receptor agonist, ameliorates autistic-like behaviors in cannabinoid CB1 receptor knockout mice

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Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disability that demonstrates impaired social interactions, social communication deficits, and restrictive/repetitive behaviors. It is reported that children with ASD and some animal models of ASD show the abnormality of endocannabinoid (eCB) system. To determine the causal role of the eCB system in the ASD, we investigated the relationship between the eCB system and ASD-like symptoms, using the cannabinoid CB1 receptor knockout (CB1KO) mice. We found that CB1KO mice demonstrated the reduced sociability and elevated repetitive grooming behaviors which reflect to core symptoms of ASD. Moreover, the CB1KO mice also showed emotional instabilities and resistance to change a learned pattern of behavior. The serum oxytocin, expected as a biomarker for ASD, significantly decreased in CB1KO mice. Moreover, the brain oxytocin also significantly decreased in the hippocampus and hypothalamus of CB1KO mice. Based on the results, we next attempted to recover the autistic-like behaviors in CB1KO mice by activation of oxytocin signaling. Then, LIT-001, a non-peptide oxytocin receptor agonist, ameliorated the reduced sociability and repetitive behaviors in CB1KO mice. These findings suggest that CB1KO mice could have potential as a novel ASD model mouse and provide the possibility of drug development using the non-peptide oxytocin receptor agonist for ASD.

2-B-P-030 一般演題（ポスター）

In vivo macroscopic analysis of Ca^{2+} activities in cortical astrocytes and pathological progression using a modified transgenic mouse model of neurodegenerative diseases

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The involvement of astrocytes in neurodegenerative diseases is attracting widespread attention in the neuroscience research field in terms of a potential therapeutic target. Astrocytic Ca^{2+} signals are affected by bioactive substances from damaged brain cells and can induce alterations of Ca^{2+}-dependent cellular processes. Such alterations may lead to functional changes in astrocytes, including gene expression profiles and secretion of neuroprotective/neurotoxic molecules. Thus, analysis of astrocytic Ca^{2+} activities may provide clues to controlling neurodegenerative diseases. However, it is still unclear how the brain pathology affects astrocytic Ca^{2+} activities. Therefore, we here applied in vivo macroscopic imaging of astrocytic Ca^{2+} signals and fluorescence labeling of a disease marker protein to neurodegenerative disease model mice expressing Ca^{2+} sensor proteins. We found a spatiotemporal correlation between astrocytic Ca^{2+} signals and accumulation sites of the disease marker. Using this relationship as an indicator, we have started to develop a machine learning-assisted protocol to predict disease progression from astrocytic Ca^{2+} activities. These results and future analyses are expected to contribute to developing therapeutic strategies for neurodegenerative diseases.

2-B-P-031 一般演題（ポスター）

Spreading of 3-repeat tau pathology in tau knock-in mice

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Neurodegenerative diseases with tau protein deposition are collectively known as tauopathies, and a prion-like propagation hypothesis has recently been proposed for their pathogenesis. Pick's disease is one of these diseases that shows the accumulation of only 3-repeat tau isoform (3R tau). However, it has been impossible to recapitulate the pathology of Pick's disease in adult wild-type mice because they do not express 3R tau.

In this study, we attempted to generate a mouse model of 3R tau accumulation by intracerebral injection of recombinant 3R tau aggregates into tau knock-in mice expressing 3R tau (Hosokawa et al. Brain 2022). Recombinant tau aggregates consisting of full-length 3R tau or tau fragments (244-391a.a.) were injected into the hippocampus of tau knock-in mice, and tau pathology was analyzed by immunohistochemistry.

As a result, AT8-positive tau pathology was observed in mice injected with tau aggregates, and tau pathology spread from the injection sites to the other brain regions over time. These results indicate that 3R tau accumulation can be induced by intracerebral injection of synthetic tau seeds into tau knock-in mice by a prion-like mechanism.

2-B-P-032 一般演題（ポスター）

The production of hydrogen sulfide in glioblastoma

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Hydrogen sulfide (H_2S) is endogenously produced from L-cysteine (L-Cys) by cystathionine beta-synthase (CBS) and cystathionine gamma-lyase (CSE). The third enzyme 3-mercaptopyruvate sulfurtransferase (3-MST) produces H_2S from 3-mercaptopyruvate (3-MP), which is provided from L-Cys and α-ketoglutarate (α-KG) by cysteine aminotransferase (CAT). Recent studies have shown that H_2S promotes growth and proliferation of tumor cells. H_2S is expected to provide a new perspective in elucidating the pathology of malignant tumors; however, the production of H_2S in glioblastoma, the most aggressive and malignant type of astrocytoma, is largely unknown. In this study, we examined the production of H_2S in human glioblastoma cell line U-251 MG. Cell lysates of U-251 MG produced H_2S from 3-MP, and the production of H_2S was inhibited by a selective inhibitor of 3-MST HMPSNE. Because 3-MP is provided by CAT from L-Cys and α-KG, we then examined the possibility that 3-MST with CAT produces H_2S. The lysates produced H_2S from L-Cys in the presence of α-KG. In the absence of α-KG H_2S from L-Cys markedly reduced, suggesting that H_2S production is highly dependent on the activity of CAT. This conclusion is supported by the observation that H_2S production from L-Cys and a-KG was suppressed by L-aspartate, a substrate with higher affinity than L-Cys. These results suggest that 3-MST with CAT may function as the major H_2S-producing enzyme in glioblastoma cells.

2-B-P-033 一般演題（ポスター）

Involvement of hematopoietic PGD_2 synthase and DP1 receptor for delayed wound healing in streptozotocin-induced diabetic mice

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Delayed wound healing is a major problem in patients with diabetes, which significantly impairs their quality of life. Prostaglandin (PG) D_2 is a major inflammatory lipid mediator synthesized by hematopoietic PGD_2 synthase (HPGDS) from PGH_2, a common precursor of all of PGs. We have previously shown that HPGDS produced PGD_2 is involved in delayed wound healing in diabetic skin. In this study, we investigated the involvement of DP1 receptor in cutaneous wound healing in streptozotocin (STZ)-induced diabetic mice. C57BL/6 mice were injected intraperitoneal with 50 mg/kg of STZ daily for 5 days. Four weeks after the injection of STZ, a full thickness wound was created with an 8-mm diameter biopsy punch on the dorsal of mice. Wound healing was significantly decelerated in diabetic mice compared with non-diabetic mice. HPGDS mRNA was significantly increased in diabetic mouse skin compared to nondiabetic mouse skin. On the other hand, there was no significant change in the amount of DP1 receptor mRNA. Furthermore, immunohistochemically analysis revealed that HPGDS was expressed in epidermal Langerhans cells of diabetic mice, and the DP1 receptor was expressed in keratinocytes. These results suggest that in hyperglycemic skin, PGD_2 produced by Langerhans cells acts on DP1 receptors on keratinocytes and may be involved in delayed wound healing.

2-B-P-034 一般演題（ポスター）

A discussion of Auranofin, a gold compound, on its repurposing (Part 3)

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This is a presentation of the effects of auranofin (AF), a disease modifying anti rheumatic drug (DMARD) containing gold in its molecule, which is able to oral administration, on the treatment of cancer, based on a review article in 2021 [9].

I and colleagues have reported that AF (10 μM) inhibited the productions of prostaglandin E_2 (PGE_2) [1-3] and nitric oxide (NO) [4], in the culture medium of rat peritoneal macrophage under inflammatory stimulation, which is concurrently lowered with levels of their producing enzymes and mRNAs, cyclooxygenase(COX)-2 and inducible NO synthase (iNOS) [5]. AF (1～10 μM) strongly lowered the translocation of inflammation-related transcription factor, NF-κB into the nuclear fraction [6]. We also find that there is no change of the protein level of COX-1 [1] though the PGE_2 production is increased [2]. We had been discussed the increase or activation of cytosolic type of PGE synthase [7-9], which still could not be revealed.

AF as a DMARD over 40 years, have been replaced by biological pharmacies, and decreased and finished its sales in 2023. Many reports tried to applicate AF on the other diseases, because of its risk-managed properties.

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9 Int Immunopharmacol. 2021;101:108272

2-B-P-035 一般演題（ポスター）

Deficiency of PLCL in tumor-associated macrophages induces cancer malignancy by exacerbating the tumor microenvironment

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Background: Tumor-associated macrophages (TAMs) are major inflammatory cells in tumor microenvironment (TME). PLCL (phospholipase C-like protein) suppresses the PI3K-AKT-mTORC1 signaling, which negatively regulates tumor cell proliferation and survival. In this study, we investigated the relationship between the expression of PLCL in human tumor tissues or TAM and tumor metastasis.

Methods: Renal cell carcinoma (RCC) tissues (30 cases, Approval of Kyusyu Univ. Hospital) were used. PLCL-positive areas were automatically calculated by a hybrid cell count. PLCL-positive TAMs in TME were performed by fluorescent multiplex immunostaining. TAM induction was analyzed when bone marrow derived macrophages (BMDMs) of *PLCL*-KO or WT mice were co-cultured with Caki1 (renal cancer cells without metastasis) or OSRC2 (renal cancer cells with metastasis).

Results: PLCL expression in tumor area was markedly decreased in the cases with metastasis compared to non-tumor area. This was not seen in non-metastatic cases. PLCL-positive TAM rate in the TME was different between the cases with and without metastasis. In addition, BMDM from *PLCL*-KO mice co-cultured with OSRC2 was most induced to TAM.

Conclusion: We indicated that decreased expression of PLCL in RCC mediates tumor malignancy, and less PLCL expression in TAMs exacerbates TME leading to tumor progression and malignant transformation. Hence, a drug enhancing PLCL expression can be effective new cancer therapeutics for RCC.

2-B-P-036 一般演題（ポスター）

Effect of CDK8/19 inhibition on IL-4-induced arginase-1 expression in macrophages.

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Macrophages polarize into anti-inflammatory macrophages by interleukin (IL)-4, and they express arginase-1. Arginase-1 promotes the function of anti-inflammatory macrophages and is crucial for maintaining tissue homeostasis. Both cyclin-dependent kinase (CDK) 8 and its paralog CDK19 are members of the transcriptional CDK family. CDK8/19 inhibitors have garnered attention as novel drugs for autoimmune diseases. However, the role of CDK8/19 inhibitors in IL-4-induced anti-inflammatory macrophage function remains unclear. In this study, we examined the effects of the CDK8/19 inhibitor BRD6989 on IL-4-induced arginase-1 expression.

RAW264.7 cells were pretreated with BRD6989, followed by stimulation with IL-4. BRD6989 enhanced IL-4-induced arginase-1 expression. Moreover, the increase in arginase-1 expression by BRD6989 was inhibited by the p38 MAPK inhibitor SB203580. On the other hand, BRD6989 increased the expression of phosphorylated p38 MAPK following IL-4 stimulation compared to the control. We then examined mRNA expression of C/EBP β and found that BRD6989 increases C/EBP β mRNA expression in comparison to controls. In summary, inhibition of CDK8/19 was found to contribute to the enhancement of IL-4-induced arginase-1 expression through the activation of p38 MAPK, suggesting the involvement of C/EBP β as a signaling pathway. Elucidating the role of CDK8/19 in the regulation of arginase-1 expression is believed to contribute to the development of novel methods for inducing anti-inflammatory macrophages.

2-B-P-037 一般演題（ポスター）

Abnormal behavior and glial responses in an animal model of tau pathology

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Tau hyperphosphorylation has been considered a major contributor to neurodegeneration in Alzheimer's disease and related tauopathies and has gained prominence in the development of therapies for Alzheimer's disease. Neuroinflammation plays an important role in the progression of neurodegenerative disorders, and activated astrocytes and microglia strongly influence Aβ and tau pathologies. Numerous transgenic mouse models that recapitulate critical Alzheimer's disease-like pathology have been developed to examine the pathogenic mechanisms underlying Alzheimer's disease and evaluate therapeutic approaches targeting tau, but the relevant mechanisms remain unknown. In this study, we investigated changes in gene expression related to neuroinflammation in glial cells of rTg4510 mice, an animal model of non-Alzheimer's disease tauopathy. First, we analyzed 4- and 6-month-old rTg4510 mice in terms of cognition and behaviors that mimic the behavioral and psychological problems of dementia. Deterioration of executive functions and impairment of daily life activities are early signs of Alzheimer's disease. In the present study, nest-building behavior, which represents active interaction with the environment, evaluated the executive functions that are the basis of daily life activities. Both 4- and 6-month-old rTg4510 mice displayed significantly impaired nesting behavior compared with control mice. Moreover, rTg4510 mice of both age groups exhibited abnormal exploratory behavior, and these mice spent a greater amount of time in the open arm of the plus-maze test than control mice. We also used magnetic-activated cell sorting to analyze the expressions of genes related to neuroinflammation, phagocytosis, and amyloid synthesis in the prefrontal cortex of rTg4510 mice. Axl, Cd11c, and CD68 expression levels were increased in microglial cells, and H2-D1, Psmb8, and H2-T23 expression levels in astrocytes were also increased in 6-month-old rTg4510 mice compared with control mice. In conclusion, neuroinflammation may be related to neuronal degeneration and abnormal behavior in rTg4510 mice.

2-B-P-038 一般演題（ポスター）

Efficacy of novel $P2Y_6$ receptor inhibitor on LPS-induced acute lung injury model in mice

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Extracellular nucleotides are released from injured cells and act as immunomodulators in various inflammatory conditions through diverse purinergic receptors. Recently, $P2Y_6$ receptor gene knockout was reported to suppress pathogenesis in a mouse model of lipopolysaccharide (LPS)-induced acute lung injury (ALI). In this study, we investigated effects of a novel inhibitor of $P2Y_6$ receptor TIM-38 on LPS-induced ALI model mice.

In ALI model mice generated by intratracheal administration of LPS, UDP concentration was increased in bronchoalveolar lavage fluid (BALF). In addition, mRNA levels of inflammatory mediators such as TNF α, IL-6, and CXCL-2 were increased in lung tissues and the associated protrin secretions in BALF were observed in ALI model mice. Administration of TIM-38 significantly inhibited the elevation of inflammatory mediator mRNA expression and associated protein secretions induced by LPS. In addition, TIM-38 suppressed LPS-induced increase in infiltrated neutrophils in BALF. In the experiment using mice macrophage cell line RAW264.7, TIM-38 inhibited LPS-induced TNF α and IL-6 production, accompanied by inhibiting ERK and Akt, and NF- κ B pathway.

These results suggest that TIM-38 may be a potential therapeutic agent for the lung tissue inflammatory responses induced by LPS *in vivo* through suppression of neutrophil migration and production of various inflammatory mediators.

2-B-P-039 一般演題（ポスター）

Altered gene expression in colon in P2X4 receptor-deficient mice

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【Background】The gastrointestinal tract is a barrier between the outside environment and the inside of the organism and is home to an enormous amount of symbiotic bacteria. In addition, various substances derived from bacteria have been shown to influence biological functions. ATP is one of such substance released by bacteria in the digestive tract, and it is known to modulate colonic immune cell functions via the ionotropic P2X4 receptor (P2X4R). However, role of P2X4R in colonic epithelial cells has not been known. Therefore, we analyzed role of P2X4R in colonic epithelial cells.

【Method】We obtained single cell RNA sequence (scRNA-seq) data (GSE148693) from NCBI GEO and analyzed cells expressing P2X4R using Seurat. DNA microarrays were used to identify genes in the colon whose expression is altered by P2X4R deficiency. The expression of P2X4R in colon was investigated by immunostaining.

【Results】scRNA-seq analysis revealed that the P2X4R is expressed in the secretory cell lineage expressing Atoh1 and Muc2. Various gene expressions were altered in the colon of P2X4R-deficient mice compared to that of wild-type mice. Among them Reg4 was expressed in P2X4R-expressing cells. Reg4 has been reported as a marker or deep crypt secretory (DCS) cells, which constitute the tem cell niche in the colon. The expression level of Relm-β, another marker for DCS cells, was also elevated in P2X4R deficient mice. These results suggest that the P2X4R may be involved in the regulation of a stem cell niche in colon epithelial cells.

2-B-P-040 一般演題（ポスター）

Establishment of a Thioacetamide and Liver X Receptor Agonist-Induced Nonalcoholic Steatohepatitis Model

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In our previous research, we successfully established a nonalcoholic steatohepatitis (NASH) model in high fat diet (HFD)-fed mice by multiple intraperitoneal administrations of carbon tetrachloride (CCl4) and liver X receptor agonist (LXR-a) over a 28-day period, allowing effective evaluation of drug efficacy. However, future usage of CCl4 in research may become restricted because it is an ozone-depleting substance. Therefore, in this study, we aimed to develop a NASH model with rapid onset of liver fibrosis using thioacetamide (TAA) as a substitute for CCl4.

Male C57BL/6J mice (7 weeks old) were fed with a high fat diet (HFD-60) for 28 days. TAA at 100 mg/kg, TAA at 200 mg/kg, or CCl4 was administered on days 16, 20, 24, and 28 of HFD feeding. Additionally, LXR-a was administered consecutively for 5 days from day 24 of HFD feeding. On the final day of HFD feeding, insulin tolerance testing was performed. Blood samples and the liver were then collected for measurements of AST, ALT, and TG concentrations in the serum and histopathological examination of the liver.

Administering TAA and LXR-a during the 28-day HFD feeding led to increased AST, ALT, and TG levels, indicating heightened insulin resistance. Additionally, liver fat accumulation and fibrosis were observed. Therefore, a NASH model with rapid-onset of liver fibrosis was established using TAA as a substitute for CCl4.

2-B-P-041 一般演題（ポスター）

Periodic changes in testicle size and pain thresholds in cynomolgus monkeys

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Cynomolgus monkeys, like humans, are continuous breeders and while the female sexual cycle has been extensively studied, little is known about male sexual cycles, such as periodic changes in the testicle size. Although testosterone, the primary male hormone secreted by the testicles, has been demonstrated to increase pain thresholds in humans and animals, whether testicle size affects pain thresholds is unknown. In the present study, we measured the testicle size in 7 matured male cynomolgus monkeys (6-8 years old) once a week for 23 weeks and observed that there were periodic changes in testicle size, with an average interval between peak points of 36.5 days. We also examined pain thresholds in these animals by measuring paw withdrawal temperatures (PWTs) in the dorsal hind paws once a week for 22 weeks. Similar to the testicles, PWTs underwent periodic changes with an average interval between peak points of 27.6 days. Although their cycles were not synchronized, the mean values of testicle size and PWTs showed a significant correlation ($P = 0.019$). These results demonstrate the presence of male sexual cycle and indicate the relationship between testicle size and pain thresholds in cynomolgus monkeys, providing new insights into research not only in primates, but also in other continuous breeders, including humans.

2-B-P-042 一般演題（ポスター）

A case of administrating an anticancer tablet through a gastrostomy to a castration-resistant prostate cancer patient undergoing tubal feeding –Our experiences preventing exposure during crushing the tablet in in-home healthcare-

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We report a case of administrating an anticancer tablet to a castration-resistant prostate cancer (CRPC) patient undergoing tubal feeding. The patient was undergoing tubal feeding due to progressive bulbar palsy and impaired consciousness with dysphagia. The patient was doing well on maximum androgen blockade at home for prostate cancer. However, his prostate-specific antigen (PSA) was elevated, and the disease was progressing into CRPC. Since imaging tests showed no metastasis, we prescribed apalutamide. we needed to administer it via gastrostomy because the drug was in tablet form. The tablet was crushed and mixed with warm water, and the administration was performed by a facility nurse and the patient's family at home. The patient‘s PSA decreased after three months of administration and was below the measurement limit after nine months, indicating significant efficacy.

Occupational exposure prevention measures have been promoted for the handling of anticancer drugs to ensure the safety of healthcare workers. As home care advances, more people are expected to receive cancer chemotherapy at home or in facilities for long-term care. Not only ward nurses but also outpatient nurses, visiting nurses, and facility nurses need to be fully aware of possible effects due to exposure to anticancer treatments. In addition, it is necessary to promote education and prepare manuals to prevent exposure and provide guidance to patients and their families. Collaboration among multiple professions is also essential in in-home healthcare. In the present case, the daughter who administered the drug at home was a former caregiver. In living environments such as homes and facilities for long-term care, there is a possibility of exposure to active anticancer drugs contained in the excretions of patients.

2-B-P-043 一般演題（ポスター）

Impact of Polypharmacy in Elderly Care Facility Residents: Clinical and Gut Microbiota Analysis

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Background: Polypharmacy in the elderly can lead to falls, memory impairment, decreased appetite, and constipation, which are commonly observed in frail and care-dependent elderly residents. This study aimed to investigate the impact of polypharmacy in elderly care facility residents on clinical presentation and gut microbiota based on various data.

Methods: The study included 62 elderly residents (aged 87.4±7.9) who were assessed for medication usage, clinical status, and caregiving needs. Concurrently, fecal samples were collected and analyzed using next-generation sequencing. Participants were categorized into the polypharmacy group (n=30) if they were taking six or more medications habitually, and the non-polypharmacy group (n=32) if they were taking five or fewer medications.

Results: There were no significant differences between the two groups in terms of constipation, laxative use, frailty degree, or caregiving level. Regarding gut microbiota, no significant differences were observed in diversity or phylum levels between the two groups. However, at the genus level, *Ruminococcaceae UCG 014*, associated with enhanced intestinal barrier function, was significantly more abundant in the polypharmacy group (p=0.036), and *Lachnospiraceae NK4A136* group showed a positive correlation with the number of medications taken (r=0.274).

Discussion: The polypharmacy group in this study showed a reduced incidence of drug-related adverse events, suggesting no apparent association with gut microbiota dysbiosis.

2-B-P-044 一般演題（ポスター）

Thermal nociception and capsaicin induced thermal hypersensitivity in dorsal hind paws of cynomolgus monkeys

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The measurement of tail withdrawal latencies to thermal stimuli has been a predominant behavioral assay for analgesic efficacies of drugs in non-human primates. Nevertheless, there are two major concerns. One is to evaluate analgesia using the tail, which is not present in humans, and the other is the presence of individual variations in the motor function of the tail. The present study performed the thermal probe test in 8 male cynomolgus monkeys (6-8 years old) to examine thermal nociception and capsaicin-induced thermal hypersensitivity in the dorsal hind paws (DHPs). Thermal stimuli (cutoff: 60℃) applying to the DHPs increased by 1℃ per second starting from 35℃ and paw withdrawal temperature (PWT) was recorded when the animal withdrew the hind paw. Buprenorphine (0.01 and 0.05 mg/kg i.m.), morphine (1 and 3 mg/kg i.m.) and medetomidine (0.02 and 0.1 mg/kg i.m.) dose-dependently increased PWT, indicating their analgesic efficacies. Topical application of 1% capsaicin to the DHPs resulted in a reduction in PWT, and this capsaicin-induced thermal hypersensitivity was reversed by buprenorphine and medetomidine. These results demonstrate the value of PWT measurements in DHPs for assessing pain and the efficacies of analgesics in cynomolgus monkeys.

2-B-P-045 一般演題（ポスター）

Quantitative evaluation of drug metabolism and transport of human iPS cell-derived intestinal epithelial cells cultured in microperfusion devices as a model of the human small intestine

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[Purpose] The development of in vitro models that can quantitatively evaluate the bioavailability of drugs is an important issue in drug development. In this study, human iPS cell-derived intestinal epithelial cells (F-hiSIEC) were cultured on a chip manufactured by Emulate, a microperfusion device and evaluate their usefulness as a small intestine model for ADME study.

[Method] Emulate chips were coated with Matrigel and F-hiSIEC (2.4×105 cells/chip) were seeded. Cell culture was performed by stretching at 2% 0.15 Hz with flow rate of 30 μL/hr. Probe drugs were added to assess function of CYP3A4, P-gp and BCRP. The drug solution was collected from the outlet 6, 24, 30, and 36 hours after the start of perfusion.

[Results and Discussion] Formation of 1-OH form, which is a metabolite of midazolam produced by CYP3A4, was inhibited by ketoconazole, while it did not affect the midazolam cell permeability. Fg of midazolam in F-hiSIEC was estimated to be 0.97. Directional transport of quinidine, a P-gp substrate, was observed in the secretion direction (BtoA) over the absorption direction (AtoB) (ER 1.7~2.6). Similarly, sulfasalazine, a BCRP substrate, also showed in the secretory direction (ER 7). The permeability of antipyrine was not directional and affected by the inhibitors.

【Conclusion】 We succeeded in culturing F-hiSIEC on the Embed chip while maintaining the barrier function, CYP3A4, P-gp and BCRP functions. CYP3A4 activity is not sufficient to explain the metabolic capacity of in vivo, and correction using scaling factor is necessary.

2-B-P-046 一般演題（ポスター）

TNFα Promotes Vascular Endothelial Cell Tube Formation via Integrin α3-β8

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【Aim】

The role of tumor necrosis factor (TNF)-α in angiogenesis was first reported in 1987 in a rat corneal model, but the exact mechanism is not fully understood. In this study, we investigated the mechanism of TNFα-induced tube formation in human endothelial cells.

【Method】

The mechanism of TNFα-induced tube formation was analyzed by the Matrigel assay using the human vascular endothelial cell line EA.hy926. To examine the effects of TNFα, vascular endothelial cells were stained with calcein-AM and observed under a microscope. Various gene expression levels were determined by real-time PCR, flow cytometry (FACS), and Western blot (WB). In addition, RNAi experiments were performed to investigate the involvement of integrin.

【Result】

TNFα induced endothelial tube formation in a dose-dependent manner. TNFα also significantly upregulated integrin α3 and β8 at both mRNA and protein levels, whereas other integrin subunits, vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMP-2 and MMP-9) were not altered at the mRNA level. In addition, TNFα-induced tube formation was effectively blocked by integrin α3 and β8 RNAi.

【Conclusion】

Our results suggest that TNFα promotes tube formation of vascular endothelial cells through integrin α3/β8, and RNAi of α3/β8 may be an inhibitor of TNFα-induced angiogenesis, and integrin α3/β8 may be a potential target for TNFα-induced angiogenic diseases.

2-B-P-047 一般演題（ポスター）

The difference in blood triglyceride levels after oral administration of olive oil in obesity-prone and obesity-resistant mouse strains is abolished by administration of lipoprotein lipase inhibitor.

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The obesity-prone ddY-H mice spontaneously develop hyperglycemia and hepatic steatosis along with a significant increase in body weight and fat mass even when fed with a normal diet, whereas the obesity-resistant ddY-L mice maintain lean and hardly develop these metabolic syndrome-like phenotypes even on a high-fat diet (HFD). To investigate differences in lipid metabolism between ddY-H and ddY-L mice, we examined whether there was any difference in fat absorption. After overnight fasting, the mice (6 weeks old) were orally administered olive oil with or without inhibiting lipoprotein lipase (LPL) by intraperitoneal injection of tyloxapol or saline. Without tyloxapol, plasma triglyceride (TG) levels increased significantly in the olive oil-administered ddY-H mice. In contrast, the TG levels in ddY-L mice remained lower; however, the TG levels in both mice were almost the same in the presence of tyloxapol. When intralipos, a soy-bean fat emulsion, was injected intraperitoneally, the increase in plasma TG levels of ddY-L mice was considerably attenuated, and again, the TG levels in both mice became the same in the presence of tyloxapol. We also found HFD-induced higher expression of LPL transcripts in epididymal fat tissue of ddY-L mice. These results suggest enhanced LPL expression may attenuate plasma TG increase in ddY-L mice.

2-B-P-048 一般演題（ポスター）

Decreased Fe^{2+} amount returns to the parental levels when clinically relevant radioresistant cells lose their radio-resistance

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We have established “clinically relevant radioresistant (CRR) cells” that can survive exposure to 2 Gy/day X-rays for more than 30 days to overcome cancer treatment resistance. CRR cells show resistance against not only radiation but also docetaxel and hydrogen peroxide which is one of the reactive oxygen species (ROS). CRR cells produce less Fe^{2+}, ROS, and lipid peroxidation compared to the parental cells. Recently, we found that this radioresistance is reversible. CRR cells that did not receive maintenance irradiation (MI; 2 Gy/day X-rays), for more than a year lost their radioresistance. We designated these CRR-NoIR cells and analyzed the mechanism of losing radioresistance. As a result, the morphology of NoIR cells maintained that of CRR cells, but the amount of both mitochondria and cytoplasm Fe^{2+} returned to the parental level. In addition, the expression of miR7-5p, which had been upregulated in CRR cells, decreased, and the expression of mitoferrin, which regulates mitochondrial iron levels downstream of miR7-5p, returned to the parental level. Furthermore, mitochondrial membrane potential and oxygen consumption also returned to parental levels. These results suggest that the amount of Fe^{2+} and the functions of mitochondria, the major iron-utilizing organelles, contribute to radio-resistance.

2-B-P-049 一般演題（ポスター）

Celecoxib inhibited the skin fibrosis via suppression of preadipocyte-myofibroblasts transition by downregulating YAP/TAZ signaling pathway

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産医大・医・薬理

Systemic sclerosis (SSc) is a connective tissue disorder characterized by skin fibrosis. Currently, there are no topical treatments available for the skin manifestations. We previously reported that celecoxib, a selective inhibitor of COX-2, suppressed cardiac fibrosis. In this study, therefore, we explored the potential of celecoxib as a topical treatment for SSc-related skin fibrosis. We found that celecoxib reduced skin fibrosis and maintained the subcutaneous fat layer in bleomycin-induced scleroderma model mice by topical treatment. To understand the underlying mechanism, we conducted in vitro experiments using 3T3-L1 murine preadipocyte cells. We found that celecoxib inhibited transforming growth factor β (TGF-β)-induced α-smooth muscle actin expression as well as extracellular matrix (ECM) genes, indicating that celecoxib hindered the differentiation of preadipocytes into myofibroblasts. Notably, celecoxib achieved it by downregulating YAP/TAZ signaling pathway, rather than TGF-β/SMAD signaling pathway. The involvement of YAP/TAZ signaling pathway was further confirmed by siRNA-based knockdown of YAP/TAZ. Our findings suggest that topical celecoxib application could be a promising treatment for SSc-related skin fibrosis.

2-B-P-050 一般演題（ポスター）

Histological evaluation of the propagation of α-synuclein into grafted human induced pluripotent stem cell-derived neurons

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Clinical trials of cell transplantation therapy using fetal mesencephalic tissue provided a proof-of-concept for regenerative therapy for the patients with Parkinson's disease. Postmortem studies of the patients who received fetal grafts revealed that α-synuclein (α-syn)$^{+}$ Lewy body-like inclusions were emerged in long-term transplantation and may reduce the clinical outcomes even the grafts were well survived in the recipients. Various studies were conducted to reveal that the host derived α-syn are transferred to the grafted neurons to assess the exsert of α-syn^{+} inclusions in the grafts. However, these studies remain the possibility to detect the intrinsic expression of α-syn in the grafted neurons. Here, we demonstrated that the human α-syn preformed fibrils inoculated into the cerebral cortex were transferred to *SNCA*$^{-/-}$ human induced pluripotent stem cell-derived midbrain dopaminergic (mDA) neurons grafted into the striatum of rats. Because grafted *SNCA*$^{-/-}$ hiPSC-derived mDA neurons lack the intrinsic expression of α-syn protein, the human α-syn-immunoreactivity found in the grafted cells should be derived from the host brain. Our results clearly showed that host-to-graft propagation of α-syn was occurred, and this work contributes to understand the molecular mechanisms to form the α-syn inclusions in the grafted mDA neurons.

2-B-P-051 一般演題（ポスター）

Effects of cigarette smoke extract derived from heated tobacco products on the endoderm differentiation of human induced pluripotent stem cells.

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In recent years, heated tobacco products (HTPs), which heat at a non-burning temperature and produce aerosols containing chemicals, have been widely used. HTPs have been reported to reduce levels of harmful chemicals, such as nicotine, tar and carbonyl compounds, compared with traditional burnt tobacco products, while epidemiological study revealed that HTP smoking may be associated with low birth weight (LBW) incidence. However, the underlying molecular mechanisms are incompletely understood. In this study, we investigated the effects of cigarette smoke extract (CSE) from HTPs on the endoderm differentiation of human induced pluripotent stem cells (iPSCs). We found that the HTP-CSE decreased the endoderm markers SOX17 and FOXA2 during endoderm differentiation of iPSCs. Next, we focused on the phosphorylation of Smad2, which is phosphorylated by activation of ALK4/7 during endoderm differentiation. The HTP-CSE inhibited the phosphorylation of Smad2 during endoderm differentiation. These results suggest that HTP-CSE disrupts endoderm differentiation of iPSCs via inhibition of Smad2 phosphorylation. Thus, HTPs during pregnancy might induce LBW and poor fetal organ growth via a delayed endoderm differentiation.

2-B-P-052 一般演題（ポスター）

Inhibitory effect of Taraxacum coreanum ethanol extract on human platelet aggregation

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Platelets play a crucial role in hemostasis, an essential physiological process that prevents excessive blood loss. In cases of hemorrhage, circulating platelets adhere to sites of injury in blood vessels, become activated upon exposure to adhesive proteins or soluble agonists, and form a platelet plug to achieve hemostasis. However, excessive platelet activation and aggregation can lead to thrombus formation and subsequent vessel occlusion. Korean dandelion (Taraxacum coreanum Nakai) is used in traditional Korean medicine to treat inflammatory diseases such as gastritis, gastric ulcer, and tonsillitis. In this study, the anti-platelet effects of an ethanol extract of T. coreanum (TCE) were investigated in vitro using collagen-, thrombin-, TPA-, or ADP-stimulated platelet aggregation. TCE significantly inhibited platelet aggregation induced by collagen, thrombin, and ADP. Furthermore, TCE exhibited obvious inhibitory effects on granule secretion, TXA2 production, integrin αIIbβ3 activation, and clot retraction. We found that TCE attenuated PI3K/Akt and MAPK (p38 and ERK) signaling pathways, and increased cAMP level. The data presented here demonstrate that TCE inhibited agonist-induced platelet aggregation and thrombus formation. These inhibitory effects may be associated with the inhibition of COX-1 activity, integrin αIIbβ3 activation, and increase of cAMP level. Therefore, we suggest that TCE may have therapeutic potential as an antiplatelet and antithrombotic agent.

2-B-P-053 一般演題（ポスター）

The effects of cardiotonic glycoside digoxin on arterial elasticity in the aortic and femoral arterial segments of anesthetized rabbits

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We have shown that arterial elasticity can be acutely modified by vasoactive drugs in rabbits. To clarify the functional role of the heart in the regulation of arterial elasticity in vivo, we analyzed the effects of cardiotonic glycoside digoxin on arterial elasticity in rabbits using the stiffness parameter β of the aortic (aortic β) and femoral arterial (femoral β) regions by measuring blood pressures at the right brachial artery, aortic bifurcation and tibial artery, electrocardiogram and phonocardiogram. Intravenous administrations of digoxin (0.03, 0.1, and 0.3 mg/kg) increased blood pressure, and carotid and femoral arterial blood flow in a dose-dependent manner. Digoxin increased femoral β in a dose-dependent manner, whereas it decreased aortic β. Meanwhile, vascular resistance was unaffected by digoxin. The current results of decreased arterial elasticity in the femoral artery region and increased aortic elasticity by digoxin at cardiotonic doses were similar to our previous study with β_1-adrenergic receptor agonist dobutamine, suggesting that cardiotonic drugs' actions can affect arterial elasticity independently of direct action on vascular smooth muscle.

2-B-P-054 一般演題（ポスター）

Effects of adrenaline administration on abnormal electrocardiographic electrical signals and myocardial H-FABP protein expression during anaphylaxis in rats.

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This study aimed to investigate the effects of adrenaline treatment on abnormal electrical signals in the ECG of anaphylaxis-induced rats. Wistar male rats were anesthetized, ventilated, and placed on a multi-electrode probe array at the heart to record the ECG and measure blood pressure. Anaphylaxis was induced with compound 48/80, and saline or adrenaline was injected intravenously 15 minutes after anaphylaxis and observed until 30 minutes after anaphylactic induction. Hypotension associated with anaphylactic shock was transiently recovered by adrenaline administration. QTc shortening was observed during anaphylaxis induction and transiently improved with adrenaline administration. The frequency of PVCs increased during anaphylaxis induction and decreased with adrenaline administration. Although there was no difference in the expression of gap junction protein Connexin40 protein in myocardial tissue at 30 minutes of anaphylaxis induction in the adrenaline-treated group compared with the untreated group, H-FABP protein expression in myocardial tissue was significantly lower in the adrenaline-treated group. Thus, the reduction in QTc shortening and PVC frequency with adrenaline administration during anaphylaxis induction may be associated with a decrease in H-FABP protein expression in myocardial tissue.

2-B-P-055 一般演題（ポスター）

miR-1914-5p regulates vascular endothelial cell-monocyte adhesion in the inflammatory environment of atherosclerosis

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[Background]Atherosclerosis results from vascular endothelial cell injury caused by lipid metabolites deposition in the vessel wall, leading to myocardial and cerebral stroke. Early detection and treatment are difficult because of the lack of clinical symptoms in the early stages. MicroRNAs (miRNAs) regulate the expression of target mRNAs and have attracted attention as diagnostic biomarkers. In this study, we focused on the adhesion between monocytes and endothelial cells in the early stage of atherosclerosis. We examined the relationship between miRNAs and changes in endothelial-monocyte adhesion induced by IL-1β.

[Results]We found that hsa-miR-1914-5p was decreased by IL-1β stimulation in human vascular endothelial cell line EA.hy926 or monocytic cell line THP-1. Endothelial-monocyte adhesion and expression of adhesion factors were significantly increased under both IL-1β-stimulated and hsa-miR-1914-5p inhibitory conditions. Furthermore, overexpression of hsa-miR-1914-5p significantly suppressed IL-1β-induced adhesion.

[Conclusion] These results suggest that hsa-miR-1914-5p may suppress endothelial-monocyte adhesion, thereby reducing the progression of early lesions in atherosclerosis. These findings are expected to lead to the development of novel therapies for atherosclerosis targeting hsa-miR-1914-5p as well as its application as a biomarker.

2-B-P-056 一般演題（ポスター）

Cigarette smoke extract (CSE) directly induces cardiomyocyte dysfunction in rat and human iPS-derived cardiomyocytes thorough abnormal intracellular Ca^{2+} dynamics and mitochondrial dysfunction.

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Smoking is well known as a major risk factor of cardiovascular diseases. However, the direct effects of smoking substances on cardiomyocyte and its cellular mechanism have not been fully clarified. In this study, we examined the effects of CSE on the contractile function, intracellular Ca^{2+} dynamics, and mitochondrial function using cultured or freshly isolated rat ventricular myocytes and human iPS-derived cardiomyocytes. CSE at concentration above 0.1% decreased the spontaneous beating rate of cultured rat cardiomyocytes in a time- and concentration-dependent manner. 1% CSE reduced the cell shortening of freshly isolated cardiomyocytes. Similar contractile dysfunctions were also observed in human iPS-derived cardiomyocytes. In contrast, 1% CSE increased intracellular Ca^{2+} transient amplitude, often triggered spike-shaped Ca^{2+} transients. Kinetic analysis indicated that CSE enhanced Ca^{2+} uptake into the SR. These results suggest that abnormal Ca^{2+} entry/release in the SR occurred upon CSE treatment. Furthermore, CSE induced a decrease in mitochondrial membrane potential, an early event in cellular damage that can be caused by abnormal intracellular Ca^{2+} dynamics. Taken together, these results demonstrate that CSE weakens contractile function of cardiomyocytes via abnormal Ca^{2+}-medicated dysregulation of mitochondrial function.

2-B-P-057 一般演題（ポスター）

Development of cardiotoxicity evaluation method using HD-CMOS-MEA

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Cardiotoxicity is a common reason for drug discontinuation in new drug development. In vitro microelectrode array (MEA) method using hiPSC-CMs is expected to be an alternative method to animal experiments, but there is a problem that the hiPSC-CMs cannot mature sufficiently in the case of two-dimensional culture. In addition, the evaluation method of MEA is mainly based on the field potential duration (FPD) as an index, and the mechanism of action based on conduction velocity and propagation pattern has not been predicted. The purpose of this research is to construct an evaluation method focusing on conduction velocity and propagation pattern as an evaluation index of MEA. In order to enable detailed conduction velocity and conduction pattern analysis, hiPSC-CMs were measured using a 240,000-electrode HD-CMOS-MEA with high-density microelectrodes instead of conventional MEA. HD-CMOS-MEA has a resolution that can record 1 cell with several tens of electrodes, and was able to accurately detect the origin of pulsation and conduction direction. We detected 6 drug responses, and by using new parameters that can be evaluated by CMOS-MEA measurement, it has become possible to detect the toxicity of mexiletine, which was difficult to detect with the conventional evaluation method. The cardiotoxicity assessment using HD-CMOS-MEA has the potential to detect cardiotoxicity risks that could not be detected by conventional MEA analysis based on new parameters. It is expected to become a new evaluation method with the development of a method for maturation of hiPSC-CMs.

2-B-P-058 一般演題（ポスター）

Cytotoxic effect of gas phase extract of mainstream smoke derived from heated tobacco products on vascular smooth muscle cells.

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Ferroptosis is an iron-dependent regulated form of regulated cell death that is caused by excessive lipid peroxidation-mediated cell membrane damage. The present study compared the cytotoxic effects of nicotine- and tar-free cigarette smoke extract (CSE) prepared from heated tobacco products (HTPs) and a ferroptosis inducer erastin on vascular smooth muscle cells. Cigarette smoke of HTPs (Ploom X, IQOS 3, and IQOS ILUMA) was generated according to the HTP-259-CTR smoking regime (55 mL puff volume, 30 s puff interval, 2 s puff duration, bell-shaped puff profile, and not blocking the ventilation holes) using an analytical vaping machine LM5E (Körber Technologies Instruments GmbH). The cytotoxicity of CSE and erastin to rat vascular smooth muscle cells (A7r5 cells) was evaluated by measuring mitochondrial metabolic activity and lactate dehydrogenase (LDH) leakage. Erastin and CSE induced a decrease in mitochondrial metabolic activity and an increase in LDH leakage. The cytotoxic effects of erastin were almost completely inhibited by a radical trapping antioxidant UAMC-3203, an iron chelator deferoxamine mesylate (DFO), a 12/15-lipoxygenase (12/15-LOX) inhibitor baicalein, and a selective 15-LOX-1 inhibitor ML351. On the other hand, CSE-induced cell damage was partially attenuated by UAMC-3203, baicalein, and ML351, but not DFO. These results suggest that although erastin induces iron-dependent, 15-LOX-mediated ferroptosis, CSE primarily causes cell damage through a ferroptosis-independent mechanism..

2-B-P-059 一般演題（ポスター）

Directionality in the EAD-evoked triggered activity as an initiator of lethal arrhythmias brought about by proarrhythmic effects of class III antiarrhythmic agents

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Prolongation of action potential duration by class III antiarrhythmic agents increases the risk of early afterdepolarization (EAD) developments, which in turn increases the risk of developing ventricular tachycardia (VT) such as torsades de pointes, and/or ventricular fibrillation. Recently, our group has reported that the concave shape (in the localized region) of repolarization potential difference boundary due to EAD clustering occurrence within the tissue may be involved in the triggered activity formation that initiates VT. In the present study, we further investigated how anisotropy of excitation propagation in the ventricle contributes to the triggered activity formation. Simulating excitation propagation in a two-dimensional (2D) ventricular tissue model in which a cardiac rapidly activating delayed rectifier K^+ channel current (I_{Kr}) was inhibited by 87% of control by class III antiarrhythmic agents, we examined whether the EAD-induced triggered activity was formed when one side of the 2D tissue was stimulated in the parallel or transverse direction to fiber orientation. By stimulation in the direction parallel to fiber orientation, the EAD-induced triggered activity was formed along the fiber orientation by parallel placing of two EAD clusters of appropriate size at appropriate distances along the fiber orientation. Under the same EAD cluster configuration, even with perpendicular stimulation to the myocardial fiber orientation, triggered activity was formed along the fiber direction. However, triggered activity did not occur when EAD clusters were placed perpendicular to the fiber direction. We concluded that there exists a certain directionality in the triggered activity formation following EAD development. The triggered activity as the initiator of lethal arrhythmias caused by proarrhythmic properties of class III antiarrhythmic agents may exhibit anisotropy-based directionality within the ventricle.

2-B-P-060 一般演題（ポスター）

Liver-specific LPLAT10/LPCAT4/LPEAT2 overexpression increases glucose-stimulated insulin secretion

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Type 2 diabetes mellitus (T2DM) incidence is increasing worldwide. The fatty acid composition of phospholipids—a major component of biological membranes—has received research attention owing to their involvement in T2DM onset and progression. We hypothesized that changes in the fatty acid composition of phospholipids in the liver, the central organ of energy metabolism, alleviate their aberrant metabolism. We focused on lysophospholipid acyltransferase 10 (LPLAT10/LPCAT4/LPEAT2), an enzyme that changes the fatty acid composition of phospholipids, and examined the effect of LPLAT10 on glucose metabolism. To overexpress LPLAT10 in mouse liver, we generated an LPLAT10-expressing adenovirus (Ad) vector (Ad-LPLAT10) using an improved Ad vector. Hepatic LPLAT10 mRNA and protein expression in Ad-LPLAT10-treated mice was much higher than that in mice treated with control Ad vector. Induction of glucose-stimulated insulin secretion (GSIS) suppressed postprandial hyperglycemia in Ad-LPLAT10-treated mice compared with that in control Ad-vector-treated mice. Hepatic and serum levels of phosphatidylcholine, containing polyunsaturated fatty acid, were elevated in Ad-LPLAT10-treated mice. Elevated phosphatidylcholine levels increased GSIS in mouse insulinoma cells. Our study suggests that LPLAT10 is a promising new therapeutic target for T2DM.

2-B-P-061 一般演題（ポスター）

Nardilysin in hepatocyte regulates brown adipose tissue activity

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Brown adipose tissue (BAT) plays a critical role in diet-induced thermogenesis, a process by which excess energy intake is consumed as heat. Several studies have suggested that hepatocytes regulate diet-induced thermogenesis in response to changes in nutritional status. However, the precise mechanisms by which hepatocytes contribute to this process are not yet fully understood.

We have previously demonstrated that mice systemically deficient in nardilysin (NRDC), a member of the M16 family of metalloendopeptidase, exhibit decreased adiposity, enhanced energy expenditure and BAT activity. To clarify the tissue-specific role of NRDC, we generated multiple lines of tissue-specific NRDC knockout mice. Among them, to our surprise, hepatocyte-specific NRDC knockout mice (LKO) showed 1) increased mRNA expression of thermogenic genes in BAT, 2) less fat accumulation in BAT, 3) increase in whole-body energy expenditure. Mechanistically, the deletion of NRDC in the liver enhances BAT theremogenesis at least partly thorough changes in humoral factors. We also found that liver nardilysin (NRDC) expression is modulated by nutrient availability with upregulation during fasting and downregulation during re-feeding. Furthermore, liver NRDC levels decrease upon high-fat diet feeding.

Taken together, these findings suggest that NRDC functions as a potential nutrition sensor in the liver and regulates diet-induced thermogenesis.

2-B-P-062 一般演題（ポスター）

MICU1 tightly regulates the mitochondrial Ca^{2+} signal in pancreatic β-cells.

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Glucose-induced stimulation of β-cells results in oscillatory changes in cytosolic Ca^{2+} concentration, which lead to insulin secretion and may also cause flux of Ca^{2+} into the mitochondrial matrix. Although recent reports suggest that Ca^{2+} uptake by mitochondria via mitochondrial Ca^{2+} uniporter (MCU) facilitates insulin secretion, mitochondrial Ca^{2+} dynamics in β-cells require further clarification. Using the recently developed CEPIA*mt* Ca^{2+} indicators, we analyzed mitochondrial Ca^{2+} dynamics in high glucose-stimulated β-cells. Contrary to our expectation, oscillatory increases in cytosolic Ca^{2+} concentrations in response to high glucose stimulation induced minimal changes in mitochondrial Ca^{2+} concentration. Upon shRNA-mediated knockdown of MICU1, one of the essential regulators of MCU, we observed markedly increased oscillatory changes in the mitochondrial Ca^{2+} concentration. In addition, MICU1-depleted β-cells showed a significant decrease in glucose-induced insulin secretion. These results indicate that MICU1 limits mitochondrial Ca^{2+} uptake in β-cells, and such tight regulation of mitochondrial Ca^{2+} signals is required to maintain insulin secretion. To further understand the physiological and pathophysiological significance of the MICU1-dependent mitochondrial Ca^{2+} regulations in insulin secretion, we plan to use β-cell-specific MICU1 knockout genome-edited mice.

2-B-P-063 一般演題（ポスター）

Clinical evaluation of Graves‘ disease using TSH receptor autoantibody (TRAb)-isotype and TRAb-IgM/TRAb-IgG (the MG ratio)

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Objective

Thyrotropin receptor antibodies (TRAbs) are causative antibodies of Graves‘ disease. TRAbs are heterogeneous antibodies containing stimulating type (thyroid stimulating antibody: TSAb), blocking type, neutral type, and they could be classified into immunoglobulin isotype (TRAb-IgG and TRAb-IgM). Although the currently used methods are not suitable to measure TRAb-IgM, it may be the important antibody produced prior to TRAb-IgG. In the present study, we measured TRAb-isotype and calculated TRAb-IgM/TRAb-IgG (MG ratio), and report the possibility that MG ratio may be a predictor of clinical state of Graves‘ disease

Methods

We prepared total of 50 serial samples from 14 patients of Graves‘ disease (7 hyperthyroidism, 8 hypothyroidism, and 35 euthyroidism), and single samples from 50 patients of Graves‘ disease and 10 healthy subjects. TRAb-IgG, TRAb-IgM, and MG ratio of these samples were measured by ELISA system.

Results & Discussion

MG ratio could separate euthyroid from hyper or hypothyroidism (Cut off: 9.532, AUC: 0.947, 95% confidence interval: 0.889-1). Both TRAb-IgG and TRAb-IgM could diagnose Graves‘ disease with good positive or negative percent agreement with conventional RRA.

In some cases, increases of MG ratio were observed before severe hypothyroidism suggested the appearance of blocking TRAb.

Conclusions

MG ratio could detect exacerbation of Graves‘ disease, and TRAb-IgG and TRAb-IgM can be used for conventional TRAb. TRAb isotype and MG ratio may become necessary to know pathophysiology of Graves‘ disease.

Zagmutt Sebastian, Sabina Quader, Rosalía Rodríguez-Rodríguez

Obesity has become an important health problem and its prevalence is highly increasing. Pharmacotherapy alone or in combination with either lifestyle modification or surgery, is consistent in maintaining a healthy body weight, and preventing progression to obesity-related diseases. However, the anti-obesity drugs are limited by non-specificity and unsustainable weight loss effects. Therefore, further research is needed to develop new preventive and treatment approaches against obesity. Here we targeted the brain lipid metabolism as a promising strategy. In the brain, the hypothalamus harbors specialized functional circuits in the physiological regulation of calories intake and spend. Importantly, some enzymes involved in fatty acid metabolism are highly expressed in the hypothalamus. We took advantage of the C75-CoA, a strong competitive inhibitor of carnitine palmitoyltransferase 1A (CPT1A) that catalyzes the rate-limiting step of fatty-acid oxidation.

Nanomedicine-based approaches hold promise for improved brain distribution of drugs. We developed an ingenious crosslinked polymeric micelle that can stably load the C75-CoA for in vivo application.

Intracerebroventricular administration of the nanomedicine resulted in the reduction of food intake and body weight compared with the free drug. This satiating effect seemed to be regulated by the appetite-related neuropeptides and specific hypothalamic nuclei activation. Altogether, this study might contribute to the development of a new generation of nanomedicine-based approaches targeting brain to prevent obesity.

The purpose of this study was to confirm the effect of natural minerals on hyperglycemia and glucose intolerance in streptozotocin (STZ)-induced diabetic mice. The STZ with natural mineral group exhibited lowered fasting plasma glucose levels than the STZ-induced diabetic group. Oral glucose tolerance tests showed that natural minerals improves impaired glucose tolerance in STZ-induced diabetic mice. Histopathological evaluation of the pancreas showed that natural minerals restores the morphology of the pancreatic islets of Langerhans and increases the secretion of insulin in STZ-induced diabetic mice. These results suggest that natural minerals is a potential anti-diabetic agent, owing to its ability to suppress hyperglycemia and improve glucose intolerance by modulating glucose metabolism and increasing glucose uptake. .(“This research was a part of the project titled 'Global multi-combination product development and export utilizing deep seawater extracted minerals (FDA notification) funded by the Ministry of Oceans and Fisheries, South Korea.")

2-B-P-066 一般演題（ポスター）

The Effects of cGMP on the filtration barrier function reinforcement in cultured glomerular podocytes by mechanical and receptor-mediated stimulation

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Glomerular podocytes are continuously exposed to neurohumoral factors and blood flow and pressure. We recently found that mechanical stimulation immediately suppressed the receptor-evoked responses of canonical transient receptor potential 6 (TRPC6) channel in podocytes using the intracellular Ca^{2+} imaging and whole-cell patch clamp techniques. This effect coincided with diminished leak of FITC-labelled albumin across the cell-culture insert membrane. In this study, we investigated the effects of cyclic GMP (cGMP) on the albumin leak, the agent reportedly suppressing the heterologously expressed TRPC6 channel activity through protein kinase G-mediated phosphorylation (Takahashi *et al., J.Physiol.*, 586, p4209-4223, 2008). We cultured immortalized mouse podocytes stably expressing wild-type TRPC6 on the cell-culture membrane inserts and induced their differentiation. The podocytes were then simultaneously stimulated by angiotensin II (AgII) and a membrane-expanding agent 2,4,6-trinitrophenol (TNP) for 48 hr, in the presence or absence of 8Br-cGMP, an analogue of cGMP. In 8Br-cGMP-treated cells, the suppression of albumin leakage by AgII and TNP was attenuated. These results imply the complex actions of cGMP on the barrier function which may not be accounted for solely by the inhibition of TRPC6 channel.

2-B-P-067 一般演題（ポスター）

The role of circadian clock gene Bmal1in TGF-β-induced renal fibrosis

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Introduction: Kidney fibrosis is the hallmark of chronic kidney disease progression. At present, no pharmacological therapy against kidney fibrosis exists. The circadian clock gene Bmal1 has been implicated in the multiple diseases including cancer and atherosclerosis. However, the role of Bmal1 in kidney fibrosis remain largely unknown. We therefore examined whether Bmal1 modulates TGF-β-induced renal fibrosis.

Methods: We used the unilateral ureteric obstruction (UUO) mouse model to examine the alteration of Bmal1 expression during renal fibrosis. Also, this study used TGF-β-stimulated NRK-49F renal cells as an in-vitro model of renal interstitial fibrosis. Masson's trichrome stain was applied to identify the degree of kidney fibrosis. Real-time PCR and western blotting techniques were used for gene expression and protein expression, respectively.

Results: Kidneys from UUO mice displayed marked renal fibrosis compared with the control mice. Western blotting analyses revealed increased Bmal1 protein expression in the kidney after UUO, with increase in NRK-49F cells following TGF-β treatment, accompanied by increased fibrotic markers including α-SMA and collagen I. In addition, Bmal1 mRNA expression was increased in TGF-β stimulation and the increase of Bmal1 inhibited by the pretreatment with MEK inhibitor, U0126. Furthermore, knockdown of Bmal1 or SR9009, a REV-ERB agonist that inhibits the transcriptional activity of the Bmal1 expression alleviated TGF-β-induced α-SMA and collagen I mRNA expression. Knockdown of Bmal1 or SR9009 also TGF-β-induced NOX4 mRNA and phosphorylation of p38 that is involved in TGF-β-induced fibrotic markers.

Conclusion: These data suggest that increased Bmal1 expression plays a crucial role in the induction of fibrotic markers, which leads to the development of kidney fibrosis. Thus, Bmal1 may be a potential therapeutic target for the prevention or treatment of kidney fibrosis.

2-B-P-068 一般演題（ポスター）

Comparison of fibrosis levels and drug effects on fibrosis among various mouse models of ischemic acute kidney injury

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【Background and Purpose】The number of patients who progress from acute kidney injury to chronic kidney disease is increasing. Glomerulosclerosis and fibrosis of the renal tubular interstitium are commonly observed in end-stage renal disease. In this study, we compared the fibrosis levels after ischemia-reperfusion treatment in mice with acute kidney injury to develop a pathological model suitable for drug evaluation. The effects of drugs on fibrosis after ischemia-reperfusion were also examined.【Methods】Male C57BL/6J mice were divided into three groups: the left kidney ischemia for 25 min group (left kidney ischemia), left kidney ischemia for 25 min and right kidney removal one week later group (left kidney ischemia-right nephrectomy), and left and right kidney ischemia for 25 min group (left and right kidney ischemia). The fibrosis levels after ischemia-reperfusion treatment were compared.【Results】All groups showed an increased kidney hydroxyproline and area fraction of positive Sirius Red stain. The degree of increase in hydroxyproline and Sirius Red stain positivity was similar in the left ischemia-right nephrectomy group and the left and right ischemia group, and was more than twice as high in the left ischemia group compared to the two groups. Mixed feeding of pirfenidone significantly suppressed fibrosis after ischemia-reperfusion treatment.

2-B-P-069 一般演題（ポスター）

Effects of an immunosuppressive agent on the anti-glomerular basement membrane nephritis model rat

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Mediford Corporation

Anti-glomerular basement membrane (GBM) nephritis, caused by the production of autoantibody against GBM, is characterized by rapidly progressive glomerulonephritis. Novel agents which prevent the progression of anti-GBM nephritis are expected. The rat model induced by administration of anti-GBM antibody is considered as one of the useful experimental models of this disease. In the present study, effects of a typical immunosuppressive agent were evaluated using this model. The anti-GBM nephritis model was induced by single intravenous injection of anti-GBM antibody to male WKY rats on Day 0. As the treatment, cyclophosphamide (CYP) was orally administrated once daily from Day 0 to 27. The urine was collected on Day 7, 14, and 28. On Day 28, the kidney was harvested and wet weight was measured. In the nephritis group, the index of the score of occult hematuria was elevated. Moreover, the urinary protein excretion and the ratio of kidney weight to body weight were increased. In the CYP treated group, the urinary protein excretion level and the kidney weight ratio were significantly suppressed. These results suggest that the anti-GBM nephritis model is useful to evaluate the effects of the therapeutic agents. This model could contribute to the development of the novel agents of anti-GBM nephritis.

2-B-P-070 一般演題（ポスター）

Protective effect of sulfur-containing molecules against cisplatin-induced nephrotoxicity

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Cisplatin is one of the most widely used chemotherapeutic agents in the treatment of various types of tumors. The efficacy is dose-dependent, but at higher doses the risk of nephrotoxicity limits its therapeutic potential. Several sulfur-containing molecules function as a cytoprotectant against cisplatin-induced nephrotoxicity. However, the mechanism of cisplatin toxicity in dividing cells differs from that in non-dividing cells, and it is unclear which state of cells are protected by sulfur-containing molecules. In this study, we examined the effect of sulfur-containing molecules against cisplatin toxicity at pre- and post-confluence of renal cell line LLC-PK1. Cytotoxicity was assessed by MTT assay. Sulfite and thiosulfate attenuated cisplatin toxicity at both pre- and post-confluence. Their oxidation products sulfuric acid and tetrathionic acid had no effect, suggesting the reducing potency of sulfite and thiosulfate provides cytoprotection. Tetrasulfide, which is proposed as a novel therapeutic agent to prevent cisplatin toxicity, did not show as much protection as sulfite or thiosulfate in either the pre- or post-confluence state. In addition, tetrasulfide exacerbated cisplatin toxicity at relatively low concentrations. These results suggest that sulfite and thiosulfate can be used as a therapeutic agent against cisplatin-induced nephrotoxicity.

2-B-P-071 一般演題（ポスター）

Exploration of Oncogenic Functions Targeting the DNA Methylation Factors UTX/UTY in Renal Cell Carcinoma

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DNA methylation and histone modifications are crucial for tissue homeostasis, and mutations in methylation-related genes like UTX and UTY are closely associated with cancer development. In renal cell carcinoma (RCC), defects in UTX and UTY have provided new therapeutic directions and insights into the understanding of the cancer's pathogenic mechanisms.

In this study, we used dual siRNA (siUTX/siUTY) to knock down the UTX and UTY genes in three cell lines, resulting in enhanced cell proliferation, invasion, and migration abilities. Clinical data for RCC (n=312) were obtained from the TCGA database. The results indicated that, compared to the high UTX expression group, the low UTX expression group exhibited a significantly shortened progression-free survival (PFS). Similarly, the low UTX expression group also showed a significantly shortened overall survival (OS) compared to the high UTX expression group.

In order to elucidate the role of UTX and UTY loss of function in the pathogenesis of RCC, we generated genetically engineered mice with kidney-specific deletions of Utx and Uty (Utx Δ, Uty Δ). These mice were then crossbred with mice carrying mutations closely associated with RCC, namely p53+/- and Vhl+/- mice. Ultimately, Utx Δ, Uty Δ, p53 +/-, and Vhl+/- compound mice were created. To promote cancer development, mice were subcutaneously injected with iron (Fe) starting from 8-9 weeks of age. Kidney tissues were collected at regular intervals to observe the onset of cancer and changes in malignancy.

Future research may focus on developing new treatment methods, diagnostic tools, and prevention strategies to improve the survival rate and quality of life for kidney cancer patients.

Effect of α2-adrenoceptor antagonist on puromycin aminonucleoside-induced nephrosis in rats

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Nephrotic syndrome is characterized by proteinuria and hypoalbuminemia, results from the dysregulation of glomerular podocytes and is a significant cause of chronic kidney disease. We previously reported that urinary protein excretion decreased by administration of yohimbine, α2-adrenoceptor antagonist, in 5/6 nephrectomy-induced chronic kidney disease rat model. Here, we examined the effect of yohimbine on puromycin aminonucleoside (PAN)-induced nephrosis rats. Male Sprague Dawley rats were randomly divided into the following groups: sham-operated, PAN (50 mg/kg, i.v.) and PAN + yohimbine (3 mg/L in drinking water). We found that proteinuria increased in the PAN group in a time-dependent manner. In immunostaining studies, PAN group showed decreased expressions of nephrin and podocin as well as increased desmin expression compared with sham group. Treatment with yohimbine improved proteinuria and increased expression of desmin in PAN group. Furthermore, yohimbine restored the expressions of nephrin and podocin. The α2C-adrenoceptors were partly distributed in some compartments of the podocytes. In contrast, α2A- and α2B-adrenoceptors were not detected in the glomeruli. These findings suggest that the yohimbine can regulate glomerular filtration and proteinuria through the induction of morphological changes in podocytes via α2C-adrenoceptors.

2-B-P-073 一般演題（ポスター）

Development of repeatable acute kidney injury model in cynomolgus monkey due to noninvasive ischemia-reperfusion of the renal artery

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Backgrounds: Conventional models of acute kidney injury due to ischemia-reperfusion of renal artery require opening the abdomen and clipping the renal artery for ischemia. We developed a method of noninvasive ischemia-reperfusion of the left renal artery after the animal's general condition had recovered by placing a vascular cuff occluder in the left renal artery at the operation of right nephrectomy.

Materials and methods: After removal of the right kidney of a male cynomolgus monkey, a vascular cuff occluder was implanted in the left renal artery. One week after placement of the cuff occluder, the cuff was inflated via catheter and renal artery was ischemic for 90 minutes, and then the cuff was deflated to resume blood flow. Ultrasonography also confirmed the cessation and resumption of blood flow. A total of four 60- or 90-min ischemia-reperfusion were performed at 2-week intervals. The plasma BUN and Cre were measured before ischemia and after reperfusion.

Results and discussion: In a 60-minute ischemia-reperfusion study, Cre increased from a pre value; 0.89 mg/dL to 1.40 mg/dL at 6 hours after reperfusion, and BUN increased from a pre value;32.9 mg/dL to 46.2 mg/dL at 6 hours after reperfusion. In the 90-minute ischemia-reperfusion studies, mean Cre increased from a pre value; 1.01 mg/dL to 2.05 mg/dL at 6 or 24 hours after reperfusion, and mean BUN increased from a pre value; 36.0 mg/dL to 66.2 mg/dL at 24 hours after reperfusion.
Our study allowed noninvasive and repeatable modeling of acute kidney injury due to the ischemia-reperfusion of the renal artery.

2-B-P-075 学生セッション（ポスター）

Analysis of energy metabolism in olanzapine-induced weight gain rats using long-term indirect calorimetry measurements

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Second Generation antipsychotics, including olanzapine are commonly prescribed for the treatment of schizophrenia. Olanzapine is recognized for its superior therapeutic efficacy; however it may cause adverse effects such as weight gain. The underlying mechanisms responsible for this side effect have not been fully elucidated in basic research. In this study, we investigated metabolic profiles of olanzapine-treated rats, using long-term indirect calorimetry system. This system allowed us to assess several metabolic parameters. These included energy expenditure(EE), respiratory quotient(RQ), carbohydrate oxidation(CHO) , and fat oxidation(FO).

The results showed that olanzapine at 3 mg/kg once daily increased energy expenditure(EE) per body weight in rats. In addition, respiratory quotient(RQ) indicated high values.

These results suggest that olanzapine alter the metabolism of carbohydrates and lipids in rats, which may contribute to olanzapine-induced weight gain. The development of targeted interventions to mitigate the adverse effects associated with olanzapine treatment may be facilitated by an understanding of these mechanisms.

Comparative study of drug prescriptions and disease diagnoses based on analysis of claims databases in Japan and the USA

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When conducting research using multiple claims databases, it should be noted that there may be bias among databases due to intrinsic preferences in drug prescription and disease diagnosis. For precise data analysis, it is important to understand the reality of drug prescriptions and disease diagnoses in each database; however, comparative analysis of claims databases in different countries have been rarely performed. In this study, we analyzed drug prescriptions and disease diagnoses recorded in JMDC Claims Databases (Japan) and IBM® MarketScan® Databases (USA) to explore the significance of using different databases with multiple examples. In each database, drug ingredient names were mapped to anatomical therapeutic chemical (ATC) codes and disease names were mapped to international classification of diseases, 10th edition (ICD-10) codes, so that 95.4% of prescriptions and all outpatient diagnoses were linked to nonredundant codes. In this analysis, 198 drugs including benzodiazepine hypnotics and 590 disease names such as interstitial lung disorder were significantly more frequent in Japan, while 220 drugs including TNF-alpha inhibitors and 785 disease names such as type 2 diabetes mellitus were significantly more frequent in the USA. These results suggest that a comprehensive comparative analysis of drug prescriptions and disease diagnoses in claims data may reveal previously unreported facts leading to a better understanding of national differences in pharmacotherapy.

2-B-P-077 学生セッション（ポスター）

Estimation of disease preventive drugs and the mode-of-action using clinical big data

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Once a disease develops, it is difficult to restore to health. Therefore, disease prevention is important to extend healthy life expectancy. In this study, we propose a computational method to estimate the potential preventive drugs that are effective in preventing the onset of the target disease by calculating the reporting odds ratio based on the reports of clinical medication history (more than 40 million reports on drug responses and adverse events). Since the potential preventive drugs were overlooked by conventional odds ratios, we calculated a variant of the odds ratio in order to overcome the problem of the conventional odds ratio, and analyzed the mode-of-action of the preventive drugs based on the chemical structures and chemical-protein interactome. We applied the proposed method to various diseases including hypertension, and evaluated its performance in terms of reproducibility for known therapeutic targets. We confirmed that the proposed odds ratio worked better than the conventional odds ratio. The proposed method is applicable to any diseases and is expected to useful for prevention for various diseases.

2-B-P-078 学生セッション（ポスター）

Functional analysis of CHAMP1, a gene product associated with intellectual disability, in circadian rhythms

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Circadian rhythms are biological phenomena that regulate the behavior of organisms, fluctuating in approximately 24-hour cycles, and regulate basic physiological functions such as sleep, metabolism, and hormone secretion. Chromosome alignment-maintaining phosphoprotein 1 (CHAMP1) is known as one of the candidate causative genes of intellectual disability (ID). Recent clinical studies have found that patients with ID harboring de novo mutations on the *CHAMP1* gene have often sleep difficulties. In this study, we focused on the molecular and cellular mechanisms underlying sleep difficulties. First, we found that the *Champ1* gene expression exhibited a time-dependent rhythm when clock genes were synchronized by serum shock in NIH3T3 cells. We also found that the expression level of the Clock gene, one of the clock genes, was decreased when the *Champ1* expression was suppressed. Furthermore, we found that the period of a mouse model heterozygous for a *CHAMP1* mutation identified in a patient with ID was significantly longer under constant dark conditions in actogram analysis. Our results suggest that sleep difficulties in patients with ID harboring *CHAMP1* gene mutations may be due to circadian rhythm dysregulation.

Functional Analysis of the Autism Spectrum Disorder-Associated Gene Product, POGZ Using Patient-Derived iPS Neurons

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Autism Spectrum Disorders (ASDs) are likely to be associated with impaired central nervous development, however the molecular and cellular pathogenesis of ASDs remains largely unknown. In this study, we focused on *POGZ*, one of the most recurrently mutated genes in patients with ASDs. To elucidate the molecular and cellular pathogenesis underlying POGZ mutation-mediated neural developmental abnormalities, comprehensive RNA expression analysis was conducted using samples derived from neural stem cells and neurons from a patient with POGZ mutation and control individuals. We performed gene ontology (GO) enrichment analysis on genes that exhibited significant differences in expression between patients and healthy individuals and found that the differentially expressed genes in neural stem cells and neurons were enriched for GO terms involving neural development. Furthermore, phospho-proteome analysis suggested that several signal transduction pathways potentially implicated in neural development were impaired in iPS cell-derived neurons from the patient. Our current results will help to clarify the pathogenesis of the *de novo* mutation in the *POGZ* gene locus, providing crucial insights into understanding the molecular and cellular pathogenesis underlying ASDs.

2-B-P-080 学生セッション（ポスター）

Phenotypic Analysis of ASD-related POGZ-Y594C Mutant Mice

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Autism Spectrum Disorders (ASDs) are neurodevelopmental conditions characterized by impairments in social interaction and repetitive behavior. Recent studies suggest that *de novo* mutations play a pathological role in ASDs, highlighting the importance of analyzing the functional consequences and their impact on individual phenotypes of these mutations. In this study, we focused on POGZ (Pogo transposable element with ZNF domain), a gene product, on which many ASDs-associated mutations have been identified. We generated a mouse model heterozygous for a Y594C mutation in *POGZ* (Y594C mice), which identified in patients with ASDs and found that Y594C mice showed ASDs-related behavioral abnormalities, including impaired social behavior. We previously generated a mouse model heterozygous for a Q1038R mutation in POGZ (Q1038R mice), which identified in patients with ASDs and found that Q1038R mice also showed ASDs-related behavioral abnormalities as seen in Y594C mutant mice. Interestingly, while Q1038R mice exhibited developmental abnormalities, such as smaller brain, Y594C mice had a generally normal brain size and structure. Comprehensive research using these model mice will help to unravel the molecular and cellular pathogenesis of *de novo POGZ* mutations in ASD.

2-B-P-081 学生セッション（ポスター）

Functional analysis of CHAMP1, a gene product associated with intellectual disability.

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Intellectual disability (ID) is one of neurodevelopmental disorders characterized by a limitation in intellectual functioning and adaptive behavior. The prevalence of ID is as relatively high as several percent of the population. Genetic factors as well as environmental factors play an important part in ID, and a significant number of candidate disease-associated genes have been identified. However, given clinical and genetic heterogeneity, the cellular and molecular mechanisms of ID remain largely unknown. Among candidate disease-associated gene products, Chromosome alignment-maintaining phosphoprotein 1 (CHAMP1), which was originally identified a regulator of kinetochore-microtubule attachment, is involved in neuronal development. Importantly, *CHAMP1* is one of the most recurrently mutated genes in patients with ID. To explore the pathological roles of CHAMP1 in ID, we generated a mouse model heterozygous for a *CHAMP1* mutation identified in a patient with ID and identified impaired fear memory formation in the mice. Our results provide insight into how disease-associated mutations on *CHAMP1* lead to impaired memory formation, which underlies the pathogenesis of ID.

2-B-P-082 学生セッション（ポスター）

Understanding the mechanism of memory impairment in POGZ-Q0138R mutant mice

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Autism Spectrum Disorders (ASDs) are neurodevelopmental conditions characterized by impairments in social interaction and repetitive behavior. Abnormalities in synaptic function and resultant memory deficits have been frequently associated with ASDs. In this study, we examined a possible association between impaired memory formation and the candidate disease-associated mutations on the *POGZ* (Pogo transposable element with ZNF domain) gene. We found that the freezing time was significantly lower in a mouse model heterozygous for a Q1038R mutation in POGZidentified in a patient with ASDs (POGZ-Q1038R mice) 24 hours after electric foot shock in the fear-conditioning contextual learning task. We also found that POGZ-Q1038R mice showed significantly less interest in the novel object than wild-type mice. These results suggest that the ASDs-associatedPOGZ-Q1038R mutation causes impaired memory formation. Further studies on the molecular, cellular, and neural-circuit level mechanisms of impaired memory formation caused by the POGZ-Q1038Rmutation will help to clarify the pathogenic mechanism of memory impairment in patients with ASDs.

Administration of the SIRT1 activator attenuates skeletal muscle atrophy induced by doxorubicin in mice.

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Background:Doxorubicin (DOX), a widely used anti-cancer drug, induces skeletal muscle atrophy. We recently reported that aging-related skeletal muscle atrophy is attenuated by treatment of mice with resveratrol (RSV), an activator of an NAD^+-dependent protein deacetylase SIRT1. In this study, we examined the effects of RSV on DOX-induced skeletal muscle atrophy.

Methods and Results:Male mice were randomly grouped into three: vehicle, DOX, and RSV+DOX groups. In the DOX and RSV+DOX groups, mice were treated with DOX (5 mg/kg, IP) 4 times every 7 days. In the RSV+DOX group, mice were fed RSV-containing diet (0.4 g/kg) for 6 weeks starting 1 week before first DOX treatment. Body weight was measured every 7 days. Tibialis anterior muscles (TA) and gastrocnemius muscles (GAS) were obtained at 1 week after the last DOX administration. Body weight was gradually decreased in the DOX group compared to the vehicle group but was partially maintained in the RSV+DOX group. TA and GAS weights were reduced in the DOX group, indicating skeletal muscle atrophy. RSV treatment blocked the reductions in TA and GAS weights induced by DOX. In GAS, Western blot analysis showed an increase in acetylated lysine levels in the DOX group compared to the vehicle group. This DOX-induced increase in lysine acetylation was suppressed by RSV, suggesting that a decrease in SIRT1 activity by DOX was recovered by RSV.

Conclusion:These results suggest that activation of SIRT1 by RSV prevented skeletal muscle atrophy induced by DOX.

2-B-P-084 学生セッション（ポスター）

Fingolimod inhibits the inflammation and vascular remodeling in pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH) is classified as group 1 of pulmonary hypertension and designated intractable disease. The pathogenesis is mainly caused by vasoconstriction and vascular remodeling of the pulmonary artery. These abnormalities lead to sustained elevations in pulmonary arterial pressure and finally cause right heart failure. Several drugs have been recently developed, but those are mainly pulmonary vasodilators and ineffective for severe PAH. Therefore, novel drugs are awaited as therapeutic strategy of PAH. Previously, we found that the expression of sphingosine-1-phosphate (S1P) receptors was upregulated in PAH patients. Those modulator "fingolimod" blocked vascular remodeling and improved the survival rate of monocrotaline-induced pulmonary hypertensive (MCT-PH) rats. Fingolimod is an immunosuppressant drug that is used to treat multiple sclerosis, therefore, we herein examined whether fingolimod reduced inflammation in PAH. The accumulation of macrophages was detected in remodeled vascular legions of MCT-PH rats, which was reduced by the administration of fingolimod. In addition, fingolimod inhibited the proliferation of macrophages. Our results suggest that fingolimod ameliorates the development of PAH by blocking pulmonary vascular remodeling as a result of the reduction of inflammation.

2-B-P-085 学生セッション（ポスター）

The efficiency of oral leucine administration on the progressing of melanoma in a murine sarcopenic model

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It has been known that sarcopenia causes the immune dysfunction. In this study, we evaluated the influence of the sarcopenia on the progression of melanoma using a denervation-induced sarcopenic model and the efficacy of leucine-intervention was evaluated.
Initially, the influences of leucine-intake on maintaining immune homeostasis were observed. The expression of PD1 on CD4-positive T cells and CD8-positive T cells increased in the sarcopenia mice comparing those in control mice. Then, a diet-intake leucine was orally administrated to both sarcopenic mice and melanoma-implanted sarcopenic mice. The administration of leucine caused an elevation of total CD4-positive cells and significantly decreased the number of PD1+CD4+ fraction and PD1+CD8+ fraction in sarcopenic mice. The subpopulation of PD1+CD8+ fraction in leucine-treated sarcopenic mice was even much lower than that in non-sarcopenic group. The Kaplan-Meier curve also suggests that leucine-intake potentially increased the survival rates in sarcopenic mice.
Taken together, the results obtained from the present study suggests that oral administration of leucine can restore the skeletal muscle mass and may affect the onset of cancer progression via improving the function of immunological defense. It would benefit the management of melanoma in sarcopenic patients.

2-B-P-086 学生セッション（ポスター）

Construction of a screening platform based on integration of in silico and biochemical experiments

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The type 1 ryanodine receptor (RyR1) is a Ca2+ release channel in skeletal muscle. RyR1 is also expressed in brain and nonmuscle tissues and hyperactivation of RyR1 is implicated not only in skeletal muscle diseases, such as malignant hyperthermia, but also in diseases of the brain and other tissues. Therefore, pharmacological inhibition of RyR1 may have a therapeutic potential for various diseases. We have developed Cpd1, a selective RyR1 inhibitor for the treatment of malignant hyperthermia, but it has disadvantages of low oral efficacy and short half-life. To identify novel inhibitors with improved properties, in this study, we aimed to establish in silico screening platform based on the Cpd1-RyR1 complex structure predicted by molecular dynamics simulations. First, we performed amino acid substitutions and Cpd1 modifications on the computer and ran simulations with these modifications. Second, based on these results, amino acid substitution mutants and Cpd1 derivatives were evaluated by biochemical experiments. The results showed that the molecular dynamics simulations were in good agreement with the biochemical experiments. The results of in silico screening with the platform will be presented.

2-B-P-087 学生セッション（ポスター）

In silico prediction of RyR1 inhibitor binding structure

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RyR1 is a calcium release channel in the sarcoplasmic reticulum which is involved in excitation-contraction coupling of skeletal muscle. Mutations in RyR1 are known to cause malignant hyperthermia. We have recently developed a RyR1 inhibitor, Cpd1, for the treatment of malignant hyperthermia. Although Cpd1 is predicted to bind to the P1 domain of RyR1, the cryo-EM structure of RyR1 complexed with Cpd1 has not been obtained yet, due to low resolution of the P1 domain. Here, we performed molecular dynamics simulations to predict the Cpd1-bound structure of the P1 domain. The simulations were performed using the molecular dynamics program myPresto with different initial coordinates and simulation time. The stability of the output structures was evaluated by the amino acid interactions, and the convergence of the conformational change was evaluated by the RMSD plot. We obtained a stable complex structure of P1 with Cpd1from several initial coordinates with longer simulation time. Interestingly, the structure of P1 complexed with Cpd1 was significantly different from the initial conformation without Cpd1, suggesting that Cpd1 may induce a significant conformational change of the P1 domain.

S1P receptor 1-mediated odontoblastic differentiation of mouse apical papilla-derived stem cells

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Sphingosine-1-phosphate (S1P) is known as a signaling sphingolipid that regulates many cellular responses, including cellular differentiation. We have previously reported that S1P signaling pathway regulates both the promotion of osteoblastogenesis and the inhibition of adipogenesis in C3H10T1/2 cells, which are functionally similar to mesenchymal stem cells. In recent years, the idea of regenerative endodontics has been advocated. The goal of this method is to improve the regenerative capacity by activating stem cells of the apical papilla (SCAPs) present in the apical portion. However, the involvement of S1P signaling in SCAPs differentiation into odontoblast is not well understood. In this study, we investigated the roles of S1PR1-mediated odontoblastic differentiation and mineralization of SCAPs.

We used the mouse immortalized cell line of SCAPs (iSCAP). iSCAPs expressed S1PR1 and S1PR2, and S1P increased the expression of S1PR1. S1P increased mRNA expression of odontoblastic differentiation marker involving DSPP, DMP-1, and MEPE, and protein secretion of DSPP, DMP-1, which was diminished by inhibitor of S1P receptor 1 (S1PR1). S1P also induced mineralization through S1PR1. We conclude that S1PR1 signaling induces odontoblastic differentiation.

2-B-P-089 学生セッション（ポスター）

Butyrate suppresses testosterone production in mouse testicular Leydig cell

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Leydig cells in the testis produce testosterone under the control of luteinizing hormone (LH) secreted by the anterior pituitary. LH binds to the Gs protein-coupled LH receptor in Leydig cells and increases intracellular levels of cyclic AMP, which stimulates testosterone production by regulating the expression of steroidogenic enzymes and others. Testosterone is critical for male physiology, including gonadal function, muscle mass, lipids, bone formation, and erythropoiesis. Butyrate, a short-chain fatty acid (SCFA) produced by butyrate-producing bacteria in the gut, is associated with inflammasome and immune function. In this study, we investigated the role of butyrate in testosterone production in testicular Leydig cells.

In the mouse Leydig tumor cell line (MLTC1), treatment with dibutyryl (db)-cAMP increased testosterone levels in the culture medium. Treatment with butyrate suppressed the db-cAMP-stimulated testosterone production. Notably, butyrate increased the mRNA expression of the steroidogenesis repressor *Nr0b1* and decreased that of the steroidogenesis enzymes *Cyp11a1* and *Hsd3b.* In addition, treatment with butyrate increased histone H3 acetylation. Taken together, our results suggest that butyrate may decrease testosterone production via modulation of histone acetylation and steroidogenic factors expression in Leydig cells and affect the development and maintenance of male physiological function.

2-B-P-090 学生セッション（ポスター）

Role of short-chain fatty acids in placental trophoblast differentiation and fusion

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Short-chain fatty acids (SCFAs), such as butyrate, propionate, and acetate, are the major end products of bacterial fermentation of dietary fiber. SCFAs produced by intestinal bacteria have been implicated in the regulation of immune function and mucosal repair. These factors are also transferred to the placenta and affect the metabolic capacity of the fetus. The surface of the placental villi is composed of mononuclear cytotrophoblasts (CTs) and multinuclear syncytiotrophoblasts (STs) formed by differentiation and fusion of CTs. In this study, we investigate the role of SCFAs in differentiation and cell fusion. Human trophoblast cell lines, BeWo and JEG-3, were treated with acetate, propionate, and butyrate and then stimulated with dibutyl (db)-cAMP to induce cell fusion and differentiation. Treatment with butyrate or propionate increased the expression of the differentiation marker hCG alpha and beta subunits in the presence of db-cAMP. In addition, butyrate promoted the db-cAMP-induced expression of the fusion marker gene ERVFRD-1 and cell fusion. Furthermore, butyrate increased histone H3 acetylation under the differentiation stimuli. Our data suggest that butyrate promotes trophoblast cell fusion and differentiation for the formation and maintenance of placental villi during pregnancy.

Potential therapeutic agent nanaomycin A as a DNMT3B inhibitor in neuroblastoma

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Neuroblastoma is one of the most common childhood solid tumors. Despite intensive multidisciplinary treatment, the current 5-year overall survival rate for children diagnosed at an advanced stage is less than 50%, with a poor prognosis. Chemotherapeutic agents commonly used to treat high-risk neuroblastomas (e.g., those involving MYCN amplifications), such as cyclophosphamide, cisplatin and so on, are toxic to proliferating cells, including normal cells. Thus, new therapeutic strategies are needed to improve the prognosis of children with neuroblastoma. DNA methylation is an epigenetic modification that suppresses gene expression. Because tumor suppressor genes are often hypermethylated in cancers, DNA methylation has emerged as a target for cancer therapeutics. Nanaomycin A, an inhibitor of DNA methyltransferase 3B (DNMT3B) which mediates *de novo* DNA methylation and is rarely expressed in normal cells, reportedly induces death in several types of human cancer cells. In the present study, nanaomycin A decreased genomic DNA methylation levels and induced apoptosis in human neuroblastoma cells. These results suggest that nanaomycin A is an effective candidate therapeutic for treating neuroblastoma. Our findings also suggest that the inhibition of DNA methylation is a promising anti-tumor therapy strategy for neuroblastoma.

2-B-P-092 学生セッション（ポスター）

Development of information technologies to predict drug combinations for enhancing synergistic effects

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In recent years, drug combination therapy, which utilizes the synergistic effects of combining multiple drugs, has been attracting attention for medical treatment of multifactorial diseases such as cancer. The advantage of drug combination therapy is that it is expected to enhance therapeutic efficacy, but the disadvantage is that blind combination of drugs may cause harmful side effects. Therefore, it is necessary to identify the optimal combination of drugs. In this study, we develop a computational method to predict synergistic drug combinations from the viewpoint of regulation of therapeutic target molecules. We evaluate the coverage of a group of target molecules of the combined drugs, because the regulation of many diseases therapeutic target molecules may enhance therapeutic efficacy. We develop an algorithm to search for drug pairs with high coverage of therapeutic target proteins of each disease considering the potential target proteins of the drugs using machine learning models on various biomedical big data. The proposed method was applied to predicting drug combinations with synergistic effects for acute myeloid leukemia, chronic myeloid leukemia, colorectal cancer, and breast cancer. The predicted drug combinations were validated using clinical data. The proposed method is expected to contribute to the identification of optimal drug combinations for various diseases.

Curcumin analog B inhibits cell proliferation and induces cell death in glioblastoma at lower concentrations than curcumin

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PURPOSE: Glioblastoma (GBM) has a high risk of recurrence and a poor prognosis due to the difficulty of surgical resection and resistance to the standard pharmacological treatment, temozolomide. Therefore, the development of new therapeutic agents against GBM is needed. This study investigated the anti-tumor activity of the curcumin (Cur) analog Compound B (ComB) against GBM.

METHODS AND RESULTS: To evaluate anti-tumor activity against GBM, an MTT assay was performed. The human GBM cell lines U87-MG and U251 were pre-treated with Cur or ComB, then cell viability was examined using a cell counting kit, and IC_{50} values were calculated. For U87-MG, the IC_{50} values for Cur and ComB were 9.78 and 1.28 μM, respectively; for U251, they were 9.50 and 0.64 μM, respectively. To examine the effects of ComB on normal cells, the same MTT assay was performed on primary cultured astrocytes from neonatal rats. ComB did not reduce cell viability in astrocytes at concentrations that had an anti-tumor effect on GBM cells (1.5 μM). Next, a cell cycle analysis was performed with PI staining and an apoptosis assay with annexin V/PI staining using flow cytometry. ComB induced G2/M phase arrest and apoptosis at lower concentrations than Cur.

DISCUSSION: These results suggest that ComB, at lower concentrations than Cur, has an anti-tumor effect without affecting normal cells by inducing cell cycle arrest and apoptosis against GBM. Further detailed analysis and *in vivo* studies of ComB may lead to the development of novel therapeutic agents for GBM.

2-B-P-094 学生セッション（ポスター）

Pathological significance of extracellular cysteine supply in hepatic tumor tissues

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Cancer cells alter the activity of various metabolic pathways to sustain their abnormal proliferation. The reprogramming of amino acid metabolism is also observed in malignant cancer cells, characterized by enhancing de novo synthesis and/or extracellular uptake via upregulation of amino acid transporters. Our previous study demonstrated that cysteine was indispensable for proliferation of cancer cells and that the contents were increased in hepatic tumor tissues as compared with healthy liver. However, the pathological significance of this increased cysteine contents in hepatic tumor tissue has not been fully understood yet. In this study, we found that the growth ability of murine hepatoma cell line, BNL 1ME A.7 R.1 (BNL 1ME), but not of primary hepatocytes, was dependent on extracellular supply of cysteine. Cysteine deprivation induced the cell cycle arrest at G0/G1 phase in BNL 1ME cells, accompanying with decrease in the expression of Cyclin D1 and Cyclin D2 proteins. The cysteine deprivation-induced decrease in D-type cyclin expression was associated with the upregulation of eukaryotic translation initiation factor 4E binding protein (4E-BP1) that act as a translational repressor of Cyclin D1 and Cyclin D2 proteins. Although extracellularly supplied cysteine may be used for glutathione synthesis and enhance anti-oxidant capacity of cancer cells, our present findings also demonstrated its contribution to promote cell cycle progression of hepatic tumor cells.

Combination of oxidative stress and NAD^+ synthesis inhibition induces synthetic lethality in cancer cell

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NAD^+ is an important coenzyme involved in various metabolic processes. NAD^+ is also used as a substrate for poly-ADP-ribose polymerase (PARP). DNA single strand break (SSB) induces auto-ADP-ribosylation of PARP and recruits DNA repair complex. It is known that oxidative stress, such as hydrogen peroxide (H_2O_2), induces SSB DNA damage and deplete NAD^+ via PARP-mediated poly ADP-ribosylation. The treatment with low dose of H_2O_2 induces NAD^+ depletion, but cannot kill the cells. Therefore, we pursuit the method to induce the synthetic lethality with low dose of H_2O_2. We induced DNA damage in A549 cells by adding low dose of H_2O_2. We found that NAD^+ level significantly declined at 1 hour after H_2O_2 treatment, but recovered to normal level thought the resynthesis of NAD^+ at 24 hours later. Next, we investigated how NAD^+ is re-synthesized after the low dose of H_2O_2 treatment. In particular, the source of ribose moiety of NAD^+ was unknown. Using stable isotope labeled glucose, we identified that phosphoribosyl pyrophosphate (PRPP), the source of ribose moiety of NAD^+, comes from the glucose but not the ADP-ribose, a degradation product of auto-ADP-ribosylated PARP. Next, we examined the effect of these combination to induce cell death in cancer cells. Single treatment of H_2O_2 or glucose depletion did not induce cell death, but the combination of the low dose of H_2O_2 treatment and glucose depletion could induce synthetic lethality in A549 cells. These results demonstrate that the combination of oxidative stress and NAD^+ synthesis inhibition can be an optimal therapeutic option to kill the cancer cells with less invasiveness.

2-B-P-096 学生セッション（ポスター）

The RFFL antisense oligonucleotides improve the function of CFTR mutants associated with cystic fibrosis.

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Cystic fibrosis (CF) is caused by mutation of CFTR, a cAMP-regulated Cl- channel expressing at the apical PM of epithelia. The most common mutant ΔF508 CFTR induces misfolding, leading to ubiquitin-mediated degradation. The CF drug Trikafta improves the PM expression of ΔF508 CFTR. However, the functional ΔF508 CFTR is still removed from the PM in the presence of Trikafta. Previously, we demonstrated that a ubiquitin ligase RFFL directly interacts with ΔF508 CFTR at the PM and endosomes, thereby facilitating the ubiquitination, endocytosis, and degradation of the CFTR mutant. Notably, RFFL knockdown inhibited the CFTR degradation and enhanced the efficacy of Trikafta in cell lines.

In this study, we determine whether RFFL KD inhibits the degradation of endogenous ΔF508 CFTR and enhances the efficacy of Trikafta in primary bronchial epithelial cells derived from CF patients (CF-HBE), which is the final evaluation system for CF therapeutics in preclinical stages. To achieve the RFFL KD, antisense oligonucleotides (ASO) containing artificial nucleic acids with high intracellular uptake capacity and excellent nuclease resistance were designed. Our results demonstrated that the introduction of the RFFL-ASO resulted in a 1.4-fold improvement in endogenous ΔF508 CFTR function. In conclusion, our study strongly implies that suppressing RFFL enhances the efficacy of CF therapeutic agents in the CF-HBE, underscoring the genuine potential of RFFL as a viable drug target for CF.

Inhibitory effect of paxilline on Ca^{2+}-activated Cl^- channel TMEM16A

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Background

Ca^{2+}-activated Cl^- channels are expressed in smooth muscle, endothelial and secretory cells, and are involved in the smooth muscle contraction and cell growth and death. It has been reported that paxilline, a versatile inhibitor of big-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels, suppresses Ca^{2+}-activated Cl^- current, but the molecular mechanism of this suppression remains unclear. In this study, we focused on TMEM16A, which has been identified as a molecular entity of Ca^{2+}-activated Cl^- channels and examined the pharmacological effects of paxilline on the Ca^{2+}-activated Cl^- channel TMEM16A.

Method

The whole-cell patch clamp method was applied to HEK293 cells with steady-state expression of TMEM16A channel, and Ca^{2+}-activated Cl^- current was measured.

Result

In TMEM16A-HEK293 cells, outward currents slowly activated by depolarization and inward trailing currents activated by repolarization were observed. The pharmacological effects of paxilline on TMEM16A channels were examined and found that 10 mM paxilline significantly suppressed the Ca^{2+}-activated Cl^- current constituted by TMEM16A. The activation time constant of the outward current was increased by paxilline. Furthermore, the inhibitory effect of paxilline on TMEM16A channels was concentration dependent.

Summary

It has been shown that paxilline, a general-purpose BK_{Ca} channel inhibitor, suppresses Ca^{2+}-activated Cl^- channels composed of TMEM16A. These findings may lead to the elucidation of the function of the Ca^{2+}-activated Cl^- channel TMEM16A and targeted drug discovery.

2-B-P-098 学生セッション（ポスター）

In Vitro Neurotoxicity Assessment Model Considering Cell-Cell Interactions: Co-Culture of SH-SY5Y neuroblastoma and iPSC-Derived Astrocytes

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In the human brain, glial cells such as astrocytes, microglia, and oligodendrocytes interact with neurons to form a microenvironment. Astrocytes, a type of glial cells, promote the formation of synapses through which nerve cells extend axons and transmit electrical signals, and stabilize structures. We tried to develop a neurotoxicity evaluation model that can consider cell-cell interactions in a neuron co-culture system that mimics the human brain environment. Based on the cell morphology and immunostaining characteristics confirmed in the co-culture conditions of human neuroblastoma SH-SY5Y cells and human iPS cell-derived astrocytes, co-culture conditions suitable for observing neurites were determined. In order to verify the established co-culture model as a neurotoxicity evaluation model, the effect on neurodevelopment was evaluated using well-known neurotoxic substances, acrylamide and hydrogen peroxide. Quantitative analysis of neurite outgrowth and cell nuclei using high-content screening showed that the neurotoxin significantly inhibited neurite outgrowth, a hallmark of neurodevelopment. Further analysis confirmed that the neurotoxins modulate genes involved in neurodevelopment. Comparative analysis between SH-SY5Y and co-culture showed that astrocytes have neuroprotective effects on neurons. Our neurotoxicity assessment model can propose an efficient neural-based in vitro neurotoxicity assessment method and illustrate the advantages of considering the cell-cell interactions of the human brain microenvironment.

2-B-P-099 学生セッション（ポスター）

Development of TDP-43 RT-QuIC method

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TAR DNA-binding protein (TDP-43) consists of 414 amino acids and is a protein with functions related to RNA metabolism, such as mRNA production and transport of mature mRNA to the cytoplasm. On the other hand, TDP-43 has been shown to form amyloid due to abnormal aggregation in amyotrophic lateral sclerosis and frontotemporal lobar degeneration, suggesting that abnormal TDP-43 propagates between cells in these diseases and causes lesions to spread to the surrounding area. However, the mechanism of this abnormal aggregation has not been elucidated, and early diagnostic and therapeutic methods have not been established. In this study, we focused on the Real-time Quaking Induced Conversion Reaction (RT-QuIC) method, an early diagnosis method that has been developed for other neurodegenerative diseases such as prion diseases and Parkinson's disease, and investigated the development of the TDP-43 RT-QuIC method.

First, recombinant TDP-43 protein was purified and seeded in vitro. The seed was used to study the optimal conditions by changing the pH and additives of the solution. Several recombinant TDP-43 mutants were also purified and used in the experiments. As a result, we identified suitable mutants of His-tagged TDP-43 and additives which increased the detection capacity, and successfully detected 50 fg of seed. Thus, the development of the TDP-43 RT-QuIC method is progressing steadily, and we hope to enable detection in biological samples in the future, ultimately aiming for clinical application.

2-B-P-100 学生セッション（ポスター）

Construction of the ELISA assay to quantify Semaphorin 3A in the adult brain

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Extracellular soluble signals that control several aspects of neuronal development are known to play a critical role in maintaining neuronal function and homeostasis in the mature nervous system. Abnormal expression and/or secretion of these molecules are therefore thought to be associated with the onset of various types of neurological disorders. It has been reported that the expression of Semaphorin 3A (Sema3A), a secreted type of repulsive axon guidance molecule, is impaired in several neurodegenerative disorders. However, due to the lack of a reliable Sema3A antibody, our knowledge about Sema3A expression in the adult brain is still limited. Here we report the identification of a pair of Sema3A monoclonal antibodies for the sandwich ELISA assay using the Autonomously Diversifying Library system. Our Sema3A monoclonal antibodies recognize the blade 3-4 or the blade 5 of Sema domain, respectively. We can measure the concentration of recombinant human Sema3A in the range of 0-100 pM by ELISA. The specificity of this assay was confirmed by using the embryonic brains from *sema3A* deficient mice as a negative control. Moreover, this assay could measure Sema3A concentration in Tris buffered saline- and SDS-soluble lysate obtained from the adult mice brains. These data suggested that our ELISA assay is a reliable tool for the validation of Sema3A as a biomarker in neurodegenerative disorders.

Ro25-6981, an NR2B/NMDA receptor antagonist, inhibits *l*-DOPA-induced striatal GABA release in the *l*-DOPA-induced dyskinesia model rats.

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In the *l*-DOPA-induced dyskinesia model rats, direct striatopallidal GABAergic neurons respond excessively to *l*-DOPA. NMDA receptors modulating corticostriatal glutamatergic-dopaminergic interactions have been shown to be important players in LID. In this study, we examined the effect of Ro25-6981, an NR2B-containing NMDA (NR2B/NMDA) receptor antagonist, on *l*-DOPA-induced striatal GABA release in the LID model rats using *in vivo* microdialysis.

Unilaterlly 6-hydroxydopamine-lesioned rats were primed with *l*-DOPA/benserazide for three weeks. *l*-DOPA/benserazide *p.o.*administration elevated striatal GABA level in the LID model rats. This elevation was maintained over three hours and enhanced by the perfusion of 1(*S*),9(*R*)-(-)-bicuculline methiodide, a $GABA_A$ receptor antagonist, through the probe implanted in striatum. On the other hand, this elevation of striatal GABA level was completely abolished when Ro25-6981 was perfused in striatum.

These results suggest that the abnormal activity of striatal GABA neurons is modulated via NR2B/NMDA receptors in the *l*-DOPA-induced dyskinesia model rats.

2-B-P-102 学生セッション（ポスター）

Expression of TRH-R1 in the mouse hippocampal dentate gyrus is associated with stages of neuronal development

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TRH-R1 (Thyrotropin-releasing hormone receptor type 1) is recognized for its abundant expression in the anterior pituitary, where it plays a role in the release of thyroid-stimulating hormone (TSH). In addition, TRH-R1 is widely distributed in the central nervous system, including the hippocampus. Previous studies have revealed that TRH plays a significant role as a neurotransmitter/neuromodulator, influencing synaptic function related to emotions, learning, and memory in the hippocampus. However, the precise localization and expression pattern of TRH-R1 remain elusive. We conducted in situ hybridization of TRH-R1, and our analysis confirmed the expression of TRH-R1 mRNA in the granule cell layer of the ventral hippocampal dentate gyrus. Interestingly, we observed that a cell layer near the subgranular zone where neurogenesis is taking place does not express TRH-R1. Notably, we observed a lack of TRH-R1 expression in Doublecortin-positive cells. Moreover, under the influence of aging, there was an increase in the expression levels of TRH-R1 within the hippocampal dentate gyrus. The data suggest that the expression of TRH-R1 in the dentate gyrus of the hippocampus increases during the development/aging of neurons.

Primary culture of neurons derived from lactating mice to elucidate the contribution of the endocannabinoid system to neurodevelopment during lactation

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Cannabinoid CB1 receptor is known to play an important role on the neuronal development such as columnar formation in cortex or age-related changes of cognitive function. Interestingly, aged CB1 receptor-deficient mice show a marked cognitive decline, whereas young CB1 knockouts show better cognition than wild-type mice. However how CB1 influence the neuronal function in very young age like infants is unknown, majorly due to lack of in-vivo/ex-vivo evaluation systems such as behavioral tests and primary cell cultures in this particular age. Thus, this study aims to generate primary cultured neurons from infant-to-toddler mice (2-3 weeks old), which has been difficult to do so far, and to evaluate whether they can be used as an ex-vivo model to analyze the neural activity these mice.

We used modified version of manufacturer's protocol by Miltenyi Biotec. While this protocol is designed to culture the neurons from adult mice up to p60, our modified protocol could culture the neurons from neonatal (P7), young (P50), and mature (P105) mice at least for 3 to 10 days which was good enough to grow axons. This result suggests that our modified method is suitable to evaluate neuronal growth and cell activity even after a certain ageing period like P105. However, since our method is restricted to the whole brain, we are currently trying to culture neurons harvested from specific brain regions. Our final goal is to establish the primary cultures from infant CB1 knockouts as well as DAGL-alpha knockouts, to study the effects of CB1-related endocannabinoid deficiency on neuronal growth and cell activity in infants-toddlers.

2-B-P-104 学生セッション（ポスター）

Altered ultrasonic vocalizations and impaired social behaviors in a mouse model of copy number variation in the neuropeptide receptor VIPR2 gene

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Results of several large-scale genetic studies demonstrated that microduplications at 7q36.3, containing VIPR2 gene, represent risk factors for schizophrenia and autism spectrum disorder (ASD). VIPR2 encodes the VPAC2 receptor that binds two homologous neuropeptides, VIP and PACAP. To address how increased VIPR2 dosage might predispose to psychiatric disorders, we have developed a bacterial artificial chromosome (BAC) transgenic (Tg) mouse model of VIPR2 copy number variation. Here we investigated neonatal ultrasonic vocalizations (USVs), an early communicative signal of mother-pup interaction, and social behaviors in adults of VIPR2-BAC Tg mice. VIPR2 mRNA expression in the prefrontal cortex and hippocampus of VIPR2-BAC Tg mice was significantly increased compared to wild-type littermates. Toal and mean durations of USVs of VIPR2-BAC Tg pups at postnatal day 7 were significantly longer than those of wild-type pups. Additionally, qualitative analysis of USVs revealed that VIPR2-BAC Tg mice showed a low proportion of “short” calls. In adulthood, VIPR2-BAC Tg mice exhibited impaired social behaviors in the reciprocal social interaction test. These results suggest that increased VIPR2 disrupts social communication development and this might lead to form some features of ASD or schizophrenia.

3-B-P-001 一般演題（ポスター）

Functional roles of NCX1 in rhythmic contractions of the ileum

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Inflammatory bowel disease, which is associated with symptoms such as abdominal pain and abnormal bowel movements, is an intractable disease that causes severe abnormalities in gastrointestinal motility. Rhythmic contraction of the gastrointestinal tract is regulated by the myenteric plexus (interstitial cells of Cajal: ICC) distributed between the longitudinal muscle layer and circular muscle layer. It has been considered that the rhythmic contraction is controlled by Ca^{2+} signals via Ca^{2+} channels and transporters in ICCs and smooth muscle cells. The Na^{+}/Ca^{2+} exchanger type-1 (NCX1) is abundantly expressed in smooth muscle cells, which is thought to be involved in contraction and relaxation of smooth muscle cells. However, the roles of NCX1 in the rhythmic contraction of the gastrointestinal tract is still unclear. In this study, we investigated the functional role of NCX1 in rhythmic contraction of the ileum, using several types of NCX1 genetically modified mice and selective NCX1 inhibitors. As the results, it is suggested that NCX1 in ileal ICC and longitudinal muscle smooth muscle cells regulates their respective intracellular Ca^{2+} dynamics, and is complexity involved in the frequency and amplitude of rhythmic contractions.

3-B-P-002 一般演題（ポスター）

Involvement of P2X4 receptor in osteoblast differentiation

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Osteoblast growth and differentiation, which are controlled by Ca^{2+} signaling, are important for bone formation and homeostasis. ATP released from hemichannels, such as pannexins and connexins located within the cell membrane, also contributes to osteoblast differentiation. The purpose of this study is to elucidate the functional relationship between hemichannel-mediated ATP efflux and P2X receptor in osteoblast differentiation of the osteoblastic cell line MC3T3-E1, established from mouse calvaria.

We found that the pharmacological blockade of hemichannels decreased the amount of released ATP from MC3T3-E1 cells during osteoblast differentiation. The expression levels of the alkaline phosphatase ALP, an osteoblast differentiation marker, were also suppressed by the treatment with CBX, a hemichannel blocker and 5-BDBD, a P2X4 blocker. ATP stimulation induced a transient Ca^{2+} influx through ATP-sensitive Ca^{2+} permeable channels, and this ATP-stimulated $[Ca^{2+}]_i$ rises were attenuated by the treatment with 5-BDBD. In addition, the endochondral ossification of metatarsal bones was inhibited by the treatment with CBX and 5-BDBD. These results suggest that ATP efflux via hemichannels may be involved in P2X4 mediated-ATP signaling during osteoblast differentiation. The functional coupling between hemichannels and P2X4 receptors plays an essential role in maintaining the bone homeostasis via modulating osteoblast differentiation.

3-B-P-003 一般演題（ポスター）

Different sensitivity of canine, mouse, and human TRPA1 channels to menthol or cold stimulation

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Transient receptor potential ankyrin 1 (TRPA1) is a Ca^{2+}-permeable, nonselective cation channel that is activated by a wide variety of stimuli. Mouse and human TRPA1 exhibit different reactivity to some stimuli, including chemicals such as menthol as well as cold stimuli. In this study, we analyzed the reactivity of heterologously expressed canine, mouse, and human TRPA1 to menthol or cold stimulation in Ca^{2+}-imaging experiments. Canine and human TRPA1 exhibited a similar response to menthol, namely, activation in a concentration-dependent manner, even at the high concentration range, in contrast to the mouse TRPA1. In addition, the response during the removal of menthol was different; mouse TRPA1 exhibited a typical response with a rapid increase in $[Ca^{2+}]_i$ ("off-response"), whereas canine and human TRPA1 responded differently to each other. Finally, canine TRPA1 as well as mouse and human TRPA1 were activated by cold stimulation, although cold sensitivity varied among these species. These responses were suppressed by the selective TRPA1 inhibitor HC-030031. Because the concentration-dependency and "off-response" of menthol as well as the cold sensitivity were not uniform among these species, studies of canine TRPA1 might be useful for understanding the species-specific functional properties of mammalian TRPA1.

3-B-P-004 一般演題（ポスター）

Deficiency of bitter taste receptor14(TAS2R14) in oral squamous cancer cells participate in Epithelial-mesenchymal transition (EMT), regulation of circadian rhythm, histamine production and receptors, and mitochondrial protein expressions

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Introduction

The expression of TAS2R14, histamine receptors and production were reported in oral squamous cancer cell lines. Recent study showed that TAS2Rs are expressed in several cancer cells. Roles of TAS2R14 in cancer cells remain unclear to date. The current study is to clarify the roles of TAS2R14 expressed in oral cancer cells with genome editing.

Methods

Oral cancer cell line, HSC4 subjected to genome editing of TAS2R14 showed completely TAS2R14 knock-out cell lines (TAS2R14KO). We compared the expression of Epithelial-Mesenchymal Transition associated proteins, regulators of circadian rhythm, histamine producing enzyme and receptors, and mitochondrial proteins by western blot analysis. Furthermore, to investigate the role in the cell growth, scratch assay was performed.

Results

E-cadherin and three mitochondrial proteins were significantly up-regulated in TAS2R14KO cells. On the other hand, N-cadherin, MAPK(p38), HDC, H1R, H2R, circadian rhythm regulators were significantly down-regulated in TAS2R14KO cells. The wound healing process was significantly delayed in TAS2R14KO cells.

Discussion and conclusion

TAS2R14 was involved in a variety of functions such as maintenance of epithelial phenotypes and probably, circadian rhythm regulation mitochondrial oxidative phosphorylation pathway.

3-B-P-005 一般演題（ポスター）

Distinct function of heteromeric canine TRPV1 channels including the subunit variant

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Transient receptor potential vanilloid 1 (TRPV1) is a tetrameric non-specific cation channel consisting of 6 transmembrane (TM) domains. In canine brain and breast cancer AZACB cells, we found the expression of a full-length (F) as well as a splice isoform (T) missing the first TM (TM1) and a part of the second TM (TM2) domains. We investigated whether the lack of this region in any subunit generating tetramers may affect the channel function. Ca^{2+} imaging experiments were conducted using Fura-2 in AZACB cells or HEK293 cells that were transfected with each of F and T monomers, or with one of the concatemers composed of the two subunits (FF, TT, FT and TF). Western blot and immunocytochemistry confirmed the expression of these channels in the transfected cells. Capsaicin, a TRPV1 stimulator, potently increased $[Ca^{2+}]_i$ in the F- or FF-transfected cells in a concentration-dependent manner, while it exhibited a smaller $[Ca^{2+}]_i$ increase in T-, TT-, FT- and TF-transfected cells. The cells expressing the T-containing heteromers as well as AZACB cells exhibited the greater cell variability of the Ca^{2+} response. Therefore, the formation of heteromeric TRPV1 including the variant lacking TM1 and a part of TM2 appeared to modify the channel function.

3-B-P-006 一般演題（ポスター）

Protective effect of novel strain of Pleurotus sp. on UVB-induced skin damage: Involvement of antioxidant ergothioneine and its transporter SLC22A4

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Ultraviolet (UV) causes skin disorders by inducing reactive oxygen species (ROS) and inflammation. Therefore, oral intake of ergothioneine (ERGO), which has antioxidant and anti-inflammatory activities, may protect from UV-induced skin damage since ERGO is efficiently absorbed from diet via its specific transporter OCTN1/SLC22A4 expressed in various tissues including epidermis . In the present study, we investigated the effect of novel strain of Pleurotus sp. (NPS), which highly contains ERGO, on UVB-induced skin damage in mice and human keratinocyte HaCaT cells. First, HR-1 hairless mice fed with 2.5% NPS containing diet or control diet were irradiated with UVB for 10 weeks. In the NPS ingested group, ERGO concentrations were ~130 μg/g tissue in the epidermis and ~40 μM in the plasma. In the skin, an oxidative marker 8-OHdG measured by ELISA and expression of TNF☒ evaluated by western blotting were significantly lower than those in control group. In undifferentiated HaCaT cells, OCTN1 was detected in both plasma membranes and intracellular compartment. [3H]ERGO was taken up in HaCaT cells in a time-dependent manner, and transfection of siRNA targeted to OCTN1 remarkably suppressed the uptake. ERGO-containing NPS significantly suppressed intracellular ROS induced by UVB treatment. Taken together, NPS ingestion may lead to a high ERGO distribution to the skin, suppressing UVB-induced oxidative stress and inflammation possibly via protective effect exerted by ERGO taken up by its specific uptake transporter OCTN1 in keratinocytes.

3-B-P-007 一般演題（ポスター）

Computational analysis of ligands to OPRD1-OPRM1 heterodimer

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Approximately 35% of approved drugs target G protein-coupled receptors (GPCRs). GPCRs interact with each other to form homo and/or heterodimers. Their pharmacological properties are different from those of monomers. According to some previous research, the pharmacological properties of the dimers could be modulated by the administration of small molecules. There are also increasing reports on ligands that bind to hetero-dimers and their pharmacological properties. For example, δ-μ opioid receptor hetero-dimer (OPRD1-OPRM1 heterodimer) ligand, CYM 51010, was confirmed to be as effective as morphine but less likely to cause tolerance than morphine [1]. In addition, 993 ligands binding to the OPRD1-OPRM1 hetero-dimer are available in the PubChem database. However, there are some open questions, such as whether these ligands bind to OPRD1, OPRM1 or both, whether heterodimer formation is ligand-dependent or not, and what properties of the ligands contribute to binding to the heterodimer. We applied unsupervised learning to analyze the OPRD1-OPRM1 heterodimeric ligands and canonical opioid receptor ligands, and report on the characteristics of the heterodimeric ligands.

[1] Gomes *et al.*, Proc Natl Acad Sci USA. 2013;110:12072-7

3-B-P-008 一般演題（ポスター）

Expression of purinergic receptors in bovine adrenocortical fasciculata cells: suppression by cAMP accumulation

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[Background]
We have previously shown that bovine adrenocortical fasciculata cells (BAFC) expressed multiple P1 and P2 purinergic receptors (PRs), some of which are associated with enhancing ACTH-induced glucocorticoid production (GP) in BAFC. However, differences were noted in the protein expression profile of the PR subtypes depending on the individual BAFC.
[Objectives]
Therefore, we looked at whether the type of cell stimulation affects the mRNA expression of PRs in the BAFC.
[Materials & Methods]
BAFC were aseptically isolated from fresh bovine adrenal cortex and were cultured in Ham's F-10 medium supplemented with serum and antibiotics. Three-day primary cultured cells were used in all experiments. The cDNA of BAFC was constructed from total RNA and used for the qPCR templates.
[Results]
Stimulation of BAFC with A23187 (2 μM), a Ca^{2+} ionophore, significantly increased the expression of PRs more than 10-fold. Conversely, in cells stimulated with dibutyl cAMP (500 μM), a cell-permeable cAMP analog, PRs expression was suppressed less than 50%.
[Discussion]
In BAFC, it is well known that both continuous Ca^{2+} mobilization and cAMP accumulation induce GP. In the present study, however, cAMP accumulation reduced GP by suppressing the expression of PRs, which is involved in the promotion of GP.
[Conclusion]
Accumulation of cAMP in BAFC cells reduces the expression of PRs, preventing their overexpression. This suppression may be an aid in maintaining homeostasis.

3-B-P-009 一般演題（ポスター）

Involvement of nociceptive TRP channels in adverse effects of antifungal drugs

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A variety of antifungals are used for topical application. Some of them have been reported to cause adverse effects, such as pain and irritation. However, the molecular basis for these effects remains unknown. Nociceptive TRPV1 and TRPA1 are mainly expressed in sensory neurons and act as sensors for irritant chemicals. Here, we investigated whether these channels are involved in the painful adverse effects of topical antifungals. Among them, isoconazole, econazole, miconazole, clotrimazole, and ketoconazole as imidazoles; liranaftate as thiocarbamates; terbinafine as allylamines; amorolfine as morpholines; and butenafine as benzylamines were used. All the drugs used evoked $[Ca^{2+}]_i$ increases in TRPA1-expressing HEK293 cells. At high concentrations, many drugs induced $[Ca^{2+}]_i$ increases non-specifically, but clotrimazole, ketoconazole, and liranaftate evoked TRPA1-specific $[Ca^{2+}]_i$ and current responses. Clotrimazole and ketoconazole also evoked $[Ca^{2+}]_i$ and current responses in TRPV1-expressing HEK293 cells. In mouse sensory neurons, liranaftate elicited $[Ca^{2+}]_i$ increases being diminished by a TRPA1 blocker or the deletion of the TRPA1-gene. $[Ca^{2+}]_i$ responses to ketoconazole and clotrimazole were suppressed by the blockade of both TRPA1 and TRPV1. These results suggest that the pain and irritation induced by topical antifungals may be due to the activation of nociceptive TRPA1 and/or TRPV1 channels. Therefore, the concomitant use of these channel inhibitors is expected to reduce the adverse effects of these topical antifungals.

3-B-P-010 一般演題（ポスター）

Animal models of neuropathic and inflammatory pain enhance glutamatergic transmission in spinal dorsal horn *in vitro* and *in vivo*

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Background; Chronic pain is characterized by abnormal sensitivity to normal stimulation coupled with a feeling of unpleasantness. This condition afflicts people worldwide and severely impacts their QOL and has become an escalating health problem. Two major models are used to study chronic pain in animals, including nerve injury and the injection of a complete Freund's adjuvant (CFA) into the hind paw. However, how these models induce glutamatergic synaptic plasticity in the spinal cord is not fully understood. Methods; Using *in vitro* and *in vivo* whole-cell patch clamp recording, we analyzed spontaneous excitatory postsynaptic currents (sEPSCs).

Results; These models increased both the frequency and amplitude of sEPSCs in spinal dorsal horn (SDH) neurons. Next, we analyzed the active electrophysiological properties of neurons, which included; resting membrane potentials (RMPs) and the generation of action potentials (APs) *in vitro*. Interestingly, about 20% of recorded SDH neurons in this group elicited spontaneous APs (sAPs) without changing the RMPs. Furthermore, we performed *in vivo* whole-cell patch clamp recording in SDH neurons to analyze active electrophysiological properties under physiological conditions. Importantly, *in vivo* SDH neurons generated sAPs without affecting RMP in the nerve injury and the CFA group.

Conclusions; Our study describes how animal models of chronic pain influence both passive and active electrophysiological properties of SDH neurons.

3-B-P-011 一般演題（ポスター）

Spinal ADAM17 contributes to painful diabetic neuropathy associated with type 2 diabetes mellitus in mice

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Diabetic neuropathic pain is the most common symptom of diabetic neuropathy. The pathogenesis of diabetic neuropathic pain is quite complex, and existing drugs for the treatment of this complication are known to have limited efficacy. Therefore, it is desirable to identify novel therapeutic targets involved in the development and maintenance of diabetic neuropathic pain. Against this background, we found that the expression of a disintegrin and metalloproteinase 17 (ADAM17), a membrane-bound enzyme that cleaves extracellular portions of transmembrane proteins, was markedly increased in the spinal cord of leptin receptor-deficient *db/db* mice, a model of type 2 diabetes. Thus, we investigated the role of this molecule in diabetic neuropathic pain. In this study, the von Frey filament test and Hargreaves test were used to assess tactile and thermal hyperalgesia, respectively. The *db/db* mice showed marked tactile and thermal hyperalgesia at 9 weeks of age. In addition, the expression of pro- and mature-ADAM17 in the spinal cord of *db/db* mice were significantly increased at 9 weeks of age. Intrathecal injection of the ADAM17 inhibitor TAPI-1 and DNA-modified siRNA against ADAM17 (dsRDC) both significantly suppressed tactile and thermal hyperalgesia observed in *db/db* mice. Since ADAM17 was expressed on neurons and microglia in the dorsal horn of the spinal cord, upregulation of ADAM17 on these cells is suggested to be involved in the development of painful diabetic neuropathy.

3-B-P-012 一般演題（ポスター）

Role of astrocytes in the dysfunction of inhibitory dorsal horn interneurons required for neuropathic allodynia

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Mechanical allodynia is a symptom of neuropathic pain and is elicited by tactile stimulation. Recently, we have identified a spinal dorsal horn (SDH) inhibitory interneuron subset (operated by AAV vectors including a neuropeptide Y promoter; AAV-NpyP$^+$ neurons) whose dysfunction is critical for neuropathic allodynia. Indeed, after peripheral nerve injury (PNI), these neurons exhibit deeper resting membrane potentials (RMP) and reduce excitability. However, the mechanism of these changes remains unclear. In this study, we show that the PNI-induced deepened RMP and hypoexcitability of AAV-NpyP$^+$ neurons were normalized by SDH astrocyte-specific expression of a dominant negative form of STAT3 (dnSTAT3) that suppresses reactive state of astrocytes. Astrocytic dnSTAT3 expression also attenuated Aβ fiber-derived neuropathic allodynia. Conversely, induction of reactive state of SDH astrocytes by expressing a constitutive active form of STAT3 (caSTAT3) in normal rats resulted in reducing activity of AAV-NpyP$^+$ neurons and causing allodynia-like behavior. Our findings indicate that reactive astrocytes in the SDH are necessary and sufficient to cause dysfunction of AAV-NpyP$^+$ neurons after PNI and neuropathic allodynia. Thus, inhibiting reactive state of astrocytes could be a new therapeutic target for neuropathic allodynia.

3-B-P-013 一般演題（ポスター）

Voluntary wheel running alleviates and prevents mechanical hypersensitivity induced by vertical chronic restraint stress

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Physical exercise has become one of important non-pharmacological treatments of chronic pain. A number of studies have shown the benefits of exercise in chronic pain models of experimental animals: exercise improves mechanical and/or thermal hypersensitivity in the neuropathic-, inflammatory- and post-operative-pain models. However, mechanisms underlying the effects of exercise on the hypersensitivities induced by psychophysical stress instead of tissue injuries have yet to be fully elucidated. The rostral ventromedial medulla (RVM) and locus coeruleus (LC) are key output elements of the descending pain modulation system in the brain. The descending signals have major impact on spinal nociceptive neurotransmission. In the present study, we examined therapeutic and preventive effects of voluntary wheel running (VWR), and phosphorylated cAMP-response element binding protein (pCREB) expression in the RVM and LC, in mice with vertical chronic restraint stress (vCRS), which induces mechanical hypersensitivity. Ten days of vCRS elicited mechanical hypersensitivity in the hindlimb, which persisted for three weeks after the end of vCRS. Thereafter, four weeks of VWR resolved the vCRS-induced mechanical hypersensitivity, whereas the hypersensitivity lasted for the same period in vCRS-sedentary mice. There was a positive correlation between total running distance and paw withdrawal threshold in the vCRS-VWR mice. The number of pCREB-IR cells of vCRS-VWR mice was significantly larger than those of vCRS-sedentary and naive mice in the RVM but not in the LC. The mice performed with vCRS and VWR simultaneously did not develop the mechanical hypersensitivity. These results indicate that VWR can alleviate and prevent vCRS-induced mechanical hypersensitivity likely through the activities of descending pain modulation system.

3-B-P-014 一般演題（ポスター）

Model of recurrent neuropathic pain by social defeat stress with underlying inflammatory responses.

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There are sustained interests in understanding the interactions between stress response and nociception. Here we examined some combination of chronic pain models with social defeat stress model to address whether social stress modulates nociceptive behavior of mice subjected to chronic pain models.

C57BL/6Jcl male mice were subjected to peripheral nerve injury (PNI) induced neuropathic pain model. After spontaneous remission was observed at day 35 post-PNI, social defeats was loaded by aggressor mice, and 50% paw withdrawal threshold was measured with von Frey filament. In this procedure, we found that loading single social defeat stress induced a decrease of paw withdrawal threshold again. This effect similarly occurred in other chronic pain models that were induced in complete Freund's adjuvant induced inflammatory pain model and reserpine induced pain model, but was not seen in naïve mice. Intraperitoneal LPS and intravenous IL6 administration induced similar relapse. Immunohistochemical analysis of iba-1 represented a sustained microglial activation in the spinal dorsal horn of stress loaded neuropathic pain model mice.

Our results demonstrated that social defeat stress itself could induce recurrence of experienced chronic pain state in which reactivation of spinal microglia may correlated with the phenomenon.

3-B-P-015 一般演題（ポスター）

Identification of cells that release HMGB1 responsible for colonic hypersensitivity in a mouse model for irritable bowel syndrome and its prevention by azeliragon, a RAGE antagonist, and sulfasalazine, an anti-arthritis/anti-colitis medicine

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Nuclear HMGB1, once acetylated by histone acetyltransferase, is released following its cytoplasmic translocation, which is negatively regulated by histone deacetylase (HDAC). Given the involvement of HMGB1 and its membrane receptors including a receptor for AGEs (RAGE) in colonic hypersensitivity caused by butyrate able to inhibit HDAC in mice, a model for irritable bowel syndrome (IBS), we aimed at identifying cells that release HMGB1 in response to butyrate and testing whether azeliragon (AZG), a RAGE antagonist, and sulfasalazine (SSZ), an anti-arthritis/anti-colitis medicine, prevent the butyrate-induced colonic hypersensitivity. Repeated intracolonic butyrate administration caused colonic hypersensitivity, which was prevented by an anti-HMGB1-neutralizing antibody, a macrophage (Mϕ) depletor, AZG or SSZ. Butyrate treatment increased Mϕ count and cytoplasmic HMGB1 distribution in Mϕ and enteric glial cells (EGCs) in the colonic mucosa. Butyrate evoked HMGB1 release from Mϕ-like RAW264.7 and EGC-like CRL-2690 cells, which was inhibited by SSZ. Our data suggest that the accumulating Mϕ as well as EGCs in the colonic mucosa releases HMGB1 in response to butyrate, resulting in colonic hypersensitivity, and that AZG and SSZ prevent the development of colonic hypersensitivity accompanying IBS by inhibiting the HMGB1/RAGE pathway.

3-B-P-016 一般演題（ポスター）

Nociceptive TRPA1 channel involves in the analgesic action of component of lavender essential oil

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Lavender essential oil (LEO) has analgesic, sedative and antianxiety effects. Recently, we reported that analgesic effect of linalool (LL), a component of the LEO is involved in the inhibition of nociceptive transient receptor potential ankyrin 1 (TRPA1) channel (Hashimoto et al., 2023). In addition to LL, linalyl acetate (LA) is also a major component of LEO, but its analgesic mechanisms have not been clarified. In this study, we investigated the effect of LA on TRPA1 channel in mouse sensory neurons and heterologously TRPA1 expressing HEK293 cells (TRPA1-HEK). To evaluate channel activity intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured by Ca^{2+}-imaging system and membrane currents were recorded by whole-cell patch-clamp technique. In TRPA1-HEK, LA suppressed $[Ca^{2+}]_i$ and current responses to exogenous TRPA1 agonists, allyl isothiocyanate (AITC) and carvacrol, and endogenous one, Prostaglandin J_2 (PGJ_2). The inhibitory effects of LA on AITC and PGJ_2 were greater than on carvacrol. In mouse sensory neurons LA induced $[Ca^{2+}]_i$ increases, which were abolished by a TRPA1 antagonist, A967079 and disappeared in neurons from TRPA1-gene deficient mice. Pretreatment of LA suppressed subsequently applied PGJ_2-induced $[Ca^{2+}]_i$ responses. These results suggest that the inhibition of nociceptive TRPA1 channel is related to the analgesic action of LA. Therefore, the components of LEO may be promising compounds for the development of new analgesic drugs.

3-B-P-017 一般演題（ポスター）

Impairment of learning and memory via loss of drebrin from dendritic spines of neurons

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Recent studies have shown that synaptic dysfunction precedes neuronal cell death in the early stages of dementia accompanied by neurodegenerative diseases. Synaptic dysfunction is presumed as a decrease in the number of dendritic spines in neurons of the cerebral cortex and hippocampus, which are essential for learning and memory. Therefore, the risk for impairment of learning and memory can be assessed by decreased number of dendritic spines. Dendritic spines are small actin-rich projections protruding from the dendrites of neurons that form excitatory synapses in the cortex and hippocampus. Drebrin is an actin-binding protein that localizes to dendritic spines in mature neurons and plays a specific role in spine formation. Drebrin is known to decrease in Alzheimer's disease with a high correlation to symptom stage. In low-density cultures of hippocampal neurons, the number of dendritic spines can be counted as the number of drebrin clusters with immunostaining. The protocol for high-throughput imaging analysis of drebrin clusters has been developed and shown to be useful for screening chemicals that bind to the NMDA receptor. In fact, we have examined the toxicity of phencyclidine (PCP) and PCP-analogues and published results in a previous paper. We have developed not only the immunocytochemical protocol for *in vitro* assay using neuronal culture but also enzyme-linked immunosorbent assay (ELISA) kits to evaluate drebrin protein levels. Thus, decreased number of dendritic spines induced by chemicals can be assessed quantitatively as a loss of drebrin immunocytochemically and biochemically. Drebrin deficiency is directly related to synaptic dysfunction and leads to the impairment of learning and memory, even in the absence of neuronal cell death.

3-B-P-018 一般演題（ポスター）

Effects of the intrahippocampal injection of anti-Lgi1 antibody on cognitive function and seizure susceptibility in mice

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Anti-LGI1 (Leucine-rich, glioma-inactivated 1) antibody is an autoantibody detected from some patients with autoimmune epilepsy or limbic encephalitis with clinical features of focal seizures and cognitive impairment. In addition, previous study reported that *Lgi1*-knock out mice exhibited increased susceptibility to seizure induction. In this study, we evaluated the effects of intrahippocampal anti-Lgi1 antibody on cognitive function and seizure susceptibility in mice. C57BL/6J mice were intrahippocampally microinjected with anti-Lgi1 antibody (Abcam). A week later, the novel object recognition test and the seizure susceptibility test against pentylenetetrazol (PTZ, 35 mg/kg, i.p.) were performed. Areas of brain excitation were also evaluated by immunohistochemical analysis of c-Fos expression. While no cognitive impairment was observed, the intrahippocampal injection of anti-Lgi1 antibody showed a tendency to increase the seizure susceptibility to PTZ, as compared to control mice. In addition, analysis of c-Fos expression using mice without seizure showed significant increase in c-Fos expression in cerebral cortex and hippocampal CA3 area of the antibody-injected mice compared to control mice. Anti-Lgi1 antibody may promote the seizure occurrence by enhancing neural activity in cerebral cortex and hippocampus.

3-B-P-019 一般演題（ポスター）

Dopamine D2 receptor agonists prevent suppression of maternal care in lipopolysaccharide-induced postpartum depression model mice

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Postpartum depression is a prevalent mental disorder that affects mothers and has adverse effects on families. The decline in parenting quality associated with postpartum depression raises concerns about the adverse impact on children. Therefore, it is desirable to prevent deterioration in parenting quality in depressed mothers. This study aims to investigate whether antidepressive agents can prevent the decline in parenting quality associated with postpartum depression. Maternal care and sucrose palatability were evaluated in postpartum female mice with intraperitoneal lipopolysaccharide (LPS) administration 24 h before testing. In the maternal care test, LPS increased the latency to retrieving pups into the nest and to crouching over the pups in the nest and decreased the duration for crouching over the pups. Furthermore, in the sucrose preference test, the ratio of sucrose intake decreased. Next, dopamine D2 receptor agonists (quinpirole and bromocriptine), a selective serotonin reuptake inhibitor (fluoxetine), or a tricyclic antidepressant (imipramine) was intraperitoneally administered 30 minutes before LPS administration. Treatment with quinpirole and bromocriptine, but not fluoxetine and imipramine, decreased the crouching latency and increased the crouching duration in LPS-treated postpartum females. On the other hand, all of the antidepressive agents did not affect the ratio of sucrose intake. Furthermore, the dopamine D2 receptor antagonist haloperidol disturbed the effects of quinpirole and bromocriptine on maternal care. These results indicate that dopamine D2 receptor agonists can prevent the decline in parenting quality via dopamine D2 receptor in LPS-induced postpartum depression model mice.

3-B-P-020 一般演題（ポスター）

Paraxanthine-induced cysteine uptake and neuroprotection

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Purine derivatives, including caffeine and uric acid, exhibit neuroprotective properties that mitigate the risk of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Our previous research has demonstrated the facilitated cysteine uptake in hippocampal slices by caffeine, uric acid, and paraxanthine. Consistently, we have established that uric acid and paraxanthine promote cysteine uptake and intracellular glutathione synthesis in HEK293 cells.

This current investigation focused on elucidating the role of the cysteine transporter, excitatory amino acid carrier -1 (EAAC1), in mediating paraxanthine-induced cysteine uptake. In SH-SY5Y cells, the inhibition of EAAC1 using L-aspartic acid beta-hydroxamate resulted in a reduction of paraxanthine-induced cysteine uptake. Subsequently, we evaluated the neuroprotective effect against oxidative stress. Treatment with paraxanthine at concentrations of 10 and 100 mM did not show any cytotoxicity, but inhibited cell death induced by 150 mM H_2O_2 after a 20-hour exposure in SH-SY5Y cells.

Based on these findings, it might be suggested that paraxanthine facilitates cysteine uptake through EAAC1 and provides neuroprotection against oxidative stress.

3-B-P-021 一般演題（ポスター）

Glucagon-like peptide 1 receptor mediated modulation of excitatory synaptic transmission in the rat nucleus tractus solitarius

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Glucagon-like peptide 1 (GLP-1) is an enteroendocrine hormone which is released from L-cells in the intestine and stimulates insulin secretion and inhibits glucagon secretion. It also shows appetite suppressing effects by mainly central action. GLP-1 is also a neuropeptide synthesized by neurons in the nucleus tractus solitarius (NTS). These neurons project to hypothalamic nuclei and release GLP-1 which inhibit food intake. GLP-1 receptors are also expressed in the NTS which is the first gate region receiving sensory vagal inputs from peripheral organs including gastrointestinal organs. However, the physiological roles of the GLP-1 receptors in the NTS were not examined precisely. So, we examined the effects of GLP-1 receptor activation on excitatory synaptic transmission in the NTS second order neurons.

Liraglutide (1 μM: GLP-1 receptor agonist) did not changed the frequency and amplitude of spontaneous EPSCs (sEPSCs) but increased the amplitude of evoked EPSCs (eEPSCs). Paired pulse ratio of eEPSCs was decreased by liraglutide which suggested presynaptic action. Another GLP-1 receptor agonist exendin-4 (1 μM) also showed similar effects with liraglutide on both sEPSCs and eEPSCs.

These results suggest that the activation of GLP-1 receptors in the NTS facilitates evoked excitatory synaptic transmission although no effects on spontaneous synaptic transmission. These effects may partly contribute to the weight reducing effects of GLP-1 agonist.

3-B-P-022 一般演題（ポスター）

Amyloid β-protein deposition increases in the brain of MEGF10 Knockout/ AD model mouse

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Multiple-EGF like domains 10 (MEGF10) is the mammalian homologue of Draper, a phagocytosis receptor of apoptotic cells in *Drosophila*, and is the type I transmembrane protein that is expressed in the brain. Previously, we clarified that MEGF10 was expressed in neurons and astrocytes, and MEGF10-expressing neurons and astrocytes had the phagocytosis ability of Amyloid β-protein (Aβ) that is thought to be one of the causes of Alzheimer's disease (AD). However, as to whether MEGF10 is involved in Aβ phagocytosis in the brain of AD model mouse was not certain. In this study, we examined this issue using MEGF10 knockout (KO) mouse.

Our results indicated that MEGF10 expression levels in the brain of MEGF10 KO/AD model mice was decreased to less than half compared with that of AD model mice. Furthermore, Aβ deposition and Aβ42 levels were increased in the cortex and hippocampus of MEGF10 KO/ AD model mice.

These results suggested that MEGF10 was contributed to Aβ clearance in the brain of AD model mice.

3-B-P-023 一般演題（ポスター）

Decrease of spontaneous firing of striatal cholinergic interneurons in aged mice

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It has been reported that the spontaneous firing of striatal cholinergic interneurons (ChINs) increases with postnatal development. However, changes in spontaneous firing frequency and firing properties during aging has not been investigated yet. In this study, cell-attached and whole-cell patch-clamp studies were carried out to investigate changes in firing properties of ChINs during aging. Brain slices were prepared from 2–3-month-old, 11–12-month-old and 24-month-old mice of either sex. Frequencies of spontaneous firing at 2–3-month-old, 11–12-month-old and 24-month-old were 4.55 ± 1.01 Hz (n = 18), 8.73 ± 2.28 Hz (n = 8) and 2.88 ± 0.99 Hz (n = 11), respectively. Firing frequency at 24 month of age was significantly lower than that of 11–12-month-old (p = 0.028). Moreover, the voltage sag induced by hyperpolarizing current injection was recorded. Notably, the sag ratio at 24-month-old was smaller (1.1 ± 0.02, n = 6) than that observed at 2–3-month-old (1.2 ± 0.01, n = 7, P = 0.04) and 11–12-month-old (1.2 ± 0.02, n = 5, P = 0.01), which implies a decline in the h-current responsible for the voltage sag during the aging. Considering the established significance of the h-current in governing the spontaneous firing of ChINs, these findings suggest that the reduction in the h current may underlie the decrease in firing activity.

3-B-P-024 一般演題（ポスター）

Effects of chronic guanfacine administration on synaptic transmission in the prefrontal cortex of mice

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The prefrontal cortex (PFC) is considered a potential contributor to attention deficit hyperactivity disorder (ADHD), a neurodevelopmental disorder, because the PFC regulates high-order cognitive functions, including attention and planning through working memory. As a medication for ADHD, the α_{2A}-adrenergic receptor (AR) agonist guanfacine (GFC) has been shown to improve PFC cognitive function, although the mechanisms of action of GFC within the PFC neuronal functions remains unknown. Previously we showed the acute GFC-induced target cell-dependent inhibition in glutamatergic synaptic transmission in the PFC. Thus, significant effect was observed by the acute GFC administration (aGFC) only in callosal/commissural-type (COM) neurons, but not corticopontine-projecting-type (CPn) neurons. On the other hand, GABAergic IPSCs were comparably inhibited in both types of neurons. In this study, we examined whether chronic GFC administration (cGFC) mimicking clinical use affects the neuronal and synaptic properties as observed in the aGFC. cGFC (0.3 and 1 mg/kg) for ~3 weeks using osmotic pump did not alter the membrane properties of CPn neurons. GFC-induced inhibitory action on EPSC amplitude was not affected either by cGFC. Based on these results, we conclude that cGFC did not alter at least the membrane properties of CPn neurons or dynamics of α_2-AR at glutamatergic synaptic terminals. We are currently analyzing the membrane properties of COM neurons and GABAergic synaptic transmission to further clarify the effect of cGFC observed in clinical use.

3-B-P-025 一般演題（ポスター）

Inhibitory effects of the novel μ-opioid receptor non-peptide antagonist, UD -030, on the morphine-conditioned place preference

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Although opioids are widely used to treat moderate to severe pain, opioid addiction and the opioid overdose epidemic are becoming more serious. Although opioid receptor antagonists/partial agonists, such as naltrexone and buprenorphine, have relatively low selectivity for the μ-opioid receptor (MOP), they have been used for the management of opioid use disorder. The utility of highly selective MOP antagonists remains to be evaluated. Here, we biologically and pharmacologically evaluated a novel nonpeptide ligand, UD-030, as a selective MOP antagonist. UD -030 had more than 100-fold higher binding affinity for the human MOP (K_i = 3.1 nM) than for δ-opioid, κ-opioid, and nociceptin receptors (K_i = 1800, 460, and 1800 nM, respectively) in competitive binding assays. The [^{35}S]-GTPγS binding assay showed that UD-030 acts as a selective MOP full antagonist. The oral administration of UD -030 dose-dependently suppressed the acquisition and expression of morphine-induced conditioned place preference in C57BL/6J mice, and its effects were comparable to naltrexone. These results indicate the UD-030 may be a new candidate for the treatment of opioid use disorder, with characteristics that differ from traditional medications that are in clinical use.

3-B-P-026 一般演題（ポスター）

OX_2 receptors modulate dopamine efflux in nucleus accumbens in a rat chronic inflammatory pain model

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The nucleus accumbens (NAc) is a major terminal area for mesocorticolimbic dopamine (DA) projections from the ventral tegmental area. We have reported that focal injection of MK-4305, an OX_1 and OX_2 receptor (-R) antagonist, into the NAc enhanced accumbal DA efflux of rats and that this MK-4305-induced enhancement of DA efflux was inhibited by co-administration of orexin-B, a selective OX_2-R agonist (Kawashima et al., 2022). We also reported that intra-accumbal infusion of MK-4305 induced smaller increases in accumbal DA efflux in rats with intra-planter injection of carrageenan (CAR), a compound that provokes inflammatory pain, relative to injection of vehicle. Here, we examine (a) whether CAR treatment influences basal accumbal DA levels, and (b) the effects of intra-accumbal infusion of orexin-B on MK-4305-induced DA efflux in CAR-treated rats using *in vivo* microdialysis. Male Sprague-Dawley rats weighing approximately 200 g were used. The doses indicated are the total amounts infused locally into the NAc for 60 min. No significant differences in basal accumbal DA levels were found between rats treated with CAR and those treated with vehicle. Infusion of orexin-B (5 ng), which did not affect basal accumbal DA levels, inhibited the MK-4305 (50 ng)-induced increase in DA efflux in CAR-treated rats. Our findings indicate that (a) CAR-induced inflammatory pain fails to affect basal accumbal DAergic neural activity, and (b) the OX_2-R mediates MK-4305-induced DA efflux in NAc in this rat chronic inflammatory pain model.

3-B-P-027 一般演題（ポスター）

The disturbances of menthol sensitivity in a prodromal Parkinson's disease model mice with intranasal rotenone

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Parkinson's disease (PD) causes taste impairments as well as impaired senses of smell since the early-stage. We previously reported that bitter taste impairments may occur simultaneously, but independently, with olfactory impairments in a prodromal animal model of PD. Cool/cold temperature also affects the perception of bitter taste, but it remains unclear whether bitter taste impairments in this animal model result from altered cool/cold sensitivity. We examined the changes in the menthol sensitivity, such as coolness/irritation at low/high concentrations of menthol, in 1-week intranasal rotenone-administrated mice using brief-access or 48-hour 2-bottle tests. The total number of licks during the 20 trials of 10 sec in rotenone-treated mice showed a significant increase at 2.3 mM menthol compared to the data obtained before the rotenone treatment. In 2-bottle tests, mice after rotenone treatment showed a higher aversion to menthol compared to before. Interestingly, rotenone-treated mice significantly preferred 200 μg/mL nicotine solution to that with 100 μg/mL menthol. The disturbances of the menthol sensitivity in 1-week intranasal rotenone-administrated mice may be induced by the impairment of not only transient receptor potential (TRP) ankyrin 1 channel, but also TRP melastatin 8.

3-B-P-028 一般演題（ポスター）

AM404 inhibits NMDA-induced retinal neuronal injury through activation of cannabinoid CB_1 receptors and TRPV1 channels in mice

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AM404, a metabolite of acetaminophen, inhibits transporters that transport the endogenous cannabinoid anandamide and stimulates transient receptor potential vanilloid (TRPV) 1 channels. We have reported that AM404 inhibits *N*-methyl-D-aspartic acid (NMDA)-induced neuronal injury in mice. The aim of this study was to determine whether cannabinoid receptors or TRPV1 channels are involved in the protective effect of AM404 on NMDA-induced neuronal injury.

Cg-Tg (Thy1-CFP) 23Jrs/J mice were subjected to intravitreal injection of NMDA. Mixture of AM404 and SR141716A (a cannabinoid CB_1 receptor antagonist), or SR144528 (a cannabinoid CB_2 receptor antagonist), or A784168 (a TRPV1 antagonist) were intravitreally administered simultaneously with NMDA. After 7 days, the number of ECFP-positive cells in the retina was measured and the percentage of retinal ganglion cells (RGC) remaining was determined. NMDA-induced RGC loss was significantly inhibited by AM404. The protective effect of AM404 against NMDA-induced RGC injury was almost completely suppressed by SR141617A, but not SR144528. Furthermore, the effect of AM404 was almost completely inhibited by A784168. These results suggest that activation of cannabinoid CB_1 receptors and TRPV1 channels are involved in the protective effect of AM404 on NMDA-induced retinal neuronal injury.

3-B-P-029 一般演題（ポスター）

Age-dependent alterations in social behavior and brain neurotransmitter levels in mice trimmed bilateral whiskers during the neonatal period

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Tactile perception via whiskers is important in rodent behavior. Whisker trimming during the neonatal period affects mouse behaviors related to both whisker-based tactile cognition and social performance. However, the molecular basis of these phenomena is not completely understood. To solve this issue, we investigated developmental changes in transmitters and metabolites in various brain regions of male mice subjected to bilateral whisker trimming during the neonatal period (10 days after birth [BWT10 mice]). We discovered significantly lower levels of 3-methoxy-4-hydroxyphenyl glycol (MHPG), the major noradrenaline metabolite, in various brain regions of male BWT10 mice at both early/late adolescent stages (at P4W and P8W). However, reduced levels of dopamine (DA) and their metabolites were more significantly identified at P8W in the nuclear origins of monoamine (midbrain and medulla oblongata) and the limbic system (frontal cortex, amygdala, and hippocampus) than at P4W. Furthermore, the onset of social behavior deficits (P6W) was observed later to the impairment of whisker-based tactile cognitive behaviors (P4W). Taken together, these findings suggest that whisker-mediated tactile cognition may contribute to progressive abnormalities in social behaviors in BWT10 mice accompanied by impaired development of dopaminergic systems.

3-B-P-030 一般演題（ポスター）

Microglia release adenosine in a neuronal activity-dependent fashion.

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Adenosine (ADO) controls neuronal excitability in memory and sleep. Major sources of extracellular ADO (ADOo) are thought to be through the degradation of extracellular ATP and/or direct release from neurons and astrocytes. However, it is still unclear which cell regulates ADOo in physiology. To answer the question, we visualized the spatiotemporal dynamics of ADOo by using an ADO sensor, $GRAB_{ADO}$, in acute brain slices. Schaffer collateral electrical stimulation (E-stim) elevated ADO_o level in hippocampal CA1 region. The ADOo elevation by E-stim was abolished by treatment with TTX, silencing neuronal activity. Interestingly, the E-stim-induced elevation of ADOo was also abolished in brain slices from PLX 5622-fed mice, in which microglia were depleted. To clarify how neurons and microglia orchestrate ADOo level, we assessed several inhibitors. CNQX and D-AP5, antagonists of ionotropic glutamate receptors, reduced the elevation of ADOo by E-stim. In addition, treatment with JMS-17-2, a CX3CR1 inhibitor, reduced the elevation of ADOo induced by E-stim, and treatment with CX3CL1 increased ADOo. These data suggest that E-stim induces the release of CX3CL1 from postsynaptic neurons via the glutamate pathway between pre- and post-synaptic neurons, which leads to ADO release from microglia via the CX3CL1-CX3CR1 axis.

3-B-P-031 一般演題（ポスター）

Region- and circuit-specific synaptic plasticity in the prefrontal cortex determines the individuality in habit execution

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Much of our decision-making relies on habit, which is formed by repetition of motivational goal-directed action. Because of stability and low cognitive demands, most habits are beneficial, while maladaptive habits performed excessively are known to be a cause of psychiatric symptoms, such as compulsivity. Generally, the execution of the action often declines as it becomes habitual, while a subset of individuals maintains a high rate of execution. The neural mechanisms underlying such individuality remain unclear. Here, we developed a 2-step task where mice initially learn goal-directed lever press and then seamlessly moved to a task with an unpredictable rule between lever-press and reward delivery which facilitates habit formation. Whereas a half of the mice reduced the frequency in the 2nd step, the rest maintained a frequency comparable to the 1st step. We then assessed task-induced synaptic plasticity and found habit formation-related significant changes in the AMPA/NMDA ratio in the lateral orbitofrontal cortex (lOFC) and anterior cingulate cortex (ACC). Chemogenetic manipulation revealed the lOFC maintains the frequency of habitual lever-press, whereas whether the lever-press becomes habit depends on the ACC. Optogenetic erasure of the synaptic potentiation in striatum-projecting lOFC neurons disrupted the maintenance of lever-press frequency. Collectively, the formation of habit and its frequency are controlled by distinct cortical regions and synaptic potentiation in the lOFC during habit formation determines habit individuality.

3-B-P-032 一般演題（ポスター）

Brain α7 nicotinic receptor stimulation inhibits the rat micturition via brain hydrogen sulfide

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We recently reported that brain α7 nicotinic receptor (α7 nAChR) stimulation and brain hydrogen sulfide (H_2S) inhibited the rat micturition. In this study, we examined whether brain H_2S is involved in the micturition inhibition induced by brain α7 nAChR stimulation in urethane-anesthetized (0.8 g/kg, ip) male Wistar rats. A catheter was inserted into the bladder to perform cystometry (12 ml/h saline infusion). We examined effects of intracerebroventricularly (icv) pretreated GYY4137 (GYY, H_2S donor, 1 or 3 nmol/rat) or AOAA (non-selective inhibitor of H_2S synthesis, 3 or 10 μg/rat) on PHA568487 (PHA, α7 nAChR agonist, 0.3 or 1 nmol/rat, icv)-induced prolongation of intercontraction intervals (ICI), an index of micturition frequency. PHA (0.3 nmol/rat) showed no significant effect on ICI, while under pretreatment with GYY, PHA significantly prolonged ICI even at a lower dose (0.3 nmol/rat). PHA (1 nmol/rat) induced ICI prolongation and the PHA-induced prolongation was significantly suppressed by AOAA. The AOAA-induced suppression of the PHA-induced ICI prolongation was cancelled by supplementation of brain H_2S via GYY. These data suggest that brain α7 nAChR stimulation inhibits the rat micturition via brain H_2S.

3-B-P-033 一般演題（ポスター）

Characterization of human iPS cell-derived intestinal epithelial like cells (F-hiSIEC™) and their utility as pharmacological models

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[Purpose]
Currently, cultured cells such as Caco-2 cells and experimental animals are used as a model system of the human small intestine. However, these model systems show poor correlation with human small intestine. In this investigation, we generated human iPS cell-derived small intestinal epithelial like cells and attempted to develop an *in vitro* model that has properties closer to those of the human small intestine than existing models.
[Method]
We established a method for inducing differentiation of human iPS cells into intestinal epithelial cells based on a previous report (Kabeya, et al. Drug Metab. Pharmacokinet. 2020) and developed cryopreserved human iPS cell-derived intestinal epithelial cells (F-hiSIEC™). In addition to characterizing these cells, we constructed various intestinal evaluation models.
[Result]
The mRNA expression of intestinal epithelial cell markers, transporters, and metabolic enzymes was similar to that in the adult small intestine. In addition, stable transporter activity and metabolic enzyme activity were exhibited among multiple lots differentiated from iPS cells, and it was possible to evaluate the intestinal absorption of compounds by using these cells. Furthermore, it was shown that inflammatory reactions, pharmacokinetics, and toxic reactions could be predicted in various intestinal tract models constructed using these cells. These results suggest that F-hiSIEC™ may be useful for *in vitro* evaluation of the function of the human small intestine.

3-B-P-034 一般演題（ポスター）

Effects of perindopril on the swallowing reflex in a rat dysphagia model induced by ligation of bilateral common carotid arteries (BCAO)

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Dysphagia, as one cause of aspiration pneumonia, is a major complication in the patients with cerebrovascular disease, Parkinson's disease, aging, and overdose of antipsychotic drugs. To evaluate the effects of the chemicals on dysphagia, we established a rat dysphagia model by ligation of bilateral common carotid arteries (BCAO). In addition, we investigated the protective effects of perindopril on the dysfunction of the swallowing reflex in the BCAO in rats.
To induce dysphagia, BCAO rats were prepared by ligation of bilateral common carotid arteries under anesthesia with isoflurane inhalation. In the BCAO rats, perindopril was continuously administered orally for 4 weeks, and the swallowing reflex was determined by recording electromyogram (EMG) of mylohyoid muscle at 29 days of post-BCAO or sham-operation. In BCAO rats, topical administration of water to the pharyngolaryngeal region evoked a diminished number of swallowing events compared to the sham-operation rats, showing a sign of dysphagia. Oral treatment with perindopril for 4 weeks improved the swallowing reflex. These results suggested that a rat model of dysphagia was established by BCAO and the effects of chemicals on swallowing reflex can be evaluated with this model.

3-B-P-035 一般演題（ポスター）

Role of Gut Microbiota in the Homeostasis of Interstitial Cells of Cajal

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Introduction: Interstitial Cells of Cajal (ICC) act as pacemakers for gastrointestinal (GI) motility. However, their relationship with gut microbiota remains unclear. This study aimed to elucidate the effects of gut microbiota on ICC homeostasis.

Methods: Mice were given water dissolved with an antibiotic (ABX) cocktail for 4 weeks to eliminate the gut microbiota. After the administration period, the ileum was collected from the mice for various examinations.

Results: ABX treatment significantly reduced GI transit. However, no changes were observed in the spontaneous contraction frequency of the ileum. The area of the c-Kit+ ICC network significantly decreased in the ABX-treated group, and a similar trend was observed in germ-free mice. Supplementation with short-chain fatty acids did not inhibit the reduction in c-Kit+ ICC induced by ABX treatment. On the other hand, serotonin supplementation significantly inhibited the reduction in c-Kit+ ICC caused by ABX administration. The number of ICCs expressing the proliferation marker decreased in the ABX-treated group.

Summary: The results suggest that gut microbiota regulates the homeostasis of ICC through serotonin. The maintenance of serotonin levels in GI tract by gut microbiota may promote ICC proliferation.

3-B-P-036 一般演題（ポスター）

Study on measurement of internal anal sphincter movement in dogs (application as evaluation method on defecation disorder)-the 3rd report

Kazuaki Sasaki, Yasuyuki Otsuka, Hirohiko Akahoshi, Kousuke Morizumi, Seiichi Katayama, Naoyuki Hironaka, Katsuhide Nishi

Mediford Corporation

The number of potential fecal incontinence patient in Japan is estimated to be about 5 million, but no fundamental treatment has been developed so far. Therefore, development of new treatments and/or novel drugs is expected. Screening using animal models is important for development of new therapeutic agents. In the previous studies, we have developed a method of measuring the contractile activity of the internal anal sphincter (IAS) in unanesthetized, unrestrained dogs by placing a force transducer into the IAS, and acquiring the the contractile activity using a telemetry method. These studies were reported at the 91st and 92nd Annual Meeting of the Japanese Pharmacological Society. In the present study, we developed a method for measuring the IAS contractile activity in dogs under sedation. The dog fecal incontinence model was created by impairing IAS. The contractile activity was measured by manometry using a pressure sensor catheter. By periodically measuring the IAS contractile activity before and after the model creation, we obtained basic data of the contractile activity during the process of spontaneous healing. In addition, we also report the effects of comparative control substances, phenylephrine in this model, for the future evaluation of the efficacy of new drugs.

3-B-P-037 一般演題（ポスター）

Gegen Qinlian decoction restores the intestinal barrier in bacterial diarrhea piglets by promoting Lactobacillus growth and inhibiting the TLR2/MyD88/NF-κB pathway

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Acute bacterial diarrhea is a severe global problem with a particularly high incidence rate in children. The microecology inhabiting the intestinal mucosa is the key factor leading to diarrhea. Gegen Qinlian decoction (GQD) is used to treat bacterial diarrhea, however, its underlying mechanism remains unclear. Thus, this study aimed to clarify the restorative effect of GQD on the intestinal barrier from the perspective of gut microbiota. A Tibetan piglet model with bacterial diarrhea was established through orally administered Escherichia coli, and diarrheal piglets were treated with GQD for three days. After treatment, GQD significantly ameliorated the diarrheal symptoms. GQD decreased the levels of IL-6, LPS, and DAO, and increased SIgA, ZO-1, and occludin levels in intestinal mucosa, indicating the restoration of intestinal barrier. GQD modulated the microbial com positions inhabited on the intestinal mucosa, especially an increase of the Lactobacillus. Spearman analysis showed that Lactobacillus was the key genus of intestinal barrier-related bacteria. Bacterial culture in vitro validated that GQD directly promoted Lactobacillus growth and inhibited E. coli proliferation. Moreover, the expressions of TLR2, MyD88, and NF-κB in the colon decreased after GQD treatment. In conclusion, GQD may treat diarrhea and restore the intestinal mucosal barrier by facilitating Lactobacillus growth and inhibiting the TLR2/MyD88/NF-κB signaling pathway.

3-B-P-038 一般演題（ポスター）

Longitudinal analysis of intestinal 5-hydroxytryptamine synthesis and metabolism in septic mice

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Sepsis is a host extreme immune response to infection, leading to tissue and organ injury, which can be life threatening. When sepsis is severe, it can also cause diarrhea, nausea, or vomiting. Such gastrointestinal symptoms may be associated with excessive production of 5-hydroxytriptamine (5-HT). 5-HT is synthesized from tryptophan hydroxylase (TPH), a rate-limiting enzyme, in enterochromaffin cells, primarily in the intestinal mucosa, and catabolized into 5-hydroxyindole acetic acid (5-HIAA) by monoamine oxidase (MAO). In this study, we investigated the longitudinal change in intestinal 5-HT synthesis and metabolism in mice with cecal ligation and puncture (CLP)-induced sepsis. Three, 6, 12, 24 and 48 h after sepsis induction by CLP, jejunal tissues were dissected. Compared to sham-operated mice, jejunal 5-HT content was slightly but significantly increased at 24 h. The 5-HIAA content was significantly increased at 3, 6 and 24 h in CLP mice. TPH1 mRNA expression was transiently elevated in CLP mice with a peak at 12 h. MAO-A mRNA expression was elevated at 6 h but significantly declined at 12 and 24 h in CLP mice. These results demonstrate that the 5-HT metabolism can be enhanced in jejunal tissue prior to 5-HT synthesis, which may provide a basic insight into the understanding of the pathological role of 5-HT in sepsis.

3-B-P-039 一般演題（ポスター）

Deletion of RAMP1 signaling enhances diet-induced obesity and fat absorption via intestinal lacteals in mice

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Lacteals play a critical role in the absorption and transport of dietary lipids into the circulation. We examined the role of signaling for receptor activity-modifying protein 1 (RAMP1), a subunit of CGRP receptor, in lacteal morphology and function in response to a high-fat diet (HFD). RAMP1 deficient ($RAMP1^{-/-}$) or wild-type (WT) mice were fed a normal diet or HFD for 8 weeks. $RAMP1^{-/-}$ mice fed an HFD showed heavier body weights than WT mice fed an HFD, which was associated with high levels of total cholesterol, triglycerides, and glucose. HFD-fed $RAMP1^{-/-}$ mice had shorter length of lacteals and greater width of lacteals than HFD-fed WT mice. HFD-fed $RAMP1^{-/-}$ mice had lower gene expression levels of lymphatic endothelial cell markers including VEGFR3, and lymphatic vascular growth factor VEGF-C than HFD-fed WT mice. The concentration of the absorbed lipid tracer in HFD-fed $RAMP1^{-/-}$ mice was higher than that in HFD-fed WT mice. The zipper-like continuous junctions were predominant in HFD-fed WT mice, while the button-like discontinuous junctions were predominant in HFD-fed $RAMP1^{-/-}$ mice. These results suggest that deletion of RAMP1 signaling suppressed lacteal growth and VEGF-C/VEGFR3 expression and accelerated the uptake and transport of dietary fats through discontinuous junctions of lacteals, leading to excessive obesity.

3-B-P-040 一般演題（ポスター）

Analysis of Neurogenesis in Enteric Nervous System of Colitis Mice

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We aimed to investigate neurogenesis in the enteric nervous system of murine colons with experimental colitis to elucidate the mechanisms that cause colonic motility disturbances in the colitis. In the motility study, veratridine, a neuroactivator induced TTX-sensitive contractions in the normal murine colons, whereas these contractions were significantly suppressed in colitis murine colons. Immunohistochemical analyses revealed that there were no significant differences in a number of myenteric neurons (pan-neural marker HuC/D-positive neurons) in the colons between normal and colitis mice, whereas the proportion of nitrergic neurons (nNOS-positive) per ganglion was significantly increased in the colons of colitis mice compared to normal mice. Furthermore, the proportion of Sox2 (neural stem cell marker)-positive nitrergic neurons among Sox2-positive neurons per ganglion was significantly increased in the colons of colitis mice compared to normal mice. In addition, L-NAME, a NOS inhibitor significantly enhanced veratridine-induced colonic contractions in colitis mice rather than normal mice. These results suggest that the colitis cause an imbalance in the enteric neural circuit composed of excitatory neurons and inhibitory neurons in the myenteric plexus of the colon, which results in the colonic dysmotility.

3-B-P-041 一般演題（ポスター）

Involvement of transient receptor potential melastatin 4 channels in the adrenergic and cholinergic contractions in mouse prostate smooth muscles.

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In prostate gland, the genes of transient receptor potential melastatin 4 (TRPM4) channels are highly expressed. However, the role of TRPM4 channels in the contractile response in this organ has not yet been elucidated. Here, we examined if TRPM4 channels are involved in the adrenergic and cholinergic contractions in mouse prostate smooth muscle preparations. Contractile responses evoked by electrical field stimulation of intrinsic adrenergic or cholinergic nerves or exogenously applied noradrenaline (NA) or carbachol (CCh) were isometrically recorded and effects of the specific TRPM4 channel inhibitors, 9-phenanthrol and 4-chloro-2-(1-naphthyloxyacetamido) benzoic acid (NBA), on those contractile responses were investigated. 9-phenanthrol and NBA inhibited both adrenergic nerve-evoked and NA-induced contractions. Similar inhibitory effects of TRPM4 channel inhibitors were obtained in cholinergic contractions. 9-phenanthrol and NBA significantly inhibited noradrenaline-induced contractions in the abdominal aorta preparations. However, the inhibitory effects were much stronger in the prostate gland than in the arterial tissue. The present results suggest that TRPM4 channels are involved in adrenergic and cholinergic contractions in the mouse prostate gland. Thus, TRPM4 channels may be a new therapeutic target for treating benign prostatic hyperplasia without noticeable vasodilation.

3-B-P-042 一般演題（ポスター）

Acetylome analysis of acetylated proteins in the skeletal muscle increased by sarcopenia.

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Background:
Sarcopenia (age-related muscle loss) reduces healthspan. SIRT1 is a NAD^+-dependent deacetylase. We reported increased protein acetylation and sarcopenia in aging and skeletal muscle specific SIRT1 knockout mice. SIRT1 activators suppress age-related sarcopenia and protein acetylation. This study aims to identify elevated acetylated proteins in aging and SIRT1 knockout muscles.
Methods and Results:
Tibialis anterior from 10-19 week-old young wild-type (Young) and 81 week-old wild-type (Old) mice, and from 10 -19 week-old wild-type (WT) and same-week-old SIRT1 knockout mice (SIRT1-MKO) were harvested. Acetylated peptides were identified by LC/MS/MS after immunoprecipitation with acetylated lysine antibody. Young/Old had 1213 acetylated peptides, and WT/SIRT1-MKO had 1879. Old vs. Young had 98 acetylated peptides, SIRT1-MKO vs. WT had 90 peptides with ≥1.5-fold increase ($P<0.05$). KEGG pathway and Gene Ontology analyses showed Old vs. Young acetylated proteins associated with TCA cycle, metabolic pathway, and fatty acid beta-oxidation. SIRT1-MKO had acetylated proteins associated with TCA cycle, metabolic pathway, nucleosome assembly, and muscle contraction. Commonly increased acetylated proteins in Old and SIRT1-MKO included many mitochondrial proteins.
Conclusions:
Aging or SIRT1 knockout skeletal muscles show increased acetylation of proteins involved in metabolism, those associated with the mitochondria. This increased acetylation may contribute to the pathogenesis of sarcopenia via reduced mitochondrial function.

3-B-P-043 一般演題（ポスター）

Vascular contraction in response to protein kinase C activator was induced via protein kinase Cβ in rat carotid artery

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Background: Protein kinase C (PKC)β has been reported to be activated in the vasculature in diabetes and contributes to the microvascular and macrovascular complications by causing inflammation. On the other hand, PKC is also a regulator of smooth muscle contraction, thus possibly alteration of PKCβ activity may affect vascular homeostasis, resulting in contributing the complications.

Purposes: In this study, we examined role of PKCβ in vascular contractile response in the vascular site.

Methods and Results: We employed male PKCβ knockout (KO) rats and measure vascular contraction in response to PKC activator, phorbol 12-myristate 13-acetate (PMA). Treatment of thoracic aorta with PMA induced contraction, but there was no difference between genotypes (n=5-6). Treatment of carotid artery with PMA induced contraction, which was strongly abolished by PKCβ deficiency (n=6-7, $p<0.05$). Treatment of femoral arteries with PMA slightly induce contraction, but there was no difference between genotypes (n=4-5). In carotid arteries of male Wistar rats, pretreatment with LY333531, a PKCβ inhibitor (1 μM, 30 min) significantly decreased the contractile response to PMA (n=4, $p<0.05$). PKCβ mRNA levels in carotid artery were not significantly increased compared with aorta or femoral artery (n=4-6).

Conclusions: PKCβ is strongly associated with PMA-induced contraction in rat carotid artery, suggesting that PKCβ activation may enhance the contractile response in carotid arteries during conditions that involve PKCβ activation including hyperglycemia.

3-B-P-044 一般演題（ポスター）

Study on the establishment of a system for measuring muscle strength of cisplatin-induced muscle atrophy model in mice

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Cisplatin is widely used in the treatment of various cancers, but it causes atrophy of skeletal muscles. The atrophy might induce sarcopenia, which greatly reduce the quality of life of patients. Therefore, it is necessary to establish an evaluating test system of muscle weakness in muscle atrophy models. There are many reports of cisplatin-induced muscle atrophy and ameliorating effect of D-methionine in mice. However, most of reports are based only at endpoint observation. Successive observations over time might be necessary to obtain precise information on the effects of chemicals on muscle function. Therefore, we examined the possibility of quantifying the effects of D-methionine on cisplatin-induced muscle atrophy model by measuring muscle strength using 1300A, 3-in-1 Whole Animal System (Aurora scientific). D-Methionine (300 mg/kg/day, p.o.) and Cisplatin (3 mg/kg/day, i.p.) were administered to male C57BL/6J mice for a week. The muscle strength was measured at days 3 and 7 after the first administration. As the results, the muscle strength of the mice injected cisplatin was lower than that of normal mice, and D-methionine ameliorated muscle weakness at the both time points. These results indicate that the current test system would be useful to evaluate potential efficacy of newly therapeutic chemicals on myofunction.

3-B-P-045 一般演題（ポスター）

Effect of docosahexaenoic acid on interleukin-1β-induced cyclooxygenase-2 expression in cultured pulmonary artery smooth muscle cells

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Docosahexaenoic acid (DHA) has been reported to have protective effects against pulmonary hypertension (PH). The protective effect could be partially attributed to the direct effect on pulmonary artery smooth muscle cells (PASMCs). In the present study, we investigated the effect of DHA on interleukin-1β (IL-1β)-induced cyclooxygenase-2 (COX-2) expression in cultured human PASMCs. Cells were treated with DHA (30 μM) in the presence or absence of IL-1β (3 ng/ml). Atmospheric pressure of 60 mmHg which simulates severe PH was given to PASMCs. COX-2 protein expression transiently induced by IL-1β stimulation, peaking at 6–24 h and returning toward basal levels after 48 h. DHA did not affect the COX-2 protein expression after 24 h of IL-1β stimulation, but significantly prevented a decrease in the COX-2 protein expression at 48 h. This preventing effect of DHA was also observed in the pressurized cells simulating PH. DHA significantly increased a p38 mitogen-activated protein kinase (MAPK) phosphorylation at 48 h in both non-pressurized and pressurized cells. These results suggest that DHA enhances IL-1β-induced COX-2 expression time by activating of p38 MAPK in the delayed phase in PASMCs. The present study may contribute to the understanding of basic mechanisms underlying the beneficial effects of DHA on PH.

3-B-P-046 一般演題（ポスター）

Patients with cardiovascular diseases exhibit distinct characteristics in their oral microbiome and are more susceptible to infections by pathogenic bacteria.

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Background & Aim: Oral bacteria potentially affect the provocation or the disease state of various systemic diseases. In our present study, we examined relationship between cardiovascular diseases and microbiome or detection rates of pathogenic oral bacteria.

Method: Patients of atrial fibrillation (AF), angina pectoris (AP), aortic stenosis (AS) and healthy control volunteers were recruited. Subjects washed their mouse with distilled water and regurgitated it as bacterial samples. Extracted bacterial DNA was sequenced to analyze microbiome of each group. Besides, *Porphyromonas gingivalis (P.g)*, *Streptococcus mutans (S.m)* and *S.m*-derived collagen-binding protein *(cnm)*, through which *S.m* induces dysfunction of tissues or organs were detected by PCR. Percentages of bacteria in microbiome and detection rates of *P.g* and *S.m* were compared between groups. In addition, we also examined correlation between these percentages and rates and serum values of IL-6 and CRP which are indicators of the disease state.

Resutls: The number of patients with *S.m* was greater in the AF and AP groups compared to that in the group of healthy subjects. Especially, number of cnm-positive subjects was larger in AP group, that positively correlates serum IL-6 levels. Furthermore, there was a positive correlation between *P.g* infection and the presence of AP. On the contrary, significant correlation was not observed between these bacteria and the presence of AS. However, only AS but not AF and AP exhibited elevated percentages of *Gemella* genus, that was positively correlated with serum CRP levels.

Conclusion: Characteristic microbiome or infection pattern could be observed in each cardiovascular disease, that may be responsible for disease state.

3-B-P-047 一般演題（ポスター）

Involvement of the parotid fatty acid transporter CD36 in salivary secretion in mice is altered with age

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Xerostomia is common in middle-aged and elderly patients. In rodents, adipose tissue increases around the aging parotid gland (PG). Triglycerides are stored in adipose tissue and metabolized into fatty acids and glycerol as needed in the cells. It has been reported that CD36, a fatty acid transporter, is expressed on the tongue and plays a role in some taste sensations through the uptake of dietary fatty acids such as palmitic acid. However, there are few reports on CD36 in salivary glands. In this study, we investigated the role of CD36 in salivary secretion using male mice. In salivary glands of BALB/c mice, CD36 mRNA was highly expressed in the PG compared with submandibular and sublingual glands. The mice pretreated with a CD36 inhibitor showed decreased muscarinic agonist-induced salivary secretion at 8 and 48 weeks but not 72 weeks of age. In vitro [^{3}H]-palmitic acid uptake assay, the amount of [^{3}H] from isolated PG of mice at 8 weeks of age was significantly reduced in the CD36 inhibitor-pretreated group. In highly aged mice, senescence-accelerated mice of 56 weeks of age, there was no change in salivary secretion after pretreatment with CD36 inhibitor due to low protein expression of PG CD36. These results suggest that the importance of PG CD36 in mouse salivary secretion changes with age.

3-B-P-048 一般演題（ポスター）

Effect of tenidap on the expression of apoptosis-related proteins induced by phenytoin in human gingival fibroblasts

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Gingival overgrowth is caused in response to the antiepileptic drug, phenytoin. Phenytoin-induced gingival overgrowth is characterized by the proliferation of fibroblasts and increased collagen formation in gingiva. We have previously reported that tenidap inhibits cell growth, DNA and collagen syntheses, and lowered intracellular pH in human gingival fibroblasts (hGFs). The present investigation was undertaken to clarify the effect of tenidap on phenytoin-treated hGFs in respect to apoptosis-related proteins. hGFs were purchased from ScienCell Research Laboratories. The cells were cultured in DMEM containing 1% FBS (DMEM-1) without (control) or with 0.25 μM phenytoin for 24 hours, and then treated with 20 μM tenidap in DMEM-1 for 6 hours. Profiling the apoptosis-related proteins was performed using the Proteome Profiler™ Array. Phenytoin decreased the protein level of pro-apoptotic factors (cytochrome c, SMAC/Diablo, and HTRA2/Omi) compared to the control in DMEM-1, while tenidap up-regulated the reduction of protein levels of pro-apoptotic factors induced by phenytoin. These results suggest that phenytoin may induce cell proliferation by suppressing the expression of pro-apoptotic factors, while tenidap inhibits phenytoin-induced proliferation by disinhibiting the expression of pro-apoptotic factors.

3-B-P-049 一般演題（ポスター）

Hypoxic condition enhanced osteoclast differentiation via inducible nitric oxide synthase pathways.

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Purpose: The balance between the activities of bone-forming osteoblasts and bone-resorbing osteoclasts (OCs) is important for maintaining bone homeostasis. Excessive activation of OCs causes bone-destruction diseases. Our previous studies showed that hypoxic condition promoted osteoclast formation. However, the precise mechanism of osteoclast formation under hypoxia has been unclear. In the present study, we investigated the role of inducible nitric oxide synthase (iNOS) on osteoclast differentiation under hypoxia. Method: Bone marrow cells obtained from mice were stimulated with receptor activator of NF-kappa B ligand (RANKL) and macrophage colony stimulating factor (M-CSF) to induce osteoclast differentiation. Bone marrow cells were cultured with iNOS inhibitor or NO (nitric oxide) donor under normoxia (O_2 20%) or hypoxia (O_2 5%). Results and Discussion: The number of osteoclasts was increased in the culture under hypoxia compared with that in the culture under normoxia. The gene and protein expression of iNOS increased in the culture under hypoxia. The addition of iNOS inhibitor in hypoxic culture reduced the number of osteoclasts. Addition of NO donors in normoxic culture, the number of osteoclasts were increased. These results suggested that hypoxic condition could promoted osteoclast differentiation via iNOS pathway.

3-B-P-050 一般演題（ポスター）

Prenatal enzyme replacement therapy restores delayed calcification in the maxillofacial region of mice with severe infantile hypophosphatemia.

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Hypophosphatasia (HPP) is a congenital disease caused by a deficiency of tissue-nonspecific alkaline phosphatase (TNALP) gene. The pathogenesis of HPP varies, ranging from severe cases resulting in no fetal bone calcification to relatively mild cases. In recent years, the establishment of enzyme supplementation as a treatment method has prolonged survival in patients. However, the effects of enzyme therapy on the jawbone and periodontal tissues have not yet been studied in detail. Therefore, in this study, we investigated the therapeutic effects of enzyme replacement therapy on jawbone in mice. Recombinant TNALP was administered to mothers before birth and newborns immediately after birth, with the effect of treatment being evaluated. The treated HPP mice had improved mandibular bone quality and tooth quality (root length of mandibular first molar, formation of cementum), as well as improved periodontal ligament structure. Furthermore, prenatal treatment had an additional therapeutic effect on the degree of mandible and enamel calcification. These results suggest that enzyme replacement therapy is effective for the treatment of HPP, specifically in the maxillofacial region, and that early initiation of treatment may have additional beneficial therapeutic effects.

3-B-P-051 一般演題（ポスター）

Cdc42 promotes apical membrane formation by regulating the transport of Rab11a-positive vesicles

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Extensive research has focused on understanding developmental mechanisms for regenerating damaged salivary glands. However, while most studies have investigated early branching morphogenesis, the process of forming acinar cells—which completes morphogenesis after birth—remains poorly understood. In this study, we examined the role of Cdc42, a member of the Rho GTPase family, in the acinar cell formation.

By knocking out Cdc42 during acinar cell formation, we observed suppression in the formation of the luminal membrane. Remarkably, GFP, a protein with a targeting signal for plasma membrane trafficking, was detected on vesicles that had gathered near the plasma membrane. Through immunostaining aquaporin 5, a marker for the luminal membrane, we revealed its co-localization with these GFP-containing vesicles. Furthermore, we examined Rab11a, a marker protein indicating vesicles that create the luminal surface membrane within cultured epithelial cells. As a result, we observed a co-localization between Rab11a and the GFP vesicles.

In summary, these findings suggest a model in which apical membrane proteins within salivary glands are transported to the apical membrane through vesicles associated with Rab11a. Additionally, our findings suggest that Cdc42 potentially plays a role in promoting the formation of the apical membrane by regulating the targeted transport of newly synthesized apical membrane proteins.

3-B-P-052 一般演題（ポスター）

An observational study of post COVID-19 syndrome in Japan using a medical database

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In Japan, clinical information on post COVID-19 syndrome symptoms including nursing care requirements is limited. In this study, we investigated the incidence of acute (within 2 weeks after diagnosis) and post (2-12 weeks after diagnosis) COVID-19 symptoms, as well as nursing care requirements in Japan, when different SARS-CoV2 strains were prevalent and vaccination statuses changed by mass vaccination programs. Electronic health records of 122,045 patients diagnosed with COVID-19 between January 1, 2020, and June 30, 2022, were obtained and divided into three observation periods according to epidemic strains and vaccination coverage. Headache, malaise/fatigue, depression, and disuse syndrome were detected in acute and post COVID-19. The incidence of depression and disuse syndrome in post COVID-19 increased with age. Moreover, increased high-level nursing care requirements after COVID-19 was observed in elderly group. A lower incidence of acute and post COVID-19 symptoms in Japan was linked to increased vaccination coverage of the population and differences in viral strains.

3-B-P-053 一般演題（ポスター）

Permittivity measurement method of microorganisms for drug screening

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The effects of pharmacological substances on viruses in drug screening has been measured indirectly via the observation of cytopathic effects on cultured cells. To quantify directly the pharmacological effects of compounds on microorganisms, we devised a method that measures the direct impact by assessing the permittivity due to the lipid bilayer membrane. In the present study, we constructed an electrode-replaceable measuring cell using 3D printing technology to demonstrate the feasibility of permittivity measurements in microorganisms. We conducted measurements of the permittivity of a KCl solution, as well as a KCl solution containing suspended liposomes or yeast cells. We found an increase in permittivity that can be ascribed to the lipid bilayers of the liposomes and yeast cells, within a frequency range of 10^4–10^8 Hz. Following the induction of liposome lysis via the application of Triton-X100, we observed a reduction in the increased permittivity. Furthermore, when the yeast cell count was reduced by dilution, there was a corresponding decrease in the enhanced permittivity. A decrease in permittivity was also measured in yeast cells suspended in a KCl solution following heat and enzyme treatments. These results suggest that the permittivity of a lipid bilayer membrane can be measured to estimate the concentration of microorganisms in a solution and our method is expected to provide a novel assay for the preliminary screening of potential drugs aimed at microorganisms.

3-B-P-054 一般演題（ポスター）

Single cell analysis of brains of aged and young mouse models of post-ICU syndrome (PICS)

Llamascovarrubias Maraanais, Yasuha Kinugasa, Yumiko Imai

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Post-intensive care syndrome (PICS), characterized by physical and mental/cognitive symptoms occurs in some intensive care unit (ICU) survivors. We developed a mouse model of PICS by combining acute lung injury (ALI) with lower limb immobilization in both young and aged mice. Clinically, these animals exhibited characteristics of PICS including disuse muscle atrophy, signs compatible with depression, and pulmonary and systemic inflammation. Single cell transcriptomic analysis in brain demonstrated the effect of ageing in several cell types, specially microglia and endothelial cells and analysis of treatment effect showed that aged mice is more susceptible to changes in gene expression by the induction of ALI. Shared upregulated genes in aged and young treated mice were identified. Our data indicates that the combination of ALI and immobilization induces gene programs in brain associated with depression or neurodegenerative disorders and differentially affects young and aged individuals.

The possibility of nucleic acid medicine by *bmp-2* gene expression vector for alveolar bone regeneration

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Bone Morphogenetic Protein-2(BMP-2) has a high potential to induce the differentiation of mesenchymal stem cells to osteogenic cells. Moreover, recombinant BMP-2 protein can induce bone formation in many animal models for bone regeneration therapy. However, it is not successfully used for bone regeneration therapy clinically. Therefore, we have tried to delivery *bmp-2* gene into the target site safely and efficiently. We constructed non-viral vectors: pCAGGS-*bmp-2* for our gene transfer system, pCAGGS can express external genes temporarily at the injection site. Therefore, we considered that our pCAGGS constructs could be applied to the regeneration of periodontal tissues such as alveolar bone.

In this study, we applied our pCAGGS-*bmp-2* construct for alveolar bone regeneration by transferring it to the periodontal tissues of rats. We evaluated the potency for alveolar bone regeneration by bone morpho metric analyses as mineral apposition rate (MAR) and primary and secondary parameters. We revealed that MAR and parameters increased after *bmp-2* gene transfer at the target site of the alveolar bone.

3-B-P-056 一般演題（ポスター）

Exosomal microRNAs in the blood of non-recurrent cancer patients

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【Purpose】 To search for microRNAs that prevent cancer recurrence from exosomes in the blood of cancer patients undergoing IVR (Interventional Radiology), and to develop new nucleic acid drugs for cancer.

【Subjects】 Blood of recurrent and non-recurrent cancer patients after IVR, and healthy subjects.

【Methods】 Exosomes were extracted from the blood of cancer patients and healthy subjects, and RNA was purified. Furthermore, microRNA was detected by GeneChip miRNA 4.0 Array analysis. In addition, microRNA mimic was transfected into colon cancer cells (HCT116), and the viability of cancer cells was evaluated by Calcein/PI fluorescence staining.

【Results】 MicroRNA expression patterns were different in exosomes in the blood of recurrent cancer patients, non-recurrent cancer patients, and healthy subjects. Furthermore, we found 18 microRNAs with significantly more than 3-fold abundance in non-recurrent cancer patients. Among the 18 microRNA mimics, 3 microRNAs reduced cancer cell viability to less than 20%.

【Summary】 Since exosomal microRNA in the blood of non-recurrence-free cancer patients markedly decreased the viability of cancer cells, it is expected as a novel nucleic acid drug to prevent recurrence of cancer.

3-B-P-057 一般演題（ポスター）

Inhibition of miR-96-5p in the mouse brain increases glutathione levels by indirectly modifying GTRAP3-18 expression.

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Glutathione (GSH) is an important antioxidant that plays a critical role in neuroprotection. Neuronal GSH depletion induces oxidative stress causing some neurodegenerative diseases. The neuronal GSH levels are mainly regulated by excitatory amino acid carrier 1 (EAAC1) and its inhibitory protein, glutamate transporter-associated protein 3-18 (GTRAP3-18). In this study, we found that GTRAP3-18 levels were increased by the up-regulation of the miR-96-5p, which is a microRNA reported to decrease EAAC1 levels in neurons. We also discovered that neuro-oncological ventral antigen 1 (NOVA1) is an intermediate protein for GTRAP3-18 expression via miR-96-5p. We show that the intra-arterial administration of a miR-96-5p-inhibiting nucleic acid to living mice by a drug delivery system using microbubbles and ultrasound decreased the levels of GTRAP3-18 via NOVA1, while increased the levels of both EAAC1 and GSH in the mouse brain. NOVA1 was recognized as an RNA-binding protein involved in miRNA regulation as well as mRNA processing and splicing. Analysis of RNA-fold predictions shows that the predicted NOVA1-binding site on GTRAP3-18 3'-UTR is a stem-loop structure, and that the stem sequence is a target site of some miRNAs, implying that NOVA1 binds to the 3'-UTR of target genes and induces conformational changes in the RNA structure that favor association with miRNAs. These findings suggest that the delivery of a miR-96-5p inhibitor to the brain would efficiently increase the neuroprotective activity by increasing GSH levels via EAAC1, GTRAP3-18 and NOVA1.

3-B-P-058 一般演題（ポスター）

Inhibition of Tumor-Associated Macrophage Differentiation by HSP90 Inhibitors: A Potential Therapeutic Strategy for Breast Cancer

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Macrophages play a crucial role in regulating the innate immune system and maintain tissue homeostasis. In the tumor microenvironment, tumor-associated macrophages (TAMs) are known to promote tumor progression, mostly derived from recruiting monocytes. However, the molecular mechanism underlying the differentiation of monocytes into TAM remains unclear.

This study aimed to determine the intracellular signals or target molecules involved in TAM differentiation and explore potential therapies targeting this process. First, we incubated THP-1 human monocyte cell with conditional culture medium (CM) from various breast cancer cell lines. RT-PCR analysis revealed that CM from the triple negative breast cancer cell line, MDA-MB231, significantly increased TAM markers, indicating monocyte polarization into TAMs. Next, we explored molecules inhibiting monocyte-to-TAM differentiation using the Screening Committee of Anticancer Drugs (SCADS inhibitor kit). Heat shock protein 90 (HSP90) inhibitors, ganetespib and Radicicol, were found to suppress the expression of TAM markers, suggesting that HSP90 inhibition prevented TAM differentiation. Furthermore, we sought to investigate the mechanism by which Hsp90 inhibition suppresses TAM differentiation. Western blotting showed that ganetespib blocked CM-induced activation of AKT and STAT3 pathway within 3 hours, and inhibited JAK2 pathway activation within 12 hours. These results suggested that ganetespib modulated TAM differentiation via restricting AKT pathway and JAK2/STAT3 pathway activation. The inhibitory effect of ganetespib on TAM differentiation was also confirmed in mouse breast cancer cell *in vitro* and murine breast cancer cell transplantation model *in vivo*. These findings suggest that Hsp90 inhibitors could be potential therapeutic agents for breast cancer by reducing TAM differentiation and tumor progression.

3-B-P-059 一般演題（ポスター）

Oral magnesium oxide prevents cisplatin-induced renal failure in a rat model

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[Background] Cisplatin is a widely used chemotherapeutic agent used to treat solid tumors, such as ovarian cancer, testicular cancer, head and neck cancer, and non-small cell lung cancer. However, one of its side effects is renal failure. In this study, we established a cisplatin-induced renal failure model in rats and investigated the inhibitory effect of orally administered magnesium oxide against it. [Methods] Renal function was evaluated in terms of plasma creatinine (Cre) and blood urea nitrogen (BUN), and the plasma magnesium concentration and body weight were used as indicators of general condition. Cisplatin 7.0 mg/kg was administered intraperitoneally on day 0. Magnesium oxide was administered orally at 200 or 400 mg/kg/day from day -3 to day 1 or from day -3 to day 0. [Results] During the period from day -3 to day 0, the increases in the plasma levels of Cre and BUN level induced by cisplatin were suppressed more significantly by magnesium oxide at 400 mg/kg/day than at 200 mg/kg/day. Magnesium oxide at 400 mg/kg/day had a greater preventive effect on cisplatin-induced nephropathy than at 200 mg/kg/day. Also, the preventive effect of magnesium oxide was higher from day -3 to day 1 than from day -3 to day 0. Examination of parameters overall suggested that body weight improved more rapidly in rats that received magnesium oxide than in rats that did not. [Conclusion] This study revealed that oral administration of magnesium oxide at a dose of 400 mg/kg/day from 3 days before to 1 day after administration of cisplatin had a marked preventive effect against cisplatin-induced renal failure in this rat model.

3-B-P-060 一般演題（ポスター）

Influence of anticancer drug S-1 on ocular surface with histological alterations of corneal nerve in rat

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Lacrimation and corneal epithelial damage have been reported in cancer patients receiving chemotherapy with S-1: a combination of tegafur, gimeracil, and oteracil potassium. Recent studies have gradually clarified that corneal nerves contribute to homeostasis of ocular surface by facilitating protection and healing of corneal epithelium and regulating tear secretion. This study aimed to investigate the relationship between the toxic effects of S-1 on the ocular surface and alteration of corneal nerves.

S-1 (vehicle, 2 or 5 mg/kg) was administered to male Wistar rats (6 weeks old) for 28 consecutive days. As index of the ocular surface symptoms, number of blinks, tear volume, and corneal epithelial damage scores were measured. Histological analysis was performed with keratoconjunctival tissue, trigeminal ganglion, and trigeminal nucleus where corneal nerves are located.

Administration of S-1 increased the number of blinks and the corneal epithelial damage score, but unaffected the tear volume. Keratoconjunctival inflammation and corneal ulceration were absent in S-1 groups, while the density of nerve fibers in cornea, the expressions of Iba1-positive microglia in the trigeminal ganglion and trigeminal nucleus significantly increased. In conclusion, these results suggest that S-1 may induced neuromorphological changes in cornea and neuroinflammation in the corneal nerve tract with modest ocular abnormality.

3-B-P-061 一般演題（ポスター）

Anti-tumor effect of mannose-conjugated chlorin e6 photodynamic therapy targeting M2-like tumor-associated macrophages

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M2-like tumor-associated macrophages (M2-TAMs) in cancer tissues play a key role in cancer progression and immune evasion. Therefore, cancer immunotherapies targeting M2-TAMs have attracted considerable attention. Photodynamic therapy (PDT) is a minimally invasive and site-selective approach that kills target cells using a visible laser and a nontoxic photosensitizer. The photosensitizer is activated by the laser and produces reactive oxygen species (ROS) that induce cell death of target cells. We developed a novel photosensitizer, mannose-conjugated chlorin e6 (M-chlorin e6), which can be selectively delivered to M2-TAMs that highly express mannose receptors (CD206). We evaluated the anti-tumor effect of M-chlorin e6 PDT in a mouse model of allogeneic transplantation of CT26 cells, a murine colon cancer cell line. M-chlorin e6 PDT significantly reduced tumor volume and weight compared to the control group. M-chlorin e6 PDT also decreased the percentage of M2-TAMs ($CD11b^+$, $CD206^+$) and increased the percentage of M1-TAMs ($CD11b^+$, $CD80^+$ or $CD86^+$, $CD206^-$) in the tumor tissue. Moreover, M-chlorin e6 PDT directly inhibited cancer cell viability and enhanced phagocytosis by the macrophage cell line RAW264.7. These results suggest that M-chlorin e6 PDT exerts its anti-tumor effect by reduction of M2-TAMs, inducing cancer cell death, and promoting macrophage phagocytosis.

3-B-P-062 一般演題（ポスター）

The anti-tumor effect of CAR-T cell therapy is enhanced by the antagonist of Inhibitor of Apoptosis Protein, tolinapant

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Chimeric antigen receptor (CAR)-T cell therapy is a promising therapeutic approach in treating hematological malignancies. However, due to escape of CAR antigens in cancer cells, resistance and relapse occur in some patients after CAR-T cell therapy. Therefore, we investigated whether the combination with an antagonist of inhibitor of apoptosis proteins (IAP), tolinapant, currently being evaluated in a phase 2 study of peripheral T-cell lymphoma and cutaneous T-cell lymphoma, could enhance anti-tumor effect of CAR-T cell therapy. We found that tolinapant enhanced the antitumor effect of CAR-T cell therapy in a TNF-α-dependent manner. TNF-α secreted from CAR-T cells in the presence of tolinapant also induced cell death of antigen-negative cancer cells not in cell-cell contact with CAR-T cells. The significant combined effect was also observed in vivo. Even at ineffective doses of CAR-T cell monotherapy, anti-tumor effect was observed when the combination treatment was used. We propose that this is because tolinapant induced both cancer cell death and CAR-T cell proliferation. In summary, we find that combination therapy with tolinapant improved the efficacy of CAR-T cells by inducing cancer cell death and CAR-T cell proliferation. This combination therapy may overcome the current limitations of CAR-T cell therapy.

3-B-P-063 一般演題（ポスター）

Cardiotoxicity assessment of VEGFR-tyrosine kinase inhibitors by human iPSC cardiomyocyte and pharmacovigilance analysis

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The number of cancer survivors is increasing because of recent advances in cancer treatments. Molecular target drugs, such as vascular endothelial growth factor receptor tyrosine kinase inhibitors (VEGFR-TKIs) have demonstrated the encouraging efficacy. However, anti-cancer drugs are known to frequently induce severe, sometimes life-threatening cardiotoxicity, such as arrhythmias and contractile dysfunction. The assessment of drug-induced proarrhythmic risk using human iPS cell-derived cardiomyocytes (hiPSC-CMs) has been validated internationally, while it has not been established to predict the cardiac contractile dysfunction. Here, we investigated the effect of VEGFR-TKIs on contractile changes of hiPSC-CM sheets (iCell cardiomyocytes 2.0, Fujifilm Cellular Dynamics International) using an image-based motion vector system (SI8000, Sony). Chronic treatment with sorafenib or pazopanib significantly decreased in the contraction and relaxation velocity of hiPSC-CMs, whereas nintedanib did not. We also found that these *in vitro* results were correlated with the FDA Adverse Event Reporting System (FARES) analysis. Taken together, the motion analysis of hiPSC-CMs is a valuable approach for assessing drug-induced cardiac contractile dysfunction.

3-B-P-064 一般演題（ポスター）

G-CSF potentiated the antitumor activities of lipopolysaccharide without enhancing the body weight loss in mice bearing MH134 hepatoma

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Background: Severe toxicity of lipopolysaccharide (LPS) have prevented its clinical use for cancer treatment so far. We have demonstrated that LPS potentiated the activity of PMN and suggested that LPS induced antitumor effect partially depend on PMN. Then, we investigated the effects of granulocyte colony stimulating factor (G-CSF), a cytokine enhancing neutrophil function, on antitumor activity of bacterial LPS against a murine syngeneic hepatoma MH134.

Methods: G-CSF (30 μg/kg) was administered for successive 4 days intravenously and LPS (20 μg/mouse) was administered with MH134 hepatoma intradermally on day 0, and tumor growth and survival days of mice bearing MH134 hepatoma were monitored.

Results: 4 day treatment of G-CSF 30 μg/kg increased the neutrophil level with statistical significance. On the MH134 hepatoma bearing mice, LPS significantly inhibited the tumor growth. Although G-CSF pretreatment alone did not inhibit the growth, once combined with LPS, significant inhibition was observed compared with LPS group. Tumor regression was demonstrated in combination group (6/12 mice), and the mice without tumor burden survived exceeding those of the LPS monotherapy group without enhancing the body weight loss.

Conclusions: G-CSF potentiated the antitumor effect of LPS and elongated the survival days of mice bearing MH134 hepatoma without enhancing the toxic response to LPS.

3-B-P-065 一般演題（ポスター）

Induction of cancer cell-specific cell death by novel cyclic naphthalene diimide derivatives

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Recently, it became clear that guanine-rich DNA sequences could form characteristic four-stranded structure (G4). As G4 is frequently found in chromosomal telomere regions and in the promotor region of cancer-related genes, G4 is expected to be a target for developing new anticancer drugs. In this study, we compared cytotoxic effect of cyclic naphthalenediimide derivative (cNDI) that specifically bind to G4, with a widely-used anticancer drug cisplatin (CDDP).

Human oral cancer cell line SAS and the human normal oral keratinocyte cells (HOK) were treated with either cNDI or CDDP and cell viability was assessed by WST-8 assay. Both cNDI and CDDP dose-dependently decreased cell viability of the cells. The ED_{50} values of cNDI on SAS and HOK were 0.05 and 1.09 microM, respectively, while those of CDDP on the cells were 2.08 and 5.39 microM. Both cNDI and CDDP induced apoptosis that was assessed by AnnexinV-staining and cleavage of PARP and procaspase-3 assessed in Western blotting. Expression level of c-Myc and TERT mRNA was decreased in SAS cells treated by cNDI, but not by CDDP.

The results suggested that cNDI induced apoptosis with high specificity to cancer cells compared to CDDP. Thus, cNDI is considered to be promising as new anticancer agents with improved cancer specificity and low adverse effect.

3-B-P-066 一般演題（ポスター）

Evaluation of dissociative anesthetic-induced neurotoxicity in human iPSC-derived neurons

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The dissociative anesthetics, including phencyclidine (PCP), ketamine and methoxetamine (MXE), are selective and potent NMDA receptor (NMDAR) antagonists that have been reported to cause neurotoxicity, similar to another NMDAR antagonist MK801. Although NMDARs are known to be involved in synaptic transmission and plasticity, the neurotoxic mechanism of dissociative anesthetics remains poorly understood. In the present study, we evaluated the neurotoxicity of PCP, ketamine and MXE using human iPSC-derived neurons. First, we confirmed the expression levels of most NMDAR subunits in human iPSC-derived neurons. Next, we examined the effect of dissociative anesthetics on extracellular field potential using a multielectrode array (MEA) system. Treatment with each dissociative anesthetic decreased the total number of spikes and network bursts in human iPSC-derived neurons. Although we found that exposure to each dissociative anesthetic decreased cell viability in higher dosages, the decrease in total spikes and network bursts occurred at lower doses. These data suggest that dissociative anesthetics induce neurotoxicity by inhibition of the neuronal network activity.

3-B-P-067 一般演題（ポスター）

Transgenerational effects of paternal methylphenidate administration on behavior and gene expression in mouse

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The prevalence of attention-deficit/hyperactivity disorder (ADHD), one of the most common neurodevelopmental disorders, in adults is estimated to be about 5%, and the number of prescriptions is increasing. However, the transgenerational effects of methylphenidate (MPH), a first-line drug of ADHD, are unclear. Recently, it has been reported that paternal environmental factors induce epigenetic changes that affect the neurodevelopment of children and grandchildren. Therefore, we investigated the effects of paternal MPH administration on the next (F1) and subsequent generations (F2) in the mice model.

Male ICR mice (6 weeks old) were administered MPH or saline for 21 days and mated with female mice to obtain F1. At nine weeks of age, F1 males were mated with female mice to obtain F2. At six weeks of age, both F1 and F2 were subjected to the elevated plus maze test to assess impulsivity. Additionally, total RNA from the striatum of F1 and F2 was subjected to RNA-seq, and the enrichment analysis was conducted.

Both F1 and F2 of MPH groups significantly increased impulsivity. Enrichment analysis showed an enrichment of exocytosis-related genes involved in neurotransmission in both F1 and F2. These findings suggest that MPH administration to male mice may alter behavior by affecting neurofunction in the next and subsequent generations.

3-B-P-068 一般演題（ポスター）

Effect of PPARγ-K107 SUMOylation on insulin sensitivity and adiposity in mice

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Peroxisome Proliferator-Activated Receptor gamma (PPARγ) is a nuclear receptor that plays an essential role in expression of adipocyte-specific genes. The activity of PPARγ is regulated not only by ligand binding, but also by post-transcriptional modifications. In this study, we investigated the physiological function of small ubiquitin-like modifier (SUMO), a suppressive post-transcriptional modification, in PPARγ activity by generating mice with a mutation at the SUMOylation site.

[Experimental Procedures] Mice with a lysine-to-arginine substitution at codon 107 of PPARγ were generated by homologous recombination (K107R). We challenged with a high-fat diet, K107R mice and their WT littermates, measured their body weights, and performed glucose tolerance test, insulin tolerance test and hyperinsulinemic–euglycemic clamp experiments. We also evaluated gene expression patterns of their adipocytes by RNA sequencing (RNA-seq), and its outcomes were validated by quantitative RT-PCR.

[Results and Discussion] SUMOylated PPARγ levels were virtually undetectable in homozygous K107R adipocytes. We observed mild reduction of body weight gain in K107R mice on high-fat diet, compared with that of WT. In a glucose tolerance test, K107R mice had decreased plasma insulin concentrations compared with WT mice. Our hyperinsulinemic–euglycemic clamp experiments indicate that K107R mice had a doubling in both the glucose infusion rate and whole-body glucose uptake rate. Significant increases were also observed in glucose uptake into gastrocnemius and diaphragm muscle and iWAT and similar trends in soleus muscle and eWAT and iWAT. Our RNA-seq analysis and following quantitative RT-PCR data indicate that genes involved in metabolism were upregulated in K107R adipose tissues. These results demonstrate that SUMO plays a critical role in regulation of PPARγ activity in vivo.

3-B-P-069 一般演題（ポスター）

Cigarette smoke gas phase induces ferroptosis via PKCβ in J774 macrophages

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Cigarette smoking is a risk factor for various types of diseases including atherosclerosis, hypertension, chronic obstructive pulmonary disease, and respiratory infection. The respiratory infection caused by cigarette smoking is due to immune cell dysfunction by cigarette smoke, although its molecular mechanism remains to be clarified. The cigarette smoke can be divided into two phases: tar (particle) phase and gas phase. We have previously reported that gas phase extract of cigarette smoke (CSE) induces cell death. In this study, we have examined the effects of CSE on J774 macrophages. CSE and unsaturated carbonyl compounds, cytotoxic factors in the CSE, induced cell death in J774 macrophages. Ferrostatin-1 and liproxstatin-1, ferroptosis inhibitors, suppressed cell death caused by CSE and unsaturated carbonyl compounds. A broad-range protein kinase C (PKC) inhibitor Gö6983 suppressed CSE- and unsaturated carbonyl compounds-induced cell death. To identify PKC isoforms involved in the process, we have examined isoform-specific inhibitors. Enzastaurin, a PKCβ-specific inhibitor, suppressed the cell death. Enzastaurin also suppressed RSL3-induced ferroptosis. These results suggest that CSE and unsaturated carbonyl compounds induce PKCβ-dependent ferroptosis in J774 macrophages.

3-B-P-070 一般演題（ポスター）

A psychedelic substance 2,5-dimethoxy-4-iodoamphetamine induces impairments in spatial short-term memory and retrieval and mood in mice

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2,5-dimethoxy-4-iodoamphetamine (also known as DOI) is a hallucinogenic Serotonin$_{2A}$ Receptor agonist. In this presentation, we demonstrate the effects of a psychedelic substance DOI on behaviors associated with spatial memory, mood, and motor coordination. Administration of DOI (1.0 mg/kg, s,c,) induced a significant decrease in Y-maze alternations compared to saline vehicle-treated mice, with no alterations in horizontal spontaneous locomotion. The decrease in the Y-maze alternations was completely recovered by administration with volinanserin (0.1 mg/kg, i.p.), a selective and potent 5-HT$_2$ receptor antagonist. No effect of DOI on motor coordination and ambulatory activity was observed based on the observations in the rotarod and pole tests. A reduction in buried marbles was observed in DOI-treated mice measured in the marble-burying test. Reduced exploratory time was observed in mice after administration of DOI in a novel-object exploration test. These observations suggest that DOI induces impairments in spatial short-term memory and retrieval and mood but not locomotor hyperactivity or dysfunction of motor coordination. Supported by Ministry of Health, Labor and Welfare Grants-in-Aid for Scientific Research (22KC1005).

3-B-P-071 一般演題（ポスター）

Effects of a single administration with opioid receptor antagonist naloxone on motivative behavior and brain monoamine turnover in mice

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We hypothesized that opioid receptor antagonists would inhibit motivated behavior produced by a natural reward. To evaluate motivated responses to a natural reward, mice were given access to running wheels using a multi-configuration testing apparatus for 71.5 h. Mice were also tested separately for novel object exploration to investigate whether naloxone affects behavior unrelated to natural reward. In control mice daily wheel running increased from day 1 to day 3. The selective mu-opioid receptor antagonist beta-funaltrexamine (beta-FNA) (5 mg/kg) slightly decreased daily wheel running which also increased from day1 to day 3 in beta-FNA-treated mice. The non-selective opioid receptor antagonist naloxone produced a greater reduction in wheel running than beta-FNA and eliminated the increase in wheel running that occurred over time in the other groups. Analysis of food access, locomotor behavior, and behavior in the novel object test suggested that the reduction in wheel running was selective for this highly reinforcing behavior. These results indicate that opioid receptor antagonism reduces responses to the natural rewarding effects of wheel running, and that these effects involve multiple opioid receptors since the non-selective opioid receptor antagonist had greater effects than the selective-opioid receptor antagonist.

3-B-P-072 一般演題（ポスター）

Convolutional neural network model for evaluation of lasting behavioral changes in mouse with kanamycin-induced unilateral inner ear dysfunction

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In a rodent model of acute aminoglycoside ototoxicity to unilateral inner ear, physical abnormalities, such as nystagmus and postural alteration, are relieved within a few days by neural compensation. However, how exploratory behavior changes after unilateral ototoxic damage in home-cage environment in mice have not yet been elucidated. In this study, exploratory behavior after kanamycin-induced unilateral inner ear injury was examined in a cage with wood-shavings as natural bedding. To this end, we developed machine learning environment with robust detection model for evaluation of drug efficacy and toxicity. In sequential images from video recording, our deep learning model detected the mouse as an object with 99.9% accuracy. After evaluation of the detection accuracy, tracing of the mouse movement revealed that total distance moved in 15 minutes was increased 3 days after surgery. Moreover, the injured mouse turned frequently toward healthy side upto 17 days after surgery. Our mouse model of unilateral inner ear dysfunction and its analysis strategy is useful to evaluate neuronal compensatory process and screening of drug with therapeutic potency.

Effects of pretreatment with LY2090314, a potent glycogen synthase kinase -3 inhibitor, on methamphetamine-induced hyperlocomotion and stereotypy in mice

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Glycogen synthase kinase-3 (GSK-3) is one of the most essential serine/threonine kinases, which are constitutively active, multifaceted, and ubiquitous in nature. In mammalian cells, GSK-3 is formed as the two isoforms termed GSK -3a and GSK-3b. GSK-3b is present in a high concentration in the abundance of tissues in the central nervous system, regulating a crucial role in neuronal signaling pathways. The research for involvement of GSK-3b signaling in drug abuse liability has been progressed based on the studies investigating molecular and cellular mechanism of action, but few reports have been made on animal research so far. In this presentation, we will demonstrate that pretreatment with LY2090314 (2.5, 10, 25 mg/kg), a potent GSK-3b inhibitor, tended to have an inhibitory effect on methamphetamine (METH; 3 mg/kg)-induced hyperlocomotion. For stereotyped behavior (10 mg/kg of METH), LY2090314 significantly inhibited METH-induced stereotypy in a dose-dependent fashion. Stereotyped biting was significantly reduced by doses of LY2090314. These results suggest that GSK-3 signaling pathway is essential for the expression of METH-induced stereotypy.

3-B-P-076 学生セッション（ポスター）

Characteristics of behavioral abnormalities induced by a psychedelic substance 2,5-dimethoxy-4-iodoamphetamine in mice

Sho Yuze[1], Nobue Kitanaka[2], Takahiro Hamana[1], Kentaro Matsuda[1], Masanori Nakai[1], Kazuo Tomita[3], Kento Igarashi[3], Tomoaki Sato[3], Nobuyoshi Nishiyama[1], Junichi Kitanaka[1]

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For basic research towards regulation of psychedelics, it is important to examine in detail the behavioral patterns of laboratory animals by one of the typical psychedelic substance 2,5-dimethoxy-4-iodoamphetamine (also known as DOI). It has been well known that DOI induces a head-twitch response in rodents. To re-evaluate the behavioral characteristics, we investigated the effects of DOI on behaviors by observers unaware of treatments. Administration of DOI (0.1, 0.5, 1.0 and 2.0 mg/kg, s,c,) or saline vehicle induced increases in preening ("face-washing behavior"), hindlimb scratching, and prone position, but not head-twitch, in dose-dependent fashions. There was a positive correlation between preening and prone position ($r^2 = 0.5988$). Preening and hindlimb scratching behaviors occurred frequently in 10 to 15 minutes after the DOI injection, while the prone position reached a plateau level 20 minutes after dosing, suggesting that the behavior of washing the face and scratching by the hind limbs could be observed for up to 20 minutes after DOI administration, and it is thought that the behavior was replaced by the prone position after that. Supported by Ministry of Health, Labor and Welfare Grants-in-Aid for Scientific Research (22KC1005).

Wortmannin, a potent phosphatidylinositol 3-kinase inhibitor, suppresses methamphetamine-induced stereotyped sniffing and biting, ameliorating the frequency of total stereotypy in mice

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Phosphatidylinositol 3-kinase (PI3K) (EC 2.7.1.137) is an enzyme essential for inflammation as well as carcinogenesis in mammals. It has been demonstrated that a close, positive relationship between expression of cellular inflammation and action of psychostimulants such as cocaine and methamphetamine (METH). This suggests that the inhibition of PI3K might regulate METH-induced positive symptoms such as hyperlocomotion and stereotyped behavior, but few reports have been made on animal research so far. In this presentation, we will demonstrate that pretreatment of mice with wortmannin (3 and 10 mg/kg), a potent and selective PI3K inhibitor, significantly inhibited METH-induced stereotypy in a dose-dependent fashion while METH-induced hyperlocomotion was not affected by pretreatment with wortmannin. It is of interest to present that a rearing behavior, which was not exhibited when mice with/without exposure to 10 mg/kg METH after 0 or 3 mg/kg wortmannin were exposed to a novel environment, was significantly augmented after exposure to 10 mg/kg METH after 10 mg/kg wortmannin, suggesting that a relatively high dose of wortmannin might shift stereotyped biting and sniffing to rearing.

3-B-P-078 学生セッション（ポスター）

Knockdown of Teneurin-4 in the nucleus accumbens attenuates dopamine release induced by methamphetamine in mice

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[Background and Aim]

Over the past few decades, global public health issues have arisen due to the production and use of methamphetamine (METH). METH is recognized as a widely distributed psychostimulant with a strong addictive potential that impacts the central nervous system. METH primarily impacts the limbic system by triggering the release of numerous neurotransmitters, such as dopamine, in the nucleus accumbens (NAc), a key component of the reward system Previous findings from our data revealed several genes associated with METH addiction, including *ODZ4*, which encodes teneurin4 (Ten4). Ten4 belongs to the subtype of type II transmembrane tenerin proteins, comprising approximately 2800 amino acids, and exhibits significant sequence homology. Genetic studies have indicated a correlation between teneurin proteins and developmental issues, neurological disorders, and drug residence Based on the aforementioned research, it is suggested that Ten4 might play a role in neuronal plasticity and development in the context of drug addiction associated with drug use. Here we investigated the knock-down of Ten4 in NAc was involved in dopamine release from the NAc by METH of mice.

3-B-P-079 学生セッション（ポスター）

Cellulose rich food induces intestinal disturbance that leads anxiety-like behavior and amygdalar dopaminergic hyperactivity.

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It is indicated the intestinal environment affects anxiety and emotions directly in recent years. For example, improvement of intestinal environment is known to suppresses the anxiety-like behavior in mice model of depression and schizophrenia. Such gut-brain relationships, called “gut-brain axis”, are suggested to involve both endocrine and neuronal system via the vagus nerve. However, previous studies have mainly used psychiatric disease models, therefore how intestinal environment will affect on emotion in normal animals remains unclear. In the present study, we verified psychological and physiological effect of food-induced intestinal environment modification.

First, we divided mice into two groups and fed either MF (Standard diet: contains various dietary fibers) or AIN-93M (contains cellulose alone) for 16 weeks. As reported, AIN-93M reduced the level of short-chain fatty acids in the cecum. Further, we found the significant decrease in peristalsis and the change in intestinal inflammation in AIN -93M-fed animals, suggesting the exacerbation of intestinal environment in those animals comparing to MF-fed control group. Interestingly, AIN-93M-fed animals also displayed the significant increase of marble-burying behavior compared to MF-fed group, indicating the enhancement of anxiety by that 16-week exposure to AIN-93M. These effects were abolished by vagotomy, suggesting that enhanced anxiety is caused by the AIN-93M-induced intestinal disturbance through gut-brain axis. We further measured brain monoamine levels in these animals, and found the dopamine level in amygdala was significantly increased in the AIN-93M-fed group.

These results suggest that the cellulose-induced intestinal disturbance and decreased intestinal function caused overactivation of dopaminergic system, which may enhance the anxiety level.

3-B-P-080 学生セッション（ポスター）

The L-DOPA receptor GPR143 in the striatal indirect-pathway inhibitory regulates anxiety-like behavior via interaction with dopamine D2-receptor in mice

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We have previously shown that L-DOPA is not just a dopamine precursor but also a neurotransmitter. Recently, a G-protein coupled receptor GPR143, a gene product of ocular albinism1, was identified as a receptor for L-DOPA. In the present study, we examined the effects of endogenous L-DOPA on anxiety-like behavior using GPR143-deficient mice (GPR143-KO). As a quantitative measure of anxiety-like behavior, we utilized the time spent in the open arm portion of the zero-maze test. Interestingly, GPR143-KO spent less time in open arm compared to wild-type mice (WT). Moreover, striatal indirect pathway-specific GPR143-KO mice spent less time in open arm compared to its control mice. Expression of GPR143-P2A-EGFP in the dorsomedial striatum of Gpr143-KO not influenced to anxiety-like behavior. Next, we used alpha-methyl-para-tyrosine（α-MPT), a synthetic inhibitor of L-DOPA. We confirmed that intraperitoneal administration of α-MPT at the dose of 3 mg/kg decreased the release of L-DOPA in the dorsal striatum, without affecting dopamine release. The administration of α-MPT decreased the time spent in open arm in WT, while this effect was not observed in GPR143-KO. Intraventricular administration of a synthetic peptide, which inhibited the interaction between GPR143 and dopamine D2 receptor, increased anxiety-like behavior in WT. These results suggest that L-DOPA inhibitory regulates anxiety-like behavior through interaction between GPR143 and dopamine D2 receptor in the striatal indirect pathway.

Abnormalities of axon initial segments in mice with attention-deficit hyperactivity disorder (ADHD)-like behavior are recovered along with behavioral abnormalities by the drug for ADHD.

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The axon initial segment (AIS) is located at the proximal axon, which is responsible for information output by generating action potentials. Recent studies have shown that the structure of AIS can be altered based on the input to the neuronal circuit, and this can impact neural activity. We previously observed that AIS length was altered in the cortical regions of both mice and rats with attention-deficit hyperactivity disorder (ADHD)-like behavior. These findings indicate that the abnormality of neural activity due to the alteration of AIS lengths is associated with ADHD. However, the link between AIS length changes and ADHD remains unclear. To investigate this further, we examined whether pituitary adenylate cyclase-activating polypeptide (PACAP)-deficient (PACAP−/−) mice, which display ADHD-like behavior show AIS length alteration. Further, we also examined the effects of atomoxetine, a drug for ADHD on the AIS length in PACAP−/− mice. We found that PACAP−/− mice exhibited a longer AIS length compared with wild-type mice. In addition, repeated treatments with atomoxetine improved AIS abnormality along with hyperactivity in PACAP−/− mice. These results suggest that AIS abnormality is one of the phenotypes of ADHD and improving AIS abnormalities could be a novel drug target for ADHD pathophysiology.

3-B-P-082 学生セッション（ポスター）

Wheel-running-related dopamine release in the mouse nucleus accumbens

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In this study, we examined wheel-running-related dopamine (DA) release in the mouse nucleus accumbens (NAc) to examine the potential of wheel-running behavior as a model of behavioral addiction in mice. Changes in the DA level were measured by a fiber photometry technique using GRAB_DA which enables high temporal resolution measurement of DA release. Male C57BL/6J mice were divided into three groups: when the partition separating the cage into two compartments was removed , group 1 was presented with a running wheel (RW), group 2 was presented with a fixed RW that could not be rotated, and group 3 was not presented with anything. DA levels in the NAc medial shell and NAc core were significantly elevated in groups 1 and 2 compared to group 3. Furthermore, DA levels in the NAc medial shell were significantly elevated in group 1 compared to group 2. On the other hand, there was no significant difference in DA levels in the NAc lateral shell among the three groups. These results suggest that DA release in the NAc medial shell may characterize the activity of the mesolimbic dopaminergic system during behavioral addictions. Further experiments including comparisons with other rewards, such as food and social interaction that do not induce behavioral addictions, are needed.

3-B-P-083 学生セッション（ポスター）

Enriched environment modulates sharp-wave ripples in hippocampus

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Enriched environment (EE) is a key experimental paradigm, which has been widely used for studying the interactions of animals to the environment and differences to the controlled subjects. While many aspects of the influence of EE have been reported at the behavioral level, little is known about EE-induced hysteretic alterations in neural activity. We studied the acute changes in mice which had experienced an EE exposure for 30 min. Compared to control animals under standard conditions, we found increases in the event frequency of sharp wave-ripples (SWRs) in the hippocampus and the amplitude and duration of SWRs. We discovered that EE altered the event distribution of SWRs so that SWRs occurred in a series of clusters. These findings highlight the beneficial effects of EE on memory consolidation and provide insights into the relationship between the richness of the environment and the replay of memories in the hippocampus. Further research is needed to examine the detailed mechanism of EE-derived acute alterations by some types of behavioral tests.

3-B-P-084 学生セッション（ポスター）

Involvement of connective tissue growth factor in the ameliorative effect of clozapine, an atypical antipsychotic, on cognitive dysfunction

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Clozapine is the only drug with proven efficacy in schizophrenia that does not respond to other antipsychotics. However, potentially serious side effects such as agranulocytosis have limited clozapine treatment. The specific mechanism of action responsible for its superior efficacy among antipsychotics is also still elusive. We previously found that chronic treatment with clozapine, but not risperidone, improved cognitive impairments in a mouse model of psychiatric disorders induced by early postnatal activation of the neuropeptide receptor VPAC2. Then, we have identified connective tissue growth factor (CTGF) as a candidate molecule, which is upregulated by clozapine. This study aimed to clarify the involvement of CTGF in the effects of clozapine *in vivo*. Chronic administration of intranasal CTGF with L-penetratin, a cell-penetrating peptide, improved cognitive impairments in the novel object recognition test. Additionally, concomitant treatment with intranasal injection of the neutralizing antibody against CTGF and L-penetratin blocked the ameliorative effects of clozapine on cognitive impairments. These results suggest that CTGF signaling pathway might be at least partly involved in the effects of clozapine and would offer new insights into an alternative therapeutic strategy for treatment resistant schizophrenia.

3-B-P-085 学生セッション（ポスター）

Effects of bone marrow transplantation on brain function using Down syndrome model mice

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Down Syndrome, a genetic disorder resulting from trisomy of chromosome 21, is frequently associated with neurodevelopmental anomalies and vascular and hematologic pathologies. Moreover, the predisposition of Down Syndrome patients to develop early onset Alzheimer's disease post 50 years of age has been postulated to be linked with the trisomy state of the amyloid precursor protein gene located on chromosome 21. However, the pathogenesis of Alzheimer's disease remains elusive since the accumulation of amyloid beta does not necessarily cause Alzheimer's disease, and antibody therapy aimed at removing amyloid beta does not achieve remission. Recent literature has provided intriguing evidence indicating the amelioration of cognitive function in geriatric mice upon administration of plasma from younger cohorts (Castellano et al., Nature, 2017), implicating a potential connection between cerebrovascular and hematologic conditions and insinuating that blood components may exert influence on cerebral function. Consequently, this study is designed to investigate whether the transplantation of bone marrow cells from Down Syndrome model mice leads to alterations in brain functionality. In this presentation, we will present data obtained to date and discuss potential mechanisms that regulate brain function by the hemopoietic stem cell.

3-B-P-086 学生セッション（ポスター）

Expression of Vacuolar Protein Sorting in a Mouse Model of Alzheimer's Disease

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Pathological features of Alzheimer's disease (AD) include the formation of senile plaques due to extracellular amyloid-β (Aβ) accumulation and the formation of neurofibrillary tangles due to intra-neuronal accumulation of hyperphosphorylated tau. The Vacuolar Protein Sorting (VPS) 35/26/29 complex proteins are significant components of retromers associated with intracellular vesicular trafficking. It has been suggested that retromer components are involved in the pathogenesis of neurodegenerative diseases, but the details remain unknown. In this study, our objective was to investigate the expression changes of retromer components during the pathogenesis of AD using a model mice. We employed App-NL-GF knock-in mice as AD model mice, including 3-month-old and 6-month-old males. Cognitive function in the mice was assessed through the Y-maze test and the fear conditioning test. The amount of Aβ was measured via immunohistochemical staining and ELISA. Changes in the expression of each VPS were analyzed using the western blot method. The behavioral test results indicated no cognitive decline in the 3-month-old AD model mice but did show cognitive decline in the 6-month-old AD model mice. However, both 3-month-old and 6-month-old AD model mice exhibited Aβ accumulation and a reduction in certain VPS proteins. Therefore, the decreased expression of VPS proteins occurring before cognitive decline may represent a potential molecular target for AD disease-modifying drugs, with potential therapeutic applications such as retromer stabilizers.

3-B-P-087 学生セッション（ポスター）

Dextromethorphan Hydrobromide Facilitates Contextual Fear Memory Extinction in a PTSD Rat Model

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NMDA receptors have been implicated in the pathophysiological mechanisms of fear memory and impairment of fear memory extinction. The purpose of this study was to investigate the effects of dextromethorphan (DXM), an NMDA receptor antagonist, on fear memory extinction in a rat model of posttraumatic stress disorder (PTSD). Using isoflurane instead of diethyl ether, we established a rat model subjected to the Single Prolonged Stress (SPS) protocol and evaluated the effects of DXM on fear memory extinction. Rats were conditioned with fear using a conditioned stimulus (CS: chamber) and an unconditioned stimulus (US: foot shock), and their freezing time was measured for three days upon re-exposure to the chamber. The group receiving long-term DXM treatment (40 mg/kg, i.p.) after SPS loading showed no improvement in freezing time. However, the group that received a single administration of DXM (40 mg/kg, i.p.) during the experimental fear memory extinction process of SPS-loaded rats showed reduced freezing behavior. Interestingly, DXM did not affect fear memory retrieval or consolidation, suggesting its specificity for facilitating fear memory extinction. These findings suggest DXM as a potential therapeutic agent for promoting fear memory extinction in the context of PTSD symptomatology, providing insight into novel pharmacological interventions for this debilitating disorder.

3-B-P-088 学生セッション（ポスター）

Effect of the Kir4.1 blocker, quinacrine, on lipopolysaccharide-induced cognitive impairment

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Inwardly rectifying Kir4.1 channels are specifically expressed in astrocytes and involved in the pathogenesis of various brain diseases, including depression, epilepsy, and Alzheimer's disease (Int. J. Mol. Sci., 22, 10236, 2021). Previous studies reported that repeated administration of lipopolysaccharide (LPS) induced neuroinflammation and cognitive impairment in mice (Sci. Rep., 9, 5790, 2019). To explore the role of astrocytic Kir4.1 channels in development of neuroinflammatory cognitive impairment, we evaluated the effect of the Kir4.1 blocker, quinacrine, on LPS-induced cognitive impairment. Treatment of mice with LPS (0.4 mg/kg, i.p.) for 7 days significantly impaired cognitive function in the novel object recognition test. In addition, immunohistochemical analysis revealed that the LPS treatment significantly increased GFAP expression in the hippocampus. On the other hand, administration of quinacrine (30 mg/kg, s.c.) significantly improved LPS-induced cognitive impairment and also reversed the elevation of GFAP expression by LPS. These results suggest that inhibition of astrocytic Kir4.1 channels is effective for the treatment of neuroinflammatory cognitive impairment.

3-B-P-089 学生セッション（ポスター）

Mechanisms of inhibitory effects of fear memory reconsolidation in mice by opioid δ-receptor agonists.

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Blockade of fear memory reconsolidation has attracted attention as a therapeutic strategy for the treatment of PTSD. Here, we investigated the effects of delta-opioid receptor (DOP) agonists, KNT-127 and SNC80 on the reconsolidation of fear memory in mice. Male C57BL/6J mice (6-8 weeks old) were subjected to contextual fear conditioning test. On day 1, the mice were conditioned with 3 foot shocks. On day 2, DOP agonists were administered subcutaneously (s.c.) and microinjected into the basolateral amygdala (BLA), ventral hippocampus (vHPC), and prelimbic (PL) and infralimbic (IL) subregions of the medial prefrontal cortex in mice. On day 3, mice were re-exposed to the chamber for 2 min as a memory testing (test session). As a result, KNT-127 (3–10 mg/kg), but not SNC80 (1–10 mg/kg), significantly decreased the freezing rates of mice in the test session. In addition, KNT-127 (50 ng/mouse) when administered to the BLA, IL, and vHPC, but not the PL, significantly reduced the freezing response during the test session. It also tended to inhibit the phosphorylation of ERK1/2 only when administered in the amygdala. On the other hand, SNC-80 had no effect on either of them. These findings suggested that KNT-127, but not SNC80, produces inhibitory effects on the reconsolidation of fear memory via BLA, IL, and vHPC in mice.

3-B-P-090 学生セッション（ポスター）

Investigation of mechanisms underlying cognitive enhancement induced by oral ingestion of yeast derived RNA in mice

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Nucleic acids exhibit various biological functions, whereas their roles in the brain are largely unknown. We have recently reported that the ingestion of DNA hydrolysate derived from salmon milt improves cognitive function in healthy mice. However, effect of ingestion of RNA on cognitive function is still unknown. The purpose of the present study was to elucidate the effect on cognitive function and mechanisms of action of yeast-derived RNA (tyRNA) in mice. Seven-week-old ICR mice were fed a low-nucleic acid diet (AIN-93M) or that containing 2.5% tyRNA. Fourteen days later, the novel object recognition test (NORT) was performed to evaluate cognitive function. The exploration time for novel object was significantly longer than that for the familiar one in mice ingesting tyRNA, whereas the exploration time was not different between the novel and familiar objects in the control mice. Measurement of mono-nucleic acids by LC-MS/MS showed that concentrations of uridine, cytidine, UMP, and dCMP in the brain of tyRNA ingestion group were significantly higher than those in control group. Effect of exposure to uridine, cytidine, UMP, and dCMP on expression of Synapsin 1 (Syn1), a presynaptic marker in primary cultured murine cortical neurons was then evaluated by Western blotting. Only cytidine tended to increase Syn 1 expression compared to vehicle-treated group, suggesting that cytidine may promote synaptogenesis. Taken together, ingestion of tyRNA in mice increased several mono-nucleic acids including cytidine in the brain and improved cognitive function in mice.

3-B-P-091 学生セッション（ポスター）

Production of brain organoids using human iPS cell-derived neurons and microglia and analysis of cellular reactivities to amyloid-β oligomers.

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Alzheimer's disease (AD) induces amyloid-β (Aβ) accumulation and neurodegeneration in the brain. Microglia are immune cells in the brain, and their origin is macrophages in the foetal yolk sac. Aβ plaques are surrounded by microglia and a unique microglial subpopulation, disease-associated microglia (DAM), appears in the AD brain. Simple and accurate cell culture systems that can reproduce the pathological microenvironment of the AD brain are needed to elucidate the pathophysiological role of microglia, including DAMs. In this study, we established a co-culture system of human induced pluripotent stem (hiPS) cells derived cortical neurons or organoids with microglial progenitor cells (hiMacs). *O*-acyl isopeptide $Aβ_{1-42}$ stably forms highly toxic oligomers and were used to analyse Aβ stress. Although Aβ induced marked neurodegeneration in cortical neurons and organoids, adding hiMacs suppressed it. hiMacs phagocytosed Aβ and showed DAM-like transitions. Furthermore, jagged Aβ plaques were formed with hot spot-like structures in cortical organoids, but were smoother surfaces near hiMacs. hiMacs showed neuroprotective effects might be phagocytosis of Aβ and morphological modification of Aβ plaques. The co-culture model of cortical neurons and organoids with hiMacs is helpful for elucidating microglial function in AD pathology.

3-B-P-092 学生セッション（ポスター）

Influence of mitochondrial DNA- cyclic GMP-AMP synthase pathway on inflammatory response in microglia

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Microglia are immune cells in the central nervous system and contribute to brain homeostasis. Recently, it has been reported that mitochondrial dysfunction in the brain is observed with aging. However, influence of mitochondrial dysfunction in microglial function is unknown. We previously reported that rotenone-induced mitochondria DNA (mtDNA) leakage into cytosol enhances the production of interferon (IFN)-β by an inflammatory stimulus (lipopolysaccharide: LPS). Therefore, the current study focused on the cytosolic mtDNA to clarify the mechanisms underlying mitochondrial dysfunction-enhanced IFN-β production.

BV2 cells, a mouse microglial cell line, were used. Mitochondrial DNA was extracted from the cytosolic fraction at 3, 6, 18, and 24 hrs after rotenone treatment. The inhibitor of cyclic GMP-AMP synthase (cGAS) recognizing mtDNA was treated 6 hours before LPS treatment. Expression levels of mRNA and mtDNA were measured by real-time PCR. Leakage of mtDNA into the cytoplasm was increased 18 and 24, but not 3 and 6, hrs after rotenone treatment. Furthermore, rotenone-enhanced IFN-β mRNA production was only observed at the time point of mtDNA leakage. In addition, cGAS inhibitor significantly suppressed the IFNβ mRNA enhancement.As a results, mitochondrial dysfunction in microglia enhances IFN-β production via the mtDNA-cGAS pathway.

3-B-P-093 学生セッション（ポスター）

Neutrophil mobilization is crucial for chronic social stress-induced behavioral alterations in mice

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Chronic stress caused by aversive and psychological stimuli induces emotional and cognitive abnormalities, precipitating the onset and relapse of human depression. Inflammation in the brain and periphery is thought to play a role in the pathophysiology of depression. We have demonstrated that chronic social stress causes neutrophil mobilization and its maintenance in mice. However, whether neutrophil mobilization is involved in behavioral alterations induced by chronic social stress remains unknown. Here we examine the role of neutrophil mobilization in chronic social stress-induced behavioral alterations by inhibiting or deleting C-X-C Motif Chemokine Receptor 2 (CXCR2), a chemokine receptor involved in neutrophil mobilization. Administration of CXCR2 antagonist once daily during and after chronic social stress prevented behavioral alterations induced by chronic social stress concomitantly with inhibiting neutrophil mobilization. Neutrophil-selective CXCR2 deletion in mice also abolished behavioral alterations in multiple behavioral tests after chronic social stress. These findings demonstrate that neutrophil mobilization is crucial for behavioral alterations induced by chronic social stress.

3-B-P-094 学生セッション（ポスター）

Ablation and functional modulation of microglia prevent depressive-like but not sickness behaviors following systemic inflammation

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Recent studies suggest that peripheral inflammation, often seen during sickness, contributes to the onset of depression. An immediate response to infection typically induces behavioral changes characterized by decreased appetite and mobility, defined as sickness behaviors in this study. Following this response, depressive-like behavior, characterized by behavioral despair, may emerge and can persist. However, the mechanism through which sickness triggers depressive-like behavior remains unknown.

Microglia, the resident macrophages in the CNS, respond to inflammatory signals from the periphery and modulate neuronal activity. Here, we tested whether microglia contribute to the induction of depressive-like behavior post-inflammation.

To examine changes in microglia, we utilized a model of systemic inflammation in rodents through lipopolysaccharide (LPS) injection. Subsequent behavioral assays in mice validated the onset of both sickness and depressive-like behavior. Moreover, both the elimination of microglia and functional modulation through the use of PLX3397 and minocycline respectively, prevented the development of depressive-like, but not sickness behavior.

Taken together, our findings suggest that microglia are essential intermediates for the development of depressive-like, but not sickness behavior.

3-B-P-095 学生セッション（ポスター）

Microglial activity in oxytocin mutant medaka females

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RNA Biology Laboratory

Oxytocin (OXT) is a kind of neuromodulator which is known to be required for many social behaviors such as mother-kin bonding and affiliative behavior towards familiar individuals. In addition, recent studies have reported that OXT treatments improved some social defects in autism spectrum disorder (ASD) patients. Abnormal synapse pruning by microglia in the brain has been proposed as a potential mechanism of ASD.

Previously, our group reported that wild-type (WT) medaka (*Oryzias latipes*) females preferred to mate with visually familiarized males, but oxytocin (*oxt*) mutant females lost this tendency. However, the cause of this behavioral abnormality was remained to be elucidated.

In this study, we report c1qb mutant medaka females lost their sexual preference as the oxt mutant females did. In addition, ionized calcium binding adaptor molecule 1 (Iba1) staining - a microglial/macrophage marker- revealed that the sizes of Iba1+ cell bodies in *oxt* mutant females were smaller than those in WT females, although the number of Iba1+ cells was not significantly different between them. These results suggested that oxt was required for normal activity of microglia and regulated neural developmental process such as synaptic pruning, which worked via C1q pathway, leading to the female sexual preference in medaka.

3-B-P-096 学生セッション（ポスター）

The effect of microglia on behavior

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Microglia is a type of glial cell that play a multifaceted role in various functions, including the development and maintenance of neurons, phagocytosis of abnormal or dead cells, pruning of dendritic spine, and spine formation. However, there have been limited reports on the impact of microglia on the behavior of individual organisms. When orally administered the Colony Stimulating Factor 1 Receptor (CSF1R) inhibitor drug PLX3397 (referred to as PLX) to mice, microglia can be temporarily removed from the brain. In this study, we conducted an open field test using PLX to investigate the effects of microglia removal on the behavior of mice.

We provided 3-4-month-old C57BL/6 strain mice (both males and females) with food containing 600 ppm of PLX for seven consecutive days and analyzed their total distance traveled, the percentage of time spent in the center, and rearing behavior. The results showed that there were no significant differences in the total distance and the time spent in the center area. However, the microglia removal group tended to spend more time in the center area compared to the control group, and the number of rearing behaviors significantly decreased in the microglia removal group compared to the control group.

This study suggests that mice with microglia removal exhibit a reduction in anxiety-like behavior compared to the control group.

Spatio-temporal analysis of CD11c^{+} microglia in the brain of mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disease worldwide. Accumulating evidence indicates that microglia, immune cells in the brain, are involved in the AD pathology, and these cells have received much attention as a key to understanding the mechanism of AD and also to developing its therapeutic strategy. It has recently been shown that microglia are highly heterogenous and that the number of a subpopulation of microglia expressing CD11c (CD11c^{+} microglia) is increased in the brains of AD patients and mouse models. However, the time course of their appearance in AD models has not been investigated well.

In this study, we immunohistochemically analyzed CD11c expression in microglia using a mouse model harboring three familial AD mutations (*APP*$^{NL\text{-}G\text{-}F}$) that had been crossed with a CD11c reporter mouse line (*CD11c-Venus*). We found that CD11c^{+} microglia increased from 3 months of age. At this timepoint, CD11c expression in the hippocampus was observed approximately 10% of total IBA1^{+} microglia in AD models, which was higher than that in control mice (around 2.5%). Additionally, CD11c^{+} microglia further increased at 6 months of age (the onset stage). Our results indicate that CD11c^{+} microglia already respond at the early stage of AD models and suggest that these cells may have a role in the pathogenesis of AD.

Irritable bowel syndrome (IBS)-like symptoms in chronic vicarious social defeat stress model of mice are improved by the selected delta opioid receptor agonist KNT-127

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Recently, we developed that the mice chronic vicarious social defeat stress (cVSDS) model, known as validated animal model of depression, showed the irritable bowel syndrome (IBS)-like symptoms such as chronic intestinal motility changes and abdominal hyperalgesia without organic lesions. Previously, we reported that a selective delta opioid receptor (DOP) agonist KNT-127 improved the depression-like behaviors observed in mice cVSDS model. In the present study, we examined the effects of KNT-127 on the IBS-like symptoms in cVSDS model. The model mice were prepared by exposure to repeated psychological stress for 10 days in C57BL/6J mice. KNT-127 was administered subcutaneously (s.c.) and microinjected into the Insular cortex (IC) 30min before the test, respectively. KNT-127 (10mg/kg, s.c.) significantly improved increased intestinal transit ratio in the charcoal meal test and hypersensitivity symptoms in the capsaicin-induced hyperalgesia test, respectively. In addition, KNT-127 (300ng/mouse) in IC was normalized their intestinal transit ratio. These results suggested that KNT-127 improved the IBS-like symptoms in cVSDS mice via DOPs in IC. We proposed that DOP agonist have the potential to be an effective treatment for IBS, capable of breaking the vicious cycle in gut-brain interactions.

3-B-P-099 学生セッション（ポスター）

The effects of PACAP-PAC1 signal blockade in the medial prefrontal cortex on repeated social defeat stress mice

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Pituitary adenylate cyclase-activating polypeptide (PACAP) and its preferred receptor PAC1 have been associated with stress responses and psychiatric disorders such as anxiety, depression, and post-traumatic stress disorder as shown by human genetic and animal model studies. We have recently shown that a single intraperitoneal administration of a small-molecule PAC1 antagonist PA-915 improved depressive-like behaviors in repeated social defeat stress (RSDS) mice. However, it remains unknown where and how PACAP-PAC1 signaling is involved in the regulation of depressive-like behaviors in the brain. In this study, we focused on the medial prefrontal cortex (mPFC) which is known to play a pivotal role in emotional processing and stress response, and performed electrophysiological, behavioral, and pharmacological analyses. We found that the decrease in the excitation and inhibition (E/I) ratio of the layer V pyramidal neurons in RSDS mice was significantly restored by the intraperitoneal treatment of PA-915. We also found that local administration of PA-915 in the mPFC improved depression-like behaviors in RSDS mice. These results indicate that PACAP-PAC1 signaling in the mPFC may modulate depressive-like behaviors by rebalancing the activities of pyramidal neurons.

3-B-P-100 学生セッション（ポスター）

Development of Novel Psychotropic Drugs by an Alcohol Abstinence Drug Disulfiram

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Aldehyde dehydrogenase inhibitor disulfiram (DSF) is used as a treatment for alcoholism. Recently, we have reported that DSF inhibits FROUNT, a molecule that promotes inflammatory chemokine receptor CCR2/5 signaling and exhibits anxiolytic-like effects in addition to anticancer effects. However, DSF shows several side effects due to the accumulation of acetaldehyde induced by inhibition of aldehyde dehydrogenase in the liver. These unwanted effects limit the development of psychotropic drugs by DSF. On the other hand, intranasal administration has been shown to selectively deliver drugs from the nose to the brain and reduce peripheral side effects. Therefore, we focused on intranasal administration, which has the potential to reduce peripheral side effects and to deliver drugs to the brain selectively.

The anxiolytic-like effects of DSF were investigated using an elevated plus-maze (EPM) test. Oral and intranasal administration of DSF increased the amount of time spent in the open arms of the maze. However, ethanol administration after oral administration of DSF showed the significant decrease in body temperature and increase in blood acetaldehyde concentration 2 hours later. On the other hand, rats treated with intranasal DSF did not show the side effects 2 hours after ethanol administration. We propose that intranasal preparation of DSF is expected to be a novel psychotropic drug with a different mechanism of action combined efficacy and safety.

3-B-P-101 学生セッション（ポスター）

Analysis of intracellular iron homeostasis and mitochondrial damage caused by the suppression of ATP13A2 expression

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Background: *ATP13A2* have been reported as a causative gene of PARK9. ATP13A2 is a lysosomal localized ATPase and is thought to be the membrane transport of cations such as polyamines, proton, and metal ions. In addition, it has been reported that iron accumulation is observed in the brains of PARK9 patients, suggesting that ATP13A2 contributes to intracellular iron homeostasis. However, the effects of ATP13A2 for iron homeostasis is unclear. In our previous study, we reported iron increase in cell organelles by knockdown of *ATP13A2*. In this study, we attempt to reveal the mechanism of intracellular iron increase.

Method: We generated PARK9 model cells by *ATP13A2* knockdown in SH-SY5Y, and analyzed expression of iron-related genes, and function of cell organelles. Heme synthesize is examined by heme assay kit.

Result: In PARK9 model cell, expression of iron-related genes such as Transferrin Receptor (TfR) was increased, suggesting the disruption of iron homeostasis. To examine iron homeostasis, we focus on the capacity of heme synthesize. Heme is known to play a role in regulating intracellular iron concentration. The ability to heme synthesizes was decreased in PARK9 model cells. Since heme is synthesized in mitochondria, it is assumed that mitochondrial disorders are involved in the reduction of heme synthesis. Therefore, it is possible that mitochondrial dysfunction may be a factor in the abnormal intracellular iron homeostasis in PARK9 model cells. In addition, we found dysfunction of mitophagy, suggesting that accumulation of damaged mitochondria.

3-B-P-102 学生セッション（ポスター）

Effects of Albinism on Sleep/Wakefulness States in Mice

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We recently reported that blindness impairs sleep quality in mice (Iba et al. Biol Pharm Bull. 2023). Albinism is also known to cause visual impairment. In the present study, we compared locomotor activity and delayed recovery from general anesthesia induced by hypnotics during light and dark periods in pigmented C57BL/6N (B6N) and albino (B6-Albino) mice. There were no significant differences in locomotor activity between B6N and B6-Albino mice during the light and dark periods. Brotizolam significantly delayed recovery from isoflurane anesthesia in both periods in B6N mice and the dark period in B6N-Albino mice. The delayed recovery was more pronounced in the light than in the dark period in B6N mice but was similar in both periods in B6-Albino mice. Diphenhydramine delayed recovery from isoflurane anesthesia, albeit slightly, in the dark period in B6N mice and the light period in B6-Albino mice. Suvorexant delayed recovery from anesthesia induced by a mixture of medetomidine, midazolam, and butorphanol (MMB) in B6N and B6-Albino mice only in the light period. However, unexpectedly, B6-albino mice recovered from MMB anesthesia significantly faster than B6N mice in the dark period. These results indicate that sleep quality is impaired in albino mice.

3-B-P-103 学生セッション（ポスター）

Involvement of xenobiotics efflux transporter MRP5/ABCC5 in transport of a neurotransmitter and neurotoxicity

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Multidrug resistance associated protein 5 (MRP5/ABCC5) is a drug efflux transporter, and its gene expression in murine primary cultured neurons is much higher than other ABCC family members although its physiological functions are largely unknown. MRP5 also pumps out the neurotransmitter N-acetylaspartyl-glutamate (NAAG), which suppresses excitatory neurotoxicity in the neurodegenerative diseases. We therefore hypothesized that MRP5 may play a protective role in the brain by regulating NAAG transport. In this study, the effect of Mrp5 knockdown on neurotoxicity was investigated. Adeno-associated virus-PHP.eB carrying shRNA targeting Mrp5 (AAV-shMrp5) or LacZ (AAV-shLacZ, negative control) expressing fluorescent protein Zsgreen1 were first injected into the cerebellum in mice, and cerebellar sections were observed under a fluorescence microscope after 12 days, showing that the number of calbindin-positive neurons in AAV-shMrp5-treated group was lower than that in negative control. The neuronal cell line Neuro2A was next transfected with siRNA targeted to Mrp5 (siMrp5), showing a lower expression of Mrp5 and a higher intracellular NAAG concentration compared to negative control siRNA. Furthermore, the cell viability measured by MTT assay and cytotoxicity evaluated by LDH assay in the siMrp5-transfected group were lower and higher, respectively, than the control group whereas NAAG treatment recovered the siMrp5-induced decrease in cell viability. These results suggest that Mrp5 may be involved in neuroprotection by regulating NAAG level.

3-B-P-104 学生セッション（ポスター）

FAD012 reduces blood-brain-barrier damage in photothrombotic stroke rats.

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Stroke often causes severe sequelae and reduces the quality of life of patients, therefore effective preventive and therapeutic methods are desired. FAD012, a ferulic acid derivative (FAD), is derived from ferulic acid (FA) and has been shown to be potentially effective in preventing cerebral infarctionin middle cerebral artery occlusion following reperfusion (MCAO/Re) rats. In this study, we investigated the protective effects of FAD012 on blood- brain-barrier (BBB) damage using photothrombotic stroke rats.

Under isoflurane anesthesia, a bone window was opened in the left temporal bone of male Sprague Dawley rats to expose the MCA. The MCA was irradiated with green laser light and Rose Bengal was administered intravenously to form a local thrombus. FAD012 was administered intraperitoneally 1 h before laser irradiation. To evaluate BBB damage, Evans blue (EB) staining and immunostaining of collagen IV and claudin V, which are factors that constitute tight junctions (TJs), were performed.

Histochemistry revealed extensive infarction and BBB damage in the left parietal lobe of the control group. Furthermore, collagen IV and claudin V were significantly decreased in the infarct area. In FAD012 group, BBB damage and reduction in these factors were suppressed. These results suggest that FAD012 protects BBB damage in the ischemic region and that protection of TJs is involved in its cerebroprotective effects.

3-B-P-105 学生セッション（ポスター）

Pretreatment with CHIR-99021, a GSK-3 inhibitor, attenuates morphine-induced Straub tail reaction and withdrawal.

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In this presentation, we demonstrate the effects of CHIR-99021(also known as Laduviglusib), an inhibitor relatively specific for glycogen synthase kinase-3 (GSK-3), on behaviors associated with morphine withdrawal such as jumping, rearing, and head shaking. Morphine is one of the most commonly used medicines for pain relief. Moreover, morphine-induced withdrawal is clinically well known for long-term use. However, there is no established treatment for morphine withdrawal. Therefore, we focused on a possible suppression by CHIR-99021 of morphine withdrawal as well as morphine induced Straub tail reaction and thus conducted experiments using mice that were administered with morphine (10 mg/kg i.p.) or saline once daily for five consecutive days. Our data indicated that pretreatment with CHIR-99021 significantly attenuated head shaking during morphine withdrawal. However, behaviors other than head shaking observed in mice pretreated with CHIR-99021 did not differ from those observed in the vehicle injection group. In addition, the pretreatment with CHIR-99021 significantly blocked the morphine-induced Straub tail reaction. These results suggest that the GSK-3 pathway might be involved in morphine-induced positive symptoms such as Straub tail reaction and head shaking specific for morphine withdrawal.