2-P-098

Decreased expression of synapse-related proteins in the prefrontal cortex of prenatal folate deficiency mice.

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impaired social communication and repetitive/restricted behaviors. Folate is essential for normal fetal development and growth. Our previous study has described that prenatal folate deficiency (FD) mice have exhibited ASD-like decreased sociability. Also, they have showed increase in convulsant-induced seizure susceptibility and decrease in anxiety-like behavior. In this study, we examined the changes in the relative expression of N-methyl-D-aspartate (NMDA)-type glutamate receptor, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptor, postsynaptic density protein 95 (PSD-95), and GABA-synthesizing enzymes (GAD65/67) in the prefrontal cortex (PFC). We found that the expression of NMDA receptor subunit 2b, AMPA receptor 1, PSD-95, and GAD65/67 were significantly decreased in FD mice compared with control mice. These results may suggest that decrease in both of excitatory and inhibitory synapse-related proteins in the PFC could have effects on behavioral impairment of FD mice.

2-P-099

Involvement of microglia in mechanical allodynia in a mouse model of neurodevelopmental disorder

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by asynchronous development in several areas such as social behaviors, cognitive capabilities, and sensory responsiveness. Among the sensory abnormalities recognized as important features of ASD is either heightened or reduced sensitivity to pain. Individuals with ASD may experience pain in unusual ways, but mechanisms underlying altered pain sensitivity and processing in ASD remain unknown. Here we investigated the pain sensitivity in a prenatal valproic acid (VPA)-induced model of ASD and subsequently analyzed the pain signaling. Pregnant ICR mice were intraperitoneally injected with either VPA or saline on embryonic day 12.5. Male offspring of VPA-treated mothers showed mechanical allodynia. In the dorsal horn of the spinal cord in prenatal VPA-treated mice, the numbers and staining intensities of Iba1-positive cells were increased and the cell bodies became enlarged, indicating the microglial activation. Administration of PLX3397, a colony-stimulating factor 1 receptor inhibitor, resulted in a decreased number of spinal microglia and attenuated mechanical allodynia in prenatal VPA-treated mice. These findings suggest that prenatal VPA treatment causes allodynia and microglial activation might in part contribute to increased nociceptive responses.

2-P-100

Effects of adolescent social isolation and resocialization on the mOFC-BLA synaptic transmission and social behavior in mice.

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Early-life social experience is critical for the development of social cognition, and early social deprivation induces alteration of sociality in animals and human. The medial orbitofrontal cortex (mOFC) to basolateral amygdala (BLA) pathway is one of the critical circuit for social behavior. Hence, the mOFC-BLA pathway may be functionally sensitive for early social environment, and its dysfunction may cause changes in social behaviors. Here, we examined effects of social isolation (SI) and resocialization (RS) on mOFC-BLA synaptic functions in mice. First, we isolated the mice during early (3-5 weeks of age) or late (6-8 weeks of age) adolescence and assessed sociality and mOFC-BLA synaptic properties using optogenetic and patch-clamp methods. SI in early-adolescent, but not late adolescent, decreased sociality and AMPA/NMDA current ratio in the mOFC-BLA synapse. Then, we examined the effects of RI during late adolescence or adulthood (9-11 weeks of age), on SI-induced synaptic and social deficit. RS in late adolescence, but not adulthood, recovered SI-induced social deficit and mOFC-BLA synaptic change. These results suggested that SI disrupted mOFC-BLA synaptic function in early adolescence and caused social deficit, and restorative effect of RS on SI-induced behavioral and synaptic change limited until late adolescence.

2-P-101

Reduced prefrontal hub function in brain networks associated with social deficits of an ASD model mouse

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Unapproved use of some antiepileptic drugs has been reported to ameliorate core social deficits of autism spectrum disorder (ASD); however, the neural mechanisms remain unclear. Here, using brain-wide neuronal activation mapping in Arc-dVenus reporter mice, we showed that ASD model mice exhibited not only aberrant hyperactivation in multiple brain areas during social interaction but also disruption of prefrontal nodes in the brain network associated with social impairments. Three unapproved-use drugs for core ASD symptoms reversed the social deficits and dysfunction of the prefrontal nodes in the brain network. In addition, we identified a new unapproved use drug that reversed the social deficits and the dysfunction of prefrontal nodes during social interactions in ASD mice. These results suggest that prefrontal nodes in the brain network can be a diagnostic feature and a therapeutic target for the core symptoms of ASD and that its monitoring is useful to predict the therapeutic effect of ASD drug candidates.

2-P-102

Neonatal blockade of NR2A-containing NMDA receptor induces schizophrenia-related behaviors in adult rats

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It is known that N-methyl-D-aspartate (NMDA) receptor is essential for early brain development. NMDA receptor blockade during neonatal period causes various abnormal behaviors in later life. In the developing brain, NR2A- and NR2B-containing NMDA receptors show different expression patterns. However, the functions of these NMDA receptors in brain development are unknown. Therefore, we investigated the effects of pharmacological inactivation of NR2A- and/or NR2B-containing NMDA receptors on various behaviors in adulthood. We postnatally treated rats with an NR2A-preferring (PEAQX), an NR2B-selective (ifenprodil), or a nonselective NMDA receptor blocker (MK-801). Interestingly, neonatal treatment with PEAQX or MK-801 caused significant decrease in spontaneous alternation in the Y-maze test. In addition, PEAQX or MK-801 treatment increased startle response to acoustic stimuli. Neonatal PEAQX treatment also induced hypersensitivity to locomotor-stimulating effect of MK-801. On the other hand, neonatal ifenprodil treatment did not cause these behavioral alterations. Furthermore, PEAQX-treated but not ifenprodil-treated rats exhibited deformity of the hippocampal CA1 area, while neonatal NMDA receptor blockade did not alter the cell density in the hippocampus. In conclusion, our results suggest that the NR2A-containing NMDA receptor plays important roles in early brain development in rats, and hypofunction of this subunit during developmental period induces schizophrenia-related behaviors in adulthood.

2-P-103

Depressive-like behaviors and GABAergic dysfunction of the raphe nuclei in rats experienced pharmacological stress during early postnatal period.

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The aim of the present study is to clarify emotional property focusing on the depressive-like behaviors, and its neuronal mechanism in the raphe nuclei which are involved in the mediation of emotional functions, in rats repeatedly administered ACTH during the early postnatal period.

Tetracosactide, the N-terminal 24 amino acids of the naturally occurring ACTH, were administered once a day at dose of 100 μg to male rat pups for 5 days on the day 21 after birth (3wACTH). Saline-injected rats were subjected as a littermate control.

Adult 3wACTH (10-12 weeks old) showed the decrease of sucrose consumption in the sucrose preference test, and reduction of time spent grooming after spraying a sucrose solution to the dorsal coat in the splash test. These abnormal behaviors in 3wACTH indicated the depressive-like/anhedonic-like behaviors, which were ameliorated by repeated administration of fluvoxamine, a selective serotonin reuptake inhibitor. Moreover, immunohistochemical studies revealed that the expression of parvalbumin, a marker of GABAergic neurons, significantly reduced in the raphe nuclei of adult 3wACTH.

These findings suggest that pharmacological stress during early postnatal period might produce depressive-like behaviors, which possibly implicated in dysfunction of the GABAergic neuronal systems in the raphe nuclei.

2-P-104

CGRP induces anxiety-like behavior through degradation of dopamine with activating MAOb/KLF11/p-HP1γ pathway

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The neuropeptide calcitonin gene-related peptide (CGRP) stimulates anxiety-like behavior with intracerebroventricular administration in mice. However, the mechanism of this effect remains unknown. Therefore, we began an investigation on dopamine, due to its close relation to anxiety-like behavior. We evaluated the effects of intracerebroventricular administration of CGRP in the mouse hippocampus. Our results showed that CGRP (0.5 nmol) evoked anxiety-like responses in open field, elevated plus maze, and hole board tests. Dopamine levels significantly decreased with CGRP administration. Moreover, levels of the dopamine-metabolizing enzyme (MAOb) and one of its transcription factors, krüppel-like-facter11 (KLF11), both significantly increased with CGRP application. Furthermore, phosphorylated heterochromatin protein 1 gamma (HP1γ) expression was significantly increased with CGRP. HP1γ is a well-known chromatin protein that plays a role in gene expression through silencing targeted genes. Conversely, phosphorylated HP1γ impairs silencing activity. These results suggest that CGRP may phosphorylate HP1γ and elevate KLF11 levels to increase MAOb. This would enhance the dopamine metabolism, resulting in anxiety-like behavior in the mouse hippocampus.

2-P-105

Possible involvement of the hippocampal microglia in the abnormal sociability lasting after tumor resection in mice

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We have established tumor-resected mouse as a model animal to analyze the mechanism underlying the depression lasting after cancer remission. Our previous data showed that tumor-resected mouse has decreased sociability and morphological changes in hippocampal microglia on day 4 after tumor resection. Here, we aimed to elucidate whether the hippocampal microglia involved in abnormal sociability in tumor-resected mice. Male BALB/c mice were intradermally inoculated 1×10^7 cells of colon 26 into their abdomen, and surgically resected formatted tumor on day 3 after tumor inoculation. Either fluoxetine (10 mg/kg), minocycline (50 mg/kg) or vehicle was orally injected for 14 days after tumor resection. We performed the social interaction test and preparation of the brain slices for immunohistochemical analyses on day 14 after tumor resection. On day 14 after tumor resection, tumor-resected mice had the hippocampal Iba1-positive cells with shortened processes, which is a morphological change observed in microglial cells. Fluoxetine or minocycline was effective in abolishing both abnormal sociability and the morphological change in the hippocampal Iba1-positive cells in tumor-resected mice. These findings suggest the possibility that morphological and functional changes in the hippocampal microglia is involved in decreased sociability in tumor-resected mice.

2-P-106

Brexpiprazole prevents colitis-induced depressive-like behavior by regulating myelination through the activation of ERK1/2-CREB-BDNF-TrkB pathway in the prefrontal cortex

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Patients with inflammatory bowel disease (IBD) have higher rates of psychiatric pathology including depression. The dextran sulfate sodium (DSS)-treated mouse is a well-characterized animal model of colitis that exhibits both IBD- and depressive-like symptoms. Recent our study found that DSS-induced depressive-like behavior may be associated with reduction of myelin-constitute proteins in the prefrontal cortex (PFC), while these changes were prevented through activation of 5-HT_{1A} receptor by administration of brexpiprazole (Brx). However, it remains unclear about their molecular mechanisms. Therefore, the present study determined changes in proteins associated with 5-HT_{1A} receptor-mediated signaling in the PFC. Then, we also investigated whether the TrkB inhibitor ANA-12 affected the effects of Brx on DSS-treated mice. Decreased phosphorylation of ERK, CREB and TrkB levels and expression of BDNF level in the PFC of DSS-treated mice were prevented by Brx, and the effects of Brx were inhibited by the selective 5-HT_{1A} antagonist WAY100635. Furthermore, ANA-12 prevented the effects of Brx on DSS-induced depressive-like behavior and abnormal myelination in the PFC. These findings indicate that myelination regulated by activation of ERK1/2-CREB-BDNF-TrkB pathway in the PFC may be involved in the antidepressant effect of Brx.

2-P-107

Chronic ocular dryness induces depressive-like behaviors in mice

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A lot of clinical studies have reported that dry eye disease is closely associated with psychiatric disorders such as depression and anxiety. Using a forced swim test, we investigated the effect of chronic ocular dryness on a depression-like behavior. The exorbital lacrimal glands (ELG) or exorbital and intraorbital lacrimal glands (ELG+ILG) were bilaterally excised from 8-9 weeks old male C57BL/6J mice resulting in persist reduction of tear volume. The ELG +ILG excision exhibited more severe corneal damage than the ELG excision. In the forced swim test, ELG excision mice showed significant longer immobility time than sham operation mice at 12 weeks post-surgery, although there was no significant difference between the groups at 6 weeks. The ELG+ILG excision mice showed higher immobility even at 6 weeks. After treatment of wheel-running apparatus for 3 days, the longer immobility times in the forced swim disappeared in both models. Our results suggest that dry eyes might directly cause a depressive disorder that depends on the severity and duration of the symptoms, and that voluntary motor activity could help recovery from a depressive state induced by dry eyes.

2-P-108

Yokukansan, a traditional Japanese medicine, suppresses stress-induced sympathetic activation and central responses to stress in rats

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Stress exposure activates the sympathetic nervous system. We previously reported that restraint stress and stress-related neuropeptides increases plasma catecholamine levels and that brain prostanoids medicate these responses in rats. Yokukansan (YKS), a traditional Japanese medicine, has been used for neurosis, night crying and irritability. Additionally, it has been reported that YKS is also effective against the behavioral and psychological symptoms of dementia in patients with Alzheimer's disease, stress-related anxiety-like behavior and stress-induced increase in plasma corticosterone in rodents. These previous findings raise the possibility that YKS can exert its action in the brain and has suppressive effects on stress responses. In this study, we examined effects of YKS on stress-induced sympathetic activation and stress responses in the brain using rats. Repeated administration with YKS suppressed stress-induced increase in plasma adrenaline level. YKS also suppressed stress-induced elevation of prostaglandin E_2 and thromboxane B_2 levels in the paraventricular hypothalamic nucleus (PVN). In addition, YKS suppressed stress-induced increases of acetylcholine, GABA and serotonin in the PVN. Our results suggest that YKS can ameliorate stress-induced sympathetic activation via inhibition of stress responses in the brain.

2-P-109

Decreased serotonin transporter in the prefrontal cortex induces anxiety-like behavior

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We have previously shown that mice exposed to chronic social defeat stress exhibit anxiety-like behavior and decreased serotonin transporter (SERT) expression and serotonin (5-HT) uptake in the prefrontal cortex (PFC). There is a correlation between these anxiety-like behavior and 5-HT uptake, suggesting that decreased SERT in the PFC may induce anxiety. In this study, to clarify whether the decrease of SERT in the PFC induces anxiety, we investigated the effect of SERT knockdown in the PFC on anxiety-like behavior. Retrograde adeno-associated virus (AAV) vectors producing shRNA against SERT were microinjected into the PFC, and after 10 days, the elevated plus maze test was performed to assess anxiety-like behavior. Mice treated with AAV producing shRNA against SERT showed decreased SERT expression and 5-HT uptake in the PFC, but no such changes in the nucleus accumbens. The elevated plus maze test revealed that mice treated with AAV producing shRNA against SERT exhibited anxiety-like behavior such as decreased time spent in the open arms. These results suggest that the decrease of SERT in PFC could induce anxiety.

2-P-110

Decreased expression of Teneurin-4 in mouse hippocampus induces depressive-like behavior

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Numbers of patients with mental disorders have been significantly increased in the worldwide. A single nucleotide polymorphism of Odz4 of bipolar disorder patients had been reported in Genome Wide Association Study. Odz4 encodes Teneurin-4 (Tenm4). Therefore, we investigated the physiological role of Tenm4 by knocking down Tenm4 specifically in the hippocampus (Hip). The Hip is considered to be a region that is involved in mental disorders, as there are reports that the volume of the Hip shrinks in patients with depression and bipolar disorder, and that the neurons of the Hip from bipolar disorder patients show excessive excitation. In this study, we examined the effects of hippocampal-specific knockdown of Tenm4 on behavioral phenotypes using a genetic modification technique applying the CRISPR-Cas9 system. gRNA vector (knock downed Tenm4; Tenm4KD mice) or control vector (mock mice) were injected into the dorsal Hip of C57BL/6J mice. At 4 weeks after the injection, various behavioral experiments were conducted. Tenm4KD mice had no effect on locomotor activity, novel object recognition or working memory, social or anxiety-like behaviors. However, the immobility time of Tenm4KD mice (264.7 ± 10.5 sec) increased compared with mock mice (241.4 ± 4.0 sec) in the forced swimming test, and the sucrose preference of Tenm4KD mice (80.0 ± 2.5%) decreased compared with mock mice (87.6 ± 1.5%). These results suggest that knockdown Tenm4 in the mice Hip exhibited depression-like behavior. We will continue our research to clarify the depressive mechanisms.

2-P-111

Short-term exposure to cuprizone impairs the development of adaptation to stress in mice

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We previously demonstrated that repeated exposure of mice to different degrees of restraint stress could create stress-adaptive and -maladaptive models. Recently, the studies using these animal models suggested that maintenance of hippocampal myelination contributes to the development of stress adaptation. In the present study, we investigated whether cuprizone, a copper-chelating agent used to produce toxic demyelination, affects the development of stress adaptation. A single exposure to restraint stress for 1 h induced a significant decrease in the number and duration of head-dipping behaviors in the hole-board test. This stress response was not seen in mice that had been exposed to restraint stress for 1 h/day for 2 weeks, which is referred to as stress adaptation. In contrast, mice fed a diet containing 0.2 % cuprizone for 3 weeks from 1 week before stress exposure did not adapt to stress, and continued to show a decrease in head-dipping behaviors. Under this condition, the expression levels of myelin protein, MBP and CNPase, in the hippocampus of mice were significantly decreased. The present findings indicate that chemical damage to hippocampal myelin impairs the ability of mice to adapt to stress, supporting our hypothesis that the maintenance of myelination is important for the development of stress adaptation.

2-P-112

Vagus nerve spiking activity modulates prefrontal–amygdalar circuit related to anxiety

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The vagus nerve has critical for brain-body interaction. It conveys internal physiological information from peripheral organs to the brain, causing interoception. Although numerous studies have shown that an alternation in vagal sensory input affects emotional states such as anxiety and depression, the mechanism by which vagus nerve activity is correlated with anxiety-related brain activity remains unknown. We have previously established a vagal spike recording method using a cuff-shaped electrode while simultaneously recording central and peripheral bioelectrical signals in freely moving rodents. In the present study, we investigated correlation between vagus nerve and anxiety-related brain activity on an elevated plus maze. We combined the recording method of vagus nerve with recording of local field potentials (LFPs) of the prefrontal cortex and the amygdala, which are crucial for expression of anxiety. Vagus nerve activity was negatively correlated with anxiety-related 4-7 Hz activity in the prefrontal cortex and the amygdala. The correlation was abolished by left cervical vagotomy. Chronic social defeat stress decorrelated vagus nerve activity with 4-7 Hz LFP oscillations. These results imply neurophysiological mechanisms underlying anxiolytic effects of vagus nerve stimulation and provide insights into neurophysiological underpinnings of the relationship between vagus nerve and emotion-related behavior.

2-P-113

Oxytocin treatment inhibits dexamethasone-induced depression by activating the hippocampal CREB-BDNF signaling pathway in female mice

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Background

Depression is twice as common in women as in men. Glucocorticoid (GC) exposure is a major risk factor for depression. Oxytocin (OT) has been shown to exert antidepressant-like effect via enhancement of CREB-BDNF pathway in the hippocampus, a key factor of mood regulation. However, it is remains unclear whether the hippocampal CREB-BDNF pathway is related to the antidepressant-like effect of OT under conditions of GC exposure, particularly in the dexamethasone (DEX)-induced depression model of female mice.

Methods

Female C57BL/6J mice were used in this study. All mice were administered with either saline (vehicle), DEX (5 mg/kg), or OT (1 mg/kg) + DEX (5 mg/kg) for eight weeks. After the termination of drug administration, animals were assessed of depression-like behavior by forced swimming test (FST). The day after the FST, the mice were sacrificed under anesthesia and their hippocampus were evaluated for phosphorylated CREB (p-CREB) and BDNF protein levels by western blot analyses.

Results

OT+DEX mice showed a significantly lower immobility time compared to the DEX mice in the FST. The immobility time of OT+DEX group was comparable with the vehicle group. In the hippocampus, BDNF and p-CREB protein levels were significantly higher in the OT+DEX group than in the vehicle and DEX groups.

Discussion

Simultaneous OT treatment blocked the adverse effects of DEX. OT treatment upregulated p-CREB and BDNF levels of the DEX exposed hippocampus. These results suggest OT exerts its antidepressant-like effect by activating the hippocampal CREB-BDNF signaling pathway in female.

2-P-114

Study on the Evaluation System of Lipopolysaccharide - Induced Depression Model in Mice

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Neuroinflammation has been attracted attention as an underlying mechanism of major depression. New animal models based on this hypothesis have been developed. These new models could be useful to evaluate new types of therapeutic agents. Based on this hypothesis, we tried to establish a Lipopolysaccharide (LPS)-induced depression model in mice to assess the effects of antidepressants.

LPS was injected i.p. at a dose of 0.8 mg/kg to male BALB/c mice the day before behavior testing. As the behavioral tests, the tail suspension test (TST) and the forced swimming test (FST) were used.

The immobility time in the TST and FST was prolonged in the LPS treated mice compared with the normal control. Effects of various antidepressants, such as tricyclic, SSRI, and ketamine were evaluated. These drugs reduced the immobility time of the model mice in both behavioral tests.

It is considered that the depression model in mice was established using LPS. As advantageous points, this model is free from stressful manipulation to animals and is sensitive to both traditional and new antidepressant drugs. The model would be useful to evaluate potential efficacy of newly developed antidepressants.

2-P-115

Effects of intranasal administration of CGRP on contextual fear memory

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Calcitonin gene-related peptide (CGRP) suppresses fear memory retention by intracerebroventricular administration in mice. We propose that CGRP holds potential as a therapeutic drug candidate for posttraumatic stress disorder. However, brain injection is not suitable for clinical application. Intranasal administration could transfer a small molecular drug to the brain without blockage from the blood-brain barrier, delivering rapid effects. In this study, we investigated the effect of intranasal administration of CGRP on fear memory. Eight-week-old C57BL6J male mice were administered with saline or CGRP intranasally. The concentration of CGRP in the cerebrospinal fluid and hippocampus 30 minutes after nasal administration of CGRP was significantly higher than saline. Moreover, CGRP suppressed memory retention without effects to the process of reconsolidation and extinction. We found that intranasal CGRP significantly increased the expression of protein kinase D (PKD), phosphorylated histone deacetylase 5 (p-HDAC5), and neuronal PAS domain protein 4 (Npas4) in the hippocampus. CGRP-mediated impairment of fear memory and increases of Npas4 expression were attenuated significantly by the CGRP receptor antagonist, BIBN4096. Our data demonstrate that intranasal CGRP successfully entered the brain and suppressed the retention of fear memory via activating the PKD/p-HDAC5/Npas4 pathway.

2-P-116

Endogenous oxytocin neuron projecting to the hippocampus participates in the modulation of cognitive behavior

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Previous reports suggested that the administration of oxytocin (OXT) modulates cognitive function, such as learning and memory. However, little is known about the role of endogenous OXT in cognitive function. To address this issue, we utilized Oxt-iCre (*C57BL/6-Oxt*$^{tm1.1(Cre)Ksak}$) mice and chemogenetic activation of OXT neurons by Cre-dependent transfection of DREADD, hM3Dq. Male Oxt-iCre mice were injected with AAV8-hSyn-DIO-hM3Dq-mCherry into the paraventricular nucleus of the hypothalamus (PVN). Then, we examined whether the specific activation of OXT neurons influences on working memory and long-term spatial memory in Y-maze and novel object recognition (NOR) tests. After the behavioral tests, the expression of c-Fos was examined. We identified the mCherry positive axons in the hippocampus. In addition, the administration of hM3Dq-specific agonist, Clozapine-N-oxide (CNO), significantly increased novel object preference in the NOR test, while no significant effect was observed in the Y-maze test. Further, the number of c-Fos positive neurons significantly increased in the dentate gyrus in the CNO-treated mice. These results suggested that the OXT neurons in the PVN project to the hippocampus and the endogenous OXT participates in the modulation of cognitive behavior in mice.

2-P-117

Synaptic plasticity improved by $GABA_A$ receptor blockade are based on the changed protein expression in hippocampus of a mouse model of Alzheimer ‘s disease.

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We previously reported a long-term potentiation (LTP)-like facilitation *in vivo*, known as synaptic plasticity, through $GABA_A$ receptor blockade by bicuculline in the mouse dentate gyrus, and protein expression profiles in the hippocampus of mice overexpressing human APP with the E693Δ mutation (APP_{OSK}-Tg) as Alzheimer‘s disease model showing impaired synaptic plasticity. In this study, we investigated whether bicuculline ameliorated impaired synaptic plasticity, and analyzed its mechanism involved with this improvement by proteome analysis. Electrophysiological study showed that the LTP-like facilitation *in vivo* induced by bicuculline was significantly greater than impaired tetanic LTP in APP_{OSK}-Tg mice, which was improved by bicuculline. Proteomic analysis showed that significant changes 8 h after bicuculline application in expression of 11 (9 up- and 2 downregulated) proteins in the hippocampus of APP_{OSK}-Tg mice. The identified proteins could be functionally classified as chaperome, cytoskeletal protein, energy metabolism, metabolism, neuronal development, and synaptic component. Thus, these results show that bicuculline improves impaired tetanic LTP in the dentate gyrus and changes the expression of proteins in the hippocampus of APP_{OSK}-Tg mice. We propose that the improvement of impaired synaptic plasticity through $GABA_A$ receptor blockade could be mediated by alterations in expression levels of these proteins.

2-P-118

Theta phase precession induces hippocampal network plasticity

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As an animal explores an environment, hippocampal place cells are sequentially activated corresponding with the animal's ongoing trajectory. These neuronal spikes occur slightly faster than hippocampal LFP theta oscillations, termed theta phase precession, resulting in sequences of locations being encoded at different phases of theta oscillations. It remains unclear whether theta phase precession is involved in hippocampal network plasticity. Here, we designed a novel closed-loop stimulation protocol to selectively induce theta phase precession in pyramidal cells. In this system, hippocampal LFP theta power was continuously monitored online and photostimulation was applied to the hippocampus, allowing us to artificially mimic theta phase precession. We applied this stimulation protocol when rats ran on a linear track and found that place cell spikes occurred more frequently at the location where the stimulation was applied. In addition, we found that the photostimulation mimicking theta phase precession more reliably induced offline reactivation of place cells in the postrest period, compared with random phase photostimulation. These results suggest that theta phase precession in hippocampal place cells play crucial roles in both generation and stabilization of spatial maps.

2-P-119

Elucidation of the mechanism of cognitive decline in mild cognitive impairment (MCI) using causal discovery

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Mild cognitive impairment (MCI) is the stage between the expected cognitive decline of normal aging and the more serious decline of dementia. Since MCI may increase risk of later developing dementia caused by Alzheimer's disease (AD) or other neurological conditions, it is important to detect and treat MCI early in order to prevent dementia. However, MCI develops from no single cause and from a lesser degree of the same types of brain changes seen in AD or other forms of dementia. In the field of artificial intelligence (AI), causal discovery, which can visualize causal relationships (cause and effect) between data, has recently been attracting attention. One of its algorithms, Linear Non-Gaussian Acyclic Model (LiNGAM), can extract causal relationships between variables from statistical data only, using probability distributions of variables that are generally non-Gaussian. In this study, we used LiNGAM to analyze gene expression data in the hippocampus of healthy subjects or MCI patients and the Mini-Mental State Examination (MMSE) score, which assesses cognitive decline. We found that mechanistic target of rapamycin (mTOR) signaling pathway regulates MMSE scores. Our results revealed a causal relationship between gene expression changes and MMSE scores, and allowed us to identify the genes responsible for cognitive decline.

2-P-120

The effect of *trans*-banglene, the active component of Indonesian ginger (bangle), on memory impairment in tauopathy mouse model

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The extract from Indonesian ginger (Zingiber purpureum Rosc.; bangle) induces neurogenic and various neurotrophic effects like nerve growth factor. However, its effects on dementia such as Alzheimer's disease (AD) are still unknown. Since memory impairment in AD correlates with the expression of neurofibrillary tangles (NFTs), we synthesized *trans*-banglene (*t*-banglene), the active component of bangle, and investigated its effect on memory impairment using tauopathy mouse model. Five-month-old rTg4510 mice and age-matched control mice (tTA) were administered *t*-banglene (50 mg/kg, p.o.) or vehicle for 21 days, and assessed object recognition memory by using novel object recognition test. Vehicle-treated rTg4510 group showed lower exploratory ratio than vehicle-treated tTA group, whereas treatment with *t*-banglene recovered the exploratory ratio. Therefore, we demonstrated that *t*-banglene improve memory performance in tauopathy-associated memory impairment. It has been reported that bangle extracts elicit neurotrophic effects via activation of Wnt/β-catenin signaling. Dysfunction of Wnt/β-catenin signaling leads hyperphosphorylation of tau protein followed by deposition of NFTs. It is possible that *t*-banglene improves memory dysfunction in tauopathy due to decrement of NFT formation following enhancement of Wnt/β-catenin signaling.

2-P-121

Anti-amnesic effect of CUD 003, a novel synthetic derivative of curcumin, on scopolamine-induced memory impairment in rats

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Curcumin, a natural polyphenol compound which is contained in turmeric (Curcuma longa), has been reported to exert ameliorating effect in various experimental animals of amnesia. In the present study, the effect of CUD003, a novel synthetic derivate of curcumin, against scopolamine-induced memory impairment was assessed using the novel object recognition test in rats. Moreover, acetylcholine esterase (AChE), monoamine oxidase (MAO) A and B inhibitory activities of CUD003 were evaluated. Oral administration of CUD003 (30 mg/kg), but not curcumin (30 mg/kg) significantly increased exploratory preference of the novel object compared to the scopolamine treated rats in behavioral test. CUD003 (IC50 = 243.7 μM) exhibited lower AChE inhibitory activity as compared to curcumin (IC50 = 52.6 μM). MAO-A and -B inhibitory effects of CUD003 (IC50 = 66.0 and 10.7 μM, respectively) also weaker than those of curcumin (IC50 = 3.7 and 3.0 μM, respectively). These results suggest that CUD003 has more potent anti-amnesic effect than curcumin. Further analysis is needed to understand the mechanisms underlying its effectiveness.

2-P-122

Involvement of hypoxia-inducible factor in postoperative cognitive dysfunction

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Background: Postoperative cognitive dysfunction (POCD) is known as a decline in cognitive performance that begins days after the surgery and lasts for weeks or months. POCD is usually self-limited but can be long-lasting or even permanent in elderly patients. Experimental studies have shown that cognitive dysfunction can be induced solely by anesthetics. However, the detailed mechanism underlying POCD is not well addressed yet. In this study, we investigated the effects of pentobarbital on cognitive performance.
Methods: Male 5-weeks-old ddY mice were anesthetized by an intraperitoneal injection of pentobarbital and subjected to novel object recognition (NOR) test for evaluation of memory performance.
Results: Pentobarbital induced anesthesia in a dose-dependent manner, and at 100 mg/kg, anesthesia was sustained for about 2 hours. Cognitive decline occurred in the NOR test at 3 days after anesthesia, but not in the test at 1 or 6 days after anesthesia. The cognitive dysfunction was attenuated by pre-administration of the hypoxia-inducible factor (HIF) signal inhibitor, YC-1 or acriflavine.
Conclusion: Delayed cognitive dysfunction induced by pentobarbital presumably reflect the symptoms of POCD. Present results suggest that the activation of HIF signal is involved in the development of POCD.

2-P-123

Molecular mechanisms of REM sleep regulation: identifying protein-protein interactions of NALCN channel through BioID technique

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REM sleep is unique to animals with highly advanced brain structures. Molecular/cellular mechanisms of REM sleep regulation remain largely unknown. The *Dreamless* mutant mice with a single amino acid substitution (N315K) in the NALCN protein show abnormalities in the homeostasis of REM sleep (Funato et al, Nature, 2016). NALCN is a voltage-independent cation channel known to be involved in the circadian rhythm and respiratory regulation. However, the components of NALCN channel complex and molecular mechanisms of NALCN regulation in REM sleep homeostasis are unclear. We are trying to comprehensively search for unknown protein-protein interactions of NALCN by using the biotin-based proximity labeling technique, BioID. Recently, the MAC-tag (consisting of HA-tag, strepIII-tag, and promiscuous mutant biotin ligase BirA*) was developed, that enables parallel analyses of both BioID and conventional affinity purified mass spectrometry (AP-MS). In this study, we generated several lines of knock-in mice, in which a MAC-tag was fused to NALCN, UNC80, or NLF-1, currently known components of the NALCN channel complex. We confirmed no expression changes in the target alleles at the mRNA and protein levels. Using these mice, we identified several interacting proteins of each target by AP-MS. In the future, we will perform comprehensive mapping of NALCN protein interactions by proteomic analyses through BioID, in order to identify novel proteins involved in the molecular regulation of NALCN that are relevant for REM sleep homeostasis.

2-P-124

Characterization of multiple phosphorylation states of CaMKIIβ in mammalian sleep regulation

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The molecular mechanisms that regulate sleep-wake cycle in mammals remain a mystery. Our previous study based on mouse genetics showed that calcium/calmodulin-dependent protein kinase II (CaMKII), a protein kinase that is regulated by intracellular Ca2+, plays a critical role in sleep/wake regulation (Tatsuki, 2016). More recently, in vivo gene expression approaches using AAV-PHP.eB has demonstrated that (auto)phosphorylation of CaMKIIβ and its activation during wakefulness is critical for sleep induction (Tone, Ode, Zhang, 2021). Furthermore, we discovered that the CaMKIIβ plays a role not only in the sleep induction but also in sleep maintenance, depending on its multiple phosphorylation state. In this study, we focused on the multiple phosphorylation state of CaMKIIβ in the sleep-maintenance mode and investigated additional phosphorylations that can modulate the function of this mode. The results provide significant implications for understanding the further modes of CaMKIIβ following sleep induction and sleep maintenance, and for exploring the functional domains within the molecule that are crucial for each mode.

2-P-125

Analysis of a novel gene involved in the maintenance of wakefulness in mammals

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The molecular basis of mammalian sleep-wake regulation remains largely unexplored. Several studies have identified sleep-promoting kinases, strongly suggesting that protein phosphorylations play a critical role in driving sleep-wake cycle(Diering, 2017; Wang, 2018; Brüning, 2019). It has been suggested that kinases such as CaMKII are activated during wakefulness and induce sleep (Tone, Ode, Zhang, 2021), but the regulation of phosphorylations involved in the induction and/or maintenance of wakefulness remains unknown. Here, we identified a novel gene involved in the maintenance of wake. This geneX is known to control protein phosphorylation in various cellular signaling pathway. AAV-mediated exogenous expression of geneX in neurons of the whole mouse brain inhibited the transition from wakefulness to sleep, resulting in a significant increase in wake duration. Conversely, inhibition of ProteinX (encoded by geneX) function in neurons increased the transition from wakefulness to sleep and decreased wake duration. Furthermore, the ProteinX-inhibited mice also showed reduced wake response to the external stimuli during sleep. These results imply that the geneX is a key regulator of signaling involved in the wake maintenance and also in the induction of acute arousal during sleep.

2-P-126

Analysis of protein dephosphorylation underlying mammalian sleep-wake regulation

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Sleep-wake cycle is an organism-level phenomenon that is precisely controlled by multi-layered systems such as molecular, cellular, and circuits in the brain. Among these, recent studies have provided a deeper understanding of the molecular basis of mammalian sleep-wake regulation. Some studies suggest that dynamic changes in the protein phosphorylation in neurons underlie the control of the sleep-wake cycle. However, the core molecular mechanism of this phosphorylation control is still unclear. In this study, we identified a novel sleep-regulating factor in mammal using AAV-based in vivo gene expression system. This geneX is known to be involved in the dephosphorylation process in various signaling pathways. Exogenous expression of the active mutant of the proteinX (encoded by geneX) in neurons resulted in a significant increase in sleep duration. Analysis of the response to external stimuli in these mice suggests that the mice is accompanied by changes in the arousal system. These results imply that the sleep-wake cycle is modulated by a balance between phosphorylation processes that induce acute arousal and dephosphorylation processes involving the geneX that counteract the phosphorylation.

2-P-127

Development of a novel Brain-Machine Interface by wide-field microscopy

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The Brain-Machine Interface (BMI) allows us to extend the human body and sensing by communication between external devices and brain. A study in monkeys (Nicolelis et al., 2000) showed that effective motor control signals to decode arm movements appeared not only in the motor cortex but also in the frontal and parietal cortices. This result suggests that the accuracy of the BMI can be improved by expanding areas of interest where neural activity is monitored to decode the motor planning. However, current BMI systems that use electrodes to record neural activity are limited in the flexibility of the region of interest. In this study, we aim to develop an optical BMI system based on activity from multiple brain regions. We designed a BMI system in which mice obtain rewards according to a specific pattern of neural activity. We established a lever-press behavioral task in which head-fixed mice under a fluorescence macroscope gained rewards in response to auditory cues. We recorded the neural activity from the cerebral cortex to identify specific activity patterns during the task. We are constructing a real-time closed-loop system that detects the pattern and triggers a reward. This study will contribute to the development of new therapies to control and restore neural function.

2-P-128

Involvement of TRPV1 channels in hemokinin-1-induced nociceptive behaviors

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Hemokinin-1 mainly acts on the neurokinin-1 (NK1) receptors and is involved in the pain transmission like substance P. Transient receptor potential vanilloid 1 (TRPV1) channel are activated by various factors, but it has not been reported that TRPV1 channel is activated by hemokinin-1. We found that the nociceptive behaviors by intrathecally (i.t.) administered hemokinin-1 in mice were caused through TRPV1 channel.

The nociceptive behaviors induced by i.t. administered hemokinin-1 were observed directed biting and licking along with both hindlimb scratching in mice and recorded as the total response times of these nociceptive behaviors. In order to confirm that Hemokinin-1 activated TRPV1, the amount of phosphorylated TRPV1 was measured by Western blot analysis.

The nociceptive behaviors induced by i.t. administered hemokinin-1 was decreased by capsazepine, a TRPV1 channel antagonist. Pretreatment with antiserum against TRPV1 channel was eliminated the nociceptive behaviors induced by i.t. administered hemokinin-1. The phosphorylation of TRPV1 channel in the spinal dorsal horn was increased by i.t. administered hemokinin-1. These results show that hemokinin-1, unlike substance P, elicits the nociceptive behaviors by TRPV1 channel as well as NK1 receptor on spinal dorsal horn.

2-P-129

Study on the involvement of TRPA1 and TRPV4 in cholestatic pruritus model

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Cholestasis is one of the chronic diseases that leads to itching with unknown underlying mechanism. TRP channels have attracted attention as nociceptor of the primary sensory nerves related to acute itching behavior. On the other hand, the involvement of TRP channels in systemic itch-like behavior in chronic cholestatic pruritus model has not been much studied., we used a cholestatic model to elucidate the involvement of TRPA1 and TRPV4 in cholestatic pruritus. Male C57BL/6 strain Mice for wild type, TRPA1 knockout mice (A1KO) and TRPV4 knockout mice (V4KO) were used for experimental animal. Two of the bile ducts connecting the right and caudate lobes of liver were ligated with silk thread and the bile ducts between the two ligation sites were cut (BDL). The number of spontaneous scratching bouts with hindlimb is observed as an index of itch behavior. The scratching bouts significantly was augmented in 5th week after the surgery in BDL, compared to sham group. BDL significantly increased the level of mRNA in TRPA1 and TRPV4 in dorsal root ganglion, although that of TRPV4 in the skin of cervical region in BDL group was significantly decreased in comparison to sham. The BDL-induced scratching bouts were significantly decreased in A1KO, compared to in WT. The results suggest that TRPA1 were involved in scratching behavior in cholestatic pruritus model.

2-P-130

The involvement of selectin signaling in the sulfatide-induced mechanical allodynia

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Sulfatide is the major lipid component of the myelin sheath. Our previous study reported that the gene expression of sulfatide synthase was increased in the spinal cord one day after intraplantar injection of complete Freund's adjuvant (CFA), indicating the sulfatide accumulation in the spinal cord during inflammation. Intrathecal injection of sulfatide in naïve mice produced mechanical allodynia and spinal glial cell activation.

Previous reports suggest that sulfatide triggers the inflammatory responses in the immune cells and the glial cells. Moreover, sulfatide binds to the cell adhesion molecule, selectin. Thus, we investigated the contribution of selectin to the sulfatide-induced mechanical allodynia. Intrathecal pretreatment of the selectin inhibitor bimosiamose blocked the sulfatide-induced mechanical allodynia, suggesting that sulfatide caused mechanical allodynia by selectin activation. However, the involvement of the spinal selectin in inflammatory pain is unclear. After CFA treatment, the effects of intrathecal injection of bimosiamose on the mechanical threshold were measured. Bimosiamose reversed mechanical allodynia. Our results lead to the hypothesis that up-regulation of the sulfatide synthesis in the spinal cord contributes to the mechanical allodynia via selectin signaling during inflammatory pain.

2-P-131

Novel small-molecule antagonists of Nr4a1 ameliorate mechanical allodynia in neuropathic and bone cancer pain model mice

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Previously, we found that intrathecal (i.t.) injection of PACAP induces long-lasting mechanical allodynia in mice and increases the expression of Nr4a1 (Nuclear receptor subfamily 4, group A, member 1) mRNA in the spinal cord. We also developed a novel small-molecule antagonist of Nr4a1, named NRA-8, using docking-based in silico screening followed by in vitro/vivo pharmacological assays. NRA-8 (i.t.) showed to be able to inhibit the induction of PACAP-induced long-lasting mechanical allodynia. In this study, based on the structure of NRA-8, we synthesized derivative compounds with potent antagonistic activity on Nr4a1 and examined their analgesic effects on pain model mice.
We synthesized 15 novel derivative compounds of NRA-8 (NRA-801 to 815) and identified that 4 derivatives (NRA-811, NRA-813 to 815) show more potent antagonist activity than NRA-8. Among 4 derivatives, the effects of NRA-811 and 815 on mechanical allodynia were investigated. I.t. injection of Nr4a1 antagonists (1 nmol) ameliorated the spinal nerve ligation-induced mechanical allodynia. In bone cancer pain model mice which are induced by transplantation of NCTC2472 cells into the femur, oral administration of Nr4a1 antagonists (10 and 30 mg/kg) dose-dependently ameliorated the mechanical allodynia. In both cases, NRA-815 showed the strongest effects than NRA-811 or NRA-8. Inhibiting Nr4a1 may be one of the strategies in the treatment of intractable pain.

2-P-132

Neat1 lncRNA in primary sensory neurons is involved in microglial activation in neuropathic pain

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Neuropathic pain is chronic pain caused by damage of somatosensory system. However, existing analgesics are insufficient in effectiveness and have serious adverse effects. Although non-coding RNAs, especially microRNA, have been increasingly recognized as a critical modulator for neuropathic pain, involvement of long non-coding RNA (lncRNA) that is longer than 200 nucleotides is poorly understood. Here, we report a role of Neat1 lncRNA in the neuropathic pain. A rat neuropathic pain model was produced by ligation of the lumbar fifth spinal nerve. RNA sequencing of the dorsal root ganglion (DRG) revealed Neat1 was the most highly expressed among putative lncRNAs and was significantly upregulated in the injured DRG. Neat1 knockdown using AAV vector expressing shRNA alleviated mechanical allodynia and thermal hyperalgesia, and blocked expression changes in multiple genes related to inflammatory process or neuronal function. Many of these mRNAs were predicted to interact directly with Neat1. Neat1 knockdown in the DRG suppressed spinal microglial activation and elevated expressions of several pro-inflammatory cytokines after nerve injury. Neat1 may contribute to neuroinflammation in neuropathic pain through direct and indirect interaction with inflammatory genes.

2-P-133

Pretreatment with RAGE inhibitor prevents the onset of nociceptive behavior in mice with distal infraorbital nerve chronic constriction injury

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Post-traumatic trigeminal neuropathy (PTTN) is a kind of chronic pain caused by damage to the trigeminal nerve. Previous study reported that pretreatment with neutralizing antibody against high mobility group box-1 (HMGB1) prevented the onset of PTTN in mice a distal infraorbital nerve chronic constriction injury (dIoN-CCI). However, mechanisms how HMGB1 evokes the PTTN is unclear. Hence, the current study investigated whether HMGB1 evokes the PTTN through the activation of specific receptor.

Under anesthesia, silk sutures were tied loosely around the dIoN in male mice. Nociceptive-like behaviors were evaluated by measurement of face grooming time. Microglial activity in spinal trigeminal nucleus caudalis (Sp5c) was determined by immunohistochemistry. The inhibitors of Toll-like receptor 4 (TLR4) or receptor for advanced glycation end products (RAGE) were perineurally treated after dIoN-CCI.

The increased facial grooming time in dIoN-CCI mice was attenuated by pretreatment with RAGE inhibitor (FPS-ZM1), but not TLR4 inhibitor (TAK242). Additionally, the FPS-ZM1 treatment inhibited microglial activation in Sp5c. These data suggest that RAGE plays a crucial role to develop the PTTN after the nerve injury. Thus, the RAGE could be a novel therapeutic target for inhibiting the onset of PTTN.

2-P-134

Effects of naftopidil on dorsal root evoked excitatory synaptic transmissions in substantia gelatinosa neurons *in vitro*

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Purpose: Naftopidil is prescribed in several Asian countries for lower urinary tract symptoms suggestive of benign prostatic hyperplasia. Previous animal experiments showed that intrathecal injection of naftopidil abolished rhythmic bladder contraction in vivo. Naftopidil facilitated spontaneous inhibitory postsynaptic currents in substantia gelatinosa (SG) neurons in spinal cord slices. However, the effect of naftopidil on evoked excitatory postsynaptic currents (EPSCs) in SG neurons remains to be elucidated.

Methods: Male SD rats at 6 to 8 weeks old were used. Whole-cell patch-clamp recordings were made using SG neurons in spinal cord slices isolated from adult rats.

Results: Bath-applied 100 μM naftopidil significantly decreased the peak amplitudes of Aδ and C fiber-evoked EPSCs in a reversible and reproducible manner. Bath application of 10 μM prazosin did not inhibit Aδ or C fiber-evoked EPSCs.

Conclusion: The present study suggests that naftopidil reduces the amplitude of evoked EPSCs via a mechanism that apparently does not involve α1-adrenoceptors. Inhibition of evoked EPSCs may also contribute to suppression of the micturition reflex, together with nociceptive stimulation.

2-P-135

Effects of vanilloid analogues structurally related to capsaicin on the TRPV1 channel

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Transient receptor potential vanilloid 1 (TRPV1) is known as a receptor of capsaicin, a spicy ingredient of chili peppers. It is also sensitive to a variety of pungent compounds and is involved in nociception. Here, we focused on the structural characteristics of capsaicin, and investigated whether vanillylmanderic acid (VMA), vanillic acid (VAcid), vanillyl alcohol (VAlc), vanillyl butyl ether (VBE), and vanillin, containing a vanillyl skeleton similar to capsaicin, affected the TRPV1 activities. For detection of TRPV1 activity, intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured in HEK 293 cells heterologously expressing mouse TRPV1 (mTRPV1-HEK) and in mouse sensory neurons. Except for vanillin, four vanilloid analogues dose-dependently increased $[Ca^{2+}]_i$ in mTRPV1-HEK. The solutions that dissolved VMA, VAcid and vanillin at high concentrations were acidic, whereas those of VAlc and VBE were neutral. Neutralized VAcid evoked $[Ca^{2+}]_i$ increases but neutralized VMA did not. Mutation of capsaicin-sensing sites diminished $[Ca^{2+}]_i$ responses to VAcid, VAlc and VBE. VAcid, VMA, and vanillin suppressed the activation of TRPV1 induced by capsaicin. VAcid and VMA also inhibited the acid-induced TRPV1 activation. In sensory neurons, VMA diminished TRPV1 activation by capsaicin or acids. The present data indicate that these structural characteristics of chemical compounds on TRPV1 may provide strategies for the development of novel analgesic drugs targeting nociceptive TRPV1.

2-P-136

Identification of the site of production of complement C5, which is upregulated on exosomal bilayers from serum of mice with partial sciatic nerve ligation

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【Introduction】Exosomes are small (50-150 nm) membrane vesicles of endocytic origin, which are found in bodying fluids, and supporting their role in intercellular communication. Although recent studies have demonstrated that various biomarkers involved in the extent of pain from the serum exosomes, the effects of exosomes on pain have not been elucidated. We have previously demonstrated that increased expression of complement C5 on exosome bilayers from partial sciatic nerve ligation (PSNL) mouse sera enhances formalin-induced nociceptive behavior. In this study, to identify tissues producing complement C5 on exosome bilayers, we extracted 1) intracellular vesicles in tissues from PSNL and sham-operated groups, 2) organ-specific exosomes from serum-derived exosomes by immunoprecipitation, and compared the protein expression levels.

【Results and Discussion】We examined the expression levels of complement C5 in intracellular vesicles extracted from the tissues of PSNL mice were compared with those of the sham-operation group, the expression levels of complement C5 were increased in the PSNL group in the liver, but there was no difference in expression levels between the two groups in other tissues. In addition, membrane proteins expressed on the bilayer of intracellular vesicles extracted from the liver of PSNL mice were scraped off by trypsin treatment, the complement C5 expression level was significantly reduced. On the other hand, liver-derived exosomes were purified from serum-derived exosomes, and complement C5 expression levels were compared between the PSNL mouse group and the sham-operated group, resulting in a significant increase in complement C5 expression levels in the PSNL mouse group. These results suggest that complement C5, which was upregulated on exosome bilayers from PSNL mouse serum, is of liver origin.

2-P-137

Sex differences in modulatory function of arterial dilation by perivascular adipose tissue in metabolic syndrome

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Sex differences in the regulation of arterial tone by perivascular adipose tissue (PVAT) have been previously reported. We have demonstrated that PVAT compensates vascular tone in a situation in which vascular dysfunction occurs in mesenteric arteries of male SHRSP.Z-*Lepr*^{fa}/IzmDmcr (SHRSP.ZF) rats, an animal model of metabolic syndrome (MetS). However, such compensation function disappears in the late stage of MetS. In this study, we aimed to compare the effects of PVAT on arterial tone regulation between sexes and investigate the underlying mechanism. Ring preparations of superior mesenteric arteries, with and without PVAT, from 23-week-old male and female SHRSP.ZF rats were prepared. Vasodilation with acetylcholine and apelin mRNA levels were determined using the organ bath method and quantitative real-time polymerase chain reaction assays, respectively. In the absence of PVAT there were no sex differences in artery relaxation; however, in the presence of PVAT, female but not male rats had increased artery relaxation. In PVAT, apelin mRNA levels were higher in female than in male rats. Further, apelin levels were positively correlated with artery relaxation differences with and without PVAT. This study demonstrates that in female rats, PVAT has an enhancing effect on vasodilation, a function that appears to be impaired in age-matched male rats. Further, apelin may be involved in the maintenance of the favorable effects of PVAT in female rats.

2-P-138

Effect of age on regulation of arterial tone by perivascular adipose tissue in rats with metabolic syndrome

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Dysfunction of perivascular adipose tissue (PVAT) is related to increased arterial contractility that can lead to the development of cardiovascular diseases. We previously demonstrated that PVAT continued to increase vasodilation in the female SHRSP.Z-*Lepr*^{fa}/IzmDmcr (SHRSP.ZF) rats at 23 weeks (wks) of age, which is the age at which this effect is impaired in the male rats. We therefore investigated whether the compensatory function of PVAT in females disappears with increasing age.

Ring preparations of superior mesenteric artery with and without PVAT were obtained from male and female SHRSP.ZF rats aged 23 and 30 wks. Vasodilation to acetylcholine and vasoconstriction to phenylephrine were determined by organ bath methods.

In male rats, the vasodilation of arteries without PVAT at 30 wks was lesser than that at 23 wks; however, the presence of PVAT did not affect the vasodilation at either age. In contrast, there was no significant difference in vasodilation of arteries without PVAT between the female rats of either age, while the presence of PVAT increased vasodilation at 23 wks but not at 30 wks. In both sexes, vasoconstriction to phenylephrine was not affected by age or the presence of PVAT.

These results demonstrate that the positive effect of PVAT on vasodilation is impaired with ageing in metabolic syndrome, but this impairment develops earlier in the males compared to the females. Thus, sex-related differences in the dysfunction of PVAT along with arterial changes with ageing may explain the difference in incidence of cardiovascular diseases between the sexes.

2-P-139

Acamprosate attenuates atherosclerotic plaque formation and suppresses oxidized LDL uptake by decreasing expression of LOX-1 and CD36 scavenger receptors.

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Backgrounds: The uptake of oxidized low - density lipoprotein (oxLDL) in macrophages in atherosclerotic lesions progresses atherosclerosis. Acamprosate (ACAM), one of the drugs used in the treatment of alcohol-dependence, is a N-methyl-D-aspartic acid receptor (NMDAR) antagonist. Additionally, blockade of peripheral NMDAR attenuates the LPS-induced lung injury in animal model. In the present study, we investigated effects of ACAM on atherosclerotic plaque formation in apolipoprotein E knockout (ApoE KO) mice and on oxLDL uptake in macrophages.

Methods and results: ApoE KO mice (8-week-old) were fed a high-fat diet and intraperitoneally injected with vehicle (saline) or ACAM (200 mg kg^{-1} day^{-1}) for 21 days. Atherosclerotic lesions were histologically analyzed. ACAM significantly inhibited atherosclerotic plaque formation in the aortic arch and aortic root of ApoE KO mice. Additionally, by immunohistochemistry, ACAM reduced the area of monocyte/macrophage in atherosclerotic plaques.
Next, to assess the effects of ACAM on oxLDL uptake in macrophage, macrophages were treated with 300 μM ACAM for 24h and then DiI-labeled oxLDL (DiI-oxLDL) was added to the celles for 4 h. ACAM significantly decreased DiI-oxLDL uptake in cells. In addition, ACAM (0-300 μM) dose-dependently decreased the protein expression of LOX-1 and CD36 scavenger receptors in cells, by western blot analyses.

Conclusion: ACAM attenuates oxLDL accumulation in macrophages by decreasing the expression of LOX-1 and CD36 and thereby ACAM may inhibit the atherosclerotic plaque formation.

2-P-140

Effect of tissue-type plasminogen activator on atherosclerosis in a mouse model of hyper-LDL cholesterolemia

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We have shown that plasminogen deficiency significantly suppresses atherosclerosis in low-density lipoprotein receptor (LDLR) and APOBEC1 double knockout ($L^{-/-}/A^{-/-}$) mice, a model of hyper-LDL cholesterolemia. Since tissue plasminogen activator (tPA) is involved in the activation of plasminogen, it is speculated that tPA may be involved in atherosclerosis. In this study, we investigated the effect of tPA on atherosclerosis. To determine the effect of tPA on atherosclerosis, LDLR, APOBEC1, and Plat triple knockout ($L^{-/-}/A^{-/-}/Plat^{-/-}$) mice were generated by crossing Plat knockout mice with LDLR and APOBEC1 knockout mice. The mice were fed a normal diet and kept for 24, 36, and 48 weeks, after which they were analyzed for atherosclerosis. Aortic sinus sections were stained with hematoxylin-eosin and Masson's Trichrome. The plaque lesion area in the aortic sinus was significantly smaller in $L^{-/-}/A^{-/-}/Plat^{-/-}$ mice than in $L^{-/-}/A^{-/-}$ mice. Furthermore, aortas were cut longitudinally and stained with Sudan IV. The plaque area in the aorta was significantly smaller in $L^{-/-}/A^{-/-}/Plat^{-/-}$ mice than in $L^{-/-}/A^{-/-}$ mice. These results suggest that tPA is involved in atherosclerosis.

2-P-141

Moxifloxacin exacerbates aortic aneurysm and aortic dissection by weakening the aortic media

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Backgrounds: Aortic aneurysm and dissection (AAD) are caused by weaking vessel wall and depletion of vascular smooth muscle cells (VSMC). Osteopontin (OPN) causes the onset and exacerbation of AAD by increasing expression of matrix metalloproteinases (MMPs). Fluoroquinolones are the most common used antibiotics. On the other hand, clinical studies have indicated that fluoroquinolones may be associated with an increased risk of AAD. In this study, we examined the effects of moxifloxacin (MFLX), one of the fluoroquinolones, on the development of AAD in model mice.

Methods and results: C57BL/6J mice (4-week-old) were fed a high-fat diet. At 8 weeks of age, the mice were started to infuse with saline or angiotensin II (1000 ng min^{-1} day^{-1}) via osmotic minipumps for 4 weeks and then these mice were orally administered water (vehicle) or MFLX (30 and 100 mg min^{-1} day^{-1}) for last 3 weeks. AAD lesions were histologically analyzed. MFLX (30 and 100 mg min^{-1} day^{-1}) induced AAD formation and elastin degradation in aortic tissues by H&E staining and EVG staining. Additionally, by western blot analyses, MFLX (100 mg min^{-1} day^{-1}) decreased the protein expression of SM22a, one of the VSMC markers, in aortic tissues compared to vehicle and MFLX (30 mg min^{-1} day^{-1}). Furthermore, MFLX (30 and 100 mg min^{-1} day^{-1}) increased the protein expression of OPN, MMP-2, and -9 in aortic tissues.

Conclusion: MFLX decreased the expression of SM22a and increased the expression of OPN and MMPs in aortic tissues from AAD model mice. Therefore, MFLX may induce the onset of AAD by weakening the aortic media.

2-P-142

Role of secreted protein acidic and rich in cysteine (SPARC) in vascular endothelial cell dysfunction and inflammation

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Secreted protein acidic and rich in cysteine (SPARC) is reported to induce collagen deposition through a disintegrin and metalloproteinase with thrombospondin type 1 motif (ADAMTS1) production and promote pro-inflammatory cytokine release in aging myocardium. The present study investigated the roles for SPARC in vascular endothelial cell dysfunction and inflammation using deoxycorticosterone acetate (DOCA)-salt hypertensive rats and cultured aortic endothelial cells. DOCA-salt treatment increased systolic blood pressure in 2 weeks and induced further hypertensin in 3 weeks. Acetylcholine-induced vasodilatory response impaired and macrophage infiltration increased in DOCA-salt rat aortas, both of which showed time-dependent changes. Immunoblot analysis revealed that SPARC and ADAMTS1 expression increased in DOCA-salt rats and peaked at 1 week. Angiotensin II treatment enhanced SPARC and ADAMTS1 protein expression in cultured aortic endothelial cells. Angiotensin II-induced overexpression of LOX -1 and MCP-1 mRNA was further augmented by SPARC or ADAMTS1 gene silencing. In conclusion, SPARC may be induced at the early stage of vascular injury to compensate inflammation. Further investigation into roles for SPARC in inflammation and relationship between SPARC and ADAMTS1 is needed.

2-P-143

Carbonyl compounds in the gas phase extract of mainstream smoke derived from heat-not-burn and combustion cigarettes cause vascular endothelial dysfunction.

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The gas phase extract of mainstream smoke of combustion cigarettes includes many carbonyl compounds, which increase oxidative stress. The present study examined whether carbonyl compounds in heated cigarette-derived smoke extract (hCSE) and burned cigarette-derived smoke extract (bCSE) cause a reduction in endothelial nitric oxide synthase (eNOS) activity. Three types of heat-not-burn cigarettes (Ploom S, glo, and IQOS) and a combustion cigarette (hi-lite) were used to generate cigarette smoke at different heating or combustion temperatures [Ploom S (200° C), glo (240° C), IQOS (300–350° C), and hi-lite (770−870°C)]. The amounts of carbonyl compounds in hCSE/bCSE were assessed by measuring the carbonylation level of Ca^{2+}-sensing receptor (CaSR) that promotes the phosphorylation of eNOS at Ser^{1177}, which positively regulates eNOS activity. Although CaSR-mediated phosphorylation of eNOS were unaffected by hCSE from Ploom S and glo, hCSE/bCSE from IQOS and hi-lite reduced the eNOS phosphorylation. hCSE/bCSE from the cigarettes, except for that from Ploom S, facilitated carbonylation of CaSR with different potencies (rank order: glo < IQOS < hi-lite). The reduction of eNOS phosphorylation and the carbonylation of CaSR induced by hCSE/bCSE from IQOS and hi-lite were inhibited by treatment with mainstream smoke using a Carboxen-572 cartridge to scavenge carbonyl compounds. These results suggest that an increase in the heating or combustion temperature leads to an increase in the generation of carbonyl compounds, which cause endothelial dysfunction characterized by a reduction in eNOS activity.

2-P-144

Differences in vascular reactivity to soluble guanylate cyclase agonists between female and castrated male pigs

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This study investigated whether there are sex differences in vasorelaxation through the nitric oxide (NO)/soluble guanylate cyclase (sGC) pathway. The hearts of female (F) and castrated male (CM) pigs (6-month-old) were collected at a local abattoir, and left anterior descending coronary arteries were isolated to perform organ chamber experiments. Vasorelaxant activity was assessed based on the pD_2 values. Bradykinin induced a concentration-dependent relaxation, which was comparable between F [8.55±0.13] and CM [8.67±0.11]. In the presence of L-NAME (100 μM, NO synthase inhibitor), the response to bradykinin was attenuated similarly in F [7.27±0.24] and CM [7.42±0.29]. Sodium nitroprusside (SNP, reduced sGC agonist) also induced relaxation in a concentration-dependent manner, and the potency in F [6.54±0.12] was similar to that in CM [6.50±0.11]. Pretreatment with ODQ (10 μM, sGC heme oxidant) attenuated SNP-induced relaxation similarly in F [4.77±0.19] and CM [4.78±0.09]. Likewise, there were no differences in BAY 60-2770 (oxidized/apo sGC agonist)-induced relaxation irrespective of the presence [10.64±0.20 (F) vs. 10.47±0.14 (CM)] or absence [9.89±0.11 (F) vs. 9.67±0.15 (CM)] of ODQ. These findings suggest that there are no differences in the regulation of coronary artery tone in response to sGC agonists between F and CM.

2-P-145

Docosahexaenoic acid (DHA) strongly suppresses TP receptor-mediated contractions of coronary and cerebral arteries in pig

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We investigated the possible inhibitory effects of docosahexaenoic acid (DHA) on the contractions induced by U46619 (a TP receptor agonist) and $PGF_{2\alpha}$ in the isolated coronary and basilar arteries of pig and on the intracellular Ca^{2+} concentrations elevated by U46619 and $PGF_{2\alpha}$ in TP or FP receptor-expressing cells. In both coronary and basilar arteries, DHA strongly suppressed U46619- and $PGF_{2\alpha}$-induced contractions in a concentration-dependent manner without affecting 80 mM KCl-induced contractions. Particularly, the suppression mediated by DHA *vs.* U46619-induced contractions suggested a role of competitive antagonism on TP receptor in coronary artery. In coronary artery, DHA did not exert significant inhibitory effects *vs.* contractions mediated by acetylcholine, histamine, and serotonin. In human TP receptor-expressing cells, DHA almost diminished U46619- and $PGF_{2\alpha}$-induced increase in the intracellular Ca^{2+} concentrations without affecting the increase in $PGF_{2\alpha}$-induced Ca^{2+} concentration in FP receptor-expressing cells. These findings indicate that DHA selectively suppresses the contractions induced by TP receptor agonists in coronary and cerebral arteries partly via competitive antagonism on TP receptors. These actions mediated by DHA seem to partly attribute to its beneficial preventive effects in many cardiovascular diseases

2-P-146

Upregulation of NR4A1 Protects Cyclic Mechanical Stretch-Induced Cell Death in Rat Aorta Smooth Muscle Cells

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Cyclic mechanical stretch (CMS) causes vascular smooth muscle cell 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. We previously demonstrated that CMS caused cell death in rat aortic smooth muscle cells (RASMCs) in a JNK- and p38-dependent manner. To explore the causal role of CMS in initiating cell death signaling and MAPK-related events, we examined the transcript profiles of CMS-induced RASMCs using cDNA microarrays and found that NR4A1 expression levels were significantly increased in response to CMS. Quantitative polymerase chain reaction (qPCR) analysis demonstrated that this increase was p38-dependent. Moreover, NR4A1 inhibition by the inhibitor CDIM8 strongly increased CMS-induced cell death in RASMCs. Considering that hypertension acts on the vascular smooth muscle cells, we also examined NR4A1 expression in arteries using an abdominal aortic constriction (AAC) mouse model. We confirmed that the NR4A1 expression was increased by hypertension in arteries subjected to AAC as compared to sham-operated arteries. From above results, we conclude that NR4A1 protects RASMCs from CMS-stimulated cell death via the p38 signal pathway.

2-P-147

PKCβ inhibitor blocked angiotensin II-induced vascular smooth muscle proliferation and oxidative stress

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Protein kinase C (PKC) β is activated under hyperglycemia and in cancerous cells, which has been implicated in cell inflammatory activation. On the other hand, the role of PKCβ in hypertension-associated inflammatory response remains unclear. In this study, we examined the effect of PKCβ inhibitor on angiotensin II (Ang II)-induced inflammatory responses in vascular smooth muscle cells. We isolated vascular smooth cells from mesenteric arteries of male Wistar rat. Treatment of vascular smooth muscle cells with Ang II (1-1000 ng/ml) for 15 min increased phosphorylation of PKCβ at the active site at Ser 660 and Thr 641 in dose dependent manner (n=4). Treatment with Ang II (100 ng/ml) for 24 hours increased expression of proliferating cell nuclear antigen (PCNA) and the effect was attenuated by pretreatment with PKCβ inhibitor (LY333531 or CGP53353)(n=6). Also, treatment with Ang II for 48 hours induced cell proliferation and the effect was blocked by pretreatment with the PKCβ inhibitor (n=4). Treatment with Ang II for 24 hours increased reactive oxygen species production and the effect was attenuated by the PKCβ inhibitor (n=4). These results suggest that PKCβ is involved in vascular smooth muscle cell proliferation and oxidative stress in response to Ang II, and may contribute to inflammation associated with hypertension.

2-P-148

The Anticonvulsant effect of flopropione does not involve catechol-O-methyl transferase (COMT) inhibition

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Flopropione (Flo) has been used for urolithiasis and gallstone as an anticonvulsant almost exclusively in Japan. According to the package insert, its main mechanism of action is COMT inhibition, and this mechanism, as described in many pharmacology textbooks, frequently occurs in questions in state examinations for pharmacists. As this mechanism is contrary to pharmacological common sense, i.e., ureter is contracted by adrenergic α stimulation and physiological relaxant of Oddi sphincter (OS) is not adrenergic, we confirmed this by experiment. The ureter was taken from male Guinea pig and its isometric contraction was recorded. Noradrenaline (NA) elicited periodical spike contractions. Flo (30 to 100 μM) reduced both its contractile force and frequency, whereas entacapone (Ent, 10 μM), whose inhibitory effect on COMT is much more potent than Flo, did not show any effect. Isolated OS was perfused with nutrient solution 1 ml/hr, and its perfusion pressure was recorded. The OS preparation showed periodical increase in pressure without stimulants. Flo inhibited the spontaneous increase, whereas Ent did not show any effect. NA inhibited the pressure increase and its inhibition was blocked by α and β antagonists, whereas the inhibitory effect of Flo was not affected by the antagonists. These observations strongly indicate that there is no room for COMT inhibition in the mechanism of anticonvulsant effect of Flo. The true mechanism of smooth muscle relaxation by Flo is presently under investigation.

2-P-149

Protective role of epithelial TRPV4 in the pathogenesis of OVA-induced food allergy in mice

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Intestinal epithelial barrier plays a crucial role in the maintenance of gastrointestinal integrity by limiting penetration of luminal bacteria and dietary allergens. TRPV4 is a non-selective cation channel that responds to mechanical, thermal, chemical stimuli, and various endogenous ligands, such as arachidonic acid metabolites. We previously reported that TRPV4 upregulated in vascular endothelia increased vascular permeability during colonic inflammation. The present study investigated the role of TRPV4 in the pathogenesis of ovalbumin (OVA)-induced food allergy in mice. TRPV4-immunoreactivities were detected in epithelial cells but not vascular endothelial cells in the colon of normal and OVA-treated wild-type (WT) mice. TRPV4 knockout (TRPV4KO) mice showed more severe systemic symptoms consist of body weight loss, pruritus, itching, and diarrhea, accompanied by serum OVA-specific IgE levels in OVA-induced food allergy than WT mice. However, colonic permeability in normal and OVA-induced food allergy was not affected by TRPV4KO. Furthermore, TRPV4KO upregulated CD11c-, CD117-, occludin-, Ki67-positive cells, and goblet cells in the colonic mucosa of OVA-induced food allergy compared with WT. These results suggest that epithelial TRPV4 plays a protective role in food allergy via regulation of epithelium-immune interaction.

2-P-150

Anti-inflammatory role of orphan G protein-coupled receptor GPR35 in experimentally-induced murine ileitis and colitis

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Orphan G protein-coupled receptor GPR35, which can be activated by lysophosphatidic acid and kynurenic acid, is highly expressed in gastrointestinal tracts. Previous studies demonstrate the implication of GPR35 in the pathogenesis of inflammatory bowel disease but this role remains undefined. The present study investigated the pathogenic role of GPR35 in murine ileitis and colitis models. GPR35-deficient (GPR35KO) mice were generated by CRISPR-Cas9-mediated genome editing on C57BL/6 background. Colitis and ileitis were induced in GPR35KO and its wild-type (WT) mice by 7-days treatment with dextran sulfate sodium (DSS) and single injection of indomethacin, respectively. DSS-treatment produced body weight loss with diarrhea and blood feces, and caused severe colitis, characterized by shortening colon length and histological injury 7 days later. The severity of colitis with systemic symptoms was significantly augmented in GPR35KO mice compared with WT mice. Indomethacin injection produced intestinal injury 48 h later and the severity was also more higher in GPR35KO mice than WT mice. These findings suggest that GPR35 plays an anti-inflammatory role in DSS-induced colitis and indomethacin-induced ileitis in mice. Thus, GPR35 may be a promising target for treatment of inflammatory bowel disease.

2-P-151

Peptidylarginine deiminase 2 (PAD2) contributes the pathogenesis of TNBS-induced murine colitis in relation to extracellular trap formation

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Peptidylarginine deiminase 2 (PAD2), an enzyme that converts peptidyl-arginine to peptidyl-citrulline, is widely distributed in various tissues and cells. Although PAD4 is known to be involved in various inflammatory and immune diseases via neutrophil extracellular trap (NETosis) formation, the pathophysiological roles of PAD2 have not been fully understood. The present study investigated the pathogenic role of PAD2 in inflammatory bowel disease using trinitrobenzene sulfonic acid (TNBS)-induced murine colitis model. PAD2-deficent (PAD2KO) mice were generated by CRISPR-Cas9-mediated genomic editing. Colitis was induced in PAD2KO and its wild-type (WT) mice by intrarectal injection of TNBS. The extracellular trap formation was detected by triple immunohistochemical staining with myeloperoxidase (MPO), citrullinated histone H3, and DNA (Sytox Blue). TNBS injection induced body weight loss, extensive colonic erosions, and ulceration accompanied by an increase in MPO activity in WT mice, but these responses were significantly attenuated in KO mice. Daily administration of Cl-amidine (an inhibitor of pan-PADs) or DNase I (an inhibitor of extracellular trap formation) also significantly reduced the severity of TNBS-induced colitis. Furthermore, the extracellular trap formation was upregulated by TNBS injection at lesion sites, but this response was apparently attenuated by PAD2KO and Cl-amidine injection. These findings suggest that PAD2 contributes to the pathogenesis of TNBS-induced colitis probably via inhibition of extracellular traps.

2-P-152

Protective effect of nafamostat mesylate on mucosal injury and 5-hydroxytryptamine potentiation induced by a consecutive administration of methotrexate to rats

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One of the major side effects of anti-cancer chemotherapy is mucosal injury in the gastrointestinal tracts. 5-Hydroxytryptamine (5-HT) is critically involved in intestinal injury due to its role as a paracrine messenger, neurotransmitter, and inflammatory mediator. Nafamostat mesylate (nafamostat), a potent and specific serine protease inhibitor, has been reported to have a therapeutic effect on several types of gastrointestinal inflammation including experimental colitis. In this study, we investigated whether nafamostat ameliorates methotrexate-induced gastrointestinal injury and/or potentiation of 5-HT dynamics in intestine. Rats received methotrexate intraperitoneally a consecutive administration (12.5 mg/kg/day) for 4 days. Nafamostat (1 or 3 mg/kg) was given subcutaneously 10 min before first administration of methotrexate, and then every 24 h for three consecutive days. Jejunal tissues were collected to analyze. Nafamostat 1 mg/kg, but not 3 mg/kg, ameliorated methotrexate-induced villus atrophy, as well as mRNA expression of pro-inflammatory cytokines. Also, nafamostat 1 mg/kg significantly inhibited methotrexate-induced tryptophan hydroxylase1 mRNA expression and 5-HT contents. The present study suggests that nafamostat could be applied as a therapeutic agent for gastrointestinal injuries caused by anti-cancer drugs.

2-P-153

Effects of pemafibrate and bezafibrate on CDAHFD-induced murine nonalcoholic steatohepatitis (NASH) model

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In this study, 6-week-old mice were fed with a choline-deficient methionine-reduced high-fat diet (CDAHFD) for 6 and 12 weeks to confirm the development of the pathology of nonalcoholic steatohepatitis (NASH). The effects of pemafibrate (PF), a selective PPARα modulator, and bezafibrate (BF), PPAR-pan agonist were also evaluated on the progression of NASH pathology. Thus, the mice were given CDAHFD for 12weeks, with concomitant oral dose of 0.1, 0.3 mg/kg of PF, or 100 mg/kg of BF was administrated for 12 weeks.

Serum and hepatic biomarkers (e.g., serum ALT, ornithine carbamoyl transferase (OCT), and hepatic TG) and hepatic mRNA expression of SREBP-1c, TNF-α, and Col1a1 were significantly increased in mice fed with CDAHFD for 6 weeks compared to control mice. In addition, mice fed with CDAHFD for an additional 6 weeks (12 weeks total) showed higher serum ALT, OCT, hepatic TC, hydroxyproline (HP), and hepatic mRNA expressions than mice fed CDAHFD for 6 weeks, revealing the development of NASH pathology.

100 mg/kg of BF administered by gavage for 12 weeks almost suppressed the progression of NASH pathology in CDAHFD fed mice. Administration of 0.1 and 0.3 mg/kg of PF suppressed the progression of NASH pathology as much as or better than administration of BF.

These findings showed that PF might thus be effective in preventing NASH.

2-P-154

Development of new treatments based on commercial medicines for side effects in cancer chemotherapy

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[Background] Anticancer drug-induced stomatitis develops in 30-40% of cancer patients undergoing chemotherapy. However, medications for this condition are not commercially available in Japan. [Methods] The oral stomatitis model was prepared by anesthetizing male Golden Syrian hamsters i.p. with 30 mg/kg pentobarbital. The center of the cheek pouch was then exteriorized and sandwiched between ring forceps 5 mm in inside diameter, followed by submucosal injection of 25 μL of 10% acetic acid solution through the ring forceps. Rebamipide nanoparticles were prepared by the wet-milling technique using hydroxypropyl cellulose (HPC-SSL) and sodium dodecyl sulfate. [Results] The results of zeta potential measurement and evaluation of their dispersibility suggested that the prepared nanosuspensions were stable. Furthermore, adhesion of the nanoparticles to the mucous membrane of the oral cavity was evaluated using a quartz crystal microbalance with dissipation monitoring (QCM-D) technology. Application of 3.0 mg/mL rebamipide solution significantly reduced the areas of injury acetic acid-induced oral stomatitis in 5-FU-untreated animals. The application of 3.0 mg/mL rebamipide solution significantly reduced the injured areas of acetic acid-induced oral stomatitis in 5-FU-treated animals. To prevent stomatitis, it appears feasible to utilize RB nanoparticles dispersed in HPC-SSL solution in a mouthwash. [Conclusion] These results suggested that the application of rebamipide reduced the injured areas of an oral stomatitis that developed under chemotherapy-treated conditions.

2-P-155

Involvement of neutrophil and TRPV4 in the decreased colon function elicited by long-term powdered diet feeding

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Immune cells, such as neutrophils, and transient receptor potential vanilloid receptor-4 (TRPV4) are known to be associated with inflammatory bowel disease. We have previously reported the importance of masticatory habits using mice that were fed a powdered diet.

In this study, we investigated that the influence of long-term powdered diet feeding on colon function in mice as well as the relationship between neutrophils and TRPV4.

Mice fed a powdered diet for 17 weeks from weaning were compared with mice fed a control diet. RN-1734, TRPV4 antagonist, and sivelestat, neutrophil elastase inhibitor, were used. We investigated the number of fecal pellets and fecal moisture content as indicators of colon function. Further, the colonic expressions of Ly-6G (neutrophil) and TRPV4 were analyzed by western blotting.

Powdered diet feeding significantly decreased the colon function and increased the Ly-6G and TRPV4 expressions. The decreased colon function and the increased expression of TRPV4 were significantly improved by sivelestat. In addition, RN-1734 also improved the decreased colon function in powdered diet fed mice.

These results suggest that the long-term powdered diet feeding causes the decreased colonic function, which may involve activation of neutrophils and TRPV4.

2-P-156

Analysis of microsomal Prostaglandin E syntase-1(mPGEs-1)/PGE_2 axis mechanism and relationship between regulatory T cells(Tregs) in promoting granulation formation.

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Background)Microsomal prostaglandin (PG) E synthase-1 (mPGEs-1) is the enzyme responsible for the second step of Prostaglandin E2 (PGE_2). The expression of mPGEs-1 was observed in various contexts, such as inflammation, fever, pain, female reproduction, and cancer growth. Immune cells, especially regulatory T cells (Tregs) related to cancer growth, tissue repair and angiogenesis. It was reported that PGE_2 modulates Tregs cell function and differentiation.Based on these previous reports, we ypothesized mPGEs-1/PGE_2 axis contribute to wound healing and granulation formation by accumulation of Tregs.

Material and Method) Male 6-8 weeks old C57Bl/6N (wild type=WT) mPGES-1 deficient mice (mPGES-1KO) were used. Polyurethane sponge disks were implanted into dorsal subcutaneous tissue of mice. Angiogenesis was estimated by weight of granulation tissue and the expressions of CD31, VEGF and TGF-beta by immunohistochemical analysis and real time PCR. Contribution of Tregs was estimated by immunohistichemical study and real time PCR against FOXp3 specific transcript factor for Tregs. Involvement of Tregs in granulation formation was analyzed by administration of CD25 antibody or folate receptor 4 (FR4) antibody.

Results) Compared to WT, weight of granulation tissue was significantly suppressed in mPGEs-1KO ($P<0.05$). Expressions of CD31 and VEGF in the granulation tissues were significantly suppressed in mPGEs-1KO. Expression of FOXp3 in the granulation tissue was significantly suppressed in mPGEs-1KO compared to WT ($P<0.05$). The numbers of accumulated FOXp3 positive cells around granulation tissue were also impaired in mPGEs-1KO compared to WT ($P<0.05$). In addition, mRNA and protein expression of TGF-beta were significantly decreased in mPGEs-1KO. WT treated with CD25 antibody or FR4 antibody were significantly suppressed granulation formation compared to IgG treated mice but not in mPGEs-1KO.

Conclusion) These results suggested that mPGEs-1/PGE_2 axis induces angiogenesis and granulation formation by accumulating Tregs.

2-P-157

Analysis of the mechanism by which TSLP production is inhibited by PHD inhibitors

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Thymic stromal lymphopoietin (TSLP) is an epithelial cell-derived cytokine that plays an important role in the regulation of skin immunity and is upregulated in the lesions of atopic dermatitis patients. Therefore, inhibitors of TSLP production are effective as therapeutic agents for atopic dermatitis. In our laboratory, we found that hypoxia condition and DMOG, a PHD inhibitor, suppressed the expression of TSLP in keratinocytes. In this study, we analyzed the mechanism by which PHD inhibitors suppress TSLP expression.

TSLP mRNA expression was induced in human keratinocyte cell line HaCaT cells by combined stimulation with TNF α, IL-4, FSL-1, and PAR2 agonist (T4FP stimulation), which was inhibited by PHD inhibitors Roxadustat and Enarodustat. The knockdown of HIF1 α by siRNA abolished the inhibitory effect of Enarodustat on TSLP expression. Chromatin immunoprecipitation assay suggested that HIF1 α binds to the hypoxia response elements (HRE) on the TSLP promoter.

In conclusion, this study demonstrated that PHD inhibitors suppressed TSLP mRNA expression induced by T4FP stimulation in HaCaT cells. The suppression of TSLP expression was found to be HIF1 α-dependent. In addition, we found that HIF1 α binds to the HRE on the TSLP promoter, suggesting that HIF1 α may affect transcription by binding to the TSLP promoter.

2-P-158

Study of anti-inflammatory Effects of Various Drugs on a Collagen-Induced Arthritis Model in Mice

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Rheumatoid arthritis (RA) is an autoimmune disease in which immune cells attack the bone, and finally joint functions are lost. Since the etiology of RA has been elusive, it is considered that the combination of environmental (e.g. infection and stress) and genetic factors might affect the onset of RA. In this study, we investigated the anti-inflammatory efficacy of various steroids, DMARDs, anti-mouse TNF-α antibody, and JAK inhibitor, on the CIA model in mice.

CIA was induced by immunizing DBA in mice with type II collagen/Adjuvant (CFA or IFA) on day 0 (primary sensitization) and 21 (secondary sensitization). The anti-inflammatory effect on the CIA in mice was evaluated, using the arthritis score, paw volume, expression of cytokines in the joint of tarsi of the hind limb and histopathological examination as the index.

Results showed that anti-inflammatory effects of by prednisolone, leflunomide, MTX, anti-mouse TNF-α antibody and tofacitinib were confirmed. Therefore, it is considered that our experiment system used in this study is useful for investigation of RA therapeutic drugs.

2-P-159

Inhibitory effect of REV-ERB agonist on inflammatory responses in primary cultured rat chondrocytes

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Osteoarthritis (OA) is characterized by inflammation of the joints and degradation of articular cartilage matrix, but treatment remains symptomatic and effective therapies have yet to be established. In OA, various inflammatory stimuli are applied to chondrocytes, resulting in cellular damage. Therefore, elucidating the regulatory mechanisms mediating inflammatory responses in chondrocytes is crucial. REV-ERBs function as transcriptional repressors in inflammatory responses, but the roles of REV-ERBs in OA have not been clarified. Then, the current study investigated the effect of REV-ERB agonist on the expression of inflammatory cytokines and proteolytic enzymes in chondrocyte stimulated with lipopolysaccharide (LPS). The chondrocytes were prepared from the knee articular cartilage of neonatal Wistar rats. Messenger RNA expression was measured by real-time PCR. Treatment of chondrocytes with LPS increased the mRNA expression of degradation enzymes of extracellular matrix (matrix metalloproteinase (MMP)-3, MMP-13) and proinflammatory cytokines (interleukin-1β, tumor necrosis factor-α). However, pretreatment with SR9009 significantly blocked these responses. These results suggest that REV-ERBs might be involved in the regulation of inflammatory responses and degradation of extracellular matrix in chondrocytes.

2-P-160

Increase in IL-10 expression by $K_{Ca}3.1$ inhibition mediating the JNK/c-Jun signaling pathway in peripherally-induced regulatory T cells

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The Ca^{2+}-activated K^+ channel, $K_{Ca}3.1$ plays an important role in the regulation of T cell functions, and its blockades has been shown to decrease Ca^{2+} influx via Ca^{2+} release-activated Ca^{2+} channels in activated T cells. $K_{Ca}3.1$ is responsible for the pathogenesis of inflammatory bowel disease (IBD), and its blockade induced the down-regulation of the inflammatory cytokine, IFN-γ in Th1 cells and up-regulation of the anti-inflammatory cytokine, IL-10 in T_{reg} cells (Ohya et al., 2014 & 2021). The expression of IL-10 in T_{reg} cells is driven by various transcription factors (TFs) and upstream signaling pathways. We recently demonstrated that a $K_{Ca}3.1$ inhibitor up-regulated the expression of IL-10 in T_{reg} cells in the recovery phase in IBD model mice and *in vitro*-induced T_{reg} cells, together with the up-regulation of their TFs: Blimp1 and E4BP4. We here investigated the involvement of AP-1 (Fos/Jun) and NF-κB in the expression of IL-10 and its TFs in *in vitro*-induced mouse splenic T_{reg} cells. The pharmacological inhibition of JNK reversed $K_{Ca}3.1$ inhibition-induced increases in the expression of IL-10 and its TFs. The inhibition of $K_{Ca}3.1$ increased phosphorylated JNK and c-Jun levels. Therefore, the JNK/c-Jun signaling pathway may contribute to the $K_{Ca}3.1$ inhibition-induced up-regulation of IL-10 in peripherally-induced T_{reg} cells.

2-P-161

Cooperative action of IFN-γ and IL-4 to induce M2-like macrophages

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Interferon (IFN)-γ and interleukin (IL)-4 are considered to be important factors to regulate immune responses. Although the effects of IFN-γ or IL-4 on macrophage functions are well established, their cooperative action is not well documented. Inducible nitric oxide synthase (iNOS) or arginase (Arg)-1 is a representative marker of M1 or M2 macrophages and plays a role in the acceleration or suppression of inflammatory responses. At present, we examined the effect of simultaneous treatment with IFN-γ and IL-4 on macrophage expression of iNOS and Arg-1 in the murine macrophage cell line, RAW264.7. IFN-γ or IL-4 increased iNOS or Arg-1 protein production, respectively. Of note, IL-4 combined with IFN-γ synergistically increased Arg-1 protein production, whereas IL-4 inhibited IFN-γ-induced iNOS production. In addition, IL-4 combined with IFN-γ synergistically increased cell surface expression of programmed death ligand (PD-L) 2, which is involved in T cell suppression, whereas IL-4 completely inhibited IFN-γ-induced expression of CD86, which is responsible for T cell activation. It seems that macrophages highly expressing Arg-1 and PD-L2 may be induced in the presence of IFN-γ and IL-4 at the inflammatory site, and might contribute to regulate inflammatory immune responses.

2-P-162

Effects of stretch-induced mechanical stimulation on macrophage function.

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Physical changes in temperature, pH, oxygen and mechanical stimuli derived from the environment, are important for regulating cell function and survival. Although the immune cells experience mechanical forces and pressures throughout their life cycle, little is known about how such mechanical processes regulate the immune cell function. In this study, we investigated the effects of cyclical stretch (CS)-induced mechanical stress, a condition in which immune cells experience in the heart, on murine macrophage RAW264.7 (RAW) cells.

CS stimulation of RAW cells evoked a marked release of nucleotides including ATP and ADP in a strength-dependent manner. The CS stimulation also initiated elevation of mRNAs for various pro-inflammatory factors, such as IL-1β, IL-6, COX-2 and monocyte chemoattractant protein-1 (MCP-1). In the presence of gadolinium (Gd^{3+}), an inhibitor of stretch-activated channels, CS-induced ATP release and mRNA elevation of IL-1β, IL-6, COX-2 were attenuated. Under such condition, CS-induced MCP-1 mRNA expression was not affected by Gd^{3+}. Treatment with apyrase, a nucleotide hydrolysis enzyme, to eliminate the effects of extracellular nucleotides did not affect the CS-induced MCP -1 mRNA expression.

These results suggest that the CS activates macrophage function via Gd^{3+}-sensitive stretch-activated channels. In contrast, additional mechanism other than CS-activated channels and autocrine stimulation by extracellular nucleotides may be involved in the CS-induced MCP-1 mRNA expression.

2-P-163

BRD6989, a CDK8/19 inhibitor, induces M2 macrophages

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M2 macrophages have anti-inflammatory and wound healing activities and secrete anti-inflammatory cytokines. M2 macrophages are induced by Th2 cytokines such as IL-4 and IL-13. The development of therapeutics that can reduce inflammation through the induction of M2 macrophages is of considerable interest. Besides, cyclin-dependent kinase (CDK) 8 and its paralog, CDK19, are involved in regulation of gene transcription. CDK8/19 inhibition has been reported to promote regulatory T cells and thereby reduce inflammation. However, the influence of CDK8/19 inhibition on M2 macrophage polarization have not yet been investigated. We examine the role of BRD6989, a small-molecule CDK8/19 inhibitor, in induction of M2 macrophages.

RAW264.7 macrophages were pretreated with BRD6989, and then stimulated with IL-4. We analyzed the expression of CD206 and arginase-1 as markers of the M2 phenotype. BRD6989 enhanced the expression of IL-4-induced CD206 and arginase-1. Therefore, we suggested that BRD6989 enhances the effect of IL-4 on M2 macrophage polarity and we are currently investigating the mechanism in this regard.

2-P-164

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2-P-165

Establishment of a novel atopic dermatitis mouse model using C57BL/6 mice

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Atopic dermatitis (AD) is a pruritic inflammatory skin disease caused by skin barrier dysfunction and immune abnormalities. We have previously reported an AD mouse model using outbred HR-1 hairless mice. However, this mouse strain might be inapplicable to studies requiring genetically modified mice. Therefore, we examined whether AD symptom is reproduced using C57BL/6 (B6) mice, a strain widely used for gene-targeting. Four-week-old female B6 mice were fed a special diet (SD) deficient in unsaturated fatty acids and starch to induce skin barrier dysfunction systemically. Then, house dust mite (HDM) extract was repeatedly applied to the face and back skin of SD-fed mice (SD + HDM mice). SD + HDM mice showed AD-like dry and eczematous lesions and itch-related behavior. In the skin of SD + HDM mice, the numbers of eosinophils and mast cells were increased compared with normal B6 mice. Furthermore, the expression levels of *S100a8, Krt16, Il1b, Il13, Il17a, Il19, Cxcl1,* and *Ccl17* were increased, whereas that of *Lor* was decreased. The skin gene expression profile in SD + HDM mice more closely resembles that of human AD than well-known other AD mouse models (spontaneous model, NC/Nga mice, or sensitizer (ovalbumin or oxazolone)-induced models). In conclusion, our novel AD model established using B6 mice could be useful for studying AD.

2-P-166

Analysis of the pathogenesis of ICU acquired weakness (ICU-AW) in mice

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With advances in intensive cares, the prognosis for critically ill patients requiring intensive cares has improved. However, discharged patients often induce ICU acquired weakness (ICU-AW) as a sequela. Especially in the elderly, ICU-AW has become a problem that the frailty is exacerbated and the patients are required long-term cares. So far, it is unknown the pathophysiology of ICU-AW, and effective preventive and therapeutics have not been established. Here we established a mouse model of ICU-AW, in which unilateral lower limb fixation was performed on mice with acute respiratory distress syndrome (ARDS) by intratracheal administration of bleomycin. Mice were divided into 4 groups: the non-lower limb fixation + non-ARDS, the lower limb fixation + non-ARDS, the non-lower limb fixation + ARDS, and the lower limb fixation + ARDS. The body weight and survival rates were the worst in the lower limb fixation + ARDS group. The lower limb fixation + ARDS group showed the impairment of respiratory function and the lung pathology, along with increased expression of pulmonary inflammatory cytokines. In addition, muscular atrophy was induced by fixation of the lower limbs, and increased expression of muscular atrophy-related genes was observed. In addition, the lower limb fixation + ARDS group had more severe weight loss than the other groups and died earlier. In the lung, the respiratory function was significantly worse in the lower limb fixation + ARDS group than in the non-lower limb fixation + ARDS group. Muscle atrophy was higher in the lower limb fixed + ARDS group than in the non-fixed + ARDS group. Pathway analysis based on gene expression analysis in muscle revealed that the Apelin signaling pathway was suppressed in the lower limb fixed + ARDS group compared to the non-fixed + ARDS group. These results suggest that in ICU-AW, muscular atrophy and ARDS influence each other's pathological conditions, and the Apelin signaling pathway may be involved in this process.

2-P-167

Development of a noradrenergic neuron-selective AAV vector and its application to the brain-wide axonal mapping

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The locus coeruleus (LC) is the main source of noradrenaline (NA) in the brain and involved in various brain functions including arousal, cognitive and emotional regulation. Recent accumulating evidence shows that individual LC-NA neurons send segregated efferent projections in a target-specific manner; however, it is still controversial whether LC-NA neurons have fully target-specific or more global projection patterns. To identify their axonal wiring at mesoscale level, we developed a novel adeno-associated viral (AAV) vector for NA neuron-selective gene expression and conducted whole-brain mapping of LC-NA axons using the block-FAce Serial microscopy Tomography (FAST). By local injection of the AAVs to the LC, we observed 92% of cells labeled with fluorescent proteins were tyrosine hydroxylase-positive. Using an axon-targeting fluorescent protein and retrograde AAV, we found that ventral hippocampus-projecting LC-NA neurons send dense projection to the dorsal hippocampus and some collaterals to the wide cortical area. These results suggest that although LC-NA neurons have dense projection in a target-specific manner, they may globally transmit NA and regulate multiple brain regions via collaterals. These findings and the AAV vectors will contribute to more precise understanding of complex organization of LC-NA systems.

2-P-168

Establishment of an *in vivo* calcium imaging method to evaluate neuronal activity in mice carrying mutations of *Arhgap10* gene

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Recently we have identified *Arhgap10* gene mutations in Japanese schizophrenia patients by the genome-wide CNV analysis. ARHGAP10 negatively regulates Rho family small GTPases that play roles in the regulation of spine morphology. We also found that *Arhgap10* S490P/NHEJ mice which were generated to mimic the patient case were highly sensitive to methamphetamine (METH) and spine density of the secondary dendrites of medium spiny neurons (MSNs) in the striatum was increased in the mutant mice. Because spine density is well associated with neuronal activity, in this study we sought to establish wireless photometry system to measure Ca^{2+} level in the striatal MSNs of *Arhgap10* S490P/NHEJ mice. Firstly, we measured the number of the c-Fos positive cells in the striatum of *Arhgap10* S490P/NHEJ mice by immunohistochemistry 2 h after METH (0.3 mg/kg, i.p.) treatment. METH increased the number of c-Fos positive cells in the dorsomedial striatum in *Arhgap10* S490P/NHEJ mice but not wild-type mice. We generated mice expressing selectivity GCaMP6 in dopamine D1 receptor-expressing MSNs (D1-MSNs) of the striatum by Cre-loxP system. In a mean while we inserted optic fiber and detected GCaMP6 signal of the striatal D1-MSNs in Drd1-Cre mice under a free moving condition. Treatment of METH (2 mg/kg, i.p.) increased Ca^{2+} signal in striatal D1-MSNs as well as locomotor activity.

2-P-169

Effects of genetic background on the phenotypic changes of p13 knockout mice

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Mitochondrial protein p13 is widely expressed in central and peripheral tissues. We previously showed p13 knockout mice with C57BL/6 genetic background (p13-KO/B6 mice) showed several phenotypic changes including a severe early postnatal mortality. Here, we examined the effects of genetic background (C57BL/6, BALB/c, or ICR) on the phenotypic changes of p13-KO mice. Whereas more than half of p13-KO/B6 mice died during the first postnatal day (P0), more than half of p13-KO mice survived the day under a BALB/C or an ICR genetic background. At P0, p13-KO mice with all three genetic background showed significant underweight and hypoglycemia. Interestingly, approximately 10% of p13-KO/BALB and p13-KO/ICR but not p13-KO/B6 mice exhibited a blood clot on their top of the head, which is deeply associated with their mortality. Accordingly, these data suggest that p13 is important for early postnatal survival, which could be modulated by their genetic background.

2-P-170

Cardiovascular dynamics of Zucker Fatty Diabetes Mellitus (ZFDM) rats, an animal model of obesity and diabetes

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Background: A relatively novel rat stain, Zucker Fatty Diabetes Mellitus (ZFDM) rats, represent early obesity and diabetes without feeding high-fat diet. We aimed to clarify the cardiovascular dynamics of ZFDM rats.
Methods: Using 12-38-week-old male ZFDM-Lepr$^{fa/fa}$ rats (Homo), an obese and diabetic phenotype, and ZFDM-Lepr$^{fa/-}$ rats (Hetero), a normal control phenotype, body weight, body mass index (BMI), blood glucose, total cholesterol, and triglyceride were measured. Systolic blood pressure (SBP) and heart rate (HR) were measured by a tail-cuff system.
Results: In Homo compared with Hetero, body weight was heavier at 12-16-week-old, similar at 17-30-week-old, and lighter at 35-38-week-old. BMI in Homo was higher than Hetero. Blood glucose (12, 16, 21, and 36-38-week-old) and lipid (total cholesterol and triglyceride at 19-week-old) in Homo were higher than Hetero. There was no difference in SBP between Homo and Hetero, while HR in Homo was lower than Hetero.
Discussion: ZFDM-Lepr$^{fa/fa}$ rats (Homo) represent early obesity, hyperglycemia, and hyperlipidemia, while high blood pressure was not observed until 38-week-old.

2-P-171

Noninvasive monitoring of muscle atrophy and bone metabolic disorder using Dual energy X-ray absorptiometry (DXA) in diabetes mellitus model mouse

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Diabetes mellitus (DM) induces a decrease in skeletal muscle mass and bone mineral density, which trigger sarcopenia and osteoporosis. Multiple mechanisms are associated with DM-induced muscle atrophy and osteopenia, and their relative contributions may change as the disease stage progresses. Therefore, continuous monitoring of muscle mass and bone metabolism is required to investigate the functional linkage between DM and musculoskeletal disorders, which is difficult in basic research using small animals.

To address this issue, we proposed Dual energy X-ray absorptiometry (DXA), a diagnostic imaging technique, as a continuous assessment of body composition method. We induced type 1 DM in mice by streptozotocin and measured their bone density, fat mass, and lean body mass using DXA for 4 weeks non-invasively. After 4 weeks we harvested the mice and measured the weight of the muscles and fat, the bone density, and blood levels of insulin and IGF-1. These values showed the progression of DM in the mice and were consisted with the values measured using DXA. We found DXA to be useful for monitoring changes in muscle and bone metabolism during the progression of DM. The application of DXA may enable continuous and non-invasive monitoring in animal models of various metabolic diseases.

2-P-172

Evaluation of methods to analyze redox state in immune cells

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

2-P-173

Establishment of SARS-CoV-2 respiratory tract infection model in CAG promoter-driven hACE2 transgenic mice

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COVID-19, caused by SARS-CoV-2, has spread worldwide with dire consequence. Vaccine is just not enough to suppress to this pandemic, and it is desired to develop the prophylactic and therapeutic agents for preventing the spread of infection and severity. Therefore, it is extremely important to propose therapeutic strategies by using pathological models. In this study, we established an animal model with highly susceptible to SARS-CoV-2 via the intratracheal tract infection in CAG-promoter-driven human angiotensin-converting enzyme 2 transgenic (CAG-hACE2) mice that more than 15 copies of hACE2 genes were tandemly integrated into the mouse genome. The CAG-hACE2 mice showed several severe symptoms of SARS-CoV-2 infection, with definitive weight loss and subsequent death. Acute pneumonia with elevated cytokine and chemokine levels was observed at an early stage of infection in CAG-hACE2 mice infected with SARS-CoV-2. In the developed model, administration of remdesivir, which is antiviral agent, or injection of plasma from immunized mice prevented body weight loss and lethality due to infection with SARS-CoV-2. These results indicated that a highly susceptible model of SARS-CoV-2 infection in CAG-hACE2 mice via the intratracheal tract is suitable for evaluating antibody therapeutics and medicines.

2-P-174

Influence of differences in intratracheal administration methods on phenotype

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There are several methods for intratracheal administration using rodents.
Verified the oropharyngeal suction method and the micro spray method with the following contents.
2% Evans Blue was intratracheally administered by each method under anesthesia with isoflurane, and the distribution of lungs was confirmed.
Mice in which LPS-induced inflammation was induced in the same manner were also prepared. (Acute Lung Injury: ALI model)
BALF was collected 48 hours after LPS administration, and WBC, neutrophils, and monocyte/macrophages were confirmed.
And histopathological examination was also performed.
As described above, introduce the influence of differences in intratracheal administration methods on the phenotype in the acute phase.

2-P-175

Elucidation of inhibitory pathway of guanylyl cyclase-B by sphingosine-1-phosphate.

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Guanylyl cyclase-B (GC-B) is a receptor for C-type natriuretic peptide, which regulates proliferation and migration in fibroblasts in various cells. Here we report that GC-B activity is suppressed by sphingosine-1-phosphate (S1P) specifically through the type 2 S1P receptor ($S1P_2$) in Swiss 3T3 fibroblasts and the type 2 and 3 ($S1P_3$) receptors in HeLa cells. In Swiss 3T3 fibroblasts, S1P-dependent suppression of GC-B activity was not mimicked by an $S1P_1$-specific agonist, and was abrogated by an $S1P_2$-specific, but not an $S1P_3$-specific, antagonist. In HeLa cells, siRNA-mediated depletion of either $S1P_2$ or $S1P_3$ reduced S1P-dependent suppression of GC-B activity, whereas depletion of $S1P_1$ had no effect. S1P-mediated suppression of GC-B activity was blocked by the Rho inhibitor, Clostridium difficile toxin B, and by expression of a dominant-negative RhoA mutant, but not by Rho kinase (ROCK) inhibitors. Toxin B treatment also reduced S1P-induced GC-B dephosphorylation, an established regulatory mechanism of GC-B activity. Furthermore, S1P-dependent suppression of GC-B activity was partially blocked by protein phosphatase 1 and 2A inhibitors. These results are consistent with a mechanism whereby S1P, acting through $S1P_2$ and/or $S1P_3$, inhibits GC-B activity via a Rho-dependent but ROCK-independent pathway that involves activation of a subset of protein phosphatases.

2-P-176

Signaling pathway for proliferation effect of tri-deoxycytidine in neural stem cells

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Nucleic acid fraction (NAF) of hydrolyzed salmon milt extract has been reported to enhance both object recognition and location memories in healthy mice and promote proliferation of primary cultured neural stem cells (pcNSCs). NAF highly contains tri-deoxyribonucleotides, and only tri-deoxycytidine (CCC) among all 64 tri-deoxyribonucleotides significantly promotes pcNSCs proliferation although the underlying mechanisms and its proliferative effect in the brain remain unclear. The purpose of the present study was to clarify the possible mechanism and the effect on neurogenesis *in vivo*. Proteome analysis of the lysates of pcNSCs after treatment with CCC was first performed, showing that CCC up- and down-regulated 81 and 248 proteins, respectively. According to further KEGG pathway enrichment analysis, PI3K-Akt signaling was significantly enriched. Then, we checked expression of proteins related to Akt signaling including phosphorylated Akt (p-Akt) in pcNSCs by western blot analysis. Expression of p-Akt in pcNSCs treated with CCC was higher than that in vehicle-treated control. We also performed intrahippocampal injection of CCC in mice to examine whether or not CCC affects neurogenesis in hippocampal dentate gyrus (DG), which highly contains NSCs. Area of newborn neuron marker doublecortin-positive cells in the hippocampal DG injected with CCC was significantly higher than that in control. These results suggest that CCC has a potential to promote proliferation of NSCs in the brain, and Akt signaling is one of the possible signaling pathways involved.

2-P-177

Hydrolytic activity of acid ceramidase on *N*-acylethanolamines, anti-inflammatory and anorexic lipid mediators: an analysis using saposin D-knockout mice

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N-Acylethanolamines (NAEs) constitute a class of lipid mediators and include palmitoylethanolamide, oleoylethanolamide, and anandamide, which exert anti-inflammatory/analgesic, anorexic, and cannabimimetic actions, respectively. NAE acid amidase (NAAA) is a lysosomal enzyme hydrolyzing NAEs. On the other hand, acid ceramidase (AC) is another lysosomal enzyme hydrolyzing ceramide (*N*-acylsphingosine), which shows 33% amino acid identity with NAAA. We previously showed that not only NAAA but also AC is capable to hydrolyze NAEs by using purified enzyme and cultured cells. Here, we examined NAE-hydrolyzing activity of AC in several tissues by using mice lacking saposin D (SAP-D), a presumed activator protein of AC. As analyzed by RT-qPCR, AC mRNA levels in brain, kidney, and liver were not significantly different between wild-type and SAP-D$^{-/-}$ mice. In contrast, C12-ceramide-hydrolyzing activities in the homogenates of these tissues in SAP-D$^{-/-}$ mice were decreased to 16–32% of those in wild-type mice, reflecting the capability of SAP-D to activate AC. Furthermore, the C12:0-NAE-hydrolyzing activities were also lowered to 15–29% in the tissue homogenates of SAP-D$^{-/-}$ mice. These results suggest a role of AC in the degradation of NAEs in tissues.

2-P-178

Up-regulated miR-199a-3p and miR-126-3p promote adipocyte differentiation by targeting HIF-1α

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Background: Nonalcoholic fatty liver disease (NAFLD) represents the range from simple steatosis to severe steatohepatitis with inflammation and fibrosis. Dysregulated lipid metabolism and lipid deposition are well-known as early stages of NAFLD pathogenesis. Recently, we have found that miR-199a-3p and miR-126-3p are up-regulated in the liver tissue of NAFLD patients. Here we investigated the impact of the miRNAs in adipocyte differentiation and lipid metabolism-related gene expression.

Methods: Transfection of miRNAs into confluent mouse 3T3-L1 preadipocytes were performed by Lipofectamine RNAiMAX. After transfection, cells were differentiated by insulin, dexamethasone, 3-isobutyl-1-methylxanthine, and rosiglitazone for 3 days. Intracellular lipid deposition and mRNA expression were analyzed by Oil red O staining and quantitative RT-PCR, respectively.

Results: Both miR-199a-3p and miR-126-3p enhanced adipocyte differentiation compared to control miRNA. These miRNAs increased CCAAT/enhancer binding protein-α (C/EBPα) and decreased hypoxia-inducible factor (HIF)-1α expression.

Conclusion: It has been reported that HIF-1α reduces C/EBPα expression. Therefore, up-regulated miR-199a-3p and miR-126-3p might promote lipid deposition and adipocyte differentiation by targeting HIF-1α.

2-P-179

Signal transduction mechanism for autocrine secretion of insulin-growth factor type 1 by *S*-allyl-L-cysteine in primary cultures of adult rat hepatocytes.

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S-allyl-L-cysteine (SAC) is a sulfur-containing amino acid contained in garlic. Recent studies have demonstrated that the pharmacological effect of SAC is not only as an antioxidant effect, but also as a cell proliferation effect. In a previous study, we demonstrated that the hepatocyte proliferating effect of SAC was via the IGF-I receptor tyrosine kinase / ERK2 pathway. In addition, the SAC-stimulated hepatocyte proliferation was suppressed by somatostatin, which inhibits the secretion of autocrine factors such as IGF-I. The aim of this study is to further investigate the mechanism of SAC-induced IGF-I secretion in primary cultures of adult rat hepatocytes. The amount of IGF-I secreted from hepatocytes was quantified by ELISA. As a result, SAC significantly increased secretion of IGF-I from cultured hepatocytes at 20 min. The SAC-induced IGF-I secretion was completely suppressed by a JAK2 inhibitor TG101209, a phospholipase C Inhibitor U-73122, and an intracellular Ca^{2+} chelating agent BAPTA-AM. Moreover, phosphorylation of JAK2 was increased rapidly in SAC-stimulated hepatocytes, and the effect was suppressed by TG101209. These results suggest that SAC stimulates JAK2, subsequently activates PLC, which increases membrane phosphatidylinositol turnover and intracellular Ca^{2+} levels, resulting in autocrine IGF-I secretion.

2-P-180

Identification of weak-interacting proteins with Sav1 using BioID2 method

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Sav1 scaffold protein regulates Hippo signaling pathway, which plays an important role in the organ size control and the tumorigenesis. Sav1 interacts with Mst1/2 and Lats1, which comprises a core component of Hippo pathway. In this study, we tried to identify weak interacting proteins by proximity-labeling techniques. Promiscuous biotin ligase, called BioID2, biotinylates the proteins within about 10 nm. BioID2 gene was ligated to 5' end of Sav1 cDNA, which was inserted into tetracycline-inducible expression vector. This expression vector was stably introduced into HEK293 cells. Tetracycline-induced expression was confirmed by western blotting and cell immunostaining. This BioID2-Sav1 was confirmed to interact with Mst1 by co-immunoprecipitation assay. Next, biotinylated proteins was pulled down using biotin binding partner, strep-tactin, conjugated beads. The pulled down fraction contained at least BioID2-Sav1 and endogenous Sav1 (Sav1 homodimerizes). As the results of western blotting by strep-tactin-HRP and silver staining, more than 8 proteins were biotinylated and pulled down. This indicates that the BioID2 method may be useful for identification of novel Sav1 interacting proteins.

2-P-181

Cytoprotective effect by Rab and PRAF proteins

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PRAF3, which is the PRA1 (prenylated Rab acceptor 1) superfamily member, plays crucial roles in membrane traffic as a GDI displacement factor *via* physical interaction with a variety of Rab proteins, as well as in the modulation of antioxidant glutathione through its interaction with EAAC1 (SLC1A1). It is known that the overexpression of PRAF3 induces the toxicity of the host cell, however, the factors capable of cancelling the cytotoxicity remained unknown. Our findings demonstrate that Rab1a can protect from the toxicity of PRAF3-overexpressed human cells. Cytoprotective effects of Rab1a protein could further suggest that PRAF3 and Rab1a are closely related to each other physiologically and genetically.

2-P-182

Analysis of sweat secretion by PACAP using human sweat gland immortalized cells

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Sweat secretion plays an important role in the human body. It is known that the hyperhidrosis and anhidrosis are caused by abnormalities in sweat secretion and can result in severe skin conditions such as pruritus and erythema. However, no effective therapies or therapeutic agents have yet been discovered. Previously, it is reported that Pituitary adenylate cyclase-activating polypeptide (PACAP) promotes sweating, but details of the mechanism of sweat secretion has not been clarified (Sasaki et al., 2017). Therefore, we used immortalized human eccrine gland cells to investigate how sweat secretion is induced by PACAP treatment. Intracellular Ca^{2+} levels were increased in these cells with physiological concentrations of PACAP.However, they were not elevated when cells were concomitantly treated with PA-8, PAC1-R selected antagonist with PACAP. Furthermore, results of immunocytochemistry experiments showed that aquaporin-5(AQP-5) was translocated from the cytoplasm to the cell membrane by PACAP.These results suggest that PACAP promotes sweat secretion by translocation of AQP-5 to the cell membrane in response to increased levels of intracellular Ca^{2+} on the sweat glands. These findings also provide a solid basis for future research initiatives to develop new therapies and therapeutic agents to treat sweating disorders.

2-P-183

The crosstalk between Il-4/IL-13 and S1P/$S1P_2$ signaling in phorbol ester-treated THP-1 MΦs

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We reported that sphingosine 1-phosphate type2 receptor ($S1P_2$)/Rho kinase pathway is necessary for full activation of STAT6 by IL-4/IL-13 in phorbol ester-treated THP-1 macrophages (MΦs) at the 94th Annual Meeting of the Japanese Pharmacological Society. This time, we report the further results. The $S1P_2^{knockout}$ THP-1 MΦs showed lower activities of STAT6 phosphorylation in response to IL-4 or IL-13 stimulation than the wild-type THP-1 MΦs. This decrease in IL-4-induced STAT6 phosphorylation in the $S1P_2^{knockout}$ THP-1 MΦs was prevented by ectopic expression of mouse *S1pr2.* $S1P_2$ regulates the Rho/Rho kinase pathway. Pretreatment of THP-1 MΦs with Rho inhibitor Rhosin inhibited the IL-4-induced increase in STAT6 phosphorylation. STAT activation is tightly controlled by multiple negative regulators of phosphorylation such as protein tyrosine phosphatases (PTPs) and suppressor of cytokine signaling (SOCS). The expressions of SOCS3 in the $S1P_2^{knockout}$ THP-1 MΦs were higher than those in wild-type THP-1 MΦs and the PTP inhibitor vanadate enhanced IL-4-induced STAT6 phosphorylation in the $S1P_2^{knockout}$ THP-1 MΦs, suggesting that SOCS3 and PTPs are involved in the positive regulation of IL-4-induced STAT6 phosphorylation by $S1P_2$. We examined the crosstalk between IL-4/IL-13 and S1P/$S1P_2$ signaling in PMA-treated THP-1 MΦs. Although IL-4/IL-13 stimulation induced the mRNA expression of $S1P_3$, those of $S1P_1$, $S1P_2$ or RhoA were not induced by IL-4/IL-13. Since $S1P_3$ has been reported to activate Rho, it is possible that the Rho-Rho kinase pathway is enhanced by IL-4/IL-13 stimulation by this mechanism. These results suggest that pharmacological inhibition of $S1P_2$/Rho/Rho kinase pathway would attenuate Th2-type immune responses induced by IL-4/IL-13, thereby suppressing allergic diseases.

2-P-184

Development of a simple assay to measure glycerophosphodiesterase GDE4 and GDE7 activities for screening their Inhibitors

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Lysophosphatidic acid (LPA) is a lipid mediator which regulates various biological processes, including cell proliferation, platelet aggregation, and cancer metastasis. Autotaxin (ATX) is a lysophospholipase D (lyso-PLD)-type enzyme that produces LPA in plasma. In contrast, glycerophosphodiesterase GDE4 and GDE7 (GDEs) are intracellular lyso-PLDs producing LPA. ATX and GDEs may play unique roles, which need to be validated with their potent and selective inhibitors. Although fluorescent substrates are useful for high-throughput screening of enzyme inhibitors, no fluorescent substrates for these GDEs have been reported. Here, we examined whether a fluorescent ATX substrate FS-3 could be applied to such assays. The membrane fractions of GDE4- and GDE7-overexpressing cells hydrolyzed FS-3 in the presence of Mg^{2+} and Ca^{2+}, respectively. Using these assay systems, we found that several known ATX inhibitors as well as natural substrates lysophosphatidylcholine and lysophosphatidylethanolamine inhibited GDEs. Furthermore, FS-3 was hydrolyzed by the membrane fraction of LNCaP and MCF-7 cells that endogenously express GDE4 and GDE7, respectively. Finally, our assay system could selectively measure GDEs activities in a mixture of the membrane fractions of GDEs-overexpressing cells. These findings allow high-throughput assays of GDEs and lead to the development of selective inhibitors as well as a better understanding of the biological roles of these enzymes.

2-P-185

Antioxidative activity of ketamine as a direct free radical scavenger

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Purpose

Ketamine, an anesthetic used for general anesthesia, has been reported to have anti-oxidative activity; ketamine is preventive against oxidative stress including ischemia/reperfusion injury and inflammation. We hypothesized that ketamine directly scavenges free radicals thereby acting as an antioxidant.

Methods

Free radical scavenging activity of ketamine was evaluated against six species of free radicals by electron spin resonance spectroscopy with the spin-trapping method. Fluorescence-based assays of cellular viability after oxidative stress was assessed using alamarBlue. Anti-oxidative activity was assessed by TBARS assay.

Results

Ketamine significantly scavenged the following free radicals in dose-dependent manners; hydroxyl radical, *tert*-butoxyl radical, ascorbyl free radical with reaction rate constants around one order of magnitude smaller than those of edaravone, and nitric oxide with three orders of magnitude smaller. Ketamine also scavenged superoxide anion and *tert*-butyl peroxyl radical at higher concentrations. Cellular viability of MRC5 cells exposed to 300 μM hydrogen peroxide was significantly improved in the presence of 1 μM ketamine. Ketamine significantly inhibited lipid oxidation in a dose dependent manner.

Conclusions

Ketamine dose-dependently scavenged multiple free radicals including hydroxyl radical. It is speculated that ketamine is protective of perioperative oxidative stress, at least partially, via non-enzymatic free radical scavenging activity.

2-P-186

Factors affecting medication adherence of older adults in acute care hospitals receiving medication support by nurses

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Purpose

This study aimed to clarify the factors that influence medication adherence of older adults supported by nurses in acute care hospitals.

Methods

An anonymous self-administered questionnaire survey was conducted with nurses working in randomly selected acute care hospitals in Japan. The contents of the survey included 40 items, which assessed medication adherence and prescription background for one elderly case for which the nurses provided medication support. The analysis was done statistically.

Results

Responses were received from 629 nurses. Valid responses included 464 responses, with no defects in the responses to the 40 items assessing medication adherence. The average score of the medication adherence assessment tool was 129.65 points. Adherence was significantly lower in older adults, those using neuropsychiatric medications, and those with longer morbidity and prescription periods for the main illness. Additionally, significantly lower subfactors for continued medication control were noted in cases of neuropsychiatric medication use, long prescription periods, and pharmacist intervention. The result of the binomial logistic regression analysis showed that the factors related to a low total medication adherence score was ≥85 (odds ratio [OR], 2.27; 95% confidence interval [CI], 1.30–3.95) and a history of cardiovascular disease (OR, 0.60; 95% CI, 0.38–0.97).

Conclusion

Older adults exhibiting low medication adherence require careful assessment of adverse drug events and polypharmacy issues as well as active support.

2-P-187

Roxadustat enhances exercise performance in female mice

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Roxadustat (Rox) is an oral hypoxia-inducible factor prolyl hydroxylase (HIF-PH) inhibitor. HIF activating agents may be abused for doping; however, there is limited information regarding their doping effects. We herein investigated the effects of Rox on exercise performance in trained male and female FVB/N mice. Mice were loaded with forced wheel running for one hour 5 times a week. Rox (70 mg/kg) was administered 3 times a week for three weeks to one group and vehicle to the other group. Exercise performance was evaluated once a week as the number of arrivals at the halfway line of a flow rate-adjustable swimming pool. The Rox treatment significantly increased serum iron levels, but not hematocrit levels in mice. At 3 weeks of administration, the blood lactate levels immediately after forced wheel running were significantly lower in the Rox group, compared to the control group, regardless of gender. On the other hand, the Rox treatment significantly increased the number of arrivals on the forced swimming test in female mice only. These results suggested that the enhancing effect of Rox on exercise performance in mice was sex-specific, and that erythropoiesis was not related to this effect.