3-P-001

Molecular mechanism of axon collateralization in corticospinlal tract by receptor protein tyrosine phosphatase

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Corticospinal neurons project to various subcortical targets including the basilar pons via axon collateral branches. Previously, we showed that the pons produces diffusible chemoattractant to promote branch initiation. However, the molecular mechanism underlying axon collateralization is still unclear. To identify molecules that are essential for axon collateralization, we performed knockdown experiments for various receptors expressed in the corticospinal neurons. We found that knockdown of one of the receptor protein tyrosine phosphatases (RPTPs), tentatively called RPTP1, of the corticospinal neurons at E12.5 significantly suppressed the formation of axon collaterals to the pons. We purified proteins interacting with extracellular region of RPTP1 from the brain lysates by affinity chromatography in order to seek for ligands for RPTP1, and we identified several secreted proteins, proteoglycans and transmembrane proteins by mass spectrometry. We found that some candidate molecules were directly bound to RPTP1, and that the formation of axon collaterals was suppressed by knockdown of some of these molecules.

3-P-002

The effects of $GABA_A$ receptor modulators on experimental febrile seizures.

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Hyperthermia-induced febrile seizures (FSs) are the most common seizures during childhood, and prolonged complex FSs can result in the development of epilepsy. Currently, $GABA_A$ receptor modulators such as benzodiazepines and barbiturates are used as medications for FSs with the aim of enhancing GABA-mediated inhibition of neuronal activity. However, it is still up for debate whether these enhancers of GABAergic neurotransmission are effective for FSs because GABA can depolarize immature neurons with relatively higher levels of the intracellular Cl^- that overwhelms mature neurons in the developing brain. Here, we performed simultaneous video-local field potential (LFP) monitoring to determine whether benzodiazepines and barbiturates affected the phenotypes of FSs in postnatal mice. We found that both benzodiazepines and barbiturates exacerbated the behavioural and electrophysiological phenotypes of the induction phase of experimental FSs. We further found that the deteriorated phenotypes were suppressed when $Na^+K^+2Cl^-$ co-transporter isoform 1 (NKCC1), which mediates Cl^- influx, was blocked by treatment with the diuretic bumetanide. Thus, our findings suggest that pharmacological enhancement of GABAergic signalling could aggravate seizure activity in the early phase of FSs, phenomena preventable with the use of NKCC1 blockers.

3-P-003

Wide-band EEG analysis of epileptic seizures during pilocarpine-induced acute status epilepticus and chronic temporal lobe epilepsy in rats

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Ictal-direct current (DC) shifts precede high frequency oscillations (HFOs) and conventional ictal EEG patterns in human epilepsy patients. However, the role of DC shifts in ictal EEG activities and HFOs is still unknown. Here, we analyzed the wide-band EEG recorded from the PILO-induced acute SE model (400 mg/kg, i.p.) and the PILO-induced chronic TLE model (after 11-13 weeks from 450 mg/kg, i.p.). Our results showed that all seizures during PILO-induced SE yielded DC shifts, which appeared immediately after the onset of ictal EEG patterns and HFOs. Meanwhile, PILO-induced chronic TLE showed two types of ictal EEG patterns, i.e., the one starts with DC shifts followed by conventional ictal EEG patterns and HFOs whereas the another starts with the conventional ictal patterns without DC shifts. These findings highlight the difference in the ictal-DC shifts mechanisms between acute and chronic epilepsy stages, the ictal-DC shifts in acute SE are probably due to massive elevation of extracellular K^+ originated from neural firing whereas those in chronic TLE possibly due to the dysfunction of astrocytic K^+ buffering.

3-P-004

How much balance between excitatory and inhibitory neurons is suitable for detection of seizure liability in hiPSC-derived neurons ?

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Multi-electrode array (MEA) assays using human induced pluripotent stem cell (hiPSC)-derived neurons are expected to predict the convulsion toxicity of new drugs. Although the balance between excitatory and inhibitory inputs is important in convulsive seizure, the optimal proportion is not known. In this study, we evaluated the spontaneous firing properties and the responses to convulsants in hiPSC-derived cortical neuronal network, in which the ratio of Glutamatergic (Glu) and GABAergic (GABA) neurons are 88 : 12, 84 : 16, 74 : 26, 58 : 42, and 48 : 52. The network with a high percentage of excitatory neurons showed short synchronized burst firings (SBFs) in spontaneous firings. On the other hand, the network with high inhibitory neurons showed the SBF with long period. In drug-induced seizure activities, there was no remarkable dose responses in high percentage of excitatory neurons. On the other hand, the network with high inhibitory neurons showed significant activity changes with a lot of convulsants regardless of GABA receptor inhibitor. These results suggest that a higher proportion of GABA neurons compared with real brain is more effective in detecting drug-induced seizure toxicity.

3-P-005

MEK inhibitor suppressed the regeneration of dopaminergic neurons in planarian *Dugesia japonica*

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Parkinson's disease (PD) is characterized by loss of dopaminergic (DA) neurons. Our previous studies revealed that DA neurons in Planarian *Dugesia Japonica* decreased after the amputation of their heads and the injection of 6-hydroxydopamine (6-OHDA), a DA neuron specific toxin, but regeneration of DA neurons was observed within 7 days. Recently, MAPK/ERK signaling was identified as a key regulator of planarian regeneration. In this study, we checked the effect of MEK inhibitors in the regeneration of DA neurons. Loss of DA neurons in Planarians of SSP strain were induced by the amputation of their heads and injection of 6-OHDA into their pharynxes. Regeneration of DA neurons were detected by immunohistochemical analysis with DjTH antibody at 7 days after amputation and at 5 days after 6-OHDA injection. Inhibition of MEK, upstream of ERK, by treatment of U0126 (10 μM) suppressed the regeneration of DA neurons. Furthermore, 5 days after 6-OHDA injection, phosphorylated-histone H3 (H3P)-positive stem cells were increased in head region, but MEK inhibition decreased H3P-positive cells. These data suggested that MEK/ERK signaling is a key regulator in the process of regeneration of DA neurons via proliferation of intrinsic stem cells.

3-P-006
Tankyrase is involved in neurite growth and synaptogenesis.

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Poly(ADP-ribosy)lation is a posttranslational modification of proteins by transferring poly(ADP-ribose) (PAR) to acceptor proteins by the action of poly(ADP-ribose) polymerase (PARP).
Tankyrase, also known as PARP5, involved in various processes such as Wnt signaling pathway, telomere length and vesicle trafficking.
In this study, we investigated whether tankyrase regulates PAR synthesis in neurons, which affects neuronal functions. Tankyrase and PAR were localized in the soma and synapses of murine hippocampal primary neurons. Pharmacological inhibition of Tankyrase suppressed PAR production, suggesting that tankyrase participates in PAR synthesis in hippocampal neurons. In addition, pharmacological inhibition of tankyrase inhibited neurite outgrowth and the number of pre- and postsynapses in neurons. Using a pull-down assay with a GST-macrodomain, which can bind ADP-ribose, some proteins expressed in neurons were poly(ADP-ribosy)lated. These findings are consistent with the fact that tankyrase catalyzes poly(ADP-ribosy)lation to acceptor proteins, resulting in enhanced neurite growth and synaptogenesis in hippocampal neurons.

3-P-007

Aβ Production in Neurons was Promoted by Leptomeningeal cells after *Porphyromonas gingivalis* Infection

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Leptomeningeal cells play an important role in preventing brain from the peripheral virulence factors. Porphyromonas gingivalis (P.g) is the main pathogenic bacteria of periodontitis and reported involved in the linkage of systemic inflammation and Alzheimer's disease. However, the molecular mechanism underlines the transduce effect from peripheral to central nervous system of leptomeningeal cells are still not clear. In this study, we aimed to elucidate the effects of leptomeningeal cells in neurodegeneration after exposure to P.g using cultured cells. The pro-inflammatory mediators in leptomeningeal cells were significantly increased after exposure to *P.g* from 1h to 12h. Exposure to P.g (MOI=10) for 3h significantly increased the expression of pro-IL-1β, mIL-1β and secretion of IL-1β. The Aβ processing genes in neurons were significantly increase following the culture with LCM for 24h. These observations suggested that leptomeningeal cells may induce the neurodegeneration after exposure to *P.g* thus providing a bridge between peripheral infection and neuronal neurodegeneration in Alzheimer's disease.

3-P-008
Evaluation of macaque monkey promoter sequence in mice

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[Introduction]
Due to the complexity of the brain, specific expression of genetic tools is necessary for identifying the responsible circuit for mental disorders. From this perspective, identification of promoter specifically active in a certain set of neurons is important. To this end, although many promoters of rodents have been identified, those of non-human primates such as macaque have been rarely identified. Here, we isolated the macaque promoter sequences and validated their functionality in mouse brain by using lentiviral vectors (LVV).[Method]Upstream sequence to somatostatin (SST), serotonin transporter (SERT), substance P (SP), vesicular acetylcholine transporter (vAChT), cholecystokinin (CCK), enkephalin (PENK), and parvalbumin (PV), was isolated from a monkey (*Macaca fascicularis*) gDNA.[Result]One week after LVV injection, we performed histological analysis. We found that 93.8 ± 4.1% of Venus(+) cells were SST(+) when SST promoter (0.3 k) was used. Similarly, colocalization rate for LVV bearing 0.5 k CCK promoter were 88.0 ± 3.3%, 0.7k PV were 84.0 ± 1.4%, 1.9k SERT were 93.8 ± 0.9%, 1.9k vACHT were 82.8 ± 3.9%, 0.8k SP were 91.7 ± 3.8% and 0.9k PENK were 88.0 ± 1.7% with colocalization . These findings suggest relative position of promoter leading to specificity differs from gene to gene, implying the importance of evaluation in vivo. The LVV we developed may be advantageous for application of genetic tools to non-human primates.

3-P-009

Acetylcholinergic regulation of the Kir4.1 channel and brain-derived neurotrophic factor (BDNF) expression in astrocytes.

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Astrocytes regulate neuronal excitability by maintaining ion homeostasis and secreting neuroactive substances. We previously showed that blockade or expressional knockdown of inwardly rectifying potassium (Kir) 4.1 channels elevate BDNF expression in astrocytes (Int. J. Mol. Sci., 19, 3313, 2018). In order to explore the neural factors influencing on the Kir4.1-BDNF system, we investigated the effects of acetylcholinergic agents on mRNA expression of Kir4.1 and BDNF in primary cultured astrocytes. Treatment of astrocytes with acetylcholine inhibited Kir4.1 expression and increased BDNF expression in a concentration-related manner. Both inhibition of Kir4.1 and enhancement of BDNF expression by acetylcholine were antagonized by mACh antagonist atropine, but not by nACh antagonist mecamylamine. In addition, inhibition of Kir4.1 expression by acetylcholine was significantly antagonized by the selective M_1 antagonist pirenzepine, which also inhibited acetylcholine-induced BDNF expression. The present results strongly suggest that acetylcholine inhibits Kir4.1 expression and increases BDNF expression via the activation of M_1 receptor in astrocytes.

3-P-010

Changes in astrocytic Kir4.1 channel expression and microglial morphology after pilocarpine-induced status epilepticus in rats

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Glial cells (astrocytes and microglia) play important roles in modifying epileptogenesis. Especially, astrocytic Kir4.1 channels regulate neuronal excitability by buffering excessive synaptic K^+ ions, preventing convulsive seizures. To clarify the changes in astrocytes and microglia in animal model of temporal lobe epilepsy, we analyzed the changes in Kir4.1 channel expression, number and morphology of astrocytes and microglia in early stage (day 3) after pilocarpine (PILO)-induced status epilepticus (SE). Our results showed that both Kir4.1- and GFAP-immunoreactivity-positive astrocytes were markedly reduced by PILO-induced SE. In contrast, the number of microglia was elevated in most regions examined. More specifically, microglia of amoeboid form increased in most temporal lobe regions, while those of ramified, hypertrophied and rod forms elevated in the hippocampus and amygdala. Finally, seizure sensitivity of animals was already increased at day 3 after PILO-induced SE. Our results suggest that the above changes in the astrocytes and microglia features are involved in elevation of seizure susceptibility after PILO-induced SE.

3-P-011
Fstl1 mediates polyI:C-induced neuronal impairment.

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Viral infection in perinatal period may affect early brain development, which increases the risk for psychiatric disorders such as schizophrenia. Our previous findings showed that polyriboioinic-polyribocytidylic acid (polyI:C) treatment in neonatal mice, which mimics virus infection by inducing innate immune system, leads to behavioral abnormality in adulthood. Furthermore, polyI:C treatment induces the expression of interferon-induced transmembrane protein 3 (Ifitm3) in astrocytes but not neurons. Ifitm3 may mediate polyI:C-induced neuronal impairment through the induction of humoral factors secreted from astrocytes. However, it remains unknown which molecular entity leads to the neuronal impairment. We screened humoral factors secreted from astrocytes by proteomic approach. As a result, we identified Follistatin like-1 (Fstl1) as a candidate astroglial factor. Astrocyte condition medium (ACM) from secondary cultured astrocytes treated with polyI:C contained a significantly higher amount of Fstl1 protein than the level in control ACM. Increase in Fstl1 level by polyI:C treatment was not observed in astrocytes from Ifitm3 KO mice. Treatment of recombinant Fstl1 on primary cultured neurons induced an impairment of neurite elongation, whereas, knockdown of Fstl1 in astrocytes diminished the neuronal impairment caused by treatment with ACM. These results suggest that astrocyte-derived Fstl1 plays a crucial role in polyI:C-induced neuronal impairment.

3-P-012

Effects of purine and pyrimidine nucleosides on hydrogen peroxide-induced thymidine incorporation

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We have found that cultured differentiated astrocytes pretreated with N^6, $2'$-*O*-dibutyryladenosine 3',5'-cyclic monophosphate (DBcAMP), a permeable analogue of cAMP, incorporate thymidine, but not uridine, via specific nucleoside transporters, ENT2 and CNT3, into TCA insoluble fraction for repair on DNA injury in the presence of hydrogen peroxide (H_2O_2) at an early time, and these phenomena are specific in differentiated astrocytes, but not undifferentiated astrocytes and neurons. Purine and pyrimidine nucleosides are DNA and RNA precursors consisting of base and ribose.

We studied the influence of purine and pyrimidine nucleosides on H_2O_2-induced thymidine incorporation into cultured astrocytes. Adenosine and guanosine, RNA precursor purine nucleosides, decreased H_2O_2-induced thymidine incorporation. On the other hand, Deoxyadnosine and deoxyguanosine, DNA precursor purine nucleosides, increased it. Cytidine, RNA precursor pyrimidine nucleoside, did not influence it, but deoxycitidine, DNA precursor pyrimidine nucleoside, decreased it.

These results indicate that H_2O_2-induced thymidine incorporation into cultured astrocytes are increased synergistically by purine nucleosides, but not pyrimidine nucleosides.

3-P-013

Protective effects of nicotinic acetylcholine receptor ligands via different mechanisms against inflammatory microglia-induced dopaminergic neuronal death.

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Inflammatory microglial activation is implicated in progressive dopaminergic neuronal loss of Parkinson's disease. Although α7 nicotinic acetylcholine receptors (nAChRs) have been reported to be expressed in microglia, the function of them is unclear. Here, we investigated the effect of nicotine or PNU-120596 (PNU), a positive allosteric modulator of α7 nAChRs on dopaminergic neuronal death induced by microglial activation. Both nicotine and PNU inhibited LPS/IFNγ-induced dopaminergic neuronal death in primary mesencephalic cultures. PNU suppressed nitric oxide release induced by LPS/IFNγ, whereas nicotine had no effect. Methyllycaconitine, an α7 nAChR antagonist, canceled the nicotine-induced neuroprotection, but did not affect PNU-induced suppression of nitric oxide release. Nicotine induced the phosphorylation of Akt. LY294002, an inhibitor of PI3K, suppressed the neuroprotective effect elicited by nicotine. On the other hand, PNU inhibited the phosphorylation of JAK2 and STAT1 induced by IFNγ in primary microglia. These results suggest that nicotine and PNU suppress dopaminergic neuronal death mediated by microglial activation through the different mechanisms.

3-P-014

Effects of histone deacetylase inhibitor on lipopolysaccharide-induced cognitive impairment.

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[BACKGROUND]
Microglia are the major type of glial cells in the central nervous system and crucial in inflammatory response such as the release of pro-inflammatory cytokines. Several studies showed that inflammations are involved in cognitive disorders including Alzheimer's disease. However, the underlying mechanisms are unclear. Hence, we investigated whether epigenetic modifications are involved in the inflammations-evoked cognitive impairments.
[METHOD]
Male ddY-mice (6 weeks) were intraperitoneally injected with lipopolysaccharide (LPS). Twenty-four hours after LPS injection, the cognitive function was determined by novelty objective recognition test. Pretreatment with a non-selective histone deacetylase (HDAC) inhibitor suberoyl anilide hydroxamic acid (SAHA, Vorinostat) was injected 1 hour before LPS injection.
[RESULT]
The LPS treated-mice were showed the cognitive impairment and microglia activation in the hippocampus. Pretreatment with SAHA suppressed LPS-evoked cognitive impairment.
[CONCLUSION]
These data suggest that inflammation-induced cognitive disfunction were evoked by epigenetic modification such as the acetylation of histone. Furthermore, HDAC inhibitors could be the potential drug target for cognitive disorders.

3-P-015

The nuclear receptor REV-ERBs suppress the pro-inflammatory responses in cultured microglia

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The orphan nuclear receptor REV-ERBs exert as transcriptional regulatory factors, and have functions in the regulation of circadian rhythm, inflammation and metabolism. Microglia are crucial in inflammatory responses such as production of pro-inflammatory molecules in the CNS, and involved in the development of various neurodegenerative diseases. However, the role of microglial REV-ERBs in inflammatory responses has yet to be elucidated.

METHOD

Primary microglia were prepared from cerebral cortices of neonatal Wistar rats. Expression levels of mRNA or protein were examined by real-time PCR or western-blotting, respectively.

RESULT

Treatment of cultured microglia with specific REV-ERBs agonist SR9009 prevented the lipopolysaccharide (LPS)-induced mRNA expression of pro-inflammatory cytokines interleukin-1β, interleukin-6 and tumor necrosis factor. Furthermore, treatment with SR9009 inhibited LPS-induced phosphorylation of p38 and p65 subunit of NF-kB.

CONCLUSION

The current study suggests that REV-ERBs contribute to the regulation of expression of pro-inflammatory molecules through the inhibition of p38 and NF-kB in cultured cortical microglia.

3-P-016

Brazilian Green Propolis Suppresses Hypoxia-Induced Neuroinflammatory Responses in Microglia

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Hypoxia has been recently proposed to drive microglia to produce proinflammatory cytokines, including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and IL-6. Considering the fact that propolis has hepatoprotective, antitumor, antioxidative and anti-inflammatory effects, it may have protective effects against the hypoxia-induced neuroinflammation. In the present study, propolis (50 μg/ml) was found to significantly inhibit the hypoxia-induced cytotoxicity and the release of proinflammatory cytokines. Furthermore, propolis significantly inhibited the hypoxia-induced generation of reactive oxygen species (ROS) and the activation of nuclear factor-κB (NF-κB) in microglia. Moreover, systemic treatment with propolis (8.33mg/kg, 2 times/day, ip) for 7 days significantly suppressed the microglial expression of IL-1β, TNF-α, IL-6 and 8-oxo-deoxyguanosine in the somatosensory cortex of mice subjected to hypoxia (10% O_2, 4 h). These observations indicate that propolis suppresses the hypoxia-induced neuroinflammation through inhibition of the NF-kB activation in microglia. Therefore, propolis can be used as pharmacological intervention preventing cognitive deficits in aging and aging-related neurodegenerative diseases.

3-P-017

Oligodendrocytes and oligodendrocyte progenitor cells facilitate the blood-brain barrier integrity

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The blood-brain barrier (BBB) is an essential property of cerebrovasculature for maintaining the highly specialized brain microenvironment. Recent studies suggest that an interaction between brain endothelial cells (BECs) and their neighboring cells is required for BBB function. Here, we presented the oligodendrocytes and oligodendrocyte progenitor cells (OPCs) as a positive regulator of BBB integrity. We found that the treatment of AG1296 (PDGFRα inhibitor) attenuated the facilitatory effects of OPCs on BBB function, suggesting that OPCs upregulate BBB integrity via PDGF-BB/PDGFRα pathway between BECs and OPCs. On the other hand, an inhibition of PDGFRα failed to affect the oligodendrocyte-induced BBB upregulation, suggesting that oligodendrocytes enhance BBB function through pathways other than PDGF-BB/PDGFRα signaling. Further studies are required for the understanding of the mechanisms underlying BEC-OPC and BEC-oligodendrocyte interactions in regulating BBB integrity.

3-P-018

Dopamine D_{2L} receptor deficiency causes stress vulnerability through 5-HT_{1A} receptor dysfunction in serotonergic neurons.

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Mental disorders are caused by contributing to genetic and environmental factors. We here show that deficiency of a genetic risk factor $D_{2L}R$, an isoform of dopamine D_2 receptor (D_2R) causes psychosocial stress vulnerability through a serotonin (5-hydroxytryptamine, 5-HT) receptor 5-$HT_{1A}R$ dysfunction on serotonergic neurons in mouse brain. Exposure to forced swim (FS) stress significantly increases anxiety- and depressive-like behaviors in $D_{2L}R$ knockout ($D_{2L}R$-KO) male mice. 8-OH-DPAT a 5-$HT_{1A}R$ agonist, which mimics the effect of antidepressants, fails to response the stress-induced behaviors in $D_{2L}R$-KO mice. In FS-stressed $D_{2L}R$-KO mice, elevated 5-HT release in the medial prefrontal cortex (mPFC) and upregulation of 5-HT homeostasis related transcripts through Pet1 in the dorsal raphe nucleus (DRN) are observed. $D_{2L}R$ forms heteromer with 5-$HT_{1A}R$ in serotonergic neurons, and 5-$HT_{1A}R$-activated G protein-activated inwardly rectifying potassium (GIRK) conductance is inhibited in $D_{2L}R$-KO serotonergic neurons. Finally, we show that $D_{2L}R$ overexpression in serotonergic neurons of the raphe nucleus reduces the stress vulnerability in $D_{2L}R$-KO mice. Taken together, the collapse of negative feedback control in $D_{2L}R$/5-HT_{1A} inhibitory G-protein-coupled heteromer is caused stress vulnerability.

3-P-019

The deficit of quinolinic acid phosphoribosyltransferase induces hypolocomotion and cognitive impairment through impairment of dopaminergic neuronal function

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Quinolinic acid (QA) is a neurotoxic and implicated in the neurological disorders. QA is metabolized by quinolinic acid phosphoribosyltransferase (QPRT). However, the physiological roles of QPRT in central nervous system are still unclear. To investigate the roles of QPRT in emotional and cognitive functions, QPRT KO mice were subjected to several types of neurobehavioral tests. The KO mice decreased locomotor activity in novel environment, prolonged escape latency in Barns maze test, and decreased alternation behavior in Y-maze test. In the KO mice, the contents of homovanillic acid (HVA) and 3,4-Dihydroxyphenylacetic acid (DOPAC), and the ratios of HVA/ dopamine (DA) in the nucleus accumbens and DOPAC/DA in the prefrontal cortex were decrease. In the immunohistochemistry, the number of tyrosine hydroxylase (TH)-positive neuron was less compared with wild mice. Taken together, the present findings suggest a novel role of QPRT in hypolocomotion and cognitive impairment in relation to impairment of dopaminergic functions in the nucleus accumbens and prefrontal cortex, respectively.

3-P-020

Acute methamphetamine administration impairs cognitive function in a mouse model of schizophrenia with both of CNV and SNV in ARHGAP10 gene that confer high risk of schizophrenia with severe clinical symptoms.

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ARHGAP10, a member of Rho GTPase-activating protein (RhoGAP) superfamily which contributes to neuronal development, polarization and function. We developed a mouse model of schizophrenia with both a deletion-type copy-number variation (CNV) and a single-nucleotide variation (SNV) in the RhoGAP domain of ARHGAP10 gene (ARHGAP10 mutant mice). Methamphetamine (METH) is one kind of highly addictive drug which induces cognitive deficit in human and rodents. In this study, we investigated the effect of METH on performance of ARHGAP10 mutant mice and wild-type mice in the touchscreen-based visual discrimination task. Mice were initially trained to discriminate between a pair of stimuli. On the testing day, mice were injected with either saline or METH (0.3 mg/kg, intraperitoneal injection) 30 min before the test. METH-treated ARHGAP10 mutant mice showed a marked reduction of percentage of accuracy compared with METH-treated wild-type mice as well as saline-treated ARHGAP10 mutant mice. We demonstrated by using the translatable visual discrimination task that cognitive function in ARHGAP10 mutant mice are highly vulnerable to acute METH treatment.

3-P-021

Study on the relationship between Parkinson's disease and Diacylglycerol kinase θ

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Study on the relationship between Parkinson's disease and Diacylglycerol kinase θ

Parkinson's disease (PD) is one of the neurological disorders associated with aging and caused by the death of dopamine (DA) secretory cells. Recently, genome wide association study on non-Hispanic whites and Chinese Han revealed that the same single nucleotide polymorphism (SNP) was found between exon 2 and 3 of diacylglycerol kinase θ (DGKθ) in PD patients. In addition, Phosphatidic acid (PA) produced by DGKθ binds to α-synuclein (α-sync), resulting in aggregation of α-sync. These facts suggested involvement of DGKθ in PD. However, the direct relationship between PD and DGKθ is completely unknown. So, this study was aimed to clarify the relationship between PD and DGKθ.

First, mRNA and protein levels of DGKθ were compared, in SH-SY5Y cells treated with KCl, H_2O_2 to mimic PD conditions. As a result, both mRNA and protein levels of DGKθ were elevated in the PD model cells. Next, to investigate the relationship between DGKθ and α-sync, SH-SY5Y cells overexpressing GFP and DGKθ-GFP were treated with KCl, H_2O_2, and aggregation of α-sync was examined by immunofluorescent staining. As a result, overexpression of DGKθ-GFP induced α-sync aggregation without KCl, H_2O_2 treatment.

3-P-022

Enriched environment restrains autism spectrum disorder-like phenotypes in mice prenatally exposed to valproic acid.

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We have previously reported that mice prenatally exposed to valproic acid (VPA) at embryonic day 12.5 exhibit autism spectrum disorder (ASD)-like phenotypes such as social deficits and cognitive impairment, and reduced dendritic spine density in the hippocampal CA1 region. Several studies show that enriched environment (EE) ameliorates some abnormal behaviors in ASD rodent models, but it is unclear whether EE improves cognitive impairment. Here, we examined the effects of early EE on ASD-like phenotypes and neuromorphological changes in prenatal VPA-exposed mice. Mice were housed for 4 weeks from 4 weeks of age under either a standard environment or EE. EE increased BDNF mRNA levels in the hippocampus of both control and VPA-exposed mice. In addition, the EE improved social deficits and cognitive impairment, but not hypolocomotion, in VPA-exposed mice. Prenatal VPA exposure caused decreases in hippocampal mRNA levels of PSD-95 and Shank2, in addition to loss of dendritic spines in the hippocampal CA1 region. The EE improved these hippocampal changes. These findings suggest that the EE improved most ASD-like phenotypes including cognitive impairment in the VPA-exposed mice by enhancing dendritic spine function.

3-P-023

Treatment of pregnant mice with valproic acid caused an increase in miR-132 levels in the embryonic brain.

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We have previously reported that mice prenatally exposed to valproic acid (VPA) at embryonic day 12.5 (E12.5) exhibit autism spectrum disorder (ASD)-like phenotypes. On the other hand, small non-coding RNAs known as microRNA (miRNA) have emerged as potential players in the pathology of neurodevelopmental disorders such as ASD. Here, we examined effects of prenatal VPA exposure on levels of miRNAs, especially the brain specific and enriched miRNAs, in the mouse embryonic brain. VPA exposure at E12.5 immediately increased miR-132 levels, but not miR-9 or miR-124 levels. The VPA exposure at E12.5 increased mRNA levels of Arc, c-Fos and BDNF prior to miR-132 expression. In contrast, VPA exposure at E14.5 did not affect miR-132 levels. The VPA exposure at E12.5 further decreased mRNA levels of MeCP2 and p250GAP, both of which are molecular targets of miR-132. Moreover, RNA sequence analysis revealed the VPA exposure-induced changes in several miRNA levels other than miR-132. These findings suggest that the alterations in neuronal activity-dependent miRNAs levels, including increased miR-132, in the embryonic period, at least in part, underlie the ASD-like phenotypes and cortical pathology in mice prenatally exposed to VPA.

3-P-024

FABP3 in the Anterior Cingulate Cortex Modulates the Methylation Status of the Glutamic Acid Decarboxylase$_{67}$ Promoter Region

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The anterior cingulate cortex (ACC) is important for emotional and cognitive processing. However, the mechanisms that underlyingie the its involvement of the ACC in the control of behavioral responses are largely unknown. Here, we show that fatty acid-binding protein 3 (FABP3), a polyunsaturated fatty acid chaperone, regulates GABA synthesis through transcriptional regulation of *Gad67* in the ACC and that methionine restores normal *Gad67* expression and behaviors in *Fabp3* knockout mice. Future investigation into the cellular and molecular mechanisms that underlie how methionine treatment restores the phenotypes caused by FABP3 deficiency may shed light on the pathomechanism of various psychiatric diseases with abnormal behaviors. Fabp3 knockout mice are a useful animal model for exploring the involvement of epigenetics in these mechanisms.

3-P-025

SKF105111 improves the autism spectrum disorder-like symptoms in ovariectomized mice

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Autism spectrum disorder (ASD) is neurodevelopmental disorders characterized by the sociability deficit and repetitive behavior. We focus on allopregnanolone (ALLO), a neurosteroid synthesized from progesterone, as a candidate factor for the onset of ASD. In our previous reports, SKF105111 (SKF), an inhibitor of 5α-reductase type I and II, induced a decline in brain ALLO content and ASD-like behaviors in not female but male mice. We also demonstrated that brain ALLO content decline and sociability deficit also appear in ovariectomized (OVX) mice. In this study, we analyzed the effects of SKF on ASD-like symptoms in OVX mice. Six-week-old mice received ovariectomy, and SKF (40 mg/kg, i.p.) were treated before 3 hours starting each behavioral test. Contrary to our expectations, brain ALLO content decline was reversed by SKF in OVX mice. SKF also attenuated OVX-induced sociability deficits and social anxiety-like behavior from the results of the 3-chamber, resident-intruder, and mirror-chamber test. These results suggest that SKF improves ASD-like symptoms of OVX mice in contrast to male mice's results. The different susceptibilities to SKF between male and female mice may give us a new insight into the sexual difference in the incidence of ASD.

3-P-026

Vulnerability for onset of depression induced by striatal Shati/Nat8l in mice

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[Background] The number of patients that have depressive disorder is increasing. However, the mechanism of depression onsets has not been completely revealed. We have previously identified Shati/Nat8l (Shati), an *N*-acetyltransferase, in the brain using a mouse model of psychosis. We reported that Shati is related to developmental disorder and methamphetamine dependence. In this study, we revealed the involvement of Shati in the vulnerability of major depression.
[Methods] We generated the Shati-overexpressed mice by injecting an adeno-associated virus into the dorsal striatum, followed by exposed the subthreshold micro social defeat stress (MSDS).
[Results] Shati-overexpressed mice showed the impairment of social interaction and sucrose preference after the MSDS. These depression-like behaviors were restored by fluvoxamine and LY341495 injection prior these tests. Furthermore, the intracerebral administration of fluvoxamine restored.
[Conclusions] Taken together, Shati in the striatum has an important role in the vulnerability of depression onsets by regulating serotonergic neuronal system. Our study suggested the new pathways induce depression like-behaviors, and Shati in the striatum might be a new target for medical tools for depression.

3-P-027

Possibility of the β-adrenoceptor dependent synaptic plasticity in the bed nucleus of stria terminalis contribute to the lipopolysaccharide-induced depressive-like behaviors

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We previously reported that the β-adrenoceptor in the bed nucleus of stria terminalis (BNST) regulate the induction of learned despair in mice. It has been reported that the stimulation of β-adrenoceptor with NMDA receptor in BNST causes the long-term synaptic potentiation (LTP). Therefore we investigated whether the β-adrenoceptor dependent LTP in BNST contribute to the lipopolysaccharide (LPS)-induced behavioral despair. We performed bilateral intra-BNST injection of propranolol, a β-adrenoceptor blocker in awake mice 30 min prior to LPS. Bilateral intra-BNST injection of propranolol alone affected neither the immobility time during tail suspension test (TST) nor sucrose preference. However, bilateral intra-BNST injection of propranolol with MK-801, a non-competitive NMDA receptor antagonist, completely prevented the LPS-induced increase in the immobility time during TST. The LPS-decreased body weight, locomotor activity and sucrose preference were not affected by co-applications of propranolol and MK-801. These results indicated the possibility that the β-adrenoceptor and NMDA receptor-dependent LTP in BNST contribute to the LPS-induced behavioral despair.

3-P-028

The role of Ca^{2+} impermeable AMPA receptor in the bed nucleus of stria terminalis in the lipopolysaccharide-induced behavioral despair

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We previously reported that the lipopolysaccharide (LPS)-induced depressive-like behavior is associated with α_1-adrenoceptor dependent membrane AMPA receptor GluR1 subunit downregulation in the reward system. However, induction of membrane GluR1 downregulation in specifically reward system caused anhedonia, but not despair behavior. Recently, we found that α_1-adrenoceptor in the bed nucleus of stria terminalis (BNST) regulate the learned despair in mice. Since the α_1-adrenoceptor in BNST dependent long-term synaptic depression is induced via increase in the Ca^{2+} impermeable AMPA receptor by RNA editing of GluR2, we investigated whether Ca^{2+} impermeable AMPA receptor in BNST contribute to the LPS-induced despair behavior. To mimic the GluR2 RNA editing, we bilaterally injected 1-Naphthylacetyl spermine (Naspm), a Ca^{2+} permeable AMPA receptor blocker, into BNST in awake mice 24 h after LPS injection. Naspm did not affect the body weight, locomotor activity and sucrose preference in LPS-challenged mice. However, immobility time during the tail suspension was significantly increased by Naspm. We suggest that the GluR2 RNA-editing in BNST contribute to the LPS-induced behavioral despair in mice.

3-P-029

Involvement of glutamate receptors in the impairment of social behaviors induced by social defeat stress exposure as juveniles

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Glutamatergic systems play a critical role in the pathophysiology and treatment of stress-related disorders. In the present study, we conducted behavioral and neurochemical experiments to reveal involvement of glutamate receptors in the impairment of social behaviors induced by stress exposure as juveniles. Acute administration of ketamine, a non-competitive NMDA receptor antagonist and subsequent AMPA receptor stimulation attenuated the impairment of social behaviors in adolescent mice exposed to social defeat stress as juveniles. NBQX, a selective AMPA receptor antagonist prevented the attenuating effect of ketamine on the impairment of social behaviors. Although there were no significant changes in the ratios of phosphorylated protein of some NMDA subunits, that of AMPA receptor GluA1 subunit was significantly increased in the hippocampus of non-tested, defeated mice. In non-tested, defeated mice, ketamine increased the hippocampal total protein level, but not the ratio of phosphorylated protein of GluA1. These results suggest that exposure to social defeat stress as juveniles induces the impairment of social behaviors in adolescents through the functional changes in AMPA receptors.

3-P-030

Short-term but not long-term exercise ameliorates chronic mild stress-induced depression-like behavior in a comparable manner to a single administration of ketamine in mice

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Voluntary exercise has been reported to reduce depression- and anxiety-like behaviors in an animal model of depressive disorder (DD). However, the degree of appropriate voluntary exercise to improve depression-like behavior remains unknown. In the present study, we examined the effects of two different terms of voluntary exercise, short (3 days)- and long (14 days)-term freewheel running, on depression-like behavior and new cell proliferation in the dentate gyrus (DG) in a DD mouse model generated by exposure to chronic mild stress (CMS), and then compared the antidepressant effects with those of ketamine. Short-term freewheel running showed an antidepressant effect in CMS mice in the forced-swim test (FST), which was comparable to that of ketamine, but did not affect new cell proliferation in the DG. In contrast, long-term freewheel running increased new cell proliferation in the DG, but did not improve depressive behavior in the FST. These results suggest that voluntary exercise, unless taken to the point of exhaustion, could beneficially contribute to the recovery of CMS-induced depression.

3-P-031

Activation of AMPK signaling in hippocampus improves depressive-like behavior in olfactory bulbectomized mice

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Recent studies have suggested that activation of hippocampal AMPK ameliorates cognitive functions in both rodents and patients. However, roles of AMPK on emotional function are unclear. Thus, we examined the effects of 5-aminoimidazole-4-carboxamide riboside (AICAR), an AMPK activator, on depressive-like behavior in olfactory bulbectomized (OBX) mice, an animal model of depression.

Mice were injected with AICAR for 1-14 days and then subjected tail-suspension test on the 21st day after surgery. Hippocampal proteins were assessed by western blotting, and neurogenesis was measured by immunohistochemical method.

Subchronic treatment with AICAR decreased immobility time in OBX mice. Phosphorylated AMPK, protein kinase C (PKC) ζ, nuclear factor-kappa B (NF-κB), cAMP response element-binding protein (CREB) and the protein level of brain derived neurothrophic factor (BDNF) in OBX mice were increased by AICAR. AICAR reversed hippocampal neurogenesis in OBX. Anti-depressant effect of AICAR in TST was attenuated by co-administration of ZIP, a PKCζ inhibitor.

Our data suggested that AMPK activation induced antidepressant effect through PKCζ/NF-κB/BDNF/CREB pathways. Therefore, AMPK may become a new therapeutic target of depression.

3-P-032
Depression-like behavioral analysis in CGRP Transgenic mice

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【Objective】Calcitonin gene-related peptide (CGRP) is a neuropeptide consisting of 37 amino acids produced by splicing difference from the calcitonin, and has strong vasodilation. We have shown that the expression of CGRP was significantly decreased in depression-like model mice hippocampus. CGRP administration into the mice brain before the beginning of stress exposure, normalized the behavioral dysfunction with increase never growth factor. In the present study, we investigated the role of highly *CGRP* expressing gene steadily using CGRP over expressing, CGRP transgenic (Tg) mice.
【Methods】8 weeks to 9 weeks old CGRP (Tg) mice were used. Same age of C57BL/6J mice were used as the control group. Behavioral tests were conducted on open field test, forced swimming test (FST), tail suspension test (TST) and sucrose preference test to evaluate depression-like behavior.
【Results】Immobility time in the FST and TST in CGRP Tg mice were significantly longer than C57BL/6J mice. In contrast, in the sucrose preference test, which measures anhedonic-like deficits, there were no significant differences. Furthermore, in the open field test, which measures spontaneous behavior decreased in CGRP Tg mice. These results suggest that prolonged immobility time of CGRP Tg mice is not due to depressive symptoms, but spontaneous behavior may have an effect.

3-P-033
Identification of a fluoxetine-target network in major depressive disorder

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Background: While the efficacy of selective serotonin reuptake inhibitor (SSRI) has been widely accepted in major depressive disorder (MDD), mechanisms of the therapeutic action in the efficacy remain to be fully elucidated. The purpose of this study was to identify the SSRI-target network that might be relevant to the therapeutic mechanism.
Methods: From a public database, we downloaded transcriptome datasets (MDD patients and model mice) and analyzed the transcriptome response of fluoxetine (FLX). We analyzed these transcriptome datasets using weighted gene co-expression network analysis to identify networks significantly relevant to FLX-responsive MDD mice and patients. In order to ascertain whether the identified network worked also in vivo, we performed in situ hybridization using zebrafish larvae treated FLX.
Results: We were able to identify three gene co-expression networks significantly relevant to FLX-responsive MDD mice and patients. Comparison of the three networks revealed a common network consisting of 26 genes, including BDNF and RGS4. Both BDNF and RGS4 have been related to the therapeutic effects of SSRI.
Conclusions: The gene co-expression network identified in this study may be relevant to the therapeutic mechanism and the efficacy of SSRI.

3-P-034

The oral administration of HYA reduced the anxiety-like behavior in Wistar male rats

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10-hydroxy-cis-12-octadecenoic acid (HYA) is produced from linoleic acid in the hydration reaction resulted from *Lactobacillus plantarum*. HYA indicated an anti-inflammatory effect in the small intestine via GPR40 pathway. In order to examine whether the anxiety-like behavior was changed by the administration of HYA, the open field and elevated plus maze tests evaluated the changes of anxiety-like behavior in Wistar male rats given HYA orally for successive 14 days. The immunohistochemistry evaluated the expression of proteins associated with anxiety-like behavior. The elevated plus maze test indicated the increase the spent time in the open arms, although the open field test indicate the spent time in center time was not changed in HYA group. The numbers of cells indicating strong immunoreactivity against anti-cFos antibody and anti-BDNF antibody were significantly increased in the rat given HYA in the rat hippocampus. Thus, HYA may partially reduce the anxiety-like behavior, increasing the expressions of c-Fos and BDNF proteins in the hippocampus.

3-P-035
The discovery of novel gene for stabilizing wakefulness

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Sleep is regulated through intricate communication among specialized neurons in the brain. While neuromodulators and their receptors have been extensively studied, the second messenger systems downstream of the receptors remained largely uncharted. Genetic manipulation is a powerful tool to investigate the function of specific components in a system, but often it is unfeasible to use conventional reverse genetics to examine genes in a comprehensive manner. Here, we used a triple-CRISPR method to efficiently produce whole-body biallelic knockout (KO) mice, and found that gene X KO mice had an increased duration of NREM sleep. Furthermore, gene X KO mice exhibited NREM sleep accompanied by body tremors and/or occasional limb movements. Behavioral experiments revealed that gene X KO mice exhibited disturbance in motor coordination and balance. In vitro reconstruction assay revealed that protein X (protein encoded by gene X) enhanced Ca^{2+} pump activity. Subsequent AAV-mediated protein X overexpression in vivo increased wake duration. These results imply a role for gene X in consolidating a behavioral state and a potential molecular mechanism for NREM parasomnia.

3-P-036

ER-mitochondria crosstalk is regulated by NCS1 and is impaired in Wolfram syndrome

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Wolfram syndrome is a childhood onset rare genetic disease (1/180,000) featuring diabetes mellitus and optic neuropathy unavoidably progressing towards legal blindness before the age of 20. Here we show that WFS1 forms a complex with Neuronal Calcium Sensor 1 (NCS1) and IP3R to promote ER-mitochondrial Ca^{2+} transfer. In addition, we report that NCS1 localizes to mitochondria-associated membranes and regulates mitochondrial respiratory chain. Importantly, NCS1 overexpression not only restore ER-mitochondria interaction and Ca^{2+} transfer, but also induces a significant rescue of the dysfunctional mitochondrial phenotype observed in WFS1 deficient cells. Since NCS1 may be targeted by pharmacological molecules, it may be a useful target to treat Wolfram syndrome pharmacologically.

3-P-037

Suvorexant enhances the loss of righting reflex induced by medetomidine-midazolam-butorphanol anesthesia in mice.

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We investigated the influence of hypnotics, including the orexin receptor antagonist suvorexant and the melatonin receptor agonist ramelteon, on the loss of righting reflex induced by anesthesia with isoflurane or a mixture of medetomidine, midazolam and butorphanol (MMB) in mice. In the case of MMB anesthesia, male ICR mice were given a single intraperitoneal injection of MMB (0.75 / 4 / 5 mg/kg), followed by intraperitoneal injection of atipamezole (3 mg/kg), for reversal of anesthetic effects, after 1 hour. Each hypnotic agent was orally administered 1 hour before anesthesia induction. Mice lost righting reflex within 5 min after administration of MMB, and administration of atipamezole made mice recover quickly from anesthesia. While chlorpromazine prolonged significantly the loss of righting reflex induced by both isoflurane and MMB, suvorexant enhanced the loss of righting reflex induced by MMB, but not isoflurane. On the other hand, ramelteon had no effect on the loss of righting reflex. These results suggested that the simple method with these anesthetic agents may be usable to investigate the characteristics of hypnotics.

3-P-038

Establishment of pain evaluation method using gait measurement method in rat neuropathic pain and MIA-induced osteoarthritis model

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Pain of the joint in the osteoarthritis (OA) and neuropathic pain cause a significant decrease in the quality of life (QOL) of the patients. To develop analgesics, a simplified method for evaluating analgesic effect has been becoming necessary although the methods like use of von frey filament, thermal stimulation or a load difference measurement method are existed, which require expert techniques for measurement. Therefore, we tried to use Catwalk which can acquire data by the animal walks to establish a method for pain evaluation in neuropathic pain and MIA-induced OA model in this study.

As the results obtained by measuring the pain parameters using the Catwalk, filament method, thermal stimulation method or load difference measurement method, a significant low value was observed in the pain parameters in both the neuropathic pain and OA model animals compared with the normal animals. On the other hand, administration of celecoxib, tramadol and pregabalin showed a significant high value in the pain parameters in the pain model animals compared with the control animals.

Based on the above results, it is suggested that walking analysis by Catwalk is useful for evaluation of analgesics.

3-P-039
The use of rat EMG to quantify injection pain

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[Objective] Various vaccines and protein drugs including antibody drugs are mainly used as injectable drug such as subcutaneous or intramuscular injection. Pain caused by administration of these injections is a side effect in a broad sense. Therefore, it is necessary to select "less painful" in consideration of improvement of QOL of patients from multiple drugs having equivalent efficacy, but it was not easy to predict and evaluate pain at the preclinical phase. Thus, we report the quantification of pain using the electromyogram generated at the injection. [Materials, Methods] Rats were anesthetized with pentobarbital. A bipolar stainless-steel electrode was placed into the semitendinosus muscle. The EMG signals were recorded in Power Lab 8/35. After inserting the electrode, the fingertip of hind limb was pinched strongly by clamp, and confirmed the EMG amplitude was increased. 0.9% Saline (50μL, pH 6.5) was served as negative controls. 10 % salt solution (50μL), dilute hydrochloric acid (50 μL, pH 3), and 0.9% saline solution (200μL) as expected to cause pain were injected into the rat plantar aspect subcutaneously. [RESULTS] EMG amplitude was increased in each solution immediately after injection. These results suggested the quantification of pain associated with parenteral injection is a useful evaluation system.

3-P-040

Origins and targets of HMGB1 essential for bortezomib-induced peripheral neuropathy in mice: distinct profiles in the development phase and sustained period

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Given our previous evidence that MΦ-derived HMGB1 participates in paclitaxel-induced peripheral neuropathy, we studied possible role of HMGB1 in the neuropathy caused by bortezomib (BTZ), a proteasome inhibitor used for treatment of multiple myeloma. To create BTZ-induced peripheral neuropathy (BIPN) in mice, BTZ was administered i.p. 3 times weekly for 2 weeks. The development of BIPN was prevented by an anti-HMGB1-neutralizing antibody (Ab) and antagonists of RAGE or CXCR4, but not Toll-like receptor 4, among targets for HMGB1, and by minocycline, an inhibitor of MΦ/microglia, ethyl pyruvate, known to inhibit HMGB1 release from MΦ, or liposomal clodronate, a MΦ depletor. Only Ab, the RAGE antagonist or minocycline, when given once on day 14, reversed the sustained BIPN. In the dorsal root ganglion, MΦ accumulation was detected on day 3, but not 14, of BTZ treatment, and RAGE upregulation was found on day 14. BTZ directly caused HMGB1 release from MΦ-like RAW264.7 cells. Our data suggest the involvement of RAGE and CXCR4 activation by MΦ-derived HMGB1 in the development of BIPN, and of RAGE activation by HMGB1 released from non-MΦ cells in the sustained period of BIPN.

3-P-041

The inhibitory actions of methylcobalamin on mechanical allodynia in herpes murine model

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Methylcobalamin (MeCbl) is an analog of vitamin B_{12} used to relieve peripheral neuropathy. In this study, we examined whether MeCbl inhibited mechanical allodynia (acute herpetic pain -AHP- and postherpetic neuralgia -PHN-) in mice infected with herpes simplex virus-1 (HSV-1). Mice were inoculated transdermally with HSV-1. Herpes zoster-like skin lesion peaked on day 7 after the inoculation, and was completely healed by day 20. Mechanical allodynia peaked on day 7-10 (AHP) post-inoculation, and was continuously observed after rash healing (PHN). Single administration of MeCbl inhibited mechanical allodynia in AHP phase. The anti-allodynic action of MeCbl was suppressed by naloxone, an opioid receptor antagonist, but not naloxone methiodide which has limited access to the CNS. Furthermore, repetitive administration of MeCbl from day 5 post-inoculation suppressed both AHP and PHN, and also promoted recovery of the peripheral nerve fibers decreased by HSV-1 infection in the footpad skin. These results suggest that activation of endogenous opioid system in the CNS and promotion of recovery of decreased peripheral nerve fibers are involved in the anti-allodynic actions of MeCbl in HSV-1-infected mice.

3-P-042
The allodynia induced by intrathecal injection of sulfatides.

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Glycosphingolipids (GSLs) play many important roles in cellular interaction, proliferation, vesicular transport and intracellular signal transduction. Our previous study revealed that the gene expressions of six glycosyltransferases in the GSLs biosynthesis pathway were altered in the spinal cord and dorsal root ganglion one day or 15 days after inflammation caused by complete Freund's adjuvant (CFA). We focused on CFA-induced upregulation of gal3st1 glycosyltransferase that catalyzes the synthesis of sulfatides, the sulfated GSLs in myelin structures, because the roles of sulfatides in nociceptive behavior are unclear. In this study, we intrathecally injected sulfatides into naïve mice and measured mechanical thresholds using von Frey test. Indeed, sulfatides induced mechanical allodynia within 40 min.

The report that sulfatides induce cytokine production from glial cells (Jeon SB et al., 2008 J. Immunology) leads to the hypothesis of the involvement of glial activation by spinal sulfatide during allodynia. Future studies are needed to reveal the molecular mechanisms of how sulfatides are involved in the pain transduction.

3-P-043

Enhancement of excitatory synaptic inputs to GRPR$^+$ dorsal horn neurons in a mouse model of atopic dermatitis

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Chronic itch is a cardinal symptom observed in patients with atopic dermatitis. Existing treatments are largely ineffective. Recent studies have identified an interneuron subpopulation expressing gastrin-releasing peptide receptors (GRPR) in the spinal dorsal horn (SDH) that is crucial for itch transmission. However, functional alterations in GRPR$^+$ neurons under chronic itch conditions remain poorly understood. In this study, we used NC/Nga mice as a model of atopic dermatitis. These mice spontaneously displayed scratching behavior under conventional (CV) but not SPF conditions. For whole-cell patch-clamp recording of GRPR$^+$ SDH neurons of these mice, we visualized these neurons by intraspinally microinjection of adeno-associated virus (AAV) which had a construct expressing mCherry under the control of GRPR promoter. We confirmed that mCherry$^+$ cells were located in the superficial neuron in the SDH, displayed the delayed firing pattern (an index of excitatory interneurons) and were depolarized by GRP application. We recorded spontaneous and miniature EPSCs of mCherry$^+$ neurons of SPF- and CV-NC/Nga mice and found an increase in the frequency, but not the amplitude, of EPSCs in mCherry$^+$ neurons of CV-NC/Nga mice. These results suggest thst the activity of GRPR$^+$ neurons in the SDH is facilitated under chronic itch conditions.

3-P-044

Effects of naftopidil on inhibitory transmission in substantia gelatinosa neurons of the adult rat spinal dorsal horn.

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Naftopidil is used clinically for the treatment of voiding disorders in benign prostatic hyperplasia. Previous *in vivo* studies in which naftopidil was applied intrathecally abolished rhythmic bladder contraction, suggesting that naftopidil might inhibit a voiding reflex through interaction with spinal dorsal horn neurons. In this study, we aimed to clarify the mechanism of action of naftopidil on spinal dorsal horn neurons by using patch-clamp recording of rat spinal cord slices. In about 30% of neurons, naftopidil increased the frequency of miniature inhibitory postsynaptic currents (mIPSCs) tested. These naftopidil activities were reversible and concentration-dependent manner, and interestingly, bath applied another alpha-1 adrenoceptor antagonist prazosin did not effects of mIPSCs. Although naftopidil was developed as an a-1 adrenoceptor antagonist, our previous studies showed that the activation of an alpha -1 adrenoceptor in substantia gelatinosa (SG) increases the frequency of mIPSCs. This result suggested that, under our conditions, naftopidil may interact with a site(s) in the spinal dorsal horn. These data suggest that naftopidil enhances the release of GABA and/or glycine by activating inhibitory interneuron terminals in the spinal dorsal horn via an unclear site(s) other than an alpha-1 adrenoceptor and thereby modulates sensory transmission in SG.

3-P-045

Therapeutic effects of amitriptyline on osteoporosis accompanied with neuropathic pain induced by partial sciatic nerve ligation

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Background: Patients with neuropathic pain frequently express osteoporosis. We have reported that an animal model of neuropathic pain induced by partial sciatic nerve ligation (PSNL mice) exhibits osteoporosis. In this study, we investigated the effects of amitriptyline, a potent analgesic drug for neuropathic pain, on bone loss in PSNL mice.

Methods: Osmotic pumps were used for chronic treatment with amitriptyline. We implanted an osmotic pump to PSNL mice subcutaneously at 7 days after nerve ligation. Then, bone tissues were harvested after 3 weeks, and their structure were assessed by micro computed tomography.

Results: As previously reported, mechanical hypersensitivity was developed in the ipsilateral paw after 7 days of partial nerve ligation, and it sustained more than 4 weeks. Changes of trabecular bone parameters indicate that PSNL mice have osteoporosis in their legs. By chronic treatment with amitriptyline, mechanical hypersensitivity was continuously attenuated and bone loss was suppressed in PSNL mice.

Conclusion: Amitriptyline, a tricyclic antidepressant, may have a clinical benefit for the treatment of neuropathic pain accompanied with osteoporosis.

3-P-046

Elucidation of the serotonergic circuits involving in nicotine withdrawal symptoms by using optogenetics

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Abrupt tobacco cessation in chronic smokers causes various withdrawal symptoms including anxiety, depressed mood, decreased arousal, and irritability. Recent studies have shown that interpeduncular nucleus is involved in nicotine withdrawal symptoms and that the nucleus projects to serotonergic nuclei. Therefore we hypothesized that the central serotonergic system regulates some of nicotine withdrawal symptoms. To test this hypothesis, we used transgenic mice expressing archaerhodopsin (ArchT) in central serotonergic neurons only, and examined whether serotonergic inhibition could induce withdrawal symptoms. Mice drank water containing nicotine for 6 weeks, and receive mecamylamine injection to induce withdrawal symptoms. Withdrawal symptoms: somatic signs, anxiety-like behavior, and depressive-like behavior were measured by visual observation, an elevated plus maze test, and a forced swim test, respectively. Somatic sings were precipitated by mecamylamine following chronic nicotine drinking. Neither anxiety-like nor depressive-like behavior was affected by chronic nicotine drinking or mecamylamine. Central serotonergic inhibition induced somatic signs in mice received mecamylamine injection without chronic nicotine drinking.

3-P-047

Prenatal nicotine exposure induces behavioral changes of the offspring: Involvement of cytokine levels in the brain.

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It is suggested that the smoking during pregnancy might impair the neurodevelopment of offspring, emotional and cognitive function. Previously, we clarified that prenatal nicotine exposure affects the proliferation and maturation of progenitor cells to glutamatergic neuron during neurodevelopment in the medial PFC, which may be associated with cognitive deficits in the offspring. Recently, there is a hypothesis that inflammation during pregnancy may induce the brain dysfunction of offspring. In this study, we examined whether prenatal nicotine exposure might affect the expression of chemokines in mice.

Dams were exposed to nicotine solution (0.2 mg/mL) dissolved in 2 % saccharin by drinking till embryonic day 14 (E14) to birth (P0) of offspring. On E15, 16, 18 and P1, we investigated the protein levels of 23 cytokines in the brain.

In the nicotine-exposed group, IL-1alpha level decreased, but IL-5 and IL-10 increased compared to those in the control group on E15. On the contrary, the levels of IL-1alpha and IL-5 were not affected but IL-10 decreased significantly compared to those in the control group on P1. These results suggest that prenatal nicotine exposure may influence the cytokine balance in the brain of offspring.

3-P-048

Influences of prenatal nicotine exposure on behavioral functions of the offspring: Involvement of chemokine levels in the brain.

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The cigarette smoking during pregnancy is thought to induce the dysfunction of emotional and cognitive function of offspring. In our previous studies, we have reported that prenatal nicotine exposure affects the proliferation and maturation of progenitor cells to glutamatergic and dopaminergic neurons during neurodevelopment in the medial PFC, which may be associated with behavioral impairments in the offspring. Recently, there is a hypothesis that inflammation during pregnancy might impair brain function of offspring. Here, we investigated whether prenatal nicotine exposure might affect the expression of chemokines in mice.

Pregnant mice were exposed to nicotine solution (0.2 mg/mL) dissolved in 2 % saccharin by drinking till embryonic day 14 (E14) to birth (P0) of offspring. On E15, 16, 18 and P1, we examined the protein levels of 10 chemokines in the brain.

The prenatal nicotine exposure increased the expression levels of CXCL1 and CCL2, but decreased those of CCL3, CCL4, CCL11 and CCL5 compared to those in the control on P1. It is suggested that prenatal nicotine exposure may influence the chemokine balance in the brain of offspring.

3-P-049

Histamine H_3 receptor inverse agonists attenuate methamphetamine-induced hyperlocomotion in mice via histamine H_1 receptors

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A single administration with METH induced a hyperlocomotion in mice. Pretreatment of mice with pitolisant, a histamine H_3 receptor inverse agonist, for 30 min showed a significant reduction of the hyperlocomotion induced by METH, as compared with vehicle-pretreated subjects, in a dose-dependent manner. Pretreatment of mice with JNJ-10181457, another H_3 receptor inverse agonist, showed a similar inhibitory effect on METH-induced hyperlocomotion. No significant change in locomotion was observed in mice pretreated with pitolisant or JNJ-10181457 alone. Pretreatment with pitolisant prior to a high-dose METH significantly decreased the intensity of stereotyped behaviors and increased its latency to onset in a dose-dependent manner. The pitolisant action on METH-induced hyperlocomotion was completely abolished by a H_1 receptor antagonist pyrilamine, but not by H_2 receptor antagonist zolantidine. These observations suggest that pretreatment with pitolisant attenuates METH-induced hyperlocomotion via histamine receptors subtype H_1 but not H_2, and support the idea that activation of brain histamine systems may be a good strategy for the development of agents which treat METH abuse.

3-P-050

Tetrabenazine attenuates morphine-induced hyperlocomotion, but not antinociception, in mice

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

3-P-051

Role of HPA axis on the development of ethanol dependence in mice

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Our previous study indicated that the HPA (hypothalamic-pituitary-adrenal) axis plays a critical role in the expression of ethanol withdrawal symptoms, including emotional abnormality and impaired recognition, in mice. To clarify the role of HPA axis on the development of ethanol dependence, mice were chronically treated with 4% ethanol-containing milk for 14 days using liquid diet method. A significant increase in the serum corticosterone concentration was observed in ethanol-treated mice compared with control mice. As with the stress response, hypertrophy of thymus and spleen, and atrophy of adrenal glands were observed in ethanol-treated mice, respectively. The present study was also designed to ascertain the changes in the protein levels of glucocorticoid receptor (GR) and TrkB in the mouse brain were evaluated on day 0, 3, 7, 10 and 14. In the Western blot analysis, we demonstrated that the level of GR and the ratio of GR/TrkB were significant increase in hypothalamus, but not hippocampus, in ethanol-treated mice. These results suggested that the HPA axis may play an important role not only on the expression of ethanol withdrawal symptoms but also on the development of ethanol dependence in mice.

3-P-052

Regulation of maternal aggression and care via glutamatergic signals in the dorsal raphe nucleus in mice

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Maternal care and maternal aggression are representative maternal behaviors in rodents. These behaviors are displayed alternatively. The dorsal raphe nucleus (DRN) regulates both nurturing and aggressive behaviors. In the present study, we examined whether the DRN are involved in regulating alternative display of maternal care and aggression. We first examined the neuronal activity in the the medial prefrontal cortex (mPFC) and lateral habenula (LHb), which send glutamatergic inputs into the DRN, in dams with a retrograde tracer Fluorogold injected into the DRN. The number of c-Fos- and Fluorogold-positive neurons in the mPFC and LHb increased in the dams that displayed biting behavior in response to an intruder, but remained unchanged in the dams that displayed nurturing behavior. Injections of N-methyl-D-aspartic acid (NMDA) receptor antagonists or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor antagonists into the DRN inhibited biting behavior but not nurturing behavior. In contrast, injections of NMDA or AMPA into the DRN inhibited nurturing behavior. These results suggest that glutamatergic signals in the DRN, which may originate from the mPFC and/or LHb, regulate the preferential display of biting behavior over nurturing behavior in dams.

3-P-053

Percutaneous Treatment of Carbon Dioxide Gas Mist Suppresses the Development of Right Ventricular Dysfunction in Monocrotaline-Induced Pulmonary Hypertensive Rats

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Background: Highly concentrated carbon dioxide (CO_2) mist is useful for treating ischemic diseases. Therefore, we investigated whether treatment with CO_2mist could attenuate the development of right ventricular (RV) dysfunction in pulmonary hypertension (PH).

Methods and Results: PH *was induced by* subcutaneous administration of monocrotaline (MCT; 60 mg/kg) to the rats, which were subsequently treated with CO_2 mist (CM) or which were untreated (UT). The lower body of each rat was encased in a polyethylene bag, filled with the designated gaseous agent via a gas mist generator, for 30 minutes daily. Rats that received MCT without treatment began to die within 3-4 weeks of the initial administration. However, treatment with CO_2 mist extended the survival period of rats in that group. MCT-induced RV weight and RV dysfunction were significantly attenuated by treatment with CO_2 mist. Both RV phosphorylated endothelial nitric oxide synthase and heat shock protein 72 levels increased significantly in the CM group, compared to the UT group.

Conclusions: Percutaneous CO_2mist therapy may alleviate RV dysfunction in patients with pulmonary hypertension.

3-P-054

Effects of exosomes derived from organs of spontaneously hypertensive rats on reactivity of thoracic aorta from normotensive Wistar rats

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Exosomes are the smallest-sized (50-150 nm in diameter) extracellular vesicles enclosed by a lipid-bilayer with density of 1.12-1.19 g ml^{-1}. Exosomes are considered to mediate cell-cell communication, since they contain various molecules, including protein and microRNA. We previously reported that plasma-derived exosomes partly regulate systemic blood pressure in both normotensive and hypertensive rats, suggesting that exosomes could affect vascular reactivity. We thus examined the effects of exosomes derived from cultured media of organs (brain, heart, liver and kidney) isolated from spontaneously hypertensive rats (SHR, 5-week-old) on reactivity of isolated thoracic aorta from normal Wistar rats (6-9-week-old). In endothelium-intact thoracic aorta, pretreatment with exosomes for 10 min and 2 h had no big effect on contraction induced by noradrenalin. The exosomes had also no big influence on acetylcholine-induced endothelium-dependent relaxation. Further study is required to elucidate whether hypertensive animal-derived exosomes could affect vasoreactivity.

3-P-055

Antihypertensive effects of flaxseed and its mechanism of action in deoxycorticosterone acetate-salt hypertensive rats

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Flaxseed is a functional food containing α-linolenic acid, lignans, and dietary fiber, and its intake is known to lower blood pressure in hypertensive rat models and hypertensive patients. However, the mechanisms of action of flaxseed have not been fully elucidated. In this study, we examined the effects of flaxseed powder, which includes all flaxseed components, flaxseed oil, comprised mainly of α-linolenic acid, flaxseed lignan, and flaxseed fiber on hypertension induced by deoxycorticosterone acetate (DOCA)-salt to elucidate the components associated with the antihypertensive effects. Then, we investigated the mechanisms of action associated with the effects of flaxseed powder and flaxseed oil. The intake of flaxseed powder and flaxseed oil prevented the elevation of blood pressure induced by DOCA-salt treatment, whereas flaxseed lignan and flaxseed fiber had no effects. Flaxseed powder and flaxseed oil suppressed the sympathetic nerve activity in DOCA-salt rats. In contrast, neither flaxseed powder nor flaxseed oil affected plasma and kidney ACE activity or MDA levels in the kidney. These results indicate that flaxseed has antihypertensive effects in DOCA-salt hypertensive rats. These effects are likely principally exerted by α-linolenic acid, the main component of flaxseed. Furthermore, modulation of the autonomic nervous system is partly involved in the antihypertensive effect of flaxseed.

3-P-056

Acute blood pressure-lowering effects of A484954, a eukaryotic elongation factor 2 kinase inhibitor in rats

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Eukaryotic elongation factor 2 (eEF2) kinase (eEF2K) is a calmodulin-dependent protein kinase regulating protein translation through phosphorylation of eEF2. We previously demonstrated that eEF2K mediates development of systemic hypertension. We recently revealed that A484954, a selective eEF2K inhibitor induces vasorelaxation through activating smooth muscle inward rectifier K^+ (K_{ir}) channel in rat isolated mesenteric artery. Here we further explored acute effects of A484954 on blood pressure (BP). BP was measured by a carotid cannulation method in rats. A484954 potentiated isoproterenol-induced decrease of diastolic BP. A484954 potentiated adrenaline-induced decrease of diastolic BP. In summary, we for the first time demonstrated that A484954 augments isoproterenol- and adrenaline-induced BP decrease perhaps through the activation of beta-2 receptor-K_{ir} channel.

3-P-057

Ophiocordyceps sinensis may ameliorate cardiovascular remodeling in pulmonary arterial hypertension via TRPM7 inhibition

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Pulmonary arterial hypertension (PAH) comprises a multifactorial group of pulmonary vascular disorders that cause pulmonary vascular remodeling and right heart failure. We previously found that *Ophiocordyceps sinensis* (OCS) suppressed the activity of TRPM7 channel, a key molecule accelerating the fibrotic process of cardiovascular remodeling. Thus, in this study, we explored the therapeutic potential of OCS for PAH and the mechanism involved therein.

In *in vitro* experiments, OCS suppressed a TRPM7 channel activity and TGF-β2-induced endothelial-to-mesenchymal transition (EndoMT) in human pulmonary arterial endothelial cells, and abnormally enhanced proliferation of pulmonary arterial smooth muscle cells from PAH patients. Moreover, *in vivo* experiments using PAH model rats and mice showed that OCS ameliorated the development of pulmonary artery thickening, cardiac fibrosis and right ventricle hypertrophy with normalization of heightened right ventricle pressure.

These results suggest that OCS can ameliorate the pathology and cardiac dysfunction associated with pulmonary hypertension via TRPM7 inhibition. These findings could serve as an important clue to developing a new combinatorial PAH treatment.

3-P-058

Analysis of compensatory metabolic pathway for taurine deficiency in mouse heart

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Taurine is one of the most abundant free amino acids in heart, and its treatment is beneficial against chronic heart failure. While knocking out taurine transporter (TauT) in mice caused a taurine-deficient cardiomyopathy, cardiac output is normal in the young TauTKO mouse, suggesting activation of homeostatic mechanisms that compensate for detrimental effects caused by taurine depletion. In the present study, we examined the integrated analysis of transcriptome and metabolome of TauTKO mice to determine the compensatory homeostatic pathway. We identified increases in several organic osmolytes, including betaine, carnitine, glycerophosphocholine (GPC) and amino acids, in the heart of TauTKO mice. Transcriptome analysis revealed that several genes of the SLC transporter family are increased in TauTKO mice, such as amino acid transporter (Slc38a2), while the established transporters for betaine and carnitine are not altered by taurine deficiency. The integrated analysis revealed a significant increase in the genes involved in Glycerophospholipid metabolism in which GPC biosynthesis is involved. In conclusion, we identified genetic/metabolic compensatory mechanisms involved in the control of the metabolome profile in taurine-deficient cardiomyopathy.

3-P-059

Effect of chloroquine treatment on cardiac function as well as cardiac atrial natriuretic peptide level in Bcl-2-associated athanogene (BAG) 3 transgenic mice

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Bcl-2-associated athanogene 3 (BAG3) is strongly expressed in cardiac muscle. To understand the functional role of cardiac BAG3, we generated TG mice that overexpress BAG3. A decrease in fractional shortening, and the induction of cardiac ANP, were observed in BAG3 TG mice. Moreover, a marked reduction in the protein level of small HSPs was detected in BAG3 TG mouse hearts. The protein turnovers of small HSPs by the autophagy system were activated in BAG3 TG mouse hearts. Thus, BAG3 is critical for the protein turnover of small HSPs via activation of autophagy in the heart. In order to address the relationship between the reduced cardiac function as well as the induction of stress markers and autophagy activation in BAG3 TG mouse hearts, the effects of chloroquine treatment on cardiac function as well as cardiac ANP level in BAG3 TG mice were examined. Twenty hours after chloroquine treatment, reduced fractional shortening was recovered to a normal level in BAG3 TG mice concomitant with marked attenuation in induced cardiac ANP. These results suggest that inhibition of the activated autophagy in BAG3 TG mouse hearts can rescue the phenotypes by BAG3 overexpression in the hearts.

3-P-060

Sarcolipin is *O*-GlcNAcylated at Ser^4 and Thr^5 in mouse hearts to regulate SERCA activity

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Sarcolipin (SLN) is a small protein,,that regulates the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) in cardiac/skeletal muscles. Although it is reported that the Thr^5 residue of human SLN (hSLN) is the key amino acid which modulates SLN function via its phosphorylation, it is not clear whether SLN is modified with *O*-linked b-*N*-acetylglucosamine (*O*-GlcNAcylation). Here, we found that mouse SLN (mSLN) was *O*-GlcNAcylated at Ser^4 and Thr^5 residues in mouse heart homogenates, by using an enzymatic labeling and a chemical detection system of *O*-GlcNAc. We also performed co-IP experiments using an *O*-GlaNAcase inhibitor in HEK293 cells where FLAG-tagged single Ala-substituted mutants to Ser^4 /Thr^5 residues of SLN (S4A/T5A) was expressed. As a result, the *O*-GlcNAcylation of SLN-WT was significantly increased with the *O*-GlaNAcase inhibitor, whereas no *O*-GlcNAcylation was detected in S4A/T5A both with or without the *O*-GlaNAcase inhibitor. Furthermore, SERCA activity was decreased with treatment of the *O*-GlaNAcase inhibitor in SLN-WT expressing HEK293 cells. These data indicate that the inhibition of SERCA activity by SLN is regulated not only via phosphorylation, but also via *O*-GlcNAcylation.

3-P-061

Inhibition of TRPV2 prevents the progression of murine heart failure

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Transient receptor potential cation channel, subfami□ly V, member 2 (TRPV2) has been previously suggested as a principal candidate for Ca^{2+} entry pathways, and it seems to be a potential therapeutic target for heart failure, such as dilated cardiomyopathy (DCM). However, there are no known TRPV2-specific inhibitors to treat DCM. Here, we produced the antibodies against TRPV2, which interact with the TRPV2 extracellular site and inhibit the Ca^{2+} influx via TRPV2. Since these antibodies had no effects on the other members of the TRP family, such as TRPV1 and TRPC1, we tested the therapeutic efficacy of TRPV2 inhibition in the murine heart failure of the 4C30 DCM model or the transaortic constriction (TAC)-induced model. We observed that the treatment with an antibody by intraperitoneal injections at a dose of 0.25-1 mg/kg once a week for 2 weeks prevented the progression of cardiac dysfunction and myocardial injuries in DCM mice and TAC mice, as evaluated by echocardiography and tissue Masson's trichrome staining. We conclude that the cardioprotection afforded by the antibody against TRPV2 proves the importance of TRPV2 in the pathophysiology of heart failure and may be a promising treatment for patients with heart failure.

3-P-062

Attenuation of doxorubicin-induced acute cardiotoxicity by supplementation of L-histidine or organic cation transporter-3 deficiency in mice

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Doxorubicin (DOX) for treatment of patients with neoplasms shows cardiac toxicity. Recent studies reported that histamine H2 receptor antagonist improved DOX-induced cardiotoxicity, while histamine deficiency exacerbates myocardial injury. To elucidate the effects and mechanisms of histamine on DOX-induced acute cardiotoxity, we investigated the cardiac functions, pathological alterations, and protein expressions of Hsp25, LAMP-1, Beclin-1, p62, LC3B, histamine H1 and H2 receptors using both mice with organic cation transporter-3 (OCT3) deficiency and mice with supplementation of L-histidine that is converted to histamine via histidine decarboxylase. The DOX-induced acute cardiotoxicity was improved in both mice by analyzing cardiac function with echocardiography and electron microscopy. The higher content of histamine, decreased histamine H_2 receptor expression in basal levels, improvements of autophagy-lysosome flow, and enhancement of Hsp25 protein expression were shown in OCT3(-/-) deficiency and mice with supplementation of L-histidine. In conclusion, OCT3 deficiency and supplementation of L-histidine significantly contributes to DOX-induced acute cardiotoxicity.

3-P-063

Cardiotoxicity induced by doxorubicin is exacerbated by deletion of SIRT1 in mice.

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Background: Doxorubicin (DOX) is an effective anti-tumor agent; however, it causes cardiotoxicity. SIRT1 is an NAD^+-dependent protein deacetylase that plays a crucial role in cell survival.
Hypothesis: SIRT1 protects against doxorubicin-induced cardiotoxicity.
Methods and Results: In wild type (WT) and tamoxifen-inducible cardiomyocyte-specific SIRT1 knockout (SIRT1 cKO) mice, either DOX or PBS was administered (4 injections of 5 mg/kg/wk) starting at 3 months of age. Echocardiography at 1 week after final DOX showed that left ventricular dimension, thickness, and fractional shortening (FS; 32.7 vs. 33.8%) were comparable in WT-PBS and cKO-PBS groups. DOX decreased FS in both genotypes; but FS was lower in cKO-DOX than in WT-DOX (24.6% vs. 27.3%, $P<0.05$). Cardiac level of nitrotyrosine, a marker of oxidative stress, was higher in cKO-DOX by 68% than WT-DOX. At 12 weeks after DOX, cardiac level of ANP mRNA, a marker of heart failure, was significantly higher in cKO-DOX than WT-DOX. Protein levels of LC3-II, a marker of autophagosomes, and p62 that is degraded by autophagy were significantly higher in SIRT1 cKO, suggesting suppression of autophagy.
Conclusion: The findings suggest that SIRT1 affords protection against DOX-induced cardiotoxicity, probably via attenuating oxidative stress through activating autophagy.

3-P-064

The role of the circadian clock gene Bmal1 in vascular remodeling

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Introduction: We examined the role circadian clock component of Bmal1 in VSMC activation and intima hyperplasia after vascular injury.
Methods: Bmal1 protein expression was evaluated in ligated mouse carotid arteries from C57BL/6J and cultured VSMCs stimulated by platelet-derived growth factor-BB (PDGF-BB) using western blotting. VSMC proliferation was measured using the MTS assay and the manual cell counting.
Results: The mice exhibited marked intimal hyperplasia after the ligation. Western blotting analyses revealed increased Bmal1 protein expression in the freshly isolated aorta after ligation, with significant increase in VSMCs following PDGF-BB treatment. PDGF-BB-induced Bmal1 expression was inhibited through treatment with a NOX inhibitor, diphenyleneiodonium and the MEK inhibitor, U0126. Furthermore, early growth response protein-1 (EGR-1) knockdown significantly reduced Bmal1 expression induced by PDGF-BB. Moreover, Bmal1 knockdown significantly decreased PDGF-BB-induced ERK phosphorylation and cell proliferation.
Conclusions: These data suggest that Bmal1 plays a crucial role in intima hyperplasia after vascular injury through the regulation of VSMC proliferation.

3-P-065
Involvement of heat shock protein 90 in cardiac fibrosis

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Raf/Mek/Erk pathway plays a crucial role in the development of cardiac fibrosis. It is assumed that heat shock protein 90 (Hsp90) may regulate the Raf/Mek/Erk signal pathway. However, the role of Hsp90 in cardiac fibrosis under pathophysiological conditions remains unclear. In this study, effects of Hsp90 inhibitor on signal transducers in cultured cardiac fibroblasts were examined. Cardiac fibroblasts prepared from neonatal rats were treated with combination of Hsp90 inhibitor 17-(allylamino)-17-dimethoxy-geldanamycin (17-AAG) and proteasome inhibitor MG132. Proliferation of cardiac fibroblasts was attenuated by 17-AAG treatment for 48 h. 17-AAG treatment also reduced an expression of collagen I and III. c-Raf content of cardiac fibroblasts was decreased in the presence of 17-AAG. An increase in phosphorylation levels of Erk1/2 in cardiac fibroblasts attenuated by 17-AAG treatment. MG132 reversed the loss of c-Raf in cardiac fibroblasts treated with 17-AAG. These findings suggest that Hsp90 involves an activation of Raf/Mek/Erk pathway via c-Raf stability in cardiac fibroblasts, leading to the development of cardiac fibrosis.

3-P-066

Activation of Drp1 by methylmercury causes cardiac fragility to hemodynamic overload

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Protein polysulfidation occurs at reactive cysteine residues in proteins, which plays pivotal roles in the regulation of redox signaling and mitochondrial bioenergetics. A trace amount of methylmercury (MeHg) is suggested to increase cardiovascular vulnerability, but the underlying mechanism is obscure. We report that exposure to subneurotoxic dose of MeHg caused mitochondrial hyperfission in myocardium through activation of dynamin related protein 1 (Drp1), which precipitated systolic heart failure induced by pressure overload in mice. Treatment of neonatal rat cardiomyocytes (NRCMs) with cilnidipine, an inhibitor of interaction of Drp1 with its guanine nucleotide exchange factor, filamin-A, suppressed MeHg-induced mitochondrial hyperfission. MeHg targeted rat Drp1 proteins at redox-sensitive Cys^{624}, which SH residue was found to make a nucleophilic polysulfidated form. MeHg induced depolysulfidation of Cys^{624} and mitochondrial hyperfission through filamin-dependent Drp1 activation. Treatment of rat cardiomyocytes with NaHS, a sulfur substrate, significantly suppressed mechanical stress-induced cell death of NRCMs exposed by MeHg. These results suggest that depolysulfidation of Drp1 at Cys^{624} by low-dose MeHg contributes to increase of cardiac vulnerability to mechanical load via filamin-dependent mitochondrial hyperfission.

3-P-067
The role of STIM1 *O*-GlcNAcylation in mouse heart

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O-linked b-*N*-acetylglucosamine (*O*-GlcNAc) modification (*O*-GlcNAcylation) of the stromal interaction molecule 1 (STIM1) is known to impair store-operated Ca^{2+} entry (SOCE). In cardiomyocytes, STIM1 interaction with phospholamban (PLN), an inhibitor of Ca^{2+}-ATPase (SERCA2a), is reported to regulate cardiac function. However, the molecular mechanisms by which STIM1 *O*-GlcNAcylation affects SOCE and PLN activity are still unclear. Here, we found that increased STIM1 *O*-GlcNAcylation with an *O*-GlcNAcase inhibitor reduced its interaction with PLN in HEK293 cells co-expressing myc-tagged STIM1 and GFP-tagged PLN-WT or S16A (mutant of *O*-GlcNAcylation site). The STIM1 interaction with PLN was significantly decreased in heart tissues of *O*-GlcNAc transferase-transgenic mice, whereas the PLN interaction with SERCA2a was significantly increased in that of STIM1-knockout mice compared to WT mice. These data suggest that STIM1 *O*-GlcNAcylation regulates its interaction with PLN, which may possibly regulate SERCA activity. Next, to clarify the molecular mechanisms by which STIM1 *O*-GlcNAcylation affects SOCE, we established the STIM1 knockout HEK293 cells by CRISPR/Cas9 system, and then, transfected STIM1 mutants lacking each *O*-GlcNAcylation site to the cells. It is being elucidated.

3-P-068

Regulation of hypoxia-induced relaxations by perivascular adipose tissue in isolated rat aortas

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Perivascular adipose tissue (PVAT) functions not only as a structural support but a paracrine organ. However, it is still not clear how PVAT affects the arterial tensions under the hypoxic insult. The role of PVAT in the hypoxia-induced responses was examined in phenylephrine (3 μM)-precontracted rat aortas by using the in vitro blood-vessel myography and pharmacological interventions. The hypoxic exposure for 2 hours caused two phases of relaxations in both PVAT-intact and denuded aorta rings. In the early phase, the aortic rings relaxed upon hypoxia to the maximal level, whereas a sustained reduction of the relaxation level was observed in the late phase. The hypoxia-induced relaxations were enhanced by Nω-nitro-L-arginine (a NOS inhibitor) and indomethacin (a COX inhibitor) in aortic rings without PVAT, but this enhancement was less obvious in PVAT-intact aortic rings. PNU 37883, a blocker of the K_{ATP} channel, significantly decreased the hypoxic relaxations in PVAT-intact and removed aortic rings to a similar level. In contrast, the hypoxic relaxation was not affected by iberiotoxin (a BK_{Ca} channel blocker) or 4-Aminopyridine (a K_V channel blocker). Furthermore, PVAT-mediated relaxations were reduced by repeated hypoxic treatments in aortas. These results suggest that PVAT enhances the hypoxic relaxations in rodent aortas, and this enhancement is partly via the increased permeability of K_{ATP} channels.

3-P-069

The correlation of the fatty acid ester of hydroxy fatty acids (fahfas) with cardiovascular related biomarkers in healthy human

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Fatty acid ester of hydroxy fatty acids (FAHFAs) are kinds of novelty long chain fatty acids and have been uncovered their anti-inflammatory effects in several diseases. However, the role of FAHFAs in cardiovascular diseases (CVDs) prevention still not investigated. Thence, we tried to clarify the correlation among the FAHFAs and several cardiovascular-related markers. The plasma samples were collected from totally 60 healthy volunteers and determined by using liquid chromatography-mass spectrometry (LC-MS) and the cytokine assay. We found two major types of FAHFAs (9-POHSA and 9-OAHSA) in human plasma. We also found the 9-POHSA revealed the positive correlation with *L*-carnitine (*L*-Car) ($r = 0.3809$, $P = 0.0032$). Moreover, both of the 9-POHSA and 9-OAHSA were shown the strong positive correlation with interleukin-9 (IL-9) ($r = 0.3588$, $P = 0.0071$; $r = 0.3977$, $P = 0.0026$). In addition, we also observed both of the 9-POHSA and 9-OAHSA were negative correlation with *S*-adenosyl-*L*-homocysteine (SAH), but not in *L*-homocysteine (*L*-Hcy). Interestingly, the 9-OAHSA also show the negative correlation with TMAO. Our results indicated that the reasons for the anti-inflammatory effect in FAHFAs may be related to the levels of the IL-9 and *L*-Car elevation, and the SAH and TMAO reduction. Therefore, we suggest that FAHFAs have the potential for CVDs protection.

3-P-070

Identification of novel mechanisms in polycystic kidney disease using omics data

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Polycystic kidney disease (PKD) is characterized by cystic expansion of the kidneys, which can lead to kidney failure. PKD affects one in □ 1000 people worldwide and is commonly caused by defects in polycystin 1 (PKD1), polycystin 2 (PKD2), or polycystic kidney and hepatic disease 1 (PKHD1). However, there are variability in the symptom and progression of PKD caused by same mutation in these causative genes, suggesting that there may be many other genes involved in the pathogenesis of PKD. In this study, we performed comparative transcriptome analysis of three mammalian PKD datasets (PKD caused by knockout of PKD1, PKHD1, or AQP11) downloaded from a public database. We were able to identify two down-regulated and six up-regulated genes including osteopontin in common among the three different PKD models. In silico analysis of the promoters of these six up-regulated genes revealed that transcription factors HNF4 and RXRA might be involved in the up-regulation of these genes. Osteropontin, HNF4 and RXRA have been associated with the pathogenesis of PKD, supporting the validity of the approach used in this study. We now analyze the relationship between these dysregulated genes and the pathogenesis of PKD using zebrafish.

3-P-071

Localization of AGN-1, which binds to protein phosphatase 6, with tau alternative splicing variants in U1spliceosome

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We cloned AGN-1 that interacted with protein phosphatase 6 as a molecule highly expressed in nephritic rat kidney. Our previous studies showed that AGN-1 was co-localized with U1-70K, one of U1snRNP, and splicing factor SC35, which was found to participate in the synthesis of tau alternative splicing variants, 4R-tau and 3R-tau. In addition, AGN-1 siRNA treatment resulted in alteration of 4R-tau/3R-tau mRNA ratio in neuronal cells in culture. In this study, we investigated co-localization of AGN-1 and tau alternative splicing variants in U1 spliceosome, composed of U1-70K and U1-A. RNA immunoprecipitation was carried out using nuclear extract prepared from Neuro2a cells and anti-phosphorylated SC35 antibody, followed by RT-PCR to detect tau pre-mRNA/mRNA bound to phosphorylated SC35. To confirm co-localization of SC35, AGN-1, U1-70K, and U1-A, protein immunoprecipitation was also performed with anti-phosphorylated SC35 antibody. RNA immunoprecipitation showed PCR fragments derived from 4R-tau and 3R-tau. Protein immunoprecipitation revealed co-localization of U1-70K, U1-A, AGN-1, and phosphorylated SC35. These results suggest that in U1 spliceosome, AGN-1 may localize with tau alternative splicing variants, 4R-tau and 3R-tau.

3-P-072
Distribution of α2-adrenoceptor subtypes in the rat kidney.

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It has been reported that α2-adrenoceptors importantly contribute to the urinary output, renin release and water and sodium excretion. We recently revealed that yohimbine, α2-adrenoceptor antagonist, has protective effect on renal ischemia/reperfusion and cisplatin-induced acute kidney injury in rat. However, the localization of α2-adrenoceptor subtypes in kidney is unclear. The present study aimed to investigate the localization of α2-adrenoceptor subtypes in rat kidney. We used 8 weeks Sprague Dawley rats. Using immunofluorescence, both α2A and α2B-adrenoceptors expressed basolateral, but not apical, membrane of the epithelial cells of the proximal tubules. We also showed that α2A and α2B-adrenoceptors were neither expressed in the glomeruli nor in the collecting duct, thin ascending limb and vasa recta. On the other hands, we revealed that α2C-adrenoceptors localized in the glomeruli, lumen of the cortical and medullary collecting duct. Thus, we demonstrated that the α2-adrenoceptor subtypes were variously distributed in rat kidney.

3-P-073

Prorenin receptor and ERK are associated with kidney development in the fetal rat with prenatal glucocorticoid administration.

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This study aimed to investigate whether prenatal glucocorticoid (GC) administration is associated with fetal kidney maturation. We investigated the effects on the prorenin receptor (PRR) and ERK expressions for kidney development in the fetal rats. Dexamethasone (DEX) was administered to pregnant rats for 2 days on day 17 or day 19 of gestation, and the kidney of fetuses and neonates were analyzed by immunohistochemistry. The viability of HEK 293 cells treated with DEX was determined by MTT assay, and mRNA and protein expressions of the PRR, ERK, and phospho(p)-ERK were analyzed. ERK-positive areas were observed in primitive perivascular mesenchymal cells and immature glomeruli of the fetal rats. ERK-positive areas were significantly increased in the kidneys of 21-day fetuses compared with those of 19-day fetuses. DEX tended to increase ERK- and p-ERK-positive areas in the kidney of 19-day fetuses. However, the PPR-positive areas did not change with DEX. DEX also significantly increased the mRNA and protein levels of ERK, p-ERK, and PRR in HEK293 cells. These results indicate that prenatal DEX administration may contribute to kidney development through ERK increase in the fetal rat.

3-P-074

Glutathione Specific Gamma-Glutamylcyclotransferase 1, CHAC1 regulates Bcl-2 expression in acute kidney injury.

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Renal ischemia-reperfusion injury (IRI) is a common course of acute kidney injury. We have found that glutathione specific gamma-glutamylcyclotransferase 1 (CHAC1) is upregulated in kidney of wild type mice but not of hypoxia-inducible factor-1alpha heterozygous knockout (hKO) mice during IRI. Since CHAC1 is an inducer of apoptosis, we investigated apoptotic induction in kidneys during IRI. Apoptotic cells in the kidney of wild type mice are induced 12-h after ischemia/reperfusion treatment. In contrast, the induction of apoptotic cells in hKO mice was retarded. The ratio of anti-apoptotic Bcl-2 (B-cell lymphoma 2)/pro-apoptotic Bax (Bcl-2 associated X) was increased in hKO mice, indicating that dysregulation of Bcl-2 and/or BAX by CHAC1 participates in retardation of apoptotic induction in hKO mice kidney. In addition, CHAC1 knockdown with siRNA in HK2 cells, a human renal proximal tubule cell line, observed increased expression of Bcl-2 but not Bax. CHAC1 knockdown also increased intracellular glutathione concentration in HK2. Since a previous report showed the expression levels of Bcl-2 are correlated with cellular glutathione concentration, CHAC1 probably regulates Bcl-2 expression or apoptotic induction through regulation of glutathione concentration in IRI.

3-P-075

Effects of pravastatin on vascular endothelial dysfunction in acute kidney injury.

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Background: We have confirmed that indoxyl sulfate (IS), one of the uremic toxin, is involved in vascular endothelial dysfunction of ischemic acute kidney injury (IAKI). SLCO4C1, one of the organic anion transporters, expressed in kidney and is involved in urinary excretion of IS. In this study, we examined the effect of pravastatin with SLCO4C1 activating action on vascular endothelial dysfunction in IAKI.

Methods: IAKI models were divided into two groups that were administrated pravastatin (IR-Pravastatin group) or vehicle (IR group). 1, 7 and 28 day after IR, blood and urine were collected, and renal function and IS concentration were measured. Vascular endothelial function was assessed by Magnus method.

Results: 1 day after IR, renal function was attenuated in IR and IR-Pravastatin group. 7 and 28 days after IR, renal function recovered to the same extent as sham. 28 days after IR, attenuation of vascular endothelial function was observed in IR group, but it was improved in IR-Pravastatin group. Furthermore, IS clearance tends to increase in IR-Pravastatin group.

Conclusion: These results suggest that IS excretion promoting action by pravastatin with SLCO4C1 activation contributes partly to improvement of vascular endothelial dysfunction in IAKI.

3-P-076

Effect of the new preventive medicine on cisplatin-induced acute kidney injury

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OBJECTIVE: Cisplatin (CDDP)-induced acute kidney injury (AKI) is highly expressed. Forced hydration and diuresis may partially prevent nephrotoxicity, but it is still difficult to completely prevent kidney injury, so establishment of a new preventive method is required. Therefore, in this study, we elected to candidates for preventive drugs of CDDP-induced AKI using big data analysis, and to verify the effectiveness of the drugs.

METHODS: Using FAERS (FDA Adverse Event Reporting System), existing drugs that may reduce CDDP-induced AKI were extracted. C57BL/6 mice were intraperitoneally administered with CDDP. Renal function was evaluated by serum creatinine and blood urea nitrogen. Histological damage in the cortex of kidney sections was scored. The effect of preventive drugs for CDDP-induced nephropathy was evaluated.

Results: The drug X was extracted a candidate drug suggesting the protective effect of CDDP-induced AKI by FAERS analysis. It was revealed that administration of the drug X significantly suppressed CDDP-induced AKI.

Conclusions: From the results of this study, it was suggested that existing pharmaceutical products elected by FAERS could be a preventive drug for CDDP-induced AKI.

3-P-077

Effects of hypoxia inducible factor alpha prolyl hydroxylase inhibitor on the development of renal fibrosis in mouse unilateral ureteral obstruction model.

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Orally active hypoxia-inducible factor (HIF) prolyl hydroxylase inhibitors that stabilize HIF protein and stimulate the production of erythropoietin in clinical trials to treat renal anemia. We have shown that HIF-1 dependent gene expression of profibrogenic molecules, PAI-1 and CTGF was observed in mouse unilateral ureteral obstruction (UUO), an animal model of renal fibrosis (Kabei et al. J Pharmacol Sci. 2018). Present study was conducted to examine the effects of FG-4592, a prolyl hydroxylase inhibitor on the development of renal fibrosis. Male C57BL/6J mice orally given either FG-4592 (50 mg/kg/day) or vehicle were subjected to UUO. At 3 days after UUO, FG-4592 potentiated the increased mRNA expression of PAI-1 and CTGF in the obstructed kidneys but such potentiation disappeared at 7 days after UUO. FG-4592 did not affect the increased mRNA expression of collagen I, collagen III and TGF beta1 observed in the obstructed kidneys. Renal cortical collagen III positive stained area were not affected by FG-4592 either at 3days or 7 days after UUO. It is suggested that FG-4592 had little effects on renal fibrosis even though high dose of FG -4592 used in the present study transiently potentiated gene expression of PAI-1 and CTGF in the UUO kidney.

3-P-078

Iron metabolism abnormality in skeletal muscle atrophy associated with chronic renal failure

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Introduction and aims: Skeletal muscle atrophy is often observed in chronic renal failure (CRF) patients. However, the molecular mechanism of skeletal muscle atrophy in CRF has remained unclear. Iron is an essential trace metal for all living organisms. On the other hand, excessive iron catalyzes the formation of highly toxic hydroxyl radicals via the Fenton reaction. The purpose of this study was to determine whether iron is involved in CRF-related skeletal muscle atrophy.

Methods: In this study, we divided 8-weeks-old C57BL/6J mice into two groups: vehicle-treated group (control mice) and adenine-injected group (CRF mice).

Results: Iron content was elevated in the skeletal muscle in CRF mice. Although the expression of divalent metal transporter 1 did not change, the expression of transferrin receptor and ferroportin were downregulated in the skeletal muscle in CRF mice. The expression of ferritin heavy chain and ferritin light chain were upregulated in the skeletal muscle in CRF mice. CRF mice showed increased oxidative stress in the skeletal muscles.

Conclusions: These results suggest that iron accumulation mediated oxidative stress has the potential to accelerate skeletal muscle atrophy in CRF.

3-P-079

Varenicline, a smoking cessation drug, lowers the osteopontin expression in alveolar macrophages

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Backgrounds: Varenicline, a selective partial agonist of the a4b2 nicotinic acetylcholine receptor (nAChR) and a full agonist of the a7 nAChR, is a drug for smoking cessation. We previously reported that varenicline protects porcine pancreatic elastase (PPE)-induced alveolar expansion via a7 nAChR. However, the mechanism remains obscure. The aim of this study was to determine whether varenicline shows protective effect on the upregulated expression of osteopontin (OPN) in the alveolar macrophages (MH-S) obtained from lungs of patients with COPD.

Results: The expressions of OPN protein and mRNA were dose-dependently decreased by varenicline treatment for 24 hr; these decreases reached a peak at 10 uM varenicline. Next, to elucidate the mechanisms underlying these effects of varenicline, MH-S macrophages were treated with varenicline and a7 nAChR antagonist. Our results demonstrated that a7 nAChR antagonist reversed varenicline-induced lowered expressions of the OPN protein and mRNA.

Conclusion: These findings suggest that varenicline protects against PPE-damaged lung by downregulating the expressions of OPN protein and mRNA via a7 nAChR in macrophages. Varenicline may be one of the most useful medication for patients with COPD.

3-P-080

The effects of dasatinib on corticosteroid insensitive airway inflammation induced by lipopolysaccharide

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Corticosteroid resistance is observed in some patients of COPD and severe asthma, resulting in difficult to control of airway inflammation. We previously reported that repeated dosed lipopolysaccharide (LPS) induced corticosteroid insensitive airway inflammation in mice. And recently, some groups reported that Src was important to inflammatory responses in COPD and asthma models of mice. Thus, we determined the effects of dasatinib, a src inhibitor, on repeated dosed LPS-induced airway inflammation in mice.

A/J mice were intranasally exposed to LPS twice daily for 3 days, and intranasally treated with dasatinib 2 hr before each LPS exposure. One day after the last LPS exposure, bronchoalveolar lavage fluid (BALF) was collected. The number of inflammatory cells and cytokines expression levels in BALF were measured by flow cytometry and ELISA, respectively.

LPS increased the number of total cells, macrophages, and neutrophils, and CXCL1 and TNF-α levels. Dasatinib significantly reduced BALF cells and the CXCL1 level. Dasatinib also reduce the TNF-α level. These results suggested that src was involved in airway inflammation and dasatinib will provide a new therapeutic agent for corticosteroid insensitive airway inflammation.

3-P-081

Effects of Src inhibitor on the corticosteroid insensitive airway inflammation induced by polyinosinic-polycytidylic acid in mice

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RNA virus infections induce exacerbation and increase the morbidity of patients with chronic airway diseases. We have previously demonstrated that polyinosinic-polycytidylic acid (poly(I:C)), a TLR3 agonist, induced corticosteroid insensitive airway inflammation. The aim of this study is to evaluate the effects of Src inhibitor on poly(I:C)-induced corticosteroid insensitive airway inflammation in mice. A/J mice were exposed with poly(I:C) intranasally twice daily for 3 days. Dasatinib (Das; Src inhibitor) were administered intranasally at 2h before each poly(I:C) exposure. BALF was collected at 24 h after the last poly(I:C) exposure and neutrophils were quantified by FACS analysis. The level of CXCL1, TNF-α and osteopontin (OPN) in BALF was determined using a commercially available kit. Poly(I:C) exposure showed significant increase in neutrophils CXCL1 in BALF. Das showed dose-dependent inhibition of airway neutrophilia and CXCL1 production in poly(I:C)-exposed mice. In addition, Das inhibited TNF-α and OPN production induced by poly(I:C) exposure at higher dose. This profile provides new insights into corticosteroid insensitive airway inflammation induced by virus infection and future treatment.

3-P-082

Disruption of sphingosine 1-phosphate receptor-2 ($S1P_2$) inhibits lung fibrosis induced by bleomycin (BLM) in mice

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Idiopathic pulmonary fibrosis is a chronic and irreversible scarring disease in the lung with poor prognosis. Recently, S1P was implicated in fibrogenesis. However, the role of $S1P_2$ in lung fibrosis is still unknown. Here, we explored the role of $S1P_2$ in a murine model of lung fibrosis induced by repetitive intraperitoneal administration of BLM. $S1P_2$-KO mice were protected against BLM-induced lung fibrosis compared with wild-type (WT) mice. X-gal staining using $S1P_2^{LacZ/+}$ mice showed that $S1P_2$ is expressed in MΦs and other various constituent cells of lung and $S1P_2$-expressing cells accumulated in fibrotic lesion. Bone marrow chimera experiments and pharmacological inhibition of CSF1R kinase indicated the major role of MΦ $S1P_2$ in lung fibrosis. The gene expression analyses by DNA microarray and real-time qPCR showed that mRNA expression of Th2 cytokines and M2 markers was reduced in $S1P_2$-KO alveolar MΦs compared with WT alveolar MΦs. We also found impaired activation of STAT6 in $S1P_2$- KO alveolar MΦs. Finally, pharmacological $S1P_2$ blockade in WT mice alleviated BLM-induced lung fibrosis. In conclusion, $S1P_2$ promotes fibrogenesis by altering alveolar MΦ polarization and is a novel therapeutic target for lung fibrosis.

3-P-083
The role of growth/differentiation factor 15 (GDF15) in lung fibrosis

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The molecular mechanisms underlying the development of lung fibrosis remain poorly understood. We previously reported that the expression of growth/differentiation factor 15 (*Gdf15*) was considerably induced in the lung from bleomycin-treated lung fibrosis model mice. GDF15 is believed to be associated with stress responses, but the role of GDF15 in lung fibrosis is still unknown. Quantitative RT-PCR analyses confirmed that mRNA expression of GDF15 increased in lung with bleomycin-induced fibrosis. Furthermore, the protein levels of GDF15 were shown to increase in lung, bronchoalveolar lavage fluid and plasma from bleomysin-treated mice compared with those from saline-treated mice by ELISA. Both M1 and M2 macrophages have been noted to be involved in the pathogenesis of lung fibrosis. Using mouse peritoneal macrophages, we then explored the role of GDF15 in M1/M2 macrophage polarization. GDF15 inhibited LPS/IFNγ-induced M1 marker gene expression (*Tnfa* and *Nos2*), but did not affect IL-4/IL-13-induced M2 marker gene expression (*Fizz1* and *Arg1*). These results suggested that GDF15 is involved in M1 polarization. Further *in vitro* studies using other cell types and *in vivo* experiments will be required to elucidate the role of GDF15 in lung fibrosis.

3-P-084

Idebenone has preventative and therapeutic effects on pulmonary fibrosis via preferential suppression of fibroblast activity

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Background: Alveolar epithelial injury and abnormal collagen production by activated fibroblasts is involved in the onset and exacerbation of idiopathic pulmonary fibrosis (IPF). Compared with alveolar epithelial cells, lung fibroblasts exhibit an apoptosis-resistance phenotype that appears to be involved in IPF pathogenesis. Thus, we screened for chemicals eliciting preferential cytotoxicity of LL29 cells (lung fibroblastst) compared with A549 cells (lung alveolar epithelial cell) from medicines already in clinical use.

Results: We identified idebenone, a synthetic analogue of coenzyme Q10 (CoQ_{10}). Idebenone induced cytotoxicity in LL29 cells at a lower concentration than in A549 cells, a feature that was not observed for CoQ_{10} and two IPF drugs (pirfenidone and nintedanib). Administration of idebenone improved pre-developped pulmonary fibrosis and bleomycin-induced increases in lung myofibroblasts, whereas administration of CoQ_{10} had no effect. *In vitro*, treatment of LL29 cells with idebenone, but not CoQ_{10}, suppressed TGF-b–induced collagen production.

Conclusions: We propose that idebenone may be more therapeutically beneficial for IPF patients than current treatments.

3-P-085

Association between prevalence rate of asthma, outdoor nitrogen dioxide and weather conditions in Japan.

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Numerous epidemiological studies have suggested a relationship between nitrogen dioxide (NO_2) and impaired respiratory function or asthma symptoms. One of main source of NO_2 is considered to be traffics. However, according to Japanese Social and Demographic Statistics, the prevalence of pediatric asthma patients is higher in the northern regions of Japan than regions with much traffics. Asthma related factors also include temperature and solar radiation. We examined the association between prevalence rate of asthma, concentration of outdoor NO_2 and weather conditions in Japan. A temporary decrease in temperature and a decrease in solar radiation were associated with an increase in NO_2. Temperature and solar radiation do not directly affect NO_2, but affect nitrous acid which is in equilibrium with NO_2. These results suggest that the change of the temperature and the solar radiation effects the concentration of NO_2 in the environment via nitrous acid in the atmosphere.

3-P-086

Inhibition of prostaglandin D_2 signaling prevents the progression of cardiomyopathy in Duchenne muscular dystrophy.

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Background

Duchenne muscular dystrophy (DMD) is a severe X-linked muscle disease caused by mutations in the dystrophin gene. We found that hematopoietic prostaglandin (PG) D synthase (HPGDS) was induced in damaged muscle fibers in DMD patient and model mice, *mdx*. We have shown that chronic treatment of HPGDS inhibitor in *mdx* mice prevent the DMD progression. On the other hand, cardiac failures are the most common causes of death in DMD patients. In this study, we investigate role of PGD_2 to cardiac function and morphology in *mdx* mice.

Methods

Dilated cardiomyopathy was induced in *mdx* mice by thyroid hormone (T3)-treatment. T3 (2 mg/kg) was daily injected subcutaneously for 2-3 weeks. HPGDS inhibitor or D-type prostanoid receptor (DP -1) antagonist was daily administered in T3-treated *mdx* mice.

Results

We detected fibrosis and cardiac inflammation in the heart in T3-treated *mdx* mice, but not in control mice treated with T3. mRNA of HPGDS, cyclooxygenase (COX)-1, and COX-2, DP-1 and also DP-2 were significantly upregulated. After HPGDS inhibitor or DP1 antagonist treatment in T3-treated in *mdx*, the hypertrophy of heart was significantly reduced compared to the vehicle-treated *mdx* mice. HPGDS inhibitor treatment lowered the serum cardiac troponin I, prevented the expression of periostin and improved deteriorated cardiac functions.

3-P-087
The role of calcineurin B homologous protein 3 (CHP3) in myoblast

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Calcineurin B homologous protein3 (CHP3) is an EF-hand calcium-binding protein. In previous studies, we found that it is largely expressed in rat cardiomyocytes. Silencing of its expression by RNAi increased the cell size and induced the phosphorylation of glycogen synthase kinase 3beta (GSK3beta), a negative regulator of cardiac hypertrophy. Therefore, we suggested that CHP3 is one of the negative regulators of cardiomyocyte hypertrophy. In this study, we focused on the role of CHP3 in skeletal muscle. In mouse myoblast cell line, C2C12, almost no endogenous CHP3 protein was expressed. However, the expression level was increased in differentiated myotubes. Immunofluorescent studies showed that CHP3 was co-localized with GSK3beta in the myotube. We will discuss the role of CHP3 in mouse skeletal muscle.

3-P-088

Negative regulation of mitochondrial Ca^{2+} uptake by MICU1 is involved in high glucose-induced insulin secretion

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Intracellular Ca^{2+} signals regulate insulin secretion from pancreatic β-cells. Although recent reports suggest that mitochondria influence insulin secretion through their Ca^{2+} uptake activity, mitochondrial Ca^{2+} dynamics in β-cells have remained elusive due to limitations in the method for mitochondrial Ca^{2+} visualization. Using recently developed high-performance intraorganellar Ca^{2+} indicators, CEPIA, we here analyzed high glucose-induced mitochondrial Ca^{2+} dynamics in an insulinoma cell line MIN6. Unexpectedly, high glucose-stimulation evoked only limited mitochondrial Ca^{2+} signals. Moreover, shRNA-mediated knockdown of mitochondrial calcium uptake 1 (MICU1), which is one of the essential regulators of mitochondrial Ca^{2+} uptake, significantly enhanced high glucose-induced mitochondrial Ca^{2+} signals. Furthermore, MICU1 knockdown caused a decrease in high glucose-induced insulin secretion. These results suggest that MICU1 negatively regulates mitochondrial Ca^{2+} uptake to maintain insulin secretion. Further analysis is required to clarify the relationship between mitochondrial Ca^{2+} uptake and insulin secretion.

3-P-089

Effects of silibin, a flavonolignan, on insulin secretion from MIN6 mouse pancreatic β-cell line

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Milk thistle *(Silibum marianum)* has been known for more than 2,000 years as a herbal remedy for liver and gallbladder diseases. Silymarin, a complex of flavonolignans derived from the seeds of the milk thistle, contains mainly silybin A, silybin B, taxifolin, isosilybin A, isosilybin B, silichristin A, silidianin. Silybin, the principal flavonoid contained in silymarin and a mixture of almost equal amount of silybin A and silybin B, showed antioxidant, anti-inflammatory and anticarcinogenic properties. The therapeutic effect of silybin on insulin resistance has been reported in both clinical studies and experimental liver injury models. However, it has not been investigated whether silybin affects insulin secretion from pancreatic β cells. The present study was, thereby, conducted to investigate the effects of silybin on insulin secretion from MIN6 mouse pancreatic β-cell line. Silymarin and silybin suppressed glucose (3 mM, 25 mM)-stimulated insulin secretion from MIN6 cells in a concentration dependent manner (10 - 100 μM). On the other hand, silymarin and silybin enhanced glibenclamide-induced insulin secretion of MIN6 cells. We now try to clarify the mechanism by which silymarin and silybin affect insulin secretion in MIN6 cells

3-P-090

Property of pancreatic alpha cell is modified by bone matrix osteocalcin

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Osteocalcin (OC), a bone-derived protein, affects the regulation of glucose and energy homeostasis in variety of target tissues. We previously reported that the number of cells expressing GLP-1 increased in peripheral cells of mouse pancreas by long-term administration of OC, and also in isolated pancreatic islets treated with OC.

In the present study, we examined the effect of OC on the expression of genes related to the property of pancreatic α cells. When, αTC1-6, a cultured pancreatic α cells were treated with OC, gene expression of prohormone convertase 1 (PC1/3), the enzyme necessary for the proteolytic processing of proglucagon to GLP-1, was elevated. On the other hand, OC downregulated the expression of pcsk1n, the gene for proSAAS, a specific endogenous inhibitor of PC1/3. Furthermore, the expression of transcriptional regulator pax6 was increased by OC-treatment of the cells. Since pax6 is known to down-regulate pcsk1n expression, these findings suggest that OC promotes GLP-1 production in pancreatic α cells by upregulating gene expression and increasing activity of the enzyme involved in proglucagon conversion to GLP-1 through pax6.

3-P-091

Influence on bilirubin metabolizing enzyme liver function in fetus with antenatal glucocorticoid administration.

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Purpose : Bilirubin transport and metabolism in the liver of infants is insufficient, and physiological jaundice often occurs. The symptom of jaundice will become more severe in premature infants. Therefore, to investigate the bilirubin metabolism capacity in the liver of premature infants is necessary. Administration of antenatal glucocorticoid (GC) is the standard of care for women at risk of a preterm birth. The purpose of this study was to examine whether GC administration act on the development and effect on expressions of bilirubin metabolizing related factors in the fetal liver.
Methods : Dexamethasone were administered to pregnant rats for 2 days and the livers of 19-day-old fetuses, 21-day-old fetuses and 1-day-old neonates were analyzed. We evaluated mRNA levels of UGT1A1, albumin, BSEP, MRP2, ABCG5, ABCG8 and OATP1 by real-time PCR.
Results and Discussion : The mRNA levels of all bilirubin metabolism-related factors increased in 19-day fetuses of compared with those of 21-day fetuses. The mRNA expressions of UGT1A1 and albumin were increased in fetal liver administration antenatal GC. These results suggested that antenatal GC administration increases bilirubin excreting in the liver of premature infants.

3-P-092

Lower hepatic steatosis and normal glucose tolerance in the obesity-resistant subpopulation of ddY mice.

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During repeated preparations of high-fat diet (HFD)-induced obese mice, we have reproducibly observed both obesity-resistant (HFD_{lean}) and obesity-prone (HFD_{fat}) subpopulations in ddY mice, an outbred strain. To investigate metabolic characteristics of the subpopulations, we analyzed liver gene expression and glucose- and fat metabolisms. Hepatic steatosis was attenuated in HFD_{lean} even after ingestion of HFD for 15 weeks, whereas HFD_{fat} formed severe fatty liver. HFD_{lean} showed normal glucose tolerance and insulin sensitivity, whereas HFD_{fat} showed severe diabetes. Expression of genes for fatty acid transport (CD36), cytoplasmic triglyceride synthesis (DGAT2), fat synthesis (SREBP-I, PPARγ, PPARα) and β-oxidation (CPT-1a, acyl CoA oxidase) were significantly attenuated in HFD_{lean} as compared with those in HFD_{fat}. Low expression of apoB and MTP in HFD_{lean} consisted with their lower plasma LDL concentration. Also, plasma leptin concentration and liver β_2 adrenoceptor (β_2AdR) expression were significantly low, and hepatic glycogen content of HFD_{lean} was higher than that of HFD_{fat}. These results suggest that lower hepatic steatosis of HFD_{lean} was due to the decreased incorporation of fatty acid into the liver and suppression of triglyceride synthesis therein; and that inefficient glycogenolysis by less sympathetic activation in the liver of HFD_{lean}.

3-P-093
Effects of lactoferrin on liver functions in fat diet mice

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[Introduction]

Lactoferrin (LF) is an iron-binding protein contained in breast milk, particularly colostrum, and is also contained in neutrophils. In this report, we examined the effects of LF on liver function of high fat diet mice.

[Methods]

Six-week-old male ICR mice were used and divided into 3 groups. In group 1, normal food (CE-2: CLEA Japan) was given and 3 g of fat meal (HFD-32: Japan Clea) was given a day in groups 2 and 3. Tap water was orally administered to groups 1 and 2 in a volume of 10 mL/kg. 100 mg/kg of LF (provided from NRL Pharma) was orally administered to groups 3 at a dose of 10 mL/kg. 10 weeks after administration, visceral fat was measured.

[Results and Summary]

Plasma GOT and GPT were determind by Transaminase CII-Test Wako kits (Japan,Osaka). And the results obtained the significant suppression of increases of visceral fat and in LF group, although a significant increase was observed of fat feeding intake group. In addition, as a result of measurement of GOT and GPT in plasma, the possible improvement of liver functions can be seen by administration of LF as compared with fat meal. These results suggest that LF significantly suppresses the visceral fat induced by high fat diet due to the modulation of liver function.

3-P-094

Acetyl-CoA carboxylase 1 and 2 inhibition ameliorates hepatic fibrosis and steatosis in a murine model of nonalcoholic steatohepatitis

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De novo lipogenesis is increased in livers of patients with nonalcoholic steatohepatitis (NASH). Acetyl-CoA carboxylase (ACC) catalyzes the rate-limiting step in this process. Although ND-630, an inhibitor of ACC1 and ACC2, reduced hepatic steatosis and fibrosis marker serum TIMP1 in patients with NASH in a randomized-controlled trial, the influences on degree of liver fibrosis are not fully elucidated. We evaluated effects of inhibition of ACC on fibrosis in addition to hepatic steatosis using melanocortin 4 receptor-deficient (MC4R-KO) mice fed Western Diet (WD) that progressively develop hepatic steatosis and fibrosis. After pre-feeding with WD for 13 weeks, 9-week treatment with ND-630 (2 and 8 mg/kg, p.o., BID) with lowering malonyl-CoA contents in the liver decreased hepatic triglyceride contents. Furthermore, ND-630 lowered Sirius red-positive area, hydroxyproline contents, and mRNA expression levels of type I collagen, showing an improvement of fibrosis. The treatment was also accompanied by reduction of plasma ALT and AST levels. These data suggest improvement of hepatic lipid metabolism by ACC1 and ACC2 inhibition could be a new option to suppress fibrosis progression as well as improve hepatic steatosis in NASH.

3-P-095

Difference in laminin subunits of two-layer basement membrane in rat anterior pituitary gland

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Laminin is a major basement membrane protein and comprises three subunits: α, β, and γ chains. Among these chains, only laminin α chain is capable of signaling via receptors. Endocrine cells of anterior pituitary gland respond to basement membrane proteins, and there is two-layer of basement membrane, endothelial cell side and parenchymal cell side, in the gland. However, there is no information about the component of two-layer of basement membrane in the gland. In this study, we used immunohistological techniques to observe five laminin α chains (α1-α5) in rat anterior pituitary gland. Laminin α1, α3, α4 andα5 chains immunoreactivities were noted in the basement membrane of the gland. In addition, laminin α1, α3 andα5 chains were located in the basement membrane on the parenchymal cell side of the gland. The basement membrane on the endothelial cell side contained laminin α4 and α5. These findings show that two-layer of basement membrane is constituted of different components in the gland. Difference in composition implies that two-layer of basement membrane has different function in the gland.

3-P-096

Secretory phase-specific downregulation of Progesterone receptor membrane component 1 (PGRMC1) during the menstrual cycle stimulates human endometrial stromal cells decidualization

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Human endometrial stromal cells (ESCs) differentiate into decidual cells for the embryo implantation during the mid-secretory phase of the menstrual cycle. Decidualization is characterized by their enhanced production of IGF binding protein-1 (IGFBP-1) and prolactin (PRL), and transformation into more rounded cells. Progesterone (P4) receptor membrane component 1 (PGRMC1) is a member of a P4 binding complex implicated in female reproduction. In this study, we explored the physiological roles of PGRMC1 in the decidualization of human ESCs. Immunohistochemical analysis revealed that PGRMC1 was expressed in endometrial glandular and luminal epithelial cells and stromal cells throughout the menstrual cycle, but the expression was reduced in the secretory phase. In the *in vitro* study, treatment of ESCs with P4 and dibutyryl (db)-cAMP, which induces decidualization, repressed PGRMC1 expression. Both PGRMC1 knock-down using siRNA and inhibition using AG-205, an inhibitor of PGRMC1 promoted the db-cAMP-induced *IGFBP-1* and *PRL* expression in ESCs. We also found that microRNA miR-98 was increased in the process of decidualization and transfection of miR-98 mimic into ESC repressed PGRMC1 expression. These findings suggest that the secretory phase-specific downregulation of endometrial PGRMC1 which is regulated in part by miR-98 may promote decidualization for the establishment of pregnancy.

3-P-097

Inhibitory effect of MAX-PROBIO against *Helicobacter pylori* in in vitro and in vivo experiments

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Purpose: *H. pylori* is a major cause of gastritis and gastric ulcer. In recent years, it has been reported that probiotic has an inhibitory effect on *H. pylori* colonization. MAX-PROBIO, a probiotic product, contains 44 strains such as lactic acid bacteria and bifidobacteria. In this study, in vitro and in vivo experiments were performed to assess the inhibitory effect of MAX-PROBIO on *H. pylori*.
Methods: In the in vitro experiment, MAX-PROBIO was added to Brucella broth adjusted to pH 3, and the mixture was incubated. *H. pylori* cultures were incubated under microaerophilic conditions at 37°C with the broth culture supernatant. The viability of *H. pylori* after incubation was assessed by counting the number of viable colonies. In the in vivo experiment, *H. pylori* was inoculated orally to mice (C57BL/6JJms Slc). MAX-PROBIO was then orally administered to the *H. pylori*-infected mice for 4 weeks, and the number of viable *H. pylori* in the stomach was counted.
Results and Conclusion: The number of viable *H. pylori* was significantly decreased in the culture supernatant cultured (in vitro) and in the stomach of MAX-PROBIO-administered mice (in vivo). These findings revealed that MAX-PROBIO contributes to suppression of growth of *H. pylori*.

3-P-098
Daptomycin-induced necrotic cell death in skeletal muscle cells

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BACKGROUND: Daptomycin(DAP)-associated myopathy or creatine phosphokinase (CPK) elevation are known adverse drug effect. Although a few studies have suggested the possibility of correlation between skeletal muscle toxicity (such as rhabdomyolysis) and plasma DAP level (trough concentration), little has known on the direct cytotoxicity of DAP to skeletal muscle cells . **METHODS:** We determined the trough DAP level in patient's plasma and reviewed their laboratory data. Direct cell toxicity assays (caspase-3/7 acticity, MTT assay) were examined using myoblast RD cells. **RESULTS:** In the patients who presented CPK elevation, median DAP trough level was significantly high compared with that in patients did not present CPK elevation. In *in vitro* assays, DAP significantly decreased myoblast viability, due to necrosis in myoblast RD cells. Meanwhile, DAP inhibited apoptosis only in the high concentration (1000 mg/L). **CONCLUSIONS:** These findings from clinical and *in vitro* studies strongly support that DAP has a direct skeletal muscle cell toxicity.

3-P-099

Allergic activation of RBL-2H3 mast cells with L-Asparaginase and its inhibition by anti-IgE antibody

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L-Asparaginase (L-ASP) derived from *E. coli* is a key drug in the treatment of childhood acute lymphoblastic leukemia (ALL). L-Asp often causes hypersensitivity reactions, including anaphylaxis. We reported that a neutralizing antibody (Ab) of IgE was effective to inhibit L-ASP-induced allergy in the mouse model. In the present study, we used RBL-2H3 cells to establish *in vitro* model of L-ASP allergy.

Male BALB/c mice were sensitized by L-ASP with $Al(OH)_3$ on days 0 and 14. Cyclophosphamide (CY) was i.p. administrated on days –2 and 12. The serum was collected on day 26. Total IgE level in the serum was measured by ELISA. RBL-2H3 was sensitized by the serum and stimulated by L-ASP to determine β-hexosaminidase (β-Hex) release *in vitro*.

L-ASP sensitization increased serum IgE level, which was enhanced by CY at 150 mg/kg. When RBL-2H3 was sensitized by L-ASP-sensitized serum *in vitro*, L-ASP stimulated β-Hex release from the cells. The serum of CY-treated mice induced higher β-Hex release than normal. Anti-IgE Ab inhibited allergic β-Hex release both *in vitro* and *ex vivo*.

From the present results, L-ASP sensitization induced IgE production *in vivo* and this serum induced β-Hex release from RBL-2H3 cells. Using cell lines expressing FceRI, it can be possible to evaluate L-ASP allergy and efficacy of anti-IgE Ab *in vitro*.

3-P-100

Suppressive effects of IgA on IgE-enhanced primary antibody response

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We previously clarified that serum IgA suppresses primary antibody response, in contrast to IgG, IgE and IgM which enhance the response. In this study, we investigated the competitive potential of immunosuppressive IgA to IgE-enhanced immune response. Sensitization of mice with anti-ovalbumin (OVA) monoclonal IgE OE-1 (Day -1) and challenge with OVA on the next day (Day 0) induced anaphylaxis (Day 0) and subsequent immune responses including primary antibody response (Day 11) and cytokine production from cultured, antigen re-stimulated splenocytes (Day 14). Anti-OVA IgA monoclonal antibody OA-4 administration before the OVA challenge did not inhibit the OE-1-induced anaphylaxis but inhibited the OE-1-enhanced immune responses, indicating that anaphylaxis and immune responses are independent each other. Importantly, OA-4 administration after the OVA challenge could also inhibit the OE-1-enhanced immune responses. These findings indicated that IgA administration after anaphylaxis in mice is effective to diminish the IgE-enhanced sensitization. The suppression of the boosting phase by IgA is considerable to be an effective treatment to ameliorate allergic diseases.

3-P-101

High concentration of IgE antibodies induce activation of human peripheral basophils

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Basophils and mast cells have the high affinity IgE receptors (FcεRI) on their plasma membrane and play important roles for IgE antibody-FcεRI associated allergic diseases, such as pollen hypersensitivity, food allergy, urticaria and asthma. To date, several reports revealed that high concentration of IgE antibodies activate mast cells in the absence of any antigens. However, IgE antibodies-induced activation of basophils has not been reported. In this study, we investigated if IgE antibodies may regulate functions of human peripheral basophils by itself without antigen *in vitro*. We first stripped IgE antibodies which were originally bound to FcεRI on human peripheral basophils by treatment with lactic acid. We then demonstrated that high concentration of IgE antibodies (> 1μg/ml) induced histamine release, morphological change, cytokine release and CD203c up-regulation of IgE antibodies-stripped basophils. Thus, high concentration of IgE antibodies directly induce the activation of basophils which express IgE-free FcεRI on their surface. The mechanism may account for antigen-independent pathogenesis of allergic disorders in which serum concentration of IgE antibodies is higher than 1μg/ml.

3-P-102

Purinergic receptor-linked up-regulation of melatonin synthesis-related enzymes in human mast cell-derived LAD2

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[Background] Recent studies of immune cells and their roles in maintaining homeostasis in the body, and protecting it from not only viral and bacterial infections but also tumors, have suggested that mast cell cytokines may play important roles. **[Purpose]** The present study was undertaken to investigate mast cell melatonin synthesis and its role in homeostasis. **[Methods]** mRNA expression in LAD2 cells, a human mast cell-derived cell line, was examined for aralkylamine N-acetyltransferase (AANAT) and hydroxyindole-O-methyltransferase (HIOMT), key enzymes for melatonin biosynthesis. **[Results]** mRNA for both enzymes was expressed in the LAD2 cells. Enzyme levels were enhanced by stimulation with ATPγ-S (100 μM) without β-hexosaminidase (β-Hex) release, while BzATP (100 μM) stimulation resulted in β-Hex release but no increase in mRNA. **[Conclusion]** These results suggest that melatonin release from mast cells is not involved in the allergic response, but is involved in homeostasis.

3-P-103
Regulation of IL-10 production in murine activated mast cells

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Accumulating evidence suggests that murine mast cells should be involved not only in inflammatory responses but in immune tolerance. Reconstitution studies using mast cell-deficient mice transplanted with bone marrow-derived cultured mast cells indicated that mast cell-derived IL-10 should play critical roles in suppression of inflammatory responses, although few studies demonstrated IL-10 release from murine cultured mast cells. We found that IL-3-dependent murine bone marrow-derived cultured mast cells (BMMCs) could produce IL-10 when they were activated by the combination of the antigen and lipopolysaccharide. BMMCs rapidly released IL-6, which was lately followed by IL -10 under this condition. This time lag raised the possibility that activated mast cells should switch from the inflammatory type to the regulatory one. Activation of BMMCs lead to a drastic decrease in mRNA expression of CD39, which is the ectoenzyme involved in degradation of extracellular ATP, and a P_2X_7 antagonist, A740003, suppressed IL-10 release in activated BMMCs. These findings suggest that ATP should mediate the phenotypic changes of BMMCs in an autocrine manner.

3-P-104

Generation of specific aptamers targeting MRGPRX2 using synthetic proteoliposomes

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Mas-related G protein coupled receptor-X2 (MRGPRX2) is a novel G protein-coupled receptor with unique features including selective expression and response to peptidergic drug-induced activation in mast cells. With the aim of inhibiting MRGPRX2-dependent mast cell activation, a specific functional aptamer was generated using the proteoliposome-based systematic evolution of ligands by exponential enrichment. After multiple cycles of selection and evolution, potential functional aptamers with high affinity and specificity were selected by binding assay and functional screening and enriched for sequencing. Several aptamers showed dose-dependent inhibitory potency on MRGPRX-dependent mast cell activation. The feasibility and efficacy of these aptamers on drug-induced anaphylactoid reactions will be investigated in our future planned study.

3-P-105

Characterization of itch-related scratching behavior in chronic atopic dermatitis in hairless mice

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Itch is the most troublesome symptom in atopic dermatitis (AD). Although skin barrier dysfunctions and aberrant immune responses are thought to contribute to AD itch, the precise mechanism is yet to be clarified. In this study, we newly established a chronic AD mouse model to identify the mechanism of itch in AD. Hairless mice were fed a special diet deficient in polyunsaturated fatty acids and starch to induce dry skin with barrier dysfunction (dry-skin mice). Ointments containing a crude extract of house-dust mite were then repeatedly applied to the skin of the dry-skin mice. The dry-skin mice treated with a mite extract (DM mice) exhibited AD-like skin manifestations and histology. DM mice showed robust scratching behavior, which was partially attenuated by treatment with either betamethasone or tacrolimus, but not by that with olopatadine. Genome-wide gene expression analysis revealed that DM mice had a similar skin gene expression profile to that of human AD. Furthermore, increased expressions of *Chi3l3, Chi3l4* and *Ear11* in DM mice were consistent with exacerbation of scratching behavior. Thus, we will further examine the role of proteins encoded by these genes in itch-related behavior in this AD model.

3-P-106

Topical application of γ-linolenic acid ameliorates atopic dermatitis in hairless mice

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Atopic dermatitis (AD) is a common pruritic skin disease associated with skin barrier defects and immune dysregulation. Conventional therapies for AD include topical treatments with corticosteroids and calcineurin inhibitors, which exert anti-inflammatory and immune regulatory effects. Thus, it would be important to identify a new drug that can effectively restore both skin barrier and immune abnormalities. We have reported a unique diet-induced AD mouse model. Hairless mice fed a special diet (named HR-AD) show AD-like pruritic dermatitis, which is caused mainly by deficiency of polyunsaturated fatty acids. The present study aimed to examine the effects of topical γ-linolenic acid (GLA) on AD symptoms in this model. Topical application of GLA suppressed Th2-mediated skin inflammation and itch-related scratching behavior. Furthermore, GLA treatment reduced transepidermal water loss with increasing covalently bound ceramides, which are essential for skin barrier formation. Genome-wide gene expression analysis revealed that GLA primarily regulated expression of epidermal differentiation genes, such as *Sprr2d,* and *S100a8*. Therefore, GLA may play various roles in skin barrier and immune regulation and could be a therapeutic candidate for AD.

3-P-107

Suppressive effects of LAT1 specific inhibitor on allergic skin inflammation

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LAT1 (SLC7A5) is a transporter that incorporates large neutral amino acids into the cells. LAT1 has been considered to have an important role for cancer growth, but the function of LAT1 in normal tissues remains unknown. We characterized LAT1 as a major transporter of amino acids for the immune reactions in activated human T cells. Although LAT1 expression was not detected in freshly isolated human T cells, full activation of primary T cells triggered the induction of LAT1 expression. Attenuation of the LAT1 function by JPH203, a specific inhibitor of LAT1, in human T cells suppressed uptake of essential amino acids and immunological reactions. Furthermore, JPH203 exhibited significant therapeutic effects on T cell-mediated allergic skin inflammation in mice. We also uncovered previously unknown mechanisms by which human T cells put a brake on the immunological reactions in response to amino acids starvation by LAT1 inhibition.Our results suggest that pharmacological inhibition of LAT1 is a novel, potentially therapeutic approach for treating hypersensitive immune reaction.

3-P-108

The bleomycin induced scleroderma models in BALB/c mice: its usefulness in evaluating the effects of immunosuppressive agents

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Systemic sclerosis (SSc) is an autoimmune disorder characterized by progressive fibrosis in the connective tissue in multiple organs. In severe case, SSc might cause life-threatening complications. However, the current treatment methods for SSc are only conservative and more effective drugs are waited. The murine sclerosis model induced by repeated administration of BLM is known as one of the models which develops SSc-like dermal fibrosis. The aim of this study is to confirm the pathologic state of the BLM induced scleroderma model in BALB/c mice and evaluate the effectiveness of immunosuppressive agents on this model. We measured the hydroxyproline (HYP) content of the skin and conducted histopathologic examinations. The HYP content of the skin and the thickness of the dermal connective tissue increased in BLM treated groups compared to the vehicle treated group. It indicates the repeated administration of BLM induce dermal fibrosis. Effects of the immunosuppressive agents were evaluated on this model. The results indicate usefulness of the BLM induced sclerosis model in evaluating the effect of therapeutic agents, which leads to treatment of SSc, for dermal fibrosis.

3-P-109
Establishment of novel targeted alpha-radionuclide medicine

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Targeted alpha therapy is receiving much attention in the field of theranostics because of its high cytotoxic effect to the targeting cancer cells. However, physiological uptakes in non-targeted organs are also observed in the targeted alpha therapy, which might lead to the severe side effects. We should consider about both maximizing the treatment effect in the tumor and minimizing the side effects in the organs at risk. From this viewpoint, decision of targeting molecule was most important.
We selected LAT1 as molecular target. LAT1 is one of the amino acid transporters. LAT1 has highly specificity to cancer tissues.
We developed next-generation internal radiotherapy using chemicals targeting LAT1. First, we synthesized alpha-methyl-L-tylosine labeled with ^{211}At. ^{211}At was produced by the cyclotron, and then quickly purified and combined to alpha-methyl-L-tyrosine (^{211}At-AAMT). Next, we performed cytotoxicity evaluation of ^{211}At-AAMT using PANC-1 cells, Human pancreas cancer cell lines. We also detected the DNA damage by ^{211}At-AAMT. As a result, cell death and specificity were confirmed in ^{211}At-AAMT. We also found the anti-cancer effects *in vivo* study. In the immediate future, we will examine that the effects of ^{211}At-AAMT using several kinds of tumor-bearing models.

3-P-110

Novel anti-ASCT2 monoclonal antibody inhibits the growth of *KRAS*-mutated human cancer cells

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Cancer cells highly express amino-acid transporters in order to sustain their rapid metabolism and growth. ASCT2 is one of the primary glutamine transporters expressed in various cancers. Several researches have shown that ASCT2 inhibitors and disruption of ASCT2 gene suppress cell proliferation and tumor growth. Here, we generated a novel anti-human ASCT2 rat monoclonal antibody (mAb) designated as Ab3-8, and investigated whether the Ab3-8 had anti-cancer effects on *KRAS* mutant cancer cells. Rats were immunized with RH7777 rat hepatoma cells stably expressing green fluorescent protein (GFP)-fused human ASCT2 proteins. Splenocytes from these rats were fused with X63 mouse myeloma cells and Ab3-8 was selected from hybridomas by flow cytometry. Ab3-8 reacted with various human cancer cell lines, and inhibited intracellular glutamine uptake and phosphorylation of AKT and ERK in SW1116 and HCT116 human colorectal cancer cells with *KRAS*-mutation. Furthermore, *in vivo* xenograft study has shown that Ab3-8 suppress tumor growth derived from these cells and phosphorylation of AKT and ERK in the tumor. These results suggest that ASCT2-targeted cancer therapy is a promising strategy for *KRAS*-mutated cancers.

3-P-111

Exploratory research in plant-derived natural organic compounds with antitumor activity targeting choline transporter

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Choline is an organic cation that plays a critical role in the structure and function of biological membranes. Intracellular choline accumulation through choline transporters is the rate-limiting step in phospholipid metabolism, and it is a prerequisite for cell proliferation. In this study, we examined the functional characterization of choline transporters in MIA PaCa-2 pancreatic cancer cells. Furthermore, we searched for compounds that inhibit choline uptake as well as cell proliferation in a plant-derived natural organic compound library. Choline uptake is Na^+-independent and mediated by a single transport system. Choline transporter-like protein 1 (CTL1) and CTL2 mRNA are highly expressed. We found three hit compounds that inhibit choline uptake and cell proliferation from 500 plant-derived natural organic compounds. These hit compounds reduced cell survival and enhanced caspase-3/7 activity. These results suggest that CTL1 and CTL2 are functionally expressed in pancreatic cancer cells and are also involved in abnormal proliferation. Identification of this CTL1- and CTL2-mediated choline transport system provides a potential new target for pancreatic cancer therapy.

3-P-112

A novel treatment for recurrent glioblastoma after chemoradiotherapy

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【Background】
Glioblastoma is highly invasive and infiltrates the brain, which makes complete removal difficult. The cancer stem cells which resist chemoradiotherapy cause recurrence of glioblastoma. Thus, it is a promising therapy target. Recent studies have shown that Ion channels are involved in cell proliferation, metastasis, and invasion in cancer.
【Aim】
We identified inhibitors of ion channels in cancer stem cells for treatment of recurrent glioblastoma.
【Method】
Cancer stem cells were established from a surgical specimen of patients of recurrent glioblastoma by sphere culture methods. We measured whole-cell currents of cancer stem cells using patch-clamp techniques, detected the distribution of ion channel proteins using fluorescent immunostaining, and performed WST-8 cell proliferation assay.
【Result】
Two cancer stem cell lines were established from specimens of recurrent glioblastoma. The cancer stem cells expressed non-selective cation currents. We identified verapamil, an inhibitor of TRPML channels, from thirty compounds. The TRPML1 and TRPML3 proteins were localized in the cell membrane of cancer stem cells. Verapamil suppressed the cell proliferation of cancer.
【Conclusion】
The results indicate that verapamil may be a novel drug for recurrent glioblastoma.

3-P-113

Anti-tumor effects of astaxanthin and adonixanthin on glioblastoma cells

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Glioblastoma (GBM) is one of the most lethal brain tumor arised from glial cells. The chemotherapy is important to improve the prognosis of GBM. Although temozolomide has been used as a first line drug for GBM, some patients could not prolong their survivals. Thus, it is required to develop new drugs which have effectiveness on GBM. Astaxanthin was reported to have anti-tumor effects on lung cancer, liver cancer and so on. This study was performed to clarify whether both astaxanthin and its intermediate compound adonixanthin have anti-tumor effects on GBM cells.

We evaluated the ability of cell proliferation and migration by WST-8 and scratch assay. Moreover, we evaluated the expression of some proteins which were related to tumor progression by immunoblot. Furthermore, we performed ROS assay of astaxanthin and adonixanthin. Astaxanthin and adonixanthin inhibited the cell proliferation and migration of GBM cells. Moreover, the phosphorylation of ERK and Akt were decreased. The suppression of ROS by astaxanthin and adonixanthin may be related to the reductions of phospho-ERK and Akt.

In conclusion, these results indicate that astaxanthin and adonixanthin inhibit the cell proliferation and migration of GBM cells via suppression of ERK and Akt signaling pathways.

3-P-114

The effect of Dipeptide-derivate Compound in Melanoma Growth and Metastasis inhibition

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Melanoma is an aggressive skin cancer and a predominant cause of skin cancer-related deaths. This study investigates the anti-tumor effects of dipeptide-derivate compound (DDC) in a highly metastatic murine melanoma cell line. Cell viability was assessed using the MTT colorimetric assay. Metastatic capabilities including migration of B16F10 melanoma cells were examined by Transwell migration and intravasation. The Akt, ERK and IκB phosphorylation was used to investigate the possible mechanisms of DDC. Melanoma xenograft and lung metastasis mouse model were used to investigate the *in vivo* effect of DDC. We first demonstrated that DDC affects the cell viability. DDC does-dependently inhibited the migration and intravasation of B16F10 cells *in vitro*. DDC also dose-dependently affected the Akt, ERK and IκB phosphorylation in B16F10 cells. The *in vivo* effect of DDC was demonstrated in the melanoma model of B6 mice that was known as the most difficult tumor model to cure. DDC administration was significantly inhibited tumor growth and metastasis in B16F10 *in vivo* model. Taken together, these findings suggested that DDC could reduce the growth and metastasis of melanoma cells. in vitro and in vivo. DDC may be a promising therapeutic agent for melanoma.

3-P-115

The anti-metastasis effects of oroxylin-a on oral squamous cell carcinoma cells

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Oral cancer ranks the seventh in cancer-related deaths in men and also the thirteenth most common in women worldwide. The lymph node and distant metastasis are the major causes of death in oral cancer patients. Therefore, it is a critical need to identify new potential therapeutic agents against oral cancer metastasis. Oroxylin A (oro-A), a main bioactive flavonoid extracted from *Scutellaria radix*, has been reported to inhibit migration in various human cancers. Our previous study demonstrated that a long-term exposure to oro-A further suppress cell migration significantly than short-term oro-A exposure and without cytotoxicity on oral cancer cells treatment. Hence, we aimed to find out the anti-migration effects and underlying mechanisms of long-term oro-A exposure on oral cancer cells. In present study, we found that the migration abilities of the oral cancer cells long-term oro-A treatment may increased by chemokine ligand 2 (CCL2). It seems that CCL2 plays a critical role in anti-migration of long-term oro-A exposure. The CCL2 treatment was demonstrated to activate the protein levels of the p-ERK, p-JNK, NF-κB, MMP-2&9 signaling pathway. We also found that oro-A inhibit the metastasis via suppressing the expression of CCL2 *in vivo*. Our results indicate long term exposure to oro-A inhibits migration in human oral cancer cells through CCL2/ERK/NF-κB/MMP -2,9 signaling pathway.

3-P-116
Regulation of oxidative stress by Addicsin-Arl6ip1 complex

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Addicsin is a negative modulator of neural glutamate transporter EAAC1. It associates with Arl6ip1, an ER localized anti-apoptotic factor involved in onset of hereditary spastic paraplegia. Addicsin indirectly promotes EAAC1-mediated glutamate transport activity by increasing Addicsin-Arl6ip1 (AA) complex formation. However, this physiological significance and molecular mechanism remain largely unknown. Here, to clarify these questions, we examined the relationship between the dynamics of AA complex and oxidative stress sensitivity. Both Arl6ip1/Addicsin mRNA expression ratio and H_2O_2-induced cell toxicity showed a very high correlation. Moreover, H_2O_2 oxidative stress sensitivity in C6BU-1 cells was drastically increased in overexpression of Arl6ip1 and AddicsinY110AL112A lacking binding ability for Arl6ip1. Immunocytochemical analysis showed that addicsin co-localized with Arl6ip1, whereas translocated to plasma membrane by 100 μM H_2O_2 exposure. These results support that AA complex dissociationinduced by addicsin translocation from ER to plasma membrane may be an important physiological phenomenon to regulate oxidative stress sensitivity in neuronal cells. I would like to discuss Arl6ip1-depedndent apoptosis signals.

3-P-117

NOX4/NADPH oxidase regulates the expression of endoglin, a TGF-β co-receptor

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Reactive oxygen species (ROS) derived from NOX4/NADPH oxidase are known to play important roles in tissue fibrogenesis. However, signaling pathways downstream of NOX4 involved in fibrogenesis have not yet been well established. A proteomics technique with cleavable isotope-coded affinity tags was applied to TGF-β-stimulated IMR-90 cells derived from human lung fibroblasts. In cells transfected with siRNAs against NOX4, we found that levels of endoglin, a co-receptor of TGF-β, were reproducibly decreased compared with those in control siRNA-transfected cells. Quantitative PCR and Western blotting revealed that the expression level of endoglin was decreased at protein levels by knock-down of NOX4. Bafilomycin A1, an inhibitor of lysosome, disturbed down-regulation of endoglin by anti-NOX4-siRNAs. In addition, an inhibitor of protein kinase C (PKC), GF109203X, as well as a pseudosubstrate inhibitor of atypical PKC, suppressed the reduction in endoglin induced by anti-NOX4 siRNAs. These findings suggested the involvement of atypical PKC in degradation of endoglin upon depletion of NOX4. Thus, NOX4-derived ROS may stabilize endoglin by regulating atypical PKC.

3-P-118

Caffeine caused the reversion of activated hepatic stellate cells in a concentration-dependent manner.

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Background: During liver injury, quiescent hepatic stellate cells (qHSCs) are activated by various cytokines and transdifferentiated into myofibroblast-like activated HSCs (aHSCs), which produce collagen, a major source of liver fibrosis. Therefore, the reversion of aHSC is regarded as a therapeutic target for liver fibrosis. Several epidemiological reports have revealed that intake of caffeine decreases the risk of liver fibrosis. In this study, therefore, we investigated the effect of caffeine on the reversion of aHSCs isolated from mice. We used aHSC activated by culturing in DMEM supplemented with 10% FBS for 7 days.

Results: Caffeine (0.1-10 mM) decreased the positive area of α-smooth muscle actin (α-SMA) in a concentration-dependent manner. Caffeine decreased the expression of COL1a1, an index of aHSC, and increased this of MMP-9, an index of qHSC. CGS-15943, an adenosine receptor inhibitor, had no significant effect on the area of α-SMA and the expression of COL1a1 and MMP-9.

Conclusion: Caffeine caused the reversion of aHSC in a concentration-dependent manner independently of adenosine receptor.

3-P-119

Analyses for the mechanisms of *N*-acylethanolamine biosynthesis in peripheral tissues using mice lacking *N*-acylethanolamine-forming phospholipase D-type enzyme

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N-Acylethanolamines (NAEs) include palmitoylethanolamide, oleoylethanolamide and anandamide, which show anti-inflammatory/analgesic, anorexic and cannabimimetic actions, respectively. These lipid mediators are biosynthesized from *N*-acyl-phosphatidylethanolamine (NAPE) by NAPE-hydrolyzing phospholipase D (NAPE-PLD) as well as through multi-step pathways via lysoNAPE. We previously showed that genetic deletion of NAPE-PLD markedly increased NAPE levels and significantly decreased NAE levels in brain. Here, we aimed to examine the mechanisms for NAE biosynthesis in peripheral tissues. As compared to wild-type mice, NAPE-PLD$^{-/-}$ mice exhibited increased NAPE levels in heart, kidney and liver, but not in jejunum, suggesting a major role of NAPE-PLD in NAPE degradation in the three tissues. However, the deletion did not affect NAE levels in all the four tissues, showing compensation by other pathway(s). Accordingly, the tissue homogenates from NAPE-PLD$^{-/-}$ mice generated [^{14}C]NAE and [^{14}C]lysoNAPE from [^{14}C]NAPE. These results suggested that the contribution of NAPE-PLD to NAE biosynthesis varies among tissues and might imply the possible presence of the tissue-specific mechanisms.

3-P-120
Regulation of PRAF3 signals by Rab protein

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PRAF3, which is the PRA1-superfamily member, plays important 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 toxicity remained unknown. Our findings demonstrate that one of Rab proteins can relieve the cytotoxicity of PRAF3 both in yeast and a human cell expression system. The ability of Rab protein to cancel the toxicity could further imply that PRAF3 and Rab proteins are closely related to each other physiologically and genetically.

3-P-121

Axonal targeting of TrkA and its interacting molecule via transcytosis

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Neurotrophins are one of the best-known examples of target-derived instructive cues that regulate distinct aspects of neuronal development. Upon nerve growth factor (NGF) binds to its receptor TrkA at axon terminals, these complexes are internalized and then retrogradely transported back to cell bodies. We have previously found the new function of retrograde signaling by which target-derived NGF enhances soma-to-axon transcytosis of TrkA. However, it is unclear whether the transcytosis machinery is a general mechanism which NGF utilizes to recruit additional membrane proteins necessary for axon growth and synapse maturation. Here we show evidence that NGF enhances soma-to-axon transcytosis of amyloid-beta precursor protein (APP). APP interacts with TrkA at the extracellular domain just adjacent to the transmembrane domain. Ectopic expression of APP induces the formation of neurite-like processes in non-neuronal cells, suggesting that transcytosis of APP with TrkA contributes to axon growth in neurons. Since APP and its proteolytic products play an important role in pathogenesis of Alzheimer's disease (AD), our data suggests that the TrkA-APP anterograde transcytosis is involved in NGF functioning and its defects might be related to the onset of AD.

3-P-122

Generation of DAT-integrin α5 heterozygous knock-in embryonic stem cells using CRISPR/Cas9 system

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We have previously found that integrin α5β1 on dopaminergic neurons plays an important role in the neurite outgrowth on striatal neurons. This finding indicates that integrin α5 (Itga)-overexpressing dopaminergic neurons enhance functional regeneration in transplantation therapy for Parkinson disease. Here, we generated the dopamine transporter (DAT)-Itga heterozygous knock-in mouse embryonic stem cells using the CRISPR)/Cas9 system. These cells can be induced to express Itga gene after dopaminergic differentiation, avoiding the loss of DAT function. The knock-in targeting vector expressing Venus (KI-Ctrl) or Itga followed by Venus (KI-Itga) contained a transgene of 2721 bp or 6470 bp, respectively, which was flanked by the 5'- and 3'- homology arms. Homology arms of approximately 2 kbp were required to obtain heterozygous recombinant clones. Although two KI-Ctrl cloned cells with accurate chromosomal sequence in non-targeted allele were obtained, all KI-Itga cloned cells had indel mutations. By decreasing the amount of Cas9-expressing plasmid and co-transfecting with the rescue vector containing chromosomal sequences, one KI-Itga5 cloned cells with accurate DNA sequence in non-targeted allele was obtained.

3-P-123

Therapeutic effects of allogeneic fetal membrane-derived mesenchymal stem cells sheet transplantation to chronic myocardial infarction

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Aim: In this study, we investigated whether transplantation of allogeneic fetal membrane (FM)-mesenchymal stem cell (MSC) sheets could attenuate myocardial dysfunction in myocardial infarction (MI) model of rat and mini-pig.

Methods and Results: Allogeneic FM-MSC sheet or autologous bone marrow (BM)-MSC sheet stacked to two layers were transplanted onto the scarred myocardium 4 week after coronary ligation in Lewis rats. From the first day until 4 weeks after transplantation, both GFP-positive FM-MSCs and BM-MSCs were engrafted in the heart. Four weeks after transplantation, both FM-derived and BM-derived sheets significantly reversed wall thinning in the scar area and improved cardiac function. However, allogeneic FM-MSC sheet transplantation failed to improve cardiac function 3 months after transplantation in the mini-pig MI model.

Conclusions: Similar to autologous BM-MSC, allogeneic FM-MSC sheet transplantation attenuated myocardial dysfunction in the rat model of MI. However, it could not show any therapeutic effects in the mini-pig model. Therefore, the improvement of therapeutic performances of MSC sheet transplantation might be necessary for the treatment of MI.

3-P-124

FGF signaling promotes bone marrow niche remodeling in post-stroke recovery

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Bone marrow (BM)-derived hematopoietic stem/progenitor cells, such as $CD34^+$ cells (HSPC) have an important role in the process of vascular injury repair. We have showed that CXCL12/CXCR4 and FGF signaling may remodel BM niche during post-stoke recovery in exercise SHRSP, a model of chronic vascular inflammation with severe hypertension. The aim of this study was to examine FGF physiological signaling by *in vivo* challenge of CXCL12.

Recombinant CXCL12 and Diprotin A were administered at day 2 to 5 post-stroke in SHRSP. A variety of cell types in BM or brain, and FGF and ROS production of BM cells were examined.

CXCL12-treated SHRSP significantly prolonged the survival. The $CD34^+$ cells and $GFAP^+$-, $Nestin^+$-neural progenitor cells (NPC) were increased in the penumbra, and tightened BBB. Increases in BDNF production and DCX^+ NPC migration were not detected in SVZ. CXCL12 treatment increased the production of FGF1/FGF2, but not VEGF, in mesenchymal stromal cells (MSC), endothelium, and megakaryocytes (MK) of BM, and the numbers of $CD34^+$ HSPC, MSC and MK in BM vascular niche following stroke injury. ROS production of BM cells reduced in CXCL12-treated SHRSP. FGF signaling in response to CXCL12/CXCR4 in BM niche may regulate $CD34^+$ HSPC migration for stroke repair.

3-P-125
Drug Repositioning approaches for breast cancer stem cells.

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Breast cancer are known to originate from a minor population of cancer cells termed cancer stem cells (CSCs), which has drug resistance. However, novel therapeutic drugs for the eradication of CSC has not been established yet. Drug repositioning (DR), which finds new medical uses for existing drugs, is expected to facilitate drug discovery. In the present study, we tried to explore DR candidates by omics analyses. We performed the ribosome profile that comprehensively analyzes translation using the deep sequencing. Breast CSCs was isolated by a CSC marker aldehyde dehydrogenase (ALDH) from breast cancer cell line MCF-7. We found that sphingosine kinase, which produces bioactive lipid sphingosine-1-phosphate (S1P), is translationally upregulated in ALDH-positive cells, compared to ALDH-negative cells. Since S1P regulates many biological processes through S1P receptors, we next focused on the immunosuppressive agent fingolimod, a S1P receptor modulator, as a candidate. The Fingolimod blocked proliferation of ALDH-positive cells and expression of stem cell markers Oct4, Nanog and Sox2. These results suggest that fingolimod is a potential target for breast cancer stem cells.

3-P-126

Possible involvement of peroxynitrite in promotion of proliferative activity in the neural stem/progenitor cells after hippocampal dentate granule cell loss

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Our previous studies demonstrated that the trimethyltin chloride (TMT) causes the granule cell loss in the dentate gyrus (DG) of adult mouse, with being regenerated in the dentate granule cell after the neuronal loss. To elucidate the involvement of peroxynitrite in proliferation of neural stem/progenitor cells (NPCs) after neuronal degeneration, we evaluated the expression of 3-nitrotyrosine (3-NT, a product of tyrosine nitration by peroxynitrite) in the newly generated cells following neurodegeneration in the DG. Mice were given TMT to prepare slices for immunostaining using antibody against nestin (NPCs marker) and 3-NT. Cells positive for nestin and 3-NT markedly increased in the DG on day 3 after TMT treatment. In vitro experiments using the NPCs isolated from the DG on day 3 post-TMT, exposure to apocynin (NADPH oxidase inhibitor) or L-NAME (nitric oxide synthase inhibitor) significantly attenuated the cell proliferation. However, KT5823 (G kinase inhibitor) did not affect it. These results support the possibility that peroxynitrite promotes proliferative activity of the NPCs generated following neuronal degeneration in the DG.

3-P-127

Evaluation of developmental neurotoxicity using neural differentiation potency in human iPS cells

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Developmental neurotoxicity (DNT) has been assessed using experimental animals. Due to complexity of human brain development and species differences, alternative in vitro testing using human cells, such as human iPS cells (iPSCs), has been expected in terms of cost and time-consuming. In the present study, we examine the effects of a well-known anticancer agent 5-fluorouracil (5-FU) with potential DNT hazard (Mundy et al, 2015). We have focused on neural differentiation potency in iPSCs as an endpoint of DNT hazard evaluation. As a result, 5-FU reduced the expression of *OTX2*, a neurogenesis marker, in the neural induction. Since neural differentiation requires ATP as an energy, we examined the cellular ATP content. 5-FU decreased ATP levels in iPSCs. To understand the mechanisms of ATP reduction by 5-FU, we examined the effects of 5-FU on mitochondrial dynamics. 5-FU reduced mitochondrial fusion protein Mfn and induced mitochondrial fragmentation. Moreover, Mfn knockdown in iPSCs inhibited neural induction via *OTX2* downregulation. These data suggest that 5-FU induces neurotoxicity via Mfn-mediated mitochondrial dysfunction in iPSCs. Thus, neural differentiation potency of iPSCs can be used for evaluation of drugs with DNT hazard.

3-P-128

Effect of antidepressants on cytochrome P450 (CYP) 2D6-mediated dopamine formation from *p*-tyramine

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[Purpose] CYP2D catalyze dopamine formation from *p*- and *m*-tyramine in the brain, and human CYP2D6 is polymorphic Imipramine, a tricyclic antidepressant, and fluvoxamine, an SSRI, are CYP2D6 inhibitors. Dopamine formation from *p*-tyramine mediated by CYP2D6 variants, CYP2D6.2 and CYP2D6.10 was compared, and the effect of genetic polymorphism on the inhibitory effects of antidepressants was investigated.
[Methods] CYP2D6.1, CYP2D6.2, and CYP2D6.10 expressed in recombinant *Escherichia coli* were used. Dopamine formation from *p*-tyramine in the presence of antidepressants such as imipramine, desipramine, fluvoxamine, fluoxetine, and paroxetine was determined by HPLC.
[Results] CYP2D6.10 had higher Michaelis constants of dopamine formation than CYP2D6.1 and CYP2D6.2. Inhibition constant of imipramine and desipramine against CYP2D6.10 were higher than that against CYP2D6.1. Fluoxetine and paroxetine inhibited CYP2D6.1-mediated dopamine formation. The maximal velocity for all CYP2D6 variants gradually increased with increasing fluvoxamine concentrations.
[Conclusions] CYP2D6 polymorphism might affect the inhibitory effect of antidepressants on dopamine formation in the brain.

3-P-129

Comparative effect of calcium channel blockers on renal function in hypertensive patients with diabetes mellitus

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Background: We conducted a retrospective cohort study to evaluate and compare the longitudinal effect of monotherapy with L-, L/T-, L/N-, and L/N/T-type calcium channel blockers (CCBs) on estimated glomerular filtration rate (eGFR), and to investigate the association of treatment duration with eGFR in diabetic patients with hypertension.
Methods: Using data from the Clinical Data Warehouse of Nihon University School of Medicine, we identified new users of five CCBs with concomitant mild to moderate hypertension and diabetes mellitus (DM). We used a multivariable regression model to evaluate and compare the effects of the drugs on eGFR, up to 12 months after the initiation of study drug administration.
Results: There was no significant association between eGFR and treatment duration in all CCB types. There was no significant difference in mean change in eGFR among the five CCBs with any treatment duration. The mean percentage change of eGFR in L-type CCBs tended to increase during the 0-3 month period. However, there was no significant difference among treatment durations in the five CCB groups.
Conclusions: Our findings suggest that monotherapy with an L-, L/T-, L/N/T-, or L/N-type CCB may have little influence on glomerular function and may be safely used in hypertensive patients with DM, at least up to 12 months.

3-P-130

Influence of ALDH2 polymorphisms on the effect of co-administration of quercetin with nitroglycerin on endothelial function

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Nitroglycerin (GTN) has reported to induce oxidative stress and impairs endothelial function (EF). Aldehyde dehydrogenase 2 (ALDH2) is *an anti-oxidative enzyme, and its activity is lost in the Glu504Lys (*2)* genotype. We reported that this genotype impaired *EF and increased oxidative stress. We also reported that a HMG-CoA reductase inhibitor (atorvastatin), which has anti-oxidative effect, improved EF after GTN treatment. In this study, w*e examined whether another anti-oxidative agent, quercetin, improved EF impaired by GTN treatment in ALDH2 *2/*2 carriers. Eighteen volunteers (*1/*1, n=12; *2/*2, n=6) were given GTN (25 mg/day) and quercetin (1000 mg/day) or GTN alone for 7 days using a crossover design. Flow-mediated dilation (FMD) was measured to evaluate EF before and after each treatment. FMD was more reduced in ALDH2*2/*2 carriers than that in *1/*1 carriers after GTN alone treatment. However, FMD was improved in the volunteers co-administered quercetin compared with those given GTN alone in both genotypes. Therefore, co-administration of quercetin might be beneficial when GTN is continuously administered, especially in ALDH2 *2/*2 patients.

3-P-131

Topical laryngopharyngeal lisinopril enhances swallowing reflux in rats with cerebral hypoperfusion

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Dysphagia, a risk factor for aspiration pneumonia in stroke patients, is associated with reduced substance P (SP) secretion from laryngopharyngeal sensory nerves. SP is degraded by angiotensin-converting enzyme (ACE) and its action is potentiated by ACE inhibitors. In this study, we investigated whether topical lisinopril can ameliorate the swallowing dysfunction induced by bilateral common carotid artery occlusion (2VO) in male Sprague-Dawley rats. Swallowing reflux was evoked by injection of 50 μL of water or citric acid into the laryngopharyngeal region and was identified by EMG activity of the mylohyoid muscle. 2VO rats showed a smaller number of swallows with a longer onset latency compared with the sham-operated rats. Pretreatment of the laryngopharyngeal region with lisinopril (1-1000 mM) dose-dependently increased the number of swallows and reduced the latency in 2VO rats, and these effects of lisinopril were completely abolished by topical FK-888 (a selective tachykinin NK1 receptor antagonist). These results suggest that topical lisinopril ameliorates the chronic cerebral hypoperfusion-induced attenuation of swallowing reflux in 2VO rats through potentiation of SP action.

3-P-132

Consecutive arterial stiffness monitoring can be beneficial for guiding fluid management of hypovolemia

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Fluid management is performed with reference to biological indicators such as blood pressure (BP) for patients with hypovolemia. Since it is little known about elasticity of the conduit artery, contributing to effective peripheral circulation via Windkessel effects, at the onset of hypovolemia as well as during the therapeutic intervention, we investigated effects of blood removal and subsequent blood transfusion on the arterial stiffness in anesthetized rabbits. Under the monitoring of BP, blood flow of common carotid artery and arterial stiffness using the cardio-ankle vascular index (CAVI), 40 ml of blood was withdrawn from the brachial artery at a rate of 1 ml/min, and the blood was subsequently transfused at a rate of 2 ml/min. Blood removal decreased the BP and common carotid blood flow, but increased the CAVI. Blood transfusion returned all parameters to the baseline. The amount of blood requiring complete recovery of the common carotid arterial blood flow was equal to that of the CAVI, but more blood transfusion was needed when monitored the BP. These results suggest that consecutive measurement of arterial stiffness, besides the BP monitoring, can be beneficial for guiding fluid management of hypovolemia.

3-P-133

TNF-α converting enzyme (TACE) inhibitor prevents insulin resistance and diabetic peripheral neuropathy

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Background: Tumor necrosis factor-α (TNF-α) converting enzyme (TACE/ADAM17) is a key sheddase that releases TNF-α from its inactive precursor and is thought as a new drug target to inhibit TNF-α production. In the present study, pharmacological effects of a novel TACE selective inhibitor, JTP-96193, on type 2 diabetes and its complication (diabetic peripheral neuropathy; DPN) were examined.

Methods: Enzyme inhibitory activity of JTP-96193 on TACE and other ADAMs were measured in *in vitro*. Fat-induced obese mice and diabetic KK-Ay mice were used to evaluate the effect of JTP -96193 on insulin resistance. Finally, streptozotocin (STZ)-induced diabetic mice were treated JTP -96193 and sciatic nerve conduction velocities (MNCV) were measured to evaluate the effect on DPN.

Results: JTP-96193 selectively inhibited human TACE with IC_{50} value of 5.4 nM. In mice model of obesity and diabetes, JTP-96193 reduced the TNF-α release and increased glucose oxidation respectively in fat tissue. JTP-96193 prevented delay of sciatic MNCV without any effects on blood glucose or insulin levels in STZ-induced diabetic mice.

Conclusion: TACE inhibitor is effective on type 2 diabetes and DPN independent from glucose lowering effect.

3-P-134

Considering about current status and issues of pharmacology education in the education of nursing, from the viewpoint of the education about harmful effect of drugs

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In the case of pharmacotherapy, we believe that the nurse need to acquire knowledge and skill about the safety management of drugs, to prevent the occurrence of harmful effect of drugs in the current medical care. In response to the voices of the victim of harmful effect of drugs in Japan, Ministry of Education, Culture, Sports, Science and Technology have surveyed since 2010 on the education to attain the extermination of the harmful side effect induced by unsuitable using drugs in each school of medicine, dentistry, pharmacy, and nursing. In this study, we analyzed current status and issues of pharmacology education in the education of nursing, from the viewpoint of the education for harmful effect of drugs. In addition, we examined what is the requirement as a nurse to check and prevent the harmful side effect induced by unsuitable using drugs if we could find the suitable education method of harmful effect of drugs because a school hour was tight and limited on the school of nursing. We carried out this study with the approval of the Saitama Prefectural University Ethical Review Board (28095). As a result, 76% of students who obtained informed consent could explain or know thalidomide incident. Only 34% of students could understand the difference between harmful effect and drug abuse, until they took a pharmacology class. On the other hand, it became clear that 84% of students thought that it was possible to prevent or relieve harmful effect of drugs as a nurse.

3-P-135

Recognition of physicians, pharmacists, nurses and patients on good medication adherence

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Purpose: This study aimed to define good medication adherence of physicians, pharmacists, nurses, and patients.

Methods: Using an interview guide, semi-structured interviews were conducted with four physicians, six pharmacists, six nurses, and two patients. A qualitative analysis method was used to analyze the obtained data.

Results: Based on the analysis, results showed that 81 codes were extracted for the entire subjects as recognition of good medication adherence. They were divided into 4 categories, "understanding prescription medications as instructed by medical professionals," "cooperativeness with medical professionals," "autonomous self-medication," and "harmony between taking medication and daily life," and further into 15 subcategories. Nurses were shown to have the highest number of extracted codes. Comparing the results for medical professionals and patients, differences in subcategory and code contents were observed.

Conclusion: Good medication adherence is recognized differently by medical professionals and patients. It is important for medical professionals to understand that the recognition of medication adherence differs depending on one's function and to support medication in cooperation with others.