ABSTRACT

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(Oral Sessions)

O1G-1-1 cRel modulates alcohol-induced $GABA_A$ receptor α6 subunit expression via IκB kinases

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Alcohol shows multiple actions on function of various cells, especially of neurons. However, it is little known about exact mechanisms of expression of ligand gated ion channels such as $GABA_A$ receptors ($GABA_ARs$). This presentation tried to clarify the effect of ethanol on expression of GABA<$_A$Rs and Cl^- transporters using mouse cerebral cortical neurons. The neurons were treated with IκB kinase (IKK) inhibitors for NFκB related signal transduction pathways to analyze how $GABA_AR$ α6 subunit ($GABA_ARα6$) expression was regulated by transcriptional factors. Ethanol up-regulated $GABA_ARα6$ mRNA with no changes in other types of α subunits and Cl^- transporters. $GABA_ARα6$ expression was significantly inhibited by both of IKK inhibitors, which are IKK inhibitor VII and BAY117082. Immunocytochemical assessment showed that ethanol increased cRel expression in nucleus, which was suppressed by IKK inhibitors. Gel shift assay showed that ethanol-induced cRel was able to bind with $GABA_ARα6$ promoter region. These results indicate that ethanol enhances $GABA_ARα6$ gene transcription via increased bindings of cRel to $GABA_ARα6$ promoter region after IKKs activation.

O1G-1-2 TMEM16A and TMEM16B act as molecular components of Ca^{2+}-activated Cl^- channels in rat pinealocytes

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The pineal gland regulates circadian rhythm through the synthesis and secretion of melatonin. Although the activity of ion channels is known to be involved in the pineal functions, the functional expression of Cl^- channels is still unknown. We found that Ca^{2+}-activated Cl^- (Cl_{Ca}) currents were observed in rat pinealocytes and partly mediated by TMEM16B-coding protein. However, the electrophysiological properties of the Cl_{Ca} currents in rat pinealocytes differed slightly from those generated by TMEM16B channels. In addition, quantitative real-time PCR data showed TMEM16A mRNA also expressed at lower level in rat pineal glands. Here we examined whether TMEM16A involves in a Cl_{Ca} conductance in rat pineal glands. Western blot data showed the expression of TMEM16A-cording protein in rat pineal glands. In rat pinealocytes, Cl_{Ca} currents were significantly decreased by siRNA-induced knockdown of TMEM16A. Moreover, the combination of TMEM16A and TMEM16B siRNAs reduced the current amplitude in an additive fashion. The kinetics of the Cl_{Ca} currents in rat pinealocytes were mimicked by co-expression of TMEM16A and TMEM16B, which were cloned from rat pineal glands, in HEK293 cells. In conclusion, pineal Cl_{Ca} channels potentially form by TMEM16A in addition to TMEM16B.

O1G-1-3 Rhythmic oscillations of the microRNA miR-96-5p play a neuroprotective role by indirectly regulating glutathione levels

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Glutathione (GSH) is a key antioxidant that plays an important neuroprotective role in the brain. Decreased GSH levels are associated with neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease. Circadian involvement in neurodegenerative diseases has long been suggested, but evidence is still elusive. In this study, we found that a diurnal fluctuation of the GSH level is correlated with neuroprotective activity against oxidative stress in dopaminergic cells. In addition, the protein level of excitatory amino acid carrier 1 (EAAC1), a transporter of cysteine for neuronal GSH synthesis, is negatively regulated by a microRNA, miR-96-5p, which exhibits a diurnal rhythm. The intracerebroventricular administration of miR-96-5p inhibitor increased the EAAC1 expression, the GSH level and neuroprotection against oxidative stress in the mouse substantia nigra. Our results demonstrate that the diurnal rhythm of a microRNA regulates the neuronal GSH amount via EAAC1 to play a role in neuroprotection.

O1G-1-4 Analysis of Histamine N-methyltransferase deficitent mice

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Neurotransmitter clearance is an essential for normal neurotransmission. However, the mechanism of brain histamine clearance has been remained almost unclear. Here, we aimed to elucidate the importance of histamine N-methyltransferase (HNMT), a histamine-metabolizing enzymes, for brain histamine clearance. First, we prepared Hnmt knockout mice (KO) by inserting LacZ gene into Hnmt gene. LacZ reporter assay showed that Hnmt strongly expressed in cortex, ambiguous nucleus and medial vestibular nucleus. Hnmt deficiency increased the histamine content in the brain lysates, and the extracellular histamine in the hypothalamic area, indicating that Hnmt was an essential for brain histamine clearance. Although the anxiety-like behaviors, working memory and social interaction were not changed, the movement time, distance and speed of KO were significantly decreased compared to WT in open field test. The locomotor activity of KO in home cages was decreased in the dark period with prolonged immobility time. In addition, the aggressive behaviors were increased in KO. The isolation stress-induced disruption of prepulse inhibition was not observed in KO. These results indicated Hnmt was involved in locomorter activity, aggressive behavior and isolation stress.

O1G-1-5 Leptin induced GRP78 and protected against ER stress-induced cell death

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Leptin centrally acts to maintain body weight by regulating energy expenditure and eating behaviors. Endoplasmic reticulum (ER) stress has been reported to cause leptin resistance, which is one of the mechanisms responsible for the pathogenesis of obesity. To cope with the stress originating from the ER, cells trigger the unfolded protein response. With anti-apoptotic properties and its ability to control the activation of the UPR, the 78 kDa glucose-regulated protein (GRP78) is essential for the normal function of the ER. In the present study, we hypothesized that leptin may be able to activate the UPR in neuronal cells. We found the induction of GRP78 by leptin treatment in neuronal cells. We subsequently determined the mechanism by which leptin-induced GRP78 is involved. Surprisingly, leptin induced-GRP78 was not mediated through the classical IRE1-XBP1 pathway. We found that PI3K-mTOR pathway may involve in leptin-induced GRP78. CHOP, an ER-apoptotic marker, was not detected in leptin-treated cells; therefore, leptin may specifically induced GRP78 in neuronal cells. Our data suggested that leptin-induced GRP78 may serve a protective role against ER-stress associated with obesity.

O1G-2-1 Calcium oscillations are a putative pathologic response of astrocytes

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Astrocytes are spontaneously active and exhibit various types of intracellular calcium elevations. The calcium activity has been observed both in situ / in vivo and in various time and space scales. However, details on its mechanism and physiological function remain to be evaluated. Here, we utilized a long-term calcium imaging technique to visualize and analyze the somatic activity patterns of multiple astrocytes in situ. Among irregular activities, a subset of cells exhibited oscillatory activities, during which a few calcium elevations repeated at a relatively regular interval of approximately 20 s. These calcium oscillations were spatiotemporally sparse in the astrocytic networks and did not exhibit intercellular propagation or synchronization. Pharmacological and genetic approaches revealed that oscillations were dependent on both intracellular calcium store and protein kinase A, suggesting the involvement of a crosstalk between cAMP and calcium signaling. Furthermore, oscillations were likely a hallmark of pathological models in vitro and were related to enhanced hypertrophy. Thus, we propose that spontaneous oscillatory patterns are a unique response of astrocytes toward pathological states.

O1G-2-2 Astrocytic Ca^{2+} signals correlate with contraction of cerebral vascular smooth muscle cells

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Astrocyte endfeet surround blood vessels in the brain. Because astrocytes also make close contacts with neurons, astrocytes are thought to mediate the neural activity-dependent increase in cerebral blood flow. To study the role of astrocytes in the blood flow regulation, we compared the changes in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) between astrocytes and adjacent arterial vascular smooth muscle cells (VSMCs), generating transgenic mouse lines that express a genetically encoded Ca^{2+} indicator in astrocytes and VSMCs. Using *in vivo* two-photon Ca^{2+} imaging, we observed sensory stimulation-induced transient decrease in $[Ca^{2+}]_i$ (i.e., relaxation) in VSMCs, which was followed by recovery of $[Ca^{2+}]_i$ with an overshoot (i.e., contraction). We found that astrocytic $[Ca^{2+}]_i$ increase had a considerable delay to the vasorelaxation, and showed a temporal correlation with the vasocontraction. We further examined the relationship between astrocytic $[Ca^{2+}]_i$ and REM sleep, during which neural activities and cerebral blood flow are increased. We observed a coincidental increase in $[Ca^{2+}]_i$ in astrocytes and VSMCs after the end of REM sleep. These results suggest that astrocytes play a key role in contraction rather than relaxation of VSMCs.

O1G-2-3 TNF-α-stimulated brain pericytes enhance microglial activation

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Brain pericytes are involved in neurovascular dysfunction, neurodegeneration and/or neuroinflammation. In the present study, we focused on the proinflammatory properties of brain pericytes to understand their participation in the induction of inflammation at the neurovascular unit (NVU). The NVU comprises different cell types, namely, brain microvascular endothelial cells, pericytes, astrocytes and microglia. Among these, we found pericytes to be the most sensitive to tumor necrosis factor (TNF)-α, possessing a unique cytokine and chemokine release profile. This was characterized by marked release of interleukin (IL)-6 and macrophage inflammatory protein-1α. Furthermore, TNF-α-stimulated pericytes induced the expression of inducible nitric oxide synthase and IL-1β mRNAs and promoted migration in the BV-2 microglial cells, suggesting that TNF-α-sensitive pericytes target on microglia to activate. Based on these findings, the possibility that brain pericytes act specifically as TNF-α-sensitive cells and as effectors of TNF-α through the release of proinflammatory factors, and that, as such, they have a role in inducing brain inflammation, should be considered.

O1G-2-4 TLR4-activated microglia upregulate GM-CSF signaling that contributes to their survival

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Lipopolysaccharide (LPS) induced rapid death of rat primary microglia, but a subpopulation of microglia survived much longer than control cells. TAK-242, a TLR4 signaling inhibitor, suppressed LPS-induced survival of microglia, confirming a critical role of TLR4. To clarify the mechanism of TLR4-mediated survival, we investigated the possibility that LPS-stimulated microglia may produce their survival factors, such as M-CSF, GM-CSF and IL-34. Among them, the mRNA level of GM-CSF was selectively increased and its protein level was also elevated in LPS-stimulated microglia. Moreover, LPS caused the increase of mRNA expression of GM-CSF receptor (GM-CSFR) α and ß subunits. We then examined the activation of JAK2/STAT5, downstream signals of GM-CSFR which controls transcription of survival-related genes. LPS stimulation resulted in the phosphorylation of STAT5 in microglia. Furthermore, NVP-BSK805, a specific JAK2 inhibitor, suppressed both STAT5 phosphorylation and the survival of LPS-stimulated microglia. These results suggest that TLR4 activation may mediate survival of subpopulation of microglia at least through self-production of GM-CSF and upregulation of GM-CSFR signaling, leading to the activation of cytoprotective JAK2/STAT5 pathways.

O1G-2-5 Activation of glycogen metabolism by pituitary adenylate cyclase-activating polypeptide in cultured astrocyte

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Glycogen is stored in astrocyte primarily located in regions of high synaptic density, and is important for brain functions. Previously, it was reported that glycogen metabolism was activated by vasoactive intestinal polypeptide (VIP), which shared common receptors with pituitary adenylate cyclase-activating polypeptide (PACAP). Therefore, we investigated the effect of PACAP on glycogen metabolism in cultured astrocyte. PACAP or VIP promoted glycogenolysis dose-dependently 1 hr after exposure, and these EC_{50} were 0.0084 or 0.43, respectively. Interestingly, EC_{50} of PACAP was at least 50 times less than neurotransmitters previously reported to promote glycogenolysis such as VIP or noradrenaline. Although PACAP decreased glycogen content 1 hr after exposure, it was over-compensated more than control level 9 hr after exposure. NR4a3 (nuclear receptor) and protein targeting to glycogen (phosphatase) are known to be important for glycogen synthesis, and these expression were increased by PACAP. These results suggest that PACAP strongly activates glycogen metabolism including glycogenolysis and glycogenesis in cultured astrocytes.

O1G-3-1 P2X7 receptor/HIF-1α pathway is a distinctive mechanism for astrocyte-mediated ischemic tolerance

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A brief ischemic episode (preconditioning; PC) induces resistance to a subsequent severe ischemic injury. This phenomenon, known as ischemic tolerance, is an endogenous process that provides robust neuroprotection. We previously showed that ischemic tolerance was dependent on astrocytes, for which emergence of P2X7 receptors in activated astrocytes was essential (astrocyte-mediated ischemic tolerance). However, the downstream signals of P2X7 receptors responsible for the ischemic tolerance remain unknown. Here we show that HIF-1α in astrocytes has an indispensable role for this event. Using a middle cerebral artery occlusion model, we found that PC increased HIF-1α in both neurons and astrocytes. It is well-known that decrease in oxygen is a main mechanism that promotes increase in HIF-1α, and actually, the neuronal HIF-1α increase was dependent on hypoxia/ischemia. However, as for astrocytes, activation of P2X7 receptors, rather than ischemia, was important. Furthermore, the increase in HIF-1α in neurons was transient, whereas that in astrocytes lasted much longer. Thus, it is strongly suggested that such characteristic feature of HIF-1α in astrocytes should explain why astrocyte-mediated ischemic tolerance is strong and sustained.

O1G-3-2 *In vivo* Ca^{2+} imaging of cortical astrocytes during ischemia using a mouse thromboembolic disease model

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Cerebral blood flow obstructions cause disruption of local oxygen supply leading to cerebral infarction. In the core of the ischemic area neurons undergo necrosis. In the surrounding area (penumbra) of the necrotic core, neuronal electrical activities are depressed in response to insufficient oxygen supply. Neurons in the penumbra are viable in the acute phase of ischemia. However, pathological processes including abnormal neuronal depolarization, vascular constriction and local inflammation evoke further oxygen exhaustion, and result in the expansion of necrotic core. Because recent studies report that astrocytes regulate neuronal activities and blood flow, we aimed to visualize activities of astrocytes in the penumbra. We developed a mouse thromboembolic model, in which clots were infused via external carotid artery. Using high-resolution MRI, we confirmed the formation of penumbra within 1-2 days and subsequent expansion of necrotic core (~1 week). We then carried out *in vivo* two-photon Ca^{2+} imaging of astrocytes during ischemia, and found that astrocytic Ca^{2+} started to oscillate at ~10-minute intervals after the embolism. The present mouse model can be a powerful tool to clarify the pathophysiology of cerebral infarction.

O1G-3-3 A screening of TRPC channels activators identifies novel neurotrophic compounds

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Neurodegenerative disorders, which occurs as a result of progressive degeneration of neurons, present enormous medical problems as they are untreatable and incurable diseases. It has been indicated that drugs which support neuronal growth and survival would ameliorate neuronal degeneration, but little progress has been made in their development. Here, we report novel activators of transient receptor potential canonical (TRPC) channels which regulate neuronal function via modulation of Ca^{2+} signaling. We screened activators for TRPC3/6 channels from chemical compounds library which have important roles in neuronal growth and survival induced by BDNF. Screened compounds induced whole-cell currents and intracellular Ca^{2+} level increase in TRPC3, 6, or 7-overexpressed HEK293 cells in patch clamp recordings and Ca^{2+} imaging. In primary cultured cerebellar granule neurons, application of our compounds showed significant increase of CREB phosphorylation and neurite outgrowth which were suppressed by the knockdown of TRPC3/6/7. These findings indicate that our compounds support neuronal growth via acivation of Ca^{2+} signaling through TRPC3/6/7 channels, providing leads for developing new drugs for neurodegenerative disorders.

O1G-3-4 Radial migration of cerebellar granule neuron regulated by Rac through a new signaling pathway

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Rac small GTPase is a key molecule in neurogenesis by regulation of cytoskeletal dynamics. However, paradoxical findings in Rac1 and its isoform have been reported.
To elucidate definitive function of Rac in-vivo, we generated Rac-knockout (KO) mice using gene targeting in cerebellar granule neuron (CGN). In contrast to no phenotype in Rac1 or Rac3 single KO mice, Rac1/Rac3 double knockout (DKO) mice showed severe gait disturbance. Histological evaluation showed numerous apoptosis in the external granule layer (EGL) at postnatal day 8 followed by agenesis of the internal granule layer (IGL) in the medial cerebellum. In-vitro experiments using primary culture and micro-explant culture from Rac1/Rac3-DKO mice revealed impaired neuritogenesis and neuronal migration, more dominantly in migration along with dendrites. DNA microarray revealed decreased level of Midline 1 (MID1), the responsible gene of Opitz syndrome characterized by agenesis of middle organs. Immunoprecipitation analysis showed that Rac forms complex involving mTOR and MID1.
These data suggested that radial migration of CGN from EGL to IGL is Rac-dependent, and a new Rac-signaling pathway involving mTOR and MID1 may play critical role for development of the medial cerebellum.

O1G-3-5 Spaciotemoporal whole-brain imaging with single-cell resolution for the organism-level systems biology

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Circuit-level identification and analysis of neural networks in the brain will require the development of whole-brain imaging with single-cell resolution. To this end, we performed comprehensive chemical screening to develop a whole-brain clearing and imaging method, termed CUBIC (Clear, Unobstructed Brain Imaging Cocktails and Computational analysis). CUBIC is a simple and efficient method involving the immersion of brain samples in a chemical mixture which enables rapid whole-brain imaging with single-photon excitation microscopy. CUBIC is applicable to multi-color imaging of fluorescent proteins or immunostained samples in adult brains, and is scalable from a primate brain to subcellular structures. We also developed a whole-brain cell-nuclear counterstaining protocol and a computational image analysis pipeline which, together with CUBIC reagents, enable the visualization and quantification of neural activities induced by environmental stimulation. CUBIC enables time-course expression profiling of whole adult brains with single-cell resolution.

O1G-4-1 Dopamine reorganizes neuronal ensembles during hippocampal sharp save-ripples *in vitro*

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Hippocampal sharp wave (SW)/ripple complexes are thought to contribute to memory consolidation. Previous studies suggest that behavioral rewards facilitate SW occurrence *in vivo*. However, little is known about the precise mechanism underlying this enhancement. Here, we examined the effect of dopaminergic neuromodulation on spontaneously occurring SWs in acute hippocampal slices. Local field potentials were recorded from the CA1 region. A transient treatment with dopamine led to a persistent increase in the event frequency and the magnitude of SWs. This effect lasted at least for our recording period of 45 min and did not occur in the presence of a dopamine D_1/D_5 receptor antagonist. Functional multineuron calcium imaging revealed that dopamine-induced SW augmentation was associated with an enriched repertoire of the firing patterns in SW events, while the overall tendency of individual neurons to participate in SWs and the mean number of cells participating in a single SW were maintained. Therefore, dopaminergic activation is likely to reorganize cell assemblies during SWs.

O1G-4-2 Alzheimer disease therapeutic candidate, SAK3 activates T-type calcium channel $Ca_v3.1$

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The T-type voltage-gated Ca^{2+} channels (T-VGCCs) are involved in the pathophysiology of epilepsy, pain and sleep. We recently elucidated the mechanism underlying cognitive enhancement of a novel Alzheimer disease drug candidate, ST101 which stimulates T-VGCC in mouse cortical slices and neuro2A cells over-expressed Cav3.1 (Moriguchi et al., J Neurochem 2012;121:44-53). We here synthesized another series of spiroimidazopyridine derivatives (SAKs) (PCT/JP2013/51388), which were much stronger than ST101 in activation of Cav3.1 channel currents. We first confirmed that Cav3.1 is expressed in both GABAergic and glutamatergic neurons in the hippocampus. Acute ST101 treatment in the hippocampal slices failed to potentiate LTP, whereas SAK3 treatment significantly enhanced LTP induction and maintenance with concomitant CaMKII activation. We also confirmed that acute SAK3 administration enhanced hippocampal acetylcholine (ACh) release in olfactory bulbectomized (OBX) mice. Consistent with these observations, acute administration of SAK3 improved impairments of memory-related behaviors seen in OBX mice. Taken together, SAK3 acting on T-VGCC can be a novel candidate of therapeutics for Alzheimer disease.

O1G-4-3 Ameliorative effect of Sansoninto on cognitive dysfunction via improving sleep disorder in rats

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Sleep disturbance has been known to be a high incidence of the behavioral and psychological symptoms of dementia (BPSD) in Alzheimer's disease(AD). The night time wandering of the patients also thought to be related to the state that never sleeps. Present study, to elucidate the relationships between cognitive dysfunction and sleep disturbance, behavioral and electroencephalographic studies were investigated using AD's model rats. Transient cerebral ischemia(10 min) with consecutive i.c.v. injection of Aß in rats caused spatial memory disturbance in Morris water maze task. This memory disturbance was significantly ameliorated by Donepezil(10 mg/kg,p.o.). Sansoninto(1000 mg/kg,p.o.), a Kampo medicine, and common sleep inducer, Triazolam(0.2 mg/kg,p.o.) also ameliorated the Aß-induced memory disturbance in rats. In electroencephalographic studies, the total wake time and the number of nocturnal awakening in light phase were significantly increased in this model rats. Donepezil decreased the number of nocturnal awakening but not the total wake time, while Sansoninto and Triazolam improved both of them. These results suggest that Sansoninto possess an ameliorative effect of cognitive dysfunction via improving sleep disturbance.

O1G-4-4 The prostaglandin E_2 induces neuronal and behavioral impairments like psychiatric disorders

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We investigated the possibility of prostaglandin E_2 (PGE_2) as a common molecule associated with vulnerability to neurodevelopmental disruptions. PGE_2 levels in whole brain were significantly increased after exposure to viral infection [injection of polyinosinic-polycytidylic acid (polyI:C)], hypoxia (exposure to CO_2), and neglect (separation from the dams) in postnatal day (PD) 2, compared to those in non-exposure. The mice administered polyI:C or direct PGE_2 during PD 2-6 exhibited impairment of social, object recognition memory and pre-pulse inhibition (PPI) in adult at PD70, and further, the decreased dopamine turnover of the mPFC in adult mice. In the primary culture of hippocampal neurons, the PGE_2 reduced the neurite growth. These behavioral and neuronal impairments were recovered by blockade of PGE_2 receptor EP1. Our findings suggest that PGE_2 is one of potential common molecule associated with vulnerability to neurodevelopmental disruptions, and PGE_2 plays a crucial role in the development of behavioral and neuronal impairments, which are associated with activation of EP1.

O1G-4-5 Methionine treatment ameliorates alterations in GAD67 expression in cingulate cortex and the abnormal behaviors of FABP3 KO mice

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[Introduction] Fatty acid binding protein (FABP) 3 is strongly expressed in GABAergic inhibitory interneurons in cingulate cortex (CC), which is one of the important brain regions for behavioral coordination. We have so far shown that FABP3 KO mice show the increase of GAD67 expression in CC and the abnormal cognitive and emotional behaviors. In order to explore the mechanism how FABP3 regulates GAD67 expression, we studied whether methionine (MET) administration, which increases DNA methylation, affects the GAD67 expression in CC of FABP3 KO mice and their abnormal behaviors. [Method] Binding ofMeCP2 to specific GAD67 CpG-rich promoter sequence was studied by ChIP assay. Mice were treated twice a day for 6 days with MET (5.2 mmol/kg, s.c.). [Result] In the CC of FABP3 KO mice, binding of MeCP2 to GAD67 promoter was significantly decreased compared with wild-type mice. MET administration restored the elevated GAD67 mRNA expression in the CC of FABP3 KO mice back to wild-type levels, and improved their abnormal behaviors.[Conclusion] These findings suggest that DNA hypomethylation and the associated chromatin remodeling underlie the elevation of GAD67 in CC and the abnormal behaviors of FABP3 KO mice.

O1G-5-1 A role for mDia, a Rho-regulated actin nucleator, in regulating morphology of presynaptic terminals and increased anxiety-like behavior induced by social isolation stress in mice

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Neuronal plasticity in the nucleus accumbens (NAc) underlies emotional changes associated with chronic stress. However, its molecular mechanism remains unknown. We have analyzed a role for mDia in emotional changes induced by chronic stress. Conditional knockout mice lacking mDia in NAc neurons failed to show increased anxiety-like behaviors induced by social isolation, suggesting a critical role for mDia in NAc neurons in stress-induced emotional changes. Social isolation also caused shrinkage of presynaptic terminals from NAc neurons and reduced GABAergic transmission in the VTA in an mDia-dependent manner. Similar morphological and functional changes were observed in primary neurons under long-term blockade of action potential. The tetrodotoxin treatment for one day induced shrinkage of GABAergic terminals and reduced GABAergic transmission in an mDia dependent manner. Notably, we observed concomitant enrichment of mDia to presynaptic terminals. Therefore, our study demonstrates a role for mDia in activity-dependent plasticity of GABAergic presynaptic terminals and is elaborating its relevance to stress-induced emotional changes.

O1G-5-2 The role of the ventrolateral striatum in motivation

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The ventral striatum (VS) dopamine receptor type 2 expressing medium-sized spiny neuron (D2R-MSN) is critically involved in goal-directed behavior and motivational processes. The VS often subdivided into medial and lateral parts based on the difference of predominant afferents, implying a functional dissociation among VS subdivisions. To elucidate the role of ventrolateral striatum (VLS) D2R-MSN, we investigated the effects of loss of function in these cells on goal-directed behavior and motivation by using transgenic mice expressing diphtheria toxin A selectively in the VLS D2R-MSN in a time-controllable manner. We first screened the behavioral phenotype of the transgenic mice by using the 3-choice serial reaction time task, which can address attention, behavioral inhibition, and motivation. The dysfunction of the VLS D2R-MSN resulted in deficits of sustained attention and/or motivation. To examine the effects of VLS D2R-MSN dysfunction on motivational processes, we next conducted the progressive ratio task, which can address motivation and rule out the influence of attentional deficits. The VLS D2R-MSN dysfunction induced a significant decline of motivation. These results highlighted that the VLS D2R-MSN selectively regulated motivational processes.

O1G-5-3 A δ opioid receptor agonist KNT-127 as an appropriate therapeutic agent for anxiety disorder when combined with exposure therapy

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Strategies to attenuate reconsolidation and facilitate extinction of fear memory have attracted increasing attention for enhancing the effectiveness of exposure therapy for anxiety disorders. Previously, we demonstrated that a δ opioid receptor agonist KNT-127 has clear anxiolytic-like effects, without impairing memory. Therefore we hypothesized that KNT-127 could be an appropriate drug for anxiety disorders when combined with exposure therapy. In this study, we examined the effects of KNT-127 on recognition, reconsolidation and extinction of fear memory in rats. In novel object recognition test, KNT-127 did not impair recognition memory, while diazepam did. Interestingly, KNT-127 attenuated reconsolidation of contextual fear memory, while enhanced the extinction learning. D-cycloserine enhanced both the reconsolidation and the extinction learning. On the other hand, diazepam impaired both of them. The anxiolytic-like effects of KNT-127 likely helped to attenuate reconsolidation of fear memory, thereby facilitating the extinction learning. Our present results strongly suggest that KNT-127 is an effective therapeutic agent when combined with exposure therapy for treating a range of anxiety disorders.

O1G-5-4 Rational design of a high-affinity, fast, red calcium indicator R-CaMP2

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Fluorescent Ca^{2+} reporters are widely used as readouts of neuronal activities. Here, we designed R-CaMP2, a high-affinity red genetically encoded calcium indicator (GECI) with a Hill coefficient near 1. Use of the calmodulin-binding sequence of CaMKK-α/ßin lieu of a M13 sequence resulted in three-fold faster rise and decay times of Ca^{2+} transients than R-CaMP1.07. These features allowed to resolve single action potential (AP) and fast AP trains up to near 20-40 Hz in cortical slices. *In vivo* imaging of the barrel cortex layer 2/3 neurons revealed reliable recording of single APs in R-CaMP2-expressing neurons, while synaptic Ca^{2+} transients were robustly detected in individual dendritic spines with similar efficacy as previously reported sensitive green GECIs. R-CaMP2 exhibits a linear relationship between AP trains and fluorescence dynamics *in vivo*. Combining green and red GECIs, we successfully achieved dual-color monitoring of neuronal activities of distinct cell types, in the mouse cortex and in free-moving *C. elegans*. Together, R/G-CaMP imaging using R-CaMP2 provides a powerful means to interrogate orthogonal and hierarchical active ensembles, thus significantly enhancing our current capacity for functional mapping of neuronal circuits *in vivo*.

O1G-5-5 A new imaging system to detect whole brain structural and functional alterations at cellular and subcellular levels

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A comprehensive understanding of brain functions regulated by a complex neural network requires systematic analysis of the whole brain. However, whole-brain imaging with a high resolution enough to visualize the cellular and subcellular distribution is still challenging. In the present study, we developed an automatic tomographic apparatus to conduct rapid whole brain imaging with a submicrometer resolution, which consists of a Nipkow-disk-equipped confocal scanning unit, a vibration microtome, and multiaxis actuators. Using this system, we could image a mouse whole brain in 2 days. In addition, our computational image analysis processed multi-terabyte datasets to provide spatial arrangement data with less than 10 gigabytes. We performed whole-brain imaging of neuronal activity in Arc-dVenus transgenic mice which express destabilized Venus, a modified yellow fluorescent protein, in activated neurons only, and identified brain regions with altered neuronal activity at single cell levels in these mutant mice subjected to acute and chronic restraint stressors. Thus, our system offers a useful tool for detection of whole brain structural and functional alterations.

O1G-6-2 Metyhl pyruvate rescues mitochondrial injury induced by SIGMAR1 missense mutant related to ALS

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The dominant missense mutation (p.E102Q) by SIGMAR1 gene mutation was discovered in the patients of juvenile amyotrophic lateral sclerosis (ALS). The sigma-1 receptor (Sig-1R) is a chaperone protein localizing in the mitochondrial-associated endoplasmic reticulum (ER) membrane, in where it regulates Ca^{2+} transport from ER to the mitochondria through IP_3 receptor (IP_3R). Mitochondrial Ca^{2+} transport induced by IP_3R stimulation was disturbed by Sig-$1R^{E102Q}$ expression, thereby reducing mitochondrial ATP production (BBA 2014;1840:3320). The Sig-$1R^{E102Q}$ mutant expression reduced the mitochondrial membrane potential and promoted mitophagy. Moreover, the ATP reduction caused the decreased proteasome activity and in turn TAR DNA binding protein (TDP-43) accumulation in the cytosol. These events were recapitulated by pharmacological inhibition of either proteasome or mitochondrial Ca^{2+} transport. The supply of mitochondrial TCA cycle substrate, methyl pyruvate, improved the Sig-$1R^{E102Q}$-induced reduction of ATP synthesis and proteasome activity with concomitant inhibition of cytoplasmic accumulation of TDP-43. Taken together, methyl pyruvate rescues mitochondrial injury associated with ALS caused by Sig-$1R^{E102Q}$.

O1G-6-1 GPNMB has protective effects against mutant TDP-43-induced motor neuronal cell death

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We have reported that glycoprotein nonmetastatic melanoma protein B (GPNMB) has protective effect against SOD1 (G93A) induced in vivo and in vitro model of familial ALS (amyotrophic lateral sclerosis). Although about 90% of ALS patients are sporadic ALS, the effect of GPNMB against sporadic ALS remains unclear. Mutations of transactive response DNA binding protein 43kDa (TDP-43) are associated with neurodegenerative disorders including familial and sporadic ALS and frontotemporal lobar degeneration (FTLD). Therefore, we evaluated the effect of GPNMB against mutant TDP-43. We transfected mock or TDP-43 (WT, M337V, A315T) plasmid into mouse motor neuron cells (NSC-34). The expression of glycosylated-GPNMB was increased after transfection of mutant TDP-43 plasmid, compared with mock transfected. Recombinant GPNMB ameliorated motor neuron cell death induced by transfection of mutant TDP-43 plasmid and serum free stress. Furthermore, the expression of phosphorylated-Akt and -ERK1/2 was decreased by this stress, and these expressions were improved by recombinant GPNMB. These results suggest that GPNMB has protective effects against mutant TDP-43 via Akt and ERK1/2 pathway, and GPNMB may be a therapeutic target for familial and sporadic ALS and FTLD.

O1G-6-3 Analysis of metabolic dynamics under the pathology of Parkinson's disease by analyzing human disease-specific iPS cells

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iPS cells are pluripotent cells which give rise to all cells in the organism. In the present study, we generated iPSCs from two Parkinson's patients and two control subjects. All of the clones differentiated into neurons included tyrosine hydroxylase-positive neurons. Under the present condition, we found differences in gene expression between control and patients. Among those, the expression level of PGC-1α, which plays a key role in mitochondrial biogenesis and energy metabolism, in Parkinson's specific-iPS cell (PD-iPSC)-derived dopaminergic neurons was significantly lower than that in control. Using CE-MS-system, we found the change in the expression of several metabolites in glycolysis and glutathione metabolism pathways in dopaminergic neuron-derived from PD-iPSCs. Interestingly, the expression of S-adenosylmethionone (SAM), which can lead to methylation of DNA, was significantly increased in PD-iPSCs-derived dopaminergic neurons. Furthermore, the expression level of catechol-O-methyltransferase (COMT) was significantly increased in dopaminergic neuron-derived from PD-iPSCs. These findings suggest that metabolic abnormality in dopaminergic neurons could lead to neuronal dysfunction in Parkinson's disease.

O1G-6-4 The effects of anti-HMGB1 antibody on pilocarpine-induced epilepsy in mice

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Epilepsy results from abnormal excessive or hypersynchronous neuronal activity in the brain. Epilepsy is often associated with brain-blood barrier (BBB)breakdown, peripheral immune response and neuronal network reorganization in hippocampus region. Inflammatory processes in brain tissue have been described in human epilepsy of various etiologies and in experimental models of seizures. High mobility group box 1 (HMGB) is now recognized as a representative of damage-associated molecular patterns (DAMPs). To gain deeper insight into whether HMGB1 is involved in BBB disruption and brain injury during/after seizure, we examined the effects of anti-HMGB1 on pilocarpine induced epilepsy model in mice .Anti-HMGB1 mAb significantly inhibited the increase in BBB permeability induced by pilocarpine. Anti-HMGB1 also suppressed the translocation of HMGB1 from neurons in cerebral cortex and hippocampus, neuronal death in hippocampus and expression of inflammatory cytokines. In conclusion, anti-HMGB1 shows protective effects on pilocarpine-induced BBB disruption and accompanying inflammatory responses in epileptic mice and may provide a new therapeutic strategy for epileptic brain injury.

O1G-6-5 ABT-418, a selective α4β2 nicotinic acetylcholine receptor agonist,ameliorates psychiatric symptoms induced by pentylenetetrazol-kindled mice

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Epilepsy is frequently related to several psychiatric disorders,including cognitive impairment and attention deficit/hyperactivity disorder(ADHD).However,it remains unclear whether epilepsy is associated with mental function.It was reported that ABT-418 showed the effect of anti-ADHD in ADHD patients.Therefore,we evaluated the ABT-418 in kindled mice of psychiatric symptoms using behavioral pharmacological tests.In addition,we also assessed the alternation of α4 subunit and neuroligin 3(NLG3) expression induced by kindling in immunohistochemistry.Kindled mice showed impaired motor coordination,anxiolytic effect and poor social approach in pharmacological tests. ABT-418 showed an ameliorative effect on kindled induced psychiatric-like behavior in the behavioral tests.The α4 subunit was significantly decreased in the piriform cortex.Meanwhile, NLG3 was increased in the kindled mice relative to that of control.These results suggested that kindled mice have mental characteristics in psychic function.This mechanism may be involved in the change of α4 and NLG3 expression.It suggested that the ABT-418 has a therapeutic effect on epilepsy who suffer from psychiatric symptoms.

O1H-1-1 Analysis of skeletal muscle-specific Ext1 lacking mice

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Heparan sulfate, a linear polysaccharide, is essential for the developments of various organs. However, the functions of heparan sulfate in the skeletal muscle remain unclear. In the present study, we investigated the importance of heparan sulfate in skeletal muscle by deleting skeletal muscle Ext1 gene which catalyzed heparan sulfate biosynthesis. First, we generated muscle specific Ext1-deficient mice (cKO) by mating Ext1 flox mice and creatine kinase mm-Cre mice. We found that the body weight of cKO mice was significantly lower than control group at more than 10 weeks of age. The locomortor activity of cKO mice in their home cages was significantly decreased. The treadmill running tests showed the running distance of cKO group was significantly shorter than control group. The wire hang test also demonstrated that the latency to fall off in cKO group was much shorter than control group. These results strongly suggested that heparan sulfate in skeletal muscle plays an important role in motor function.

O1H-1-2 miR-34a and miR-155 overexpression suppresses neutrophil migration and activation of Cdc42

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Various neutrophil functions are impaired in myelodysplastic syndromes (MDS). However, the molecular basis of these remains unclear. We recently found that miR-34a and miR-155 were increased in neutrophils from MDS patients compared to healthy cells. To examine the effects of the aberrant microRNA expression on neutrophil functions, HL60 cells, in which miR-34a, miR-155 or control microRNA was ectopically expressed, were differentiated with 500 μM dibutyryl cAMP toward a neutrophil-like phenotype. The fMLP-induced release of both myeloperoxidase and elastase was significantly increased by overexpression of miR-34a and miR-155. In contrast, miR-34a and miR-155 reduced the migration towards 10 nM fMLP through 0.3 μm pores for 90 min to 42.7 ± 14.6% ($p < 0.05$) and 40.3 ± 9.2% ($p < 0.05$), respectively, compared to control cells (63.4 ± 13.4%). Although the microRNA overexpression did not alter the mRNA levels of the fMLP receptor subtypes, fMLP-induced GTP-bound Cdc42 became 20% and 37% of that of the controls cells in the miR-34a- and miR-155-overexpressing cells, respectively. In conclusion, overexpression of miR-34a and miR-155 may be a cause of reduced migration of neutrophils in MDS via suppression of Cdc42 activity.

O1H-1-3 Application of model-based meta-analysis to chronic heart failure: Reduced myocardial oxygen consumption by carvedilol

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[Background] Although relationships of decrease in the event frequency and the clinical observations such as blood pressure and heart rate were analyzed in various clinical studies, they have met with limited success since the clinical observations do not reflect intrinsic situation of the heart or the vessels. In this study, more intrinsic parameters such as oxygen consumption of the heart (probable index of the stress) and the systemic vascular resistance were evaluated based on the model-based meta-analysis, and therapeutic potentials of carvedilol, metoprolol, felodipine, and enalapril were compared in patients of chronic heart failure.
[Method] Various information was systematically collected from literature, and was analyzed with a new mathematical blood circulation model to estimate the oxygen consumption and the systemic vascular resistance.
[Results] The estimated myocardial oxygen consumption was most evidently reduced by carvedilol, whereas systemic vascular resistance was slightly increased.
[Conclusion] The superior therapeutic effects of carvedilol would be explained by reduced cardiac stress which was revealed by the current model-based meta-analysis for the first time.

O1H-1-4 Systems pharmacology of developmental neurotoxicity of valproic acid

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It is well known that exposure to valproic acid (VPA) during developmental period may cause various neuropsychiatric disorders. However, the detailed mechanism has been unknown. To elucidate the molecular mechanism, we exposed VPA to fertilized eggs of zebrafish and analyzed the gene expression variations comprehensively using DNA microarray comparing with publicly available transcriptome data analyzing the effect of VPA in developmental stages. As a result, we discovered that the expression of epc2 was significantly decreased by VPA exposure at developmental stages in both zebrafish and mouse. EPC2 is one of the polycomb genes and related to 2q23.1 microdeletion syndrome characterized by psychomotor retardation, seizure, and stereotypic repetitive behavior. Then, we knocked out epc2 gene in zebrafish using TALEN to analyze the functional role of epc2 downregulation by VPA exposure. We were able to identify several genes dysregulated in common between zebrafish exposed to VPA and zebrafish with epc2 knockout, suggesting that epc2 may be involved at least partly in developmental neurotoxicity of VPA.

O1H-1-5 Whole body imaging with single cell resolution by the CUBIC perfusion protocol

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To reveal sophisticated life systems of multicellular organism, we need to focus the most essential unit of life "cell. Whole-body imaging with single-cell resolution will enable to reveal these systems such as cellular dynamics which has been a fundamental challenge in biology. Previous clearing methods focused on homogenizing mismatched refractive indices of individual tissues and lipid removal, enabling reductions in opacity. However these methods is not unable to clear the whole body because of the light absorbance by endogenous chromophores. Here, we show that aminoalcohols in CUBIC reagents decolorize the blood by efficiently eluting the heme chromophore. Direct transcardial perfusion of CUBIC reagents with a 10 day to 2 week clearing protocol decolorized nearly transparent almost all organs of adult mice as well as the entire body of infant and adult mice. This CUBIC-perfusion protocol enables rapid whole-body and whole-organ imaging with single-cell resolution by using light-sheet fluorescent microscopy. The CUBIC protocol is also applicable to 3D pathology, anatomy, and immunohistochemistry of various organs. These results suggest whole-body imaging with single-cell resolution will lead to organism-level systems biology.

O1H-2-1 Stromal cell-derived factor 2, Hsp72 client protein, is a target molecule to overcome oxaliplatin resistance

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In cancer cells, Heat shock protein 72 (Hsp72) has functions such as promotion of protein folding, stabilizing proteins, and avoiding stress. We previously reported that Hsp72 is a key molecule in oxaliplatin (OXA) resistance and Hsp72-client proteins may target molecules to overcome drug resistance. In this study, we identified the Hsp72-client proteins in OXA-resistant cells and investigated the potential of these proteins as new target molecules to overcome OXA resistance. All experiments used human gastric cancer cell line, OCUM-2M (2M) and OXA resistant 2M (2M/OXA). First, Hsp72-client proteins were purified with anti-Hsp72 antibodies from 2M and 2M/OXA, and then performed proteomic analysis by LC/MS/MS. Stromal cell-derived factor 2 (SDF2) is one of the protein, which identified 16 specific Hsp72-client proteins in 2M/OXA. Hsp72 knockdown decreased the level of SDF2 protein but not mRNA. These data suggest that Hsp72 prevented the degradation of SDF2. Second, drug sensitivity was examined under SDF2 silencing condition. Suppression of SDF2 by siRNA enhanced OXA-induced cell death. Collectively, Hsp72-client protein, SDF2 is a new target molecule to overcome OXA resistance.

O1H-2-2 Magnetized methotrexate derivative for novel anti-cancer therapy

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Background: We previously reported the generation of a novel paclitaxel derivative with intrinsic magnetism. Similarly, we have synthesized a novel derivative of methotrexate, a conventional drug for cancer and rheumatic diseases,with intrinsic magnetism (M-MTX). This is a single methotrexate compound and is not a methotrexate encapsulated in micelle with magnetic particles. We have examined whether M-MTX has both the magnetic and the anti-cancer property with similar efficacy to methotrexate.
Materials & Methods: The magnetic property of M-MTX was measured by Electron Spin Resonance (ESR) and Superconducting Quantum Interference Device (SQUID). MCF7, breast cancer cells were used. Cell proliferation was assessed by a commercially available kit, XTT Cell Proliferation Assay Kit (ATCC). Apoptosis was analyzed using fluorescence activated cell sorter (FACS).
Results: M-MTX was easily attracted by a deodium magnet. Both ESR and SQUID showed that M-MTX has an intrinsic magnetism. Furthermore, M-MTX inhibited cell proliferation and induced cellular apoptosis in MCF7 cell lines.
Conclusion: M-MTX may provide us a new strategy for cancer therapy, i.e., chemotherapy with magnetic drug delivery with a single agent.

O1H-2-3 ANP attenuates angiotensin II-exacerbated hematogenous lung metastasis

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Atrial natriuretic peptide (ANP), which are clinically used for heart failure, inhibits renin-angiotensin system through binding guanylate cyclase-A (GC-A) receptor. In this study, we examined whether ANP treatment attenuates pulmonary metastasis which were exacerbated by angiotensin-II (ATII) stimulation. B16-F10 melanoma cells, which did not express the ATII type1 and GC-A receptors, were intravenously injected into C57BL/6 mice. Two weeks after injection, pulmonary metastatic colonies were counted. ATII was continuously administered for 7 days via subcutaneous osmotic pump (SOP) from 3 days before tumor cell injection. ANP was continuously administered for 14 days via SOP from 2 days before ATII stimulation. In the ATII treated group, the number of metastatic colonies was significantly increased compared with that in the control group, and these gains were prevented by the treatment of ANP. ATII stimulation increased the number of adherent A549 cancer cells to human microvascular endothelial cells (HMVEC). Pre-treatment of ANP to the HMVEC reduced the number of adherent tumor cells. In conclusion, ANP attenuated ATII-exacerbated pulmonary metastasis. These effects may be caused by the reduction of tumor-cell adhesion to the vascular endothelial cells.

O1H-2-4 Tumor endothelial cell-derived prostaglandin D_2 suppresses tumor angiogenesis

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Tumor endothelial cells (TECs) are one key component of tumor microenvironment and are known to possess irregular characteristics in motility and inflammatory reactions. In comprehensive gene expression analysis, we found drastic increase of lipocalin-type prostaglandin D synthase (L-PGDS) expression in TECs. We next investigated the contribution of L-PGDS-dependent PGD_2 production in tumor growth using genetically modified mice. Lewis lung carcinoma implanted into the back of L-PGDS deficient mice grew faster than those of WT mice. Immunohistochemical staining revealed that L-PGDS was mainly expressed by TECs. Host L-PGDS deficiency accelerated vascular hyper-permeability and angiogenesis which in turn reduced tumor necrosis and apoptosis. Following *in vitro* experiments showed that PGD_{22} strongly suppressed endothelial tube formation by activating PGD_2 receptor DP.
These observations identify L-PGDS-derived PGD_2 as a negative regulator of tumor microenvironment by restricting angiogenesis and vascular permeability.
This work is supported by Programme for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry and Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry.

O1H-2-5 Inhibition of L-type amino acid transporter 1 exerts anti-angiogenic effects

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L-type amino acid transporter 1 (LAT1) is abundantly expressed in various types of cancers and supports rapid proliferation of cancer cells by supplying amino acids. Inhibition of LAT1, therefore, is expected to be a promising approach in cancer therapeutics. We recently found that, in addition to tumor cells, LAT1 is also expressed in the stromal vascular endothelial cells of nude mouse xenograft model implying yet undescribed roles of LAT1 in angiogenesis. Consistently, treatment with LAT1 inhibitors significantly suppressed the migratory property and the formation of blood vessel-like tubular network of human umbilical vascular endothelial cells. Similar results were obtained by siRNA-mediated knockdown of LAT1. Furthermore, matrigel plug in vivo angiogenesis assay in mouse revealed that invasion of CD31-positive endothelial cells and the subsequent blood vessel formation were suppressed in the presence of LAT1 inhibitors or of siRNA designed against LAT1. Taken together, these results strongly indicate that LAT1 plays an important role in angiogenesis, at least partially, by promoting migration of endothelial cells. These results have significant implications on the mechanisms of action of LAT1 inhibitors as anti-cancer drug.

O1H-3-1 Heterocyclic organobismuth compound (bi-chlorodibenzo [c,f] [1,5] thiabismocine), a new selective anticancer drug, induces differentiation of human myelocytic leukemia cells

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Our previous study showed that heterocyclic organobismuth compounds possess anti-bacterial and anti- cancer activity. Of note, the compound shows anti-cancer activity mediated by cell cycle arrest, apoptosis induction and ROS production. We also showed that the compound causes depolymerization of microtubule network in HeLa cells, another anti-cancer activity. However the anti-cancer property of the compound remains controversial. Here we show the compound induces cellular differentiation induction activity in NB4 and K562 cells derived from human acute promyelocytic leukemia (APL) and human chronic myelocytic leukemia (CML), respectively. Some medical drugs are used for treatment of myelocytic leukemia such as ATRA (All-trans retinotic acid) or imatinib, which induce cell-differentiation and inhibit cell growth. Our study revealed that heterocyclic organobismuth compound also induces differentiation marker transcription and surface antigen expression by flow cytometry in these myelocytic leukemia cells. We are attempting to develop a more selective anti-cancer drug using both structure-based activity and molecular-based mechanism.

O1H-3-2 The involvement of prostaglandin E_2-EP2 signaling in colon cancer formation

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The involvement of prostaglandin (PG) cascade in colon cancer formation is well established. However, current problem of drug treatment is adverse effect derived from non-specific inhibition of PGs. Thus, we aimed to clarify a responsible PG receptor for colon cancer formation as a potential therapeutic target. For this purpose, we subjected mice deficient in each PG receptor to colon cancer model induced by azoxymethane, a mutagen, and dextran sodium sulfate to induce colitis. As a result, EP2 deficiency reduced tumor numbers and the EP2 antagonist, PF04418948, mimicked suppressive effect of EP2. In immunostaining, EP2-expressing cells were mainly $Gr\text{-}1^+$ neutrophils, major inflammatory cells *in situ*. Transplantation of EP2-deficient bone marrow to wild type attenuated colon cancer formation, supporting a role of EP2 on neutrophils. In cultured neutrophils, EP2 signaling induced COX-2 and CXCL1, a major chemoattractant for neutrophils, expression synergistically with TNF-α, suggesting a role of EP2 to amplify neutrophil-mediated inflammation. Finally we confirmed the expression of EP2 in neutrophils infiltrated in human lesions with Ulcerative Colitis. Our study revealed the crucial role of EP2 in colon cancer formation through regulating inflammation.

O1H-3-3 Connexin 43 associates with bax and enhances sunitinib-induced apoptosis

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[Background] Connexin (Cx) is a component of intercellular channel; gap junction (GJ), and plays a regulatory role in cellular physiology. In cancer cells, GJ function is often aberrant, associating with the decreased Cx expression. Previously, we have shown that the sensitivity of malignant mesothelioma (MM) cells to cisplatin was enhanced by increasing expression of Cx43. Hence, the restoration of Cx expression would be beneficial to recover the anti-proliferative effect of anti-cancer drugs. The present study aims to evaluate the effect of Cx43 on sunitinib (SU)-induced cytotoxicity in MM cells.
<B>[Results]</B> Increased Cx43 expression enhanced SU-induced apoptosis in an MM cell line (H28), which was not altered by a GJ inhibitor. To examine the mechanism of enhanced apoptosis by SU, the interaction of Cx43 with a pro-apoptotic factor, Bax was then investigated. It was shown that Bax was immunoprecipitated with anti-Cx43 antibody. Furthermore, Cx43 increased the protein expression of Bax and the production of a cleaved (active) form of Bax by SU treatment.
[Conclusion] These findings suggest that Cx43 directly interacts with Bax, and this interaction might be involved in enhancing SU-induced apoptosis in MM cells through a GJ function-independent mechanism.

O1H-3-4 Autophagy activation confers responsiveness to anti-LAT1 therapy in breast cancer cell lines

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LAT1 is an amino acid transporter with onco-fetal expression pattern. In many cancer cells, large neutral amino acids like leucine were taken up through LAT1 and stimulate mammalian target of rapamycin (mTOR) pathway, resulting in tumor growth or inhibition of apoptosis. Several reports including ours have shown anti-LAT1 therapy is effective against broad range of cancers. However, there is no biomarker to predict the efficacy of anti-LAT1 therapy. Because mTOR inhibition should result in increase in autophagy, we hypothesized that autophagy activation can be used for a biomarker for anti-LAT1 therapy. In 4 breast cancer cell lines, T-47D, SK-BR-3, MCF7,MDA-MB-231, anti-proliferative effect of JPH203, a specific inhibitor of LAT1, was measured. IC_{50} (μM) was 4, 20, 100, 200, respectively. Autophagy activation was assessed by the amount of LC3-II in chloroquine-treated cell lysate. JPH203 treatment increased LC3-II in T-47D and SK-BR-3 cells but not others. These results suggest that autophagy activation can be a biomarker for JPH203 responsiveness. In cancer cells, where autophagy is activated by JPH203, autophagy inhibition following JPH203 treatment could enhance effectiveness of JPH203.

O1H-4-1 Critical role of lung macrophage-derived NPY in the pathogenesis of influenza virus infection

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Cross-talk between the autonomic nervous system and the immune system is considered an important biological process in health and diseases. The sympathetic nervous system is known to release neurotransmitters such as catecholamines and the co-transmitter neuropeptide Y (NPY). It has been previously reported that phagocytes are capable of production of catecholamines, which enhance pulmonary inflammation. However, little is known about the participation of NPY-mediated neuro-immune cross talk in host immunity to influenza virus infection. In the present study, we show that NPY levels in lung macrophages were elevated in mice intratracheally infected with influenza virus (H1N1/PR8). Using chemical sympathectomy, we were able to exclude sympathetic nerve endings as sources of the virus infection-modulating NPY. Blockades of NPY or its receptor Y1R on macrophages improved the survival of mice infected with influenza viruses. These data suggest a critical role of lung macrophage-derived NPY in the pathogenesis of influenza virus infection.

O1H-4-2 Role of PI3 kinase p85α subunit on the development of acute colitis in mice

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In the present study, we examined the role of phosphoinositide 3-kinase (PI3K) p85α subunit on the development of acute colitis, focusing on the macrophage functions. PI3K p85α hetero deficient (p85α+/-) mice and WT mice were used. Experimental acute colitis was induced by giving 3% dextran sulfate sodium (DSS) in drinking water for 7 days. The severity of DSS-induced acute colitis was significantly attenuated in p85α+/- mice compared with WT mice. The proportion of intestinal macrophages in the colonic mucosa was increased equivalently in both WT and p85α+/- colitis mice. However, the LPS-upregulated mRNA expression of proinflammatory cytokines in intestinal macrophages isolated from inflamed colonic mucosa was suppressed in p85α+/- colitis mice compared with WT colitis mice. We found that p85α+/- bone marrow-derived macrophages (BMDMs) from p85α+/- mice produced higher amount of IL-10 in response to LPS stimulation than BMDMs from WT mice. Furthermore, adoptive transfer of BMDMs from p85α+/- mice improved the severity of DSS-induced acute colitis in WT mice. These results suggest that the deficiency of PI3K p85α enhances the production of IL-10 on colonic macrophages, thereby suppressing the development of DSS-induced acute colitis.

O1H-4-3 Roles of high affinity leukotriene B_4 receptor signaling in enhancement of fibrosis during unilateral ureteral obstruction in mice

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Kidney fibrosis is developed under interactions of chemokines and cytokines secreted from migrated cells and kidney cells. We investigated roles of a high affinity leukotriene B_4 receptor (BLT1) signaling that attracts leukocytes in development of kidney fibrosis. Male C57BL/6 wild type mice (WT) and BLT1 knockout mice (BLT1-/-) had unilateral ureteral obstruction (UUO) at 8 weeks old. Accumulations of immunoreactive of collagens in UUO kidneys, increased temporally in WT, were suppressed in BLT1-/- mice. mRNA levels of collagens were also reduced in BLT1-/- in comparison with WT. Expressions of BLT1 increased in renal tubules in UUO kidney in WT. Under UUO treatment, accumulations of macrophages and fibroblasts were increased temporally, but these accumulations were suppressed in BLT1-/-. The same was true in mRNA levels of TGF-ß and FGF-2. LTB_4-BLT1 signaling plays critical roles in fibrosis in UUO kidneys, via increased accumulations of macrophages and fibroblasts that secrete collagens directly in response to LTB_4, with concomitant upregulation of TGF-ßthat possibly enhances collagen biosynthesis in fibroblasts.

O1H-4-4 Role of EGF family and suppressors of Ras/ERK pathway in the dermal fibroblast proliferation of allergic contact dermatitis

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Allergic contact dermatitis represents a severe health problem with increasing worldwide prevalence. Recently, we suggested that 2,4,6-trinitrochlorobenzene (TNCB) challenges induced hyper proliferation of dermal fibroblast in the ear. Furthermore, up-regulation of EGF family and down-regulation of suppressors of Ras/ERK pathway (Spred, Sprouty and Sef) were observed in the TNCB challenged whole ear. We therefore examined the gene expression of EGF family, Spred, Sprouty and Sef in epidermis and dermis of TNCB challenged ear. Mice were sensitized with TNCB to the abdominal skin. Then, the mice were repeatedly challenged with TNCB to the ear. Twenty-four hr after last challenged, the layer of epidermis and dermis were separated from an ear and then experiments were performed. The gene expressions of EGF family were increased between epidermis and dermis in the TNCB challenged ear. On the other hand, the gene expressions of Spred, Sprouty and Sef in the dermis were decreased by TNCB challenge. These results suggest that TNCB challenges caused upregulation of EGF family in between them and the down-regulation of Ras/ERK pathway suppressors in dermis, resulting in ear swelling.

O1H-4-5 Investigation on combination therapy of histamine H_4 receptor antagonist and topical glucocorticoid for treatment of chronic skin diseases

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Topical application of glucocorticoid (GC) is the first-line therapy for treatment of chronic skin diseases. However, long-term topical GC often increases side effects. Histamine H_4 receptor (H_4R) is expressed on various immune cells and its signals are involved in aggravation of the chronic skin diseases. In this study, we investigated whether the dose of topical GC can be lowered by using oral JNJ28307474 (7474), a selective H_4R antagonist, in combination retaining sufficient anti-inflammatory effects. Dexamethasone (DEX, 0.03 or 0.01%) was topically applied once a day and 7474 (20 mg/kg) was orally administered twice a day to 2,4,6-trinitrochlorobenzene-induced chronic dermatitis mice for 7 weeks. The combination treatment of 0.01% DEX and 7474 decreased ear thickness, an index of skin inflammation, to the same level as the single treatment of 0.03% DEX. Organ weights of spleen and thymus were significantly decreased by 0.03% DEX, but were less noticeable by the combination therapy. These results suggested that the combination therapy of oral H_4R antagonist and topical GC with reduced dose would be effective in treatment of the chronic skin diseases with decreased side effects.

O1H-5-1 Effects of zoledronate and ibandronate on histamine release in RBL-2H3 cells and rat peritoneal mast cells

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Bisphosphonates are a class of drugs to treat several bone disorders, that work as pyrophosphate analogues or inhibitors of farnesyl pyrophosphate synthase. In this report, we explain the ability of bisphosphonates in inhibiting mast cells activities.We investigated the changes in histamine release in rat basophilic leukemia cells (RBL-2H3 cells), a tumor analogue of mast cells and rat peritoneal mast cells (RPMCs) after zoledronate and ibandronate treatment. Zoledronate inhibited histamine release in cells induced by DNP-BSA as an antigen, ionomycin and compound 48/80. Whereas, the inhibitory effect of ibandronate on histamine release was only shown when stimulated with DNP-BSA and compound 48/80 induced. The changes in histamine contents could not be observed after both zoledronate and ibandronate treatment. In both cells used, zoledronate showed higher potency in inhibiton compared to ibandronate. In RBL-2H3 cells the inhibitory effects of zoledronate and ibandronate could be recovered by administration of 20 μM of farnesol or geranylgeraniol, but not with 300 μM mevalonolactone. Inhibition of protein farnesylation and gernylgeranylation could be accounted for the inhibitory effects of zolendronate and ibandronate.

O1H-5-2 Mast cell-derived PGD_2 attenuates anaphylaxis

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Antigen-stimulated mast cell releases amount of inflammatory mediators which in turn cause life threatening allergic reactions; anaphylaxis. Although mast cells strongly express hematopoietic-prostaglandin D_2 synthase (H-PGDS), the role of prostaglandin D_2 (PGD_2) in anaphylaxis remains unknown. Antigen-stimulation or administration of mast cell activator (C48/80) caused anaphylactic responses; vascular hyper-permeability, hypothermia, and hypotension in wild type (WT) mice. All of these responses were almost abolished by histamine H1 receptor blockade. H-PGDS deficiency exacerbated while H-PGDS overexpression attenuated the C48/80-induced anaphylaxis. Immunohistochemistry clarified that mast cells strongly expressed H-PGDS in WT. Histamine causes NO-dependent vascular hyper-permeability. Administration of NO synthase inhibitor or PGD_2 receptor agonist abolished the C48/80-induced hyper-permeability and then hypothermia under H-PGDS deficiency. These observations suggest mast cell-derived PGD_2 negatively regulate anaphylaxis. PGD_2 attenuates anaphylaxis maybe partially by inhibiting vascular hyper-permeability.

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O1H-5-3 EPRAP exerts anti-inflammatory effects through dephosphorylation by PP2A

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We have demonstrated that EP4 receptor-associated protein (EPRAP) in macrophages functioned to prevent excess inflammation in vivo; however, the underlying mechanisms remain unknown. Because EPRAP contains several potential phosphorylation sites, we investigated the significance of posttranslational modifications of EPRAP in the anti-inflammatory functions using mouse embryo fibroblasts (MEFs) isolated from EPRAP-deficient mouse. Wild-type (WT)- or mutated EPRAP-expressing plasmids were transfected, and the levels of LPS-induced TNFα production were evaluated. Among EPRAP mutations, the MEF cells transfected with mutated EPRAP where serine 108 and 608 were substituted to alanine (S108A/S608A) showed least TNFα production. Accordingly, the MEFs with EPRAP(S108A/S608A) demonstrated stronger inhibition of LPS-induced phosphorylations of p105 and MEK1/2 than those with WT-EPRAP. Notably, the serine phosphatase 2A (PP2A) inhibitor, cantharidic acid, increased LPS-induced TNFα production in WT-EPRAP expressing MEFs cells but not in EPRAP(S108A/S608A). Besides, immunoprecipitation showed the direct interaction of EPRAP with PP2A. Taken together, EPRAP may exert anti-inflammatory effects through dephosphorylation of serine 108/608 residues by PP2A.

O1H-5-4 Effects of flavonol glycosides on catecholamine secretion induced by acetylcholine in bovine adrenal medullary cells

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Flavonol glycosides are derived from the plants of the genus Epimedium which have been used in Traditional Chinese Medicine as a tonic, antirheumatic and aphrodisiac. In the present study, we investigated the effects of four flavonol glycosides, such as ikarisoside A, icariin, epimedicin C, and epimedoside A on catecholamine secretion in cultured bovine adrenal medullary cells. We found that ikarisoside A (0.3-100 μM), but not other three flavonol glycosides, concentration-dependently inhibits catecholamine secretion, $^{22}Na^{+}$ influx, and $^{45}Ca^{2+}$ influx induced by acetycholine (ACh) in the cells. When the concentration of ACh in the incubation medium increased from 3μM to 300μM, they did not overcome the inhibitory effect of ikarisoside A. In Xenopus laevis oocytes expressing α 3β4 nicotinic ACh receptors, ikarisoside A directly inhibited the current evoked by ACh. The present findings suggest that ikarisoside A inhibits ACh-induced catecholamine secretion through suppression of Na^{+} influx and subsequent Ca^{2+} influx in bovine adrenal medulla cells. It is possible that ikarisoside A protects the hyperactive catecholamine system induced by strong stress which evokes the secretion of ACh from the splanchnic nerves.

O1H-6-1 Molecular mechanism of renal accumulation of 3-fluoro-L-α-methyltyrosine (FAMT) in ^{18}F-FAMT PET

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L-[3-^{18}F]-α-methyltyrosine (^{18}F-FAMT) has been developed as a potential amino acid probe for positron emission tomography (PET) diagnosis of cancer. The advantage of ^{18}F-FAMT compared with conventionally used ^{18}F-fluorodeoxyglucose (^{18}F-FDG) is its cancer-specificity and low physiological background in brain. However, ^{18}F-FAMT is highly accumulated and retained in kidney. To understand the molecular mechanism underlying the renal accumulation of ^{18}F-FAMT, we studied transporters in *Xenopus* oocytes expression system to reveal ones responsible for ^{18}F-FAMT uptake into renal proximal tubules. In this study, we synthesized ^{14}C-labeled FAMT and first studied amino acid transporters. Our results showed that ^{14}C-FAMT was not transported by any amino acid transporters physiologically expressed in kidney. We, then, examined organic ion transporters expressed in kidney. Among organic ion transporters tested, OAT1, OATN1 and OCTN2 transported ^{14}C-FAMT, among which OATN1 showed the prominent uptake of ^{14}C-FAMT with the K_m value of 400.8 μM. In conclusion, kidney accumulation of ^{18}F-FAMT in PET is due to the uptake of FAMT by organic ion transporters such as OATN1, OAT1 and OCTN2.

O1H-5-5 In vivo imaging of damage associated molecular patterns (DAMPs) dsDNA in mouse lungs with ARDS

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Many pathogenic non-viral and viral conditions lead to the development of the acute respiratory distress syndrome (ARDS), the most severe form of acute lung injury (ALI). ARDS is characterized in the early phase by neutrophil-mediated, excessive pulmonary inflammation. We previously reported that damage associated molecular patterns (DAMPs) derived from damaged lung tissues activate Toll like receptor 4 (TLR4)-TRIF pathway, resulting in severe lung injury (Imai et al. Cell 2008). Also, recent accumulating evidence suggests that double-stranded structure of DNA (dsDNA), possesses immunomodulatory effects when introduced into the cytosol or its homeostatic clearance is hampered. In the present study, using in vivo mouse lung imaging system with SYTOX Green, we observed massive accumulation of neutrophil extracellular traps (NETs)-like DNA structures in the alveolar space in the lungs with non-viral (acid aspiration-induced) and viral (influenza virus-induced) ARDS. Also, the concentrations of free DNA were significantly higher in the BAL fluids obtained from lungs with ARDS than controls in mice. These data suggest that DAMPs DNA were generated in the lungs with ARDS, which might be associated with the development of ARDS.

O1H-6-2 Analyses of the urate-lowring effects by zotepine and chlorprothixene

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Introduction
Zotepine and chlorprothixene are antipsychotic drugs, and they have been reported to decrease serum uric acid levels in several studies. However, the molecular mechanism underlying these effects has not yet been clarified.
Methods
1. We measured [^{14}C] urate uptake by using stable cells expressing the human urate transporter (HEK-URAT)1 and mock cells (HEK-mock), to evaluate the uricosuric action of zotepine and chlorprothixene.
2. We measured the activity of human xanthine oxidase (XO) to ascertain if zotepine and chlorprothixene have inhibitory effects on urate production.
Results
Zotepine and chlorprothixene inhibited URAT1-mediated urate uptake. In contrast, urate production mediated by XO was not inhibited.
Conclusions
URAT1, a major contributor of renal urate reabsorption and a major target of uricosuric drugs such as losartan and benzbromarone, interacted with zotepine and chlorprothixene; however, XO, a major enzyme for urate production in the body, did not interact with zotepine and chlorprothixene. These results suggest that the urate-lowering effects of zotepine and chlorprothixene are mainly associated with the inhibition of renal urate reabsorption via renal urate transporters such as URAT1.

O1H-6-3 Urat1-Uox double knockout mice are an experimental animal model of renal hypouricemia and exercise-induced acute kidney injury

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Urate transporter URAT1 gene and uricase gene (Uox) double knockout mice (DKO) were studied as an experimental animal model of Renal Hypouricemia (RHUC) and its complication of excise-induced acute kidney injury (EIAKI). The DKO mice were maintained with 27mg allopurinol / 100g feed. One week feeding with variable content of allopurinol was followed by HPLC measurement of urate (UA) and creatinine (Cr) concentrations of spot urine and blood from tail. Without allopurinol, plasma Cr and UA levels of DKO mice were higher than those of Uox-KO mice. Urinary UA excretion of DKO mice was about 30 times higher than that of humans. With allopurinol, plasma Cr levels in DKO mice were normal, however, plasma UA levels were lower than those of Uox-KO mice. There were no difference in the urinary UA excretions between DKO and Uox-KO mice, although allopurinol reduced them dose-dependently. Thus, hypouricemia and normal urinary UA excretion may indicate that the DKO with allopurinol is an animal model of RHUC. DKO mice without allopurinol show acute kidney injury with UA overexcretion, like post-exercise, is likely to be an animal model of EIAKI. Moreover, allopurinol is suggested to be a prophylactic of EIAKI.

O1H-6-4 Roles of the prostaglandin I_2-IP system in nonalcoholic steatohepatitis

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Nonalcoholic steatohepatitis (NASH) is a hepatic manifestation of metabolic syndrome. Although prostaglandin (PG) I_2 receptor IP is expressed broadly in the liver, a role of PGI_2-IP signaling in the development of NASH remains to be determined. Here we investigated the role of the PGI_2-IP system in the development of NASH using mice lacking IP (IP-KO mice) and specific IP agonists. Wild-type (WT) mice fed with methionine- and choline-deficient (MCD) diet developed NASH at 10 weeks. Interestingly, IP-KO mice had earlier development of NASH at 5 weeks with augmented steatosis and prominent inflammatory cell infiltration, and increased TNF-α expression in the liver. Furthermore, IP-KO mice had higher hepatic iron deposition, resulting in prominent oxidative stress at 10 weeks. An IP agonist improved biochemical and histological parameters of NASH in WT mice with reduced hepatic TNF-α expression. In primary cultured Kupffer cells of WT mice, an IP agonist reduced the LPS-stimulated TNF-α mRNA expression. These results indicate the PGI_2 plays a crucial role in the development of NASH, by modulating the inflammatory response involving Kupffer cells. We suggest that the PGI_2-IP signaling might be an attractive therapeutic target for the treatment of NASH.

O1H-6-5 Animal model of postoperative ileus is unique model to determine plastic property of ICC during inflammation

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Objective: Postoperative ileus (POI) is a common complication after intra-abdominal surgery. Activated leukocytes induce iNOS, which results in intestinal motility disorder. However, the pathophysiological changes of interstitial cells of Cajal (ICC) are not well understood. Aim of this study is to investigate the morphological and functional changes of ICC in POI.
Method: Intestinal manipulation (IM) was performed to the distal ileum of BALB/c mice under anesthesia. Ileal smooth muscle strip was isolated 24h or 48h after IM. Morphological changes of ICC were evaluated by immunohistochemistry. A microelectrode array was performed to determine pacemaker ability of ICC.
Result: 24h after IM, ICC network stained by anti c-kit or ANO1 antibody was disrupted, in accordance with decrease in mRNA of both genes. Decreased amplitude of the field potentials and irregular spontaneous electric activity of ICC was also observed. Morphological changes of ICC were immediately recovered within 48 h after IM.
Conclusion: IM induces disruption of ICC network accompanied with abnormal pacemaker activity. Model mice for POI are unique model to determine plastic property of ICC during intestinal inflammation.

O1I-1-1 Novel substrates for Rho kinase in the heart

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Rho kinase/ROCK is highly expressed in cardiac muscle and is activated in embryonic and failing heart. However, the specific substrate for Rho kinase in it hasn't been reported so far. We previously developed a proteomic approach for screening of protein kinase substrates by combining mass spectrometry and affinity chromatography of catalytic domain of protein kinase. Using this method, we obtained more than 200 candidate substrates in the rat heart tissue. We then confirmed that some of these candidates including heart disease-related gene products were really phosphorylated by Rho kinase in vitro. Among them, we focused on ANKRD1/CARP. CARP has been reported to play a critical role in maintaining sarcomere structure and regulating transcription dependent on its subcellular localization. We further searched for the binding partners whose interactions are affected by phosphorylation state of CARP. Among the known partners, transcriptional factor YB-1 preferentially bound to non phosphorylated CARP. In addition, we also found that 14-3-3 proteins specifically associated with phosphorylated CARP. These results suggest that Rho kinase modifies the CARP function by regulating its transcriptional activity and subcellular localization through the phosphorylation.

O1I-1-2 Oxidative stress induced LAT1/CD98 expression via PI3K/AKT signaling pathway in cholangiocarcinoma: a potential therapeutic target

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Cholangiocarcinoma (CCA) is a major public health problem in the northeast region of Thailand. The liver fluke, Opisthorchis viverrini (Ov), infection is evidenced as etiology of CCA in this area. Oxidative stress during inflammatory reaction is a key event of Ov-induced CCA genesis. The present study, therefore aimed to examine the expression of L-type amino acid transporter 1 (LAT1) and CD98 in Ov-induced CCA in a hamster model. Our results showed that increased expressions of LAT1/CD98 were found during Ov-induced CCA development in hamster tissues. In addition, H2O2-induced oxidative stress could increase them as well as the activity of pro-survival PI3K/AKT signaling in CCA cells. We also revealed that suppression of PI3K/AKT signaling by BEZ235 decreased LAT1/CD98 expression. Moreover, JPH203 inhibited cell growth of CCA. The results indicate that oxidative stress resulted in an elevation of LAT1/CD98 expression, which is one of key factor driven CCA induction caused by Ov infection. We also firstly addressed the effect of BEZ235 on LAT1/CD98 expression, indicating that LAT1/CD98 are regulated by PI3K/AKT pathway. It may be a promising drug for CCA treatment.

O1I-1-3 Death-associated protein kinase3 promotes proliferation and migration of A549 human lung cancer cells via activating the ERK/c-Myc signaling

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(Background) Death-associated protein kinases (DAPKs) are the serine/threonine protein kinases family, which regulates cell death. Although DAPK3 has been implicated as tumor suppressor, the role of DAPK3 in the lung cancer pathogenesis is still not fully understood. In this report, we examined whether DAPK3 controls proliferation and migration of lung cancer cells with focusing on cellular signals. **(Methods and results)** We generated A549 human lung cancer cells stably expressing small hairpin RNA (shRNA) targeting DAPK3. In these cells, DAPK3 protein level was significantly decreased and cell proliferation was inhibited. Cell migration and invasion were also inhibited by DAPK3 knockdown (KD) as determined by a Boyden chamber assay and an invasion assay, respectively. Moreover, DAPK3 KD inhibited anchorage-independent cell growth as determined by soft-agar colony formation assay. We also found DAPK3 KD inhibited phosphorylation of ERK and c-Myc. **(Conclusion)** The present results for the first time demonstrate that DAPK3 promotes lung cancer cell proliferation and migration through activation of ERK/c-Myc signals, suggesting DAPK3 as a novel molecular target for lung cancer therapy.

O1I-1-4 Screening of novel diacylglycerol kinase alpha (DGKα) activator to develop drugs improving diabetic nephropathy

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Diabetic nephropathy is caused by abnormal protein kinase C (PKC) activation by increased diacylglycerol (DG) in diabetic hyperglycemia. On one hand, diacylglycerol kinase (DGK) converts DG to phosphatidic acid (PA). Therefore, it is expected that activation of DGK can improve diabetic nephropathy. Actually, it was reported that vitamin E (VtE) improved diabetic nephropathy by normalizing DG level and PKC activity through DGK activation. Based on the report, we had revealed that VtE subtype-specifically activated DGKα. However, significant improvement by VtE was not observed in clinical trials using human diabetic patients. So, we tried to search for novel DGKα activator.

Ten thousand compounds having various structures were chosen, and applied to high through put screening which we newly developed using fluorescent ADP. The screening brought 11 candidates for DGKα activator. Next, we measured ability of DGKα activation of the 11 compounds by classical DGK assay methods. Finally, we identified 2 novel DGKα activators; compound 8 and 10. Both compound showed dose dependent effect on DGKα activation, and compound 8 and 10 significantly increased DGKα activity by 20% at 200 μM and 100 μM, respectively.

O1I-1-5 The phagocytosis of advanced glycation end products (AGEs) in marcrophages

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Advanced glycation end products (AGEs) are the products of a series of nonenzymatic modification of proteins with reducing sugars. The accumulation of AGEs in the vessel wall may contribute to the development of vascular lesions. In our previous study, it was demonstrated AGE might be incorporated into macrophages in a dosage dependent manner and induced caspase-3 activation. The present study showed time-course of AGE incorporation and a sub cellular localization of the endocytozed AGEs. The internalized AGEs were detected by immunofluorescence staining and confocal microscopy. The result revealed AGEs not only bind on the cell membranes but also are taken up into cytoplasm. As the incubation with AGEs was prolonged, AGEs were detected on the nuclear membrane and inside the cell nucleus. AGE-induced caspase-3 activation coincided with phosphatidylserine staining on the cell surface.

O1I-2-1 Spinal PACAP-specific receptor activation induces long-lasting mechanical allodynia through the activation of astrocytes

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Pituitary adenylate cyclase activating polypeptide (PACAP) functions as a pleiotrophic neuropeptide through three types of G protein-coupled receptors, PACAP-specific receptor (PAC1-R), and PACAP/vasoactive intestinal polypeptide (VIP)-indifferent VPAC1-R and VAPC2-R. PACAP and PAC1-Rs are present in the spinal dorsal horn and dorsal root ganglia, suggesting that PACAP-PAC1-R signaling could play an important role in the modulation of spinal nociceptive transmission, although the functional significance is still controversial. In this study we showed that a single i.t. administration of PACAP or maxadilan (a PAC1-R agonist), but not that of VIP, induced long-lasting hindpaw mechanical allodynia which persisted at least 3 months without affecting thermal nociceptive threshold in mice. Induction of this long-lasting mechanical allodynia was almost completely prevented by the pretreatment of a PAC1-R antagonist, Max.d.4, or coadministration with l-α-aminoadipate, an astroglial toxin. Our results indicate that spinal PACAP-PAC1-R signaling pathway triggers long-lasting mechanical allodynia via astroglial activation, and blocking this signaling pathway might provide a novel strategy for treating chronic pain.

O1I-2-2 Influence of change in the brain BDNF expression after early life stress on the neuropathic pain-induced depression-like behavior

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We have demonstrated that early life stress exacerbates neuropathic pain in adult mice. Interestingly, depression-like behavior after nerve injury is observed in stressed female mice at 3 weeks after surgery. Accumulating evidences suggest brain derived neurotrophic factor (BDNF) contributes to the pathogenesis of depression. In this study, we investigated the effect of early life stress on the BDNF expression.

Early life stress was induced by maternal separation during postnatal days 15-21 and social isolation (MSSI). The sample from each region of brain was collected at the end of maternal separation or 3 weeks after surgery. BDNF expression is increased in male but not female mice immediately after maternal separation. On the other hand, MSSI has no effect on the BDNF expression in male and female mice at 3weeks after surgery.

Our results suggest that BDNF expression after early life stress but not in stressed mice with nerve injury may be associated with neuropathic pain-induced depression-like behavior in adult mice. Gender difference of BDNF expression after exposure to early life stress may contributes to the vulnerability on neuropathic pain-induced emotional dysfunction.

O1I-2-3 $Ca_v3.2$ T-type Ca^{2+} channels in sensory neurons of rats with neuropathic pain

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We have reported that upregulation of $Ca_v3.2$ in nociceptors contributes to neuropathic pain in rats with L5 spinal nerve-cutting (L5SNC). $Ca_v3.2$ is transcriptionally regulated by Egr-1, an activator, and REST, a repressor, and protected by USP5, a deubiquitinating enzyme, from proteasomal degradation. Thus, we determined protein levels of $Ca_v3.2$, Egr-1, REST and USP5 in the dorsal root ganglia (DRG) and analyzed their roles in the L5SNC-induced neuropathy in rats. After L5SNC, the hyperalgesia/allodynia was first detected on day 6 and reached a plateau on days 9-14, being reversed by RQ-00311651, a T-channel blocker. Protein levels of Egr-1 and $Ca_v3.2$, but not REST, significantly increased in L4 DRG on days 6 and 14. The upregulation of USP5 was detected on day 14, but not day 6. Knockdown of Egr-1 inhibited the L5SNC-induced hyperalgesia and upregulation of $Ca_v3.2$ in L4 DRG. These data suggest that, after L5SNC, the rapidly increased Egr-1 induces $Ca_v3.2$ upregulation, and the delayed induction of USP5 contributes possibly to the maintenance of the upregulated $Ca_v3.2$, playing a key role in the pathology of neuropathic pain.

O1I-2-4 Functional role of the mesolimbic dopaminergic system in pain transmission using optogenetics and designer receptors exclusively activated by designer drug (DREADD) system

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Mesolimbic dopaminergic system has been recognized for its central role in motivated behaviors and various types of reward. On the other hand, it has been reported that pain threshold is reduced in depressed patients, but elevated in schizophrenia patients. Pain has a negative correlation with pleasure. Thus, one expect that the control of mesolimbic dopaminergic activities affects pain transmission. However, the role of the mesolimbic dopaminergic system in neuropathic pain has not been fully understood. The recent development of the optogenetic tool and designer receptors exclusively activated by designer drug (DREADD) system has provided a valuable opportunity to inhibit or stimulate activity in genetically targeted neural populations with high spatial precision. In this study, we evaluated the pain threshold when we controlled the dopaminergic activity using optogenetics and DREADD system. We found that activation of mesolimbic dopamine neurons produced analgesia, whereas suppression of them decreased morphine-induced analgesia. These findings provide evidence that the mesolimbic dopaminergic system may play a crucial role in controlling pain transmission.

O1I-2-5 Blockade of glycine transporter subtypes has distinct influences on glycinergic synaptic transmission in spinal superficial dorsal horn neurons

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Recent behavioral studies employing various animal models of chronic pain have shown pain relief after blockade of glycine transporter subtypes GlyT1 and GlyT2. We examined the effects of subtype-specific inhibition of glycine uptake on evoked or miniature glycinergic inhibitory postsynaptic currents (eIPSCs evoked at 0.1 Hz or mIPSCs) in the superficial dorsal horn of spinal slices of adult mice. Bath application of NFPS and ALX-1393 for 20 min, the selective GlyT1 and GlyT2 inhibitor, respectively, prolonged the decay phase of eIPSCs without affecting their amplitude. Any statistically significant influences were not observed on mIPSCs by NFPS. By contrast, ALX-1393 significantly increased the frequency of mIPSCs. We further explored the role of GlyTs in the maintenance of glycinergic IPSCs. To facilitate vesicular release of glycine, repetitive high-frequency stimulation (HFS) intervened at 10 Hz for 3 min during continuous recordings of eIPSCs at 0.1 Hz. We found prominent suppression of eIPSCs after HFS in the presence of ALX-1393. By contrast, NFPS did not affect eIPSCs after HFS. Thus, blockade of GlyTs exerts subtype-specific influences on glycinergic synaptic transmission.

O1I-3-1 Macrophage-derived HMGB1 contributes to the inflammatory hyperalgegia through the NF-κB pathway

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In macrophages, high mobility group box 1 (HMGB1), one of damage associated molecular patterns, when acetylated by histone acetyltransferase (HAT), is translocated from the nucleus to the cytoplasm, and secreted to the extracellular space. Given our evidence for the pronociceptive role of HMGB1, we analyzed possible involvement of macrophage-derived HMGB1 in pain signaling. The mechanical hyperalgesia caused by intraplantar (i.pl.) administration of HMGB1 in mice was inhibited by anti-HMGB1 neutralizing antibody (antiHMGB1Ab) or ammonium pyrroridine dithiocarbamate (PDTC), an NF-κB inhibitor. The hyperalgesia following i.pl. lipopolysaccharide (LPS) was blocked by antiHMGB1Ab or PDTC, and partially by macrophage depletion with liposomal clodronate. Trichostatin A (TSA) or suberoylanilide hydroxamic acid, inhibitors of histone deacetylase (HDAC), given i.pl., also caused hyperalgesia, an effect inhibited partially by antiHMGB1Ab. In RAW264.7 macrophages, LPS or TSA translocated nuclear HMGB1 to the cytoplasm, and LPS caused upregulation of CBP, a HAT member. HMGB1 is thus considered to be secreted by macrophages via acetylation by upregulated CBP in response to LPS stimulation, and cause inflammatory hyperalgesia via the NF-κB pathway.

O1I-3-2 Involvement of macrophage-derived vascular endothelial growth factor in neuropathic pain

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Chronic neuroinflammation is promoted by activated immune cells, which play a crucial role in neuropathic pain. Vascular endothelial growth factor (VEGF) is a key factor for angiogenesis in inflammatory states, such as cancer and diabetic retinopathy. Herein, we investigated the involvement of VEGF-induced angiogenesis in neuropathic pain. For the neuropathic pain model, mice were subjected to partial sciatic nerve ligation (PSL). The injured sciatic nerve (SCN) was used for biochemical and histochemical assays. To visualize dynamic image of peripheral vessels, we performed transcardiac perfusion of DiI fluorescence. Complicated neovascular network was observed in the injured SCN after PSL. Macrophages were accumulated around the vascular network. The protein of VEGFA was expressed in migrating macrophages in the injured SCN. Minocycline or liposomed-clodronate, macrophage depletion agent, attenuated PSL-induced thermal hyperalgesia and tactile allodynia, that is, neuropathic pain. VEGF neutralizing antibody or VEGF tyrosine kinase inhibitor also suppressed the PSL-induced neuropathic pain. These results suggest that macrophage-derived VEGF may facilitate peripheral neuroinflammation through angiogenesis, leading to neuropathic pain.

O1I-3-3 The involvement of free fatty acid receptor GPR40/FFAR1 signaling on the development of chronic pain

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We have demonstrated that the activation of the G protein-coupled receptor 40/free fatty acid receptor 1 (GPR40/FFAR1) signaling pathway may play an important role in the regulation of the descending pain control system. Here, we examined the involvement of supraspinal GPR40/FFAR1 signaling in the development of chronic pain. We used a complete Freund's adjuvant (CFA)-induced inflammatory chronic pain mouse model. Mechanical allodynia and thermal hyperalgesia were evaluated using von Frey filaments and plantar test, respectively. Long-lasting a hyperplasia of paw, a persistent mechanical allodynia and thermal hyperalgesia were elicited in CFA-treated mice. The intracerebroventricular (i.c.v.) injection of docosahexaenoic acid (DHA) (50 μg) and GW9508 (1.0 μg), a GPR40/FFAR1 agonist, significantly reduced mechanical allodynia and thermal hyperalgesia at day 7, but not at day 1, after CFA injection. These effects were inhibited by the i.c.v. pretreatment with GW1100 (10 μg), a GPR40/FFAR1 antagonist. Our findings suggest that the activation of the supraspinal GPR40/FFAR1 signaling might provide valuable information regarding a novel therapeutic approach for pain control.

O1I-3-4 The neurosteroid allopregnanolone induces itch in atopic dermatitis model mouse

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Allopregnanolone (ALLO) is one of neurosteroids produced in the brain, but little is known about the relationship between ALLO and itching. We have previously shown that hairless mice fed a special diet develop atopic dermatitis-like symptoms, and administration of ethanol or barbiturates markedly aggravated itch-related scratching in mice suffering from AD. In this study, we hypothesized that ALLO, which has similar actions to ethanol and barbiturates, induces scratching behavior in atopic mice. Intraperitoneal administration of ALLO significantly increased scratching in atopic mice, but not in normal ones. ALLO-induced scratching was inhibited by a $GABA_A$ receptor antagonist picrotoxin and partially suppressed by an L-type voltage-dependent calcium channel agonist BAY K 8644. Next, we examined whether ALLO is involved in ethanol-induced scratching in atopic mice, because it has been reported that ethanol increases ALLO in the brain. Pretreatment with finasteride, a synthetic inhibitor of ALLO, significantly suppressed ethanol-induced scratching. In conclusion, we first demonstrated that ALLO induced itch-related scratching in atopic mice. Furthermore, ethanol-induced scratching may be mediated through endogenously produced ALLO.

O1I-4-1 Ameliorative effect of mepenzolate bromide against pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is thought to involve lung injury caused by reactive oxygen species (ROS), which in turn is followed by abnormal fibrosis. A transforming growth factor (TGF)-β1-induced increase in myofibroblast number plays an important role in this abnormal fibrosis. We recently found that mepenzolate bromide (mepenzolate), which has been used clinically to treat gastrointestinal disorders, has ROS-reducing property. In the present study, we examined the effect of mepenzolate on bleomycin-induced pulmonary fibrosis and lung dysfunction in mice. Intratracheal administration of mepenzolate prior to bleomycin treatment reduced the extent of pulmonary fibrosis, and improved lung dysfunction. Furthermore, mepenzolate produced a therapeutic effect even when it was administered after the development of fibrosis. Administration of mepenzolate also prevented bleomycin-induced pulmonary cell death, inflammatory responses and increased myofibroblast number. Mepenzolate also decreased NADPH oxidase activity and active TGF-β1 level or increased glutathione S-transferase activity in the presence of bleomycin treatment. These results suggest that mepenzolate could be beneficial for the treatment of patients with IPF.

O1I-4-2 Selective α_{1A}-adrenoceptor blocker silodosin decreases the prostatic growth in the spontaneously hypertensive rat

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Introduction:The effect of the selective α_{1A} blocker silodosin was investigated in the spontaneously hypertensive rat (SHR) prostate as a benign prostatic enlargement (BPE) model focusing on the prostatic blood flow (PBF).
Material and Methods: Twelve-week-old male SHRs were administered silodosin per orally (0, 100, 300 μg/kg/day) once daily for 6 weeks. Wistar-kyoto (WKY) rats were used as normotensive controls. The effects of silodosin on blood pressure and PBF were estimated. Then, the tissue levels of oxidative stress marker, inflammatory cytokines, and prostatic growth factors were measured.
Results: There was a significant increase in blood pressure, MDA, IL-6, CXCL1/CINC1, TNF-α, TGF-β1, bFGF and α-SMA in the SHRs compared to WKY rats. On the other hand, the PBF was decreased in the SHRs. Both doses of silodosin significantly reversed the above parameters of SHRs to almost identical levels to the ones of the WKY group excluding the blood pressure.
Discussion: A decreased PBF could induce the proliferative factors via induction of oxidative stress and inflammatory cytokines in the SHR prostate. Silodosin may improve BPE by increasing the PBF.

O1I-4-3 The ClC-7 chloride channel is downregulated by hypoosmotic stress in human chondrocytes

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Articular chondrocytes in osteoarthritis (OA) patients are exposed to hypoosmotic stress because the osmolality of synovial fluid in OA patients is lower than normal levels. We have found that Cl^- conductance contributes to the regulations of resting membrane potential (RMP). The molecular component and pathological function of Cl^-- channels, however, remain to be determined. Here, we showed that ClC-7 was predominantly expressed in a human chondrocyte cell line (OUMS-27). DIDS, a Cl^- channels blocker, hyperpolarized cells from approximately -25 to -40 mV followed by an increase in $[Ca^{2+}]_i$. ClC-7 knockdown significantly hyperpolarized membrane potential and thus a reduced DIDS-induced hyperpolarization. Short-term culture (48 h) in hypoosmotic medium (270 mOsm) attenuated the expression of ClC-7 and the activity of ClC7-incuced currents. Interestingly, the hypoosmotic culture or ClC-7 knockdown leaded to cell death. These findings indicate that ClC-7 channels regulate RMP to avoid excessive Ca^{2+} influx via membrane hyperpolarization in chondrocytes. The downregulation of ClC-7 channels during hypoosmotic stress is a potential pathological mechanism of OA, providing a novel target of therapeutic intervention and drug development for OA.

O1I-4-4 Systems pharmacology of mitochondria-targeted drugs that can protect hair cells against toxic stress

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Dysfunction of mitochondria contributes to the pathogenesis of many diseases, including neurodegenerative diseases such as Alzheimer's disease, metabolic disorders such as Diabetes mellitus, and hearing loss caused by aging, noise or clinical drugs. Pharmacological approaches targeting mitochondria may be beneficial for patients with these diseases. These include increasing mitochondrial biogenesis, reactive oxygen species scavenging, targeting mitochondrial dynamics, the membrane fluidity and plasticity. However, the pattern of mitochondrial dysfunction can be complicated. Drug screening using in vivo models for these diseases is indispensable to discover drugs that can ameliorate diseases without detectable toxicity. In this study, we used zebrafish as an in vivo model of drug-induced hearing loss and performed screening of mitochondria-targeted drugs. We were able to identify compounds that protected auditory hair cells against drug-induced damage by modulating mitochondrial functions. These results support zebrafish's potential for in vivo efficacy and toxicity screening of mitochondria-targeted drugs.

O1I-4-5 Pharmacological properties of the oxatomide as $P2X_7$ receptor antagonist

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$P2X_7$ receptor ($P2X_7R$) is the ligand-gated ion channel activated by extracellular ATP. This receptor plays crucial role in immune responses, and has been implicated in inflammatory diseases. Although various compounds have been developed as $P2X_7R$ antagonists, they have not yet been translated into the clinical application. We previously reported that oxatomide (Oxa) has an antagonist effect at mouse $P2X_7R$. Since $P2X_7R$ antagonists are known to exhibit large species differences in their pharmacological effects, we investigated the effects of Oxa on ATP-induced membrane currents in HEK293 expressing human, rat and mouse $P2X_7R$. Oxa inhibited ATP-induced membrane current mediated by human and mouse $P2X_7R$ without affecting that mediated by rat $P2X_7R$. The inhibitory effect of Oxa on human $P2X_7R$ was more potent than that on mouse $P2X_7R$. The analysis of mode of action showed that Oxa acts as a competitive antagonist. In human cell line RPMI8226, which express functional endogenous $P2X_7R$, Oxa inhibited ATP-induced Ca^{2+} influx, pore formation and membrane protein shedding. Since Oxa has been already used safely in the clinical cure of allergic diseases, it is interesting to evaluate whether the inhibitory effects of Oxa on human $P2X_7R$ contribute to its clinical effects.

O1I-5-1 Antianginal effects of tetramethylpyrazine assessed in the vasopression-induced angina model of rats

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Tetramethylpyrazine (TMP) is an extract of the herb *Ligusticum wallichii franchat*, which has been empirically used for the treatment of ischemic heart disease in China. We assessed its antianginal efficacy compared with Ca^{2+} channel blockers. Donryu rats (n=5) were anesthetized with pentobarbital sodium (60 mg/kg, i.p.) and the surface ECG was recorded. Vasopressin (0.5 IU/kg, i.v.) was administered to obtain the responses in the absence of TMP. More than 30 min later, TMP (10 mg/kg, i.v.) was administered. After 5 min, the same dose of vasopressin was administered. At the pre-TMP, vasopressin increased the systolic blood pressure to >200 mmHg with a transient ST-segment depression of 0.14-0.17 mV,reflecting the presence of endocardial ischemia. Under the TMP treatment, vasopressin increased the systolic blood pressure, but did not depress ST-segment, indicating disappearance of endocardial ischemia. Similar antianginal effetct was observed by a dual L/N-type Ca^{2+} blocker cinidpine, but it was not obtained by the L-type Ca^{2+} channel blockers nicardipine or nifedipine. These results confirmed the efficacy of TMP against ischemic heart disease. Precise mechanisms against vasopression-induced angina need to be determined.

O1I-5-2 Inactivation of dynamin-related protein 1 underlies cardiomyocyte senescence in peri-infarct myocardial regions

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Mitochondrial fission-fusion cycle plays a crucial role in maintenance of cardiac function. Dynamin-related protein 1 (Drp1), a redox sensitive small GTP-binding protein, is a key regulator of mitochondrial fission. Although it have been reported that neural Drp1 forms disulfide-dimer concomitant with Drp1 activation in several pathological conditions, the role of Drp1 dimerization in the heart is obscure. We here demonstrate that disulfide dimer formation of Drp1 mediates cardiac cell senescence after myocardial infarction (MI) in mouse hearts. Drp1 activity and the number of mitochondria were increased in the peri-infarct myocardial regions. Four weeks after MI, the cardiac Drp1 significantly formed disulfide dimer and Drp1 activity was completely diminished. Drp1 inactivation concomitant with disulfide dimer formation could be observed in neonatal cardiomyocytes induced by hypoxia-reoxygenation (H/R). The H/R-induced cardiomyocyte senescence was significantly suppressed by the treatment with Mdivi-1, an selective inhibitor of Drp1. These results strongly suggest that Drp1 disulfide dimer formation caused by H/R is a novel therapeutic target of chronic heart failure.

O1I-5-3 Repeated remote ischemic conditioning reverses left ventricular remodeling via exosome-mediated intercellular communication

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Background: Remote ischemic conditioning (RIC) by repeated treatment of transient limb ischemia is a clinically applicable method for protecting the heart against injury at the time of reperfusion. Here, we investigated the effects of repeated RIC on cardiac dysfunction after myocardial infarction (MI). **Methods and Results**: At 4 weeks after MI, rats were separated into the untreated group or the RIC-treated group. RIC treatment was performed by 5 cycles of 5 minutes of bilateral hindlimb ischemia and 5 minutes of reperfusion once a day for 4 weeks. Despite comparable MI size, RIC treatment prevented the deterioration of left ventricular (LV) ejection fraction and diastolic function. MI-induced LV interstitial fibrosis and oxidant stress were significantly attenuated by RIC treatment. MicroRNA-29a (miR-29a), a key regulator of tissue fibrosis, was highly expressed in the exosomes and the marginal area of the RIC group. Even in the differentiated C2C12-derived exosomes, miR-29a was highly expressed under hypoxic condition. **Conclusions**: Repeated RIC reduces adverse LV remodeling and oxidative stress by MI. Exosome-mediated intercellular communication may contribute to the beneficial effect of RIC treatment.

O1I-5-4 Angiotensin II type 1 receptor/β-arrestin2 biased signaling activates L-type Ca^{2+} channels in immature cardiac myocytes

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Angiotensin II (AII) plays important roles in cardiovascular functions. Here, we examined the effect of AII on cardiac L-type Ca^{2+} channels (LTCC). Twohour treatment of AII (3 μM) doubled LTCC activity in isolated mouse neonatal ventricular cardiac myocytes (NVCM) and atrial cell line HL-1, but not in adult ventricular cardiac myocytes (AVCM). An AT_1 receptor antagonist, candesartan (10 μM), but not an AT_2 receptor antagonist, PD123319 (3 μM), abolished the effect of AII in NVCM and HL-1. Knockdown of ß-arrestin2 but not ß-arrestin1 or $G_{q/11}$ significantly inhibited the effect of AII in HL-1. It is reported that AII promotes the proteasomal breakdown of $p27^{KIP}$ (p27) in NVCM, thereby activating casein kinase 2α' (CK2α') associated with p27. Indeed, knockdown of p27 occluded AII-dependent activation of LTCC whereas knockdown of CK2α' significantly inhibited the effect of AII on LTCC in HL-1. Furthermore, the inhibition of Src tyrosine kinase, which is known to promote p27 degradation, with overexpression of C-terminal Src kinase significantly inhibited the effect of AII in HL-1. These results indicate that AT_1 receptor/ß-arrestin2/Src/p27/CK2α' strongly activates LTCC in immature but not adult cardiac myocytes.

O1I-5-5 RGS4 regulates partial agonism of the M2 muscarinic receptor-activated K^+ currents

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

O1I-6-1 Drinking citrus fruits prevents vascular remodeling in inflammatory vascular injury model

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Objective: Drinking citrus fruits is expected to have preventive effects on cardiovascular disease based on epidemiological analysis; however, there are few reports investigating their efficacy on vascular remodeling using animal models.
Methods: Male C57BL6 mice were divided as follows: 1) Control (C), 2) 10% Citrus unshiu (CU) (CU10), 3) 40% CU (CU40), 4) 10% Citrus Iyo (CI) (CI10), 5) 40% CI (CI40). After 2 week-drinking, cuff injury was induced by polyethylene cuff placement around the femoral artery.
Results: Neointima formation was significantly attenuated in CU40, CI10 and CI40 compared with C. Although inflammatory cytokine levels (MCP-1, IL-6, IL-1ß and TNF-α) were not significantly changed, increases in superoxide anion production and cell proliferation were remarkably attenuated in CI10 but not in CU10 compared with C. In contrast, increase in ERK activity and immune cell accumulation such as macrophage and neutrophil was attenuated in CI10 and CU10 compared with C without significant difference between CU10 and CI10.
Conclusion: These results indicate that drinking citrus fruits attenuates vascular remodeling. Interestingly, there is a difference in efficacy between CU and CI. The detailed mechanism is under investigation.

O1I-6-2 Interaction between AT1 and LOX-1 promote oxLDL-induced cell responses

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Objective: LOX-1-mediated actions by oxidized LDL (oxLDL) play a critical role in atherogenesis. Angiotensin II type 1 receptor (AT1) is involved in atherosclerotic development aside from regulating blood pressure. Here, we investigated interaction of these two receptors and its role in vascular dysfunction.
Methods and Results: We found that AT1 formed a complex with LOX-1 on cell surface membrane using a co-immunoprecipitation assay and an in situ PLA. Additional expression of AT1, compared with CHO cells solely expressing LOX-1, promoted oxLDL-induced cell responses such as ERK phosphorylation, NF-κB and SRF activation. In addition, an AT1 blocker (ARB), olmesartan, suppressed ERK phosphorylation by oxLDL in HUVEC. To investigate in vivo effect of AT1 and LOX-1 interaction, SHRSP were given high fat diet and olmesartan (0.1 mg/kg/day) or hydralazine (2 mg/kg/day) for a week from the age of 8 weeks. Arterial lipid accumulation in SHRSP, where LOX-1 is known to be involved, was decreased by ARB-treatment irrespective of blood pressure compared with hydralazine-treatment ($p<0.05$). Plasma total cholesterol, HDL, triglyceride and phospholipid did not differ between the groups.
Conclusion: AT1 accelerates LOX-1-mediated cellular response and vascular reactions.

O1I-6-3 The role of smooth muscle cell-derived hypoxia-inducible factor-1α in vascular remodeling

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Recently, we have reported that smooth muscle cell (SMC)-derived HIF-1α contributes to angiotensin II-induced vascular fibrosis via regulation of extracellular matrix (ECM)-related genes, PAI-1 and collagen I (Imanishi et al. Cardiovasc Res 2014). While collagen accumulation links to fibrosis, it is essential for arterial tensile strength and has protective effects against aortic aneurysm formation. We next investigate the role of SMC-derived HIF-1α in weakness of the aortic wall by using the mouse model of the pharmacologically induced aortic aneurysm. In this model, SMC-specific HIF-1α deficiency induced aneurysm formation and elastin breaks of the aortic wall. Accompanied with these results, elastin volume reduction in the aortae was induced and the activity of lysyloxidase, the enzyme involved in cross-link elastin and collagen, was lower in the mutant mice. Our results suggest that SMC-derived HIF-1α induction causes vascular remodeling via ECM metabolism elevation, however, it has protective effects against aortic aneurysm formation.

O1I-6-4 Rho-kinase and cyclophilin A are associated with inorganic phosphate-induced signaling in vascular smooth muscle cells

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Inorganic phosphate (Pi)-induced osteogenic differentiation of vascular smooth muscle cell (VSMC) plays an important role in vascular calcification. The involvement of rho-kinase in vascular calcification has been suggested, but detail mechanism has not been elucidated. CyclophilinA (CypA) is reported to be involved in vascular diseases through its rho-kinase-dependent autocrine. We have observed that the expressions of CypA as well as RUNX2, a marker for calcifying VSMC, were increased in aortic media both from atherosclerotic patients and from type II diabetic mice. In this study, we examined whether rho-kinase-CypA signaling is related to Pi-induced calcification in rat aortic smooth muscle cells (RASMCs). Pi increased rho-kinase activation and CypA secretion, peaked at 10 minutes in RASMCs. Rho-kinase inhibitor, Y-27632 suppressed Pi-induced alkaline phosphatase (ALP) activity, calcium accumulation, and phosphorylation of extracellular-signal regulated kinase (ERK) 1/2, mediating Pi-induced calcification. Moreover, CypA inhibitor also suppressed Pi-induced ERK1/2 phosphorylation in RASMCs. From these results, it was suggested that rho-kinase and CypA are involved in Pi-induced calcification in VSMC.

O1I-6-5 Purinergic P2Y6 receptor orchestrates angiotensin II-induced hypertension in mice

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Angiotensin II (Ang II) is a potent vasoconstricting peptide that can induce hypertrophic growth of vascular smooth muscle cells (VSMCs) through AT1 receptor (AT1R). We previously reported that a purinergic G protein-coupled receptor, P2Y6R, triggers cardiac fibrosis induced by pressure overload in mice. Although Ang II / AT1R signaling also participates in mechanical stress-induced cardiac fibrosis, it is obscure the relationship between AT1R and P2Y6R. In this study, we investigated the role of P2Y6R on Ang II-induced hypertension using P2Y6R-deficient (P2Y6R (-/-)) mice.
The blood pressure of P2Y6R (-/-) mice treated with Ang II was significantly lower than that of wild type (P2Y6R (+/+)) mice treated with Ang II. Deletion of P2Y6R also reduced Ang II-induced hypertrophic growth of VSMCs, suggesting that P2Y6R is involved in Ang II-induced chronic hypertension in mice. AT1R formed a protein signaling complex with P2Y6R. P2Y6R suppressed Ang II-induced recruitment of ß-Arrestin2 to AT1R and AT1R internalization independently of agonist stimulation, resulting in enhancement of AT1R / G protein-mediated hypertrophic signaling in VSMCs. These results suggest that P2Y6R orchestrates Ang II signaling through formation of AT1R/P2Y6R heteromultimers.

O2G-1-1 Pharmacotherapy based on mechanisms underlying lysophosphatidic acid-mediated neuropathic pain in mice

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Lysophosphatidic acid (LPA) and its receptor signaling initiate the molecular machineries of neuropathic pain. Most of this mechanism is closely related to the spinal cord levels of LPA. At the level of spinal cord, the initial LPA production in the spinal dorsal horn is started by c/iPLA2-catalized lysophosphatidyl choline (LPC) synthesis, followed by the extracellular release and conversion to LPA by autotaxin (ATX). LPA also causes an amplification of LPA production through LPA1/3 receptors, glial cells and cytokines/chemokines. Nerve inury or intrathecal injection of LPA, both which cause neuropathic pain, decreases the levels of K^+-Cl^--cotransporter KCC2 in the dorsal horn. As the down-regulation of KCC2 is reported to switch the GABA transmission from inhibitory to excitatory signal, this mechanism is also presumed to play roles in the LPA-mediated neuropathic pain. Here we report the pharmacotherapeutical studies of various inhibitors against key molecules involved in LPA production and neuropathic pain mechanisms.

O2G-1-2 Lysophosphatidic acid, an initiator of neuropathic pain, causes peripheral demyelination via transcriptional mechanisms

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Lysophosphatidic acid (LPA) induces neuropathic pain via induction of peripheral demyelination of dorsal root fibers. The mechanisms for LPA-induced demyelination include transcriptional and posttranscriptional down-regulation of myelin-related molecules. For instance, LPA activates calpain protease, which degrades myelin-related proteins, to induced demyelination and neuropathic pain. However, the mechanisms of LPA-induced transcriptional repression of myelin genes remain unclear. This study aimed to elucidate the transcriptional mechanisms, by which LPA down-regulates myelin genes in Schwann cells. A single intrathecal injection of LPA was found to rapidly induce expression level of c-Jun, a negative regulator of myelin gene expression, in the dorsal root. Also, we found that LPA treatment up-regulated c-Jun expression and down-regulated transcription of myelin genes in cultured Schwann cells. Inhibition of Jun-N-terminal kinase was found to block LPA-induced transcriptional repression of myelin genes and neuropathic pain. The present study demonstrated that LPA induces c-Jun to cause transcriptional repression of myelin genes and neuropathic pain.

O2G-1-3 The involvement of spinal astrocyte neuron shuttle in neuropathic pain onset

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Nerve injury induces functional changes of spinal and brain glial cells and neurons. The activation of spinal astrocytes is proposed to be involved in the expression of neuropathic pain. Astrocytes decompose glycogen into L-lactate that facilitates neurotransmission. In this study, we investigated the role of L- lactate on the hyperalgesia in neuropathic pain. Lowered mechanical withdrawal threshold on third days after the partial sciatic nerve ligation was suppressed by intrathecal administration (i.t.) of 1,4-dideoxy-1,4-imino-D-arabinitol hydrochloride (DAB). In addition, mechanical hyperalgesia was attenuated by i.t. treatment with α-cyano-4-hydroxycinnamate (4-CIN) that inhibits monocarboxylic acid transporter transporting L-lactate from astrocytes to neurons. From these results, the expression of mechanical hyperalgesia might require L-lactate from astrocytes. I.t. administration of L-lactate (50-200ng) dose-dependently lowered the mechanical withdrawal threshold. The L-lactate-induced mechanical hyperalgesia was attenuated by i.t. pretreatment with 4-CIN. These results suggest the expression of mechanical hyperalgesia in neuropathic pain may require the excessive supply of the L- lactate, which is released from the activated spinal astrocytes.

O2G-1-4 VNUT plays a crucial role in the pathogenesis of neuropathic pain

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Intrathecal administration of antagonists for purinergic receptors including P2X4 receptors produces a reversal of neuropathic pain after peripheral nerve injury (PNI), implying that the endogenous ligand ATP must be required. However, the type of cells releasing ATP within the spinal cord remains to be determined. In this study, we examined the role of vesicular nucleotide transporter (VNUT), a secretory vesicle protein responsible for the storage and release of ATP. In wild-type mice, PNI increased expression of VNUT and extracellular ATP content within the spinal cord. Interestingly, VNUT deficiency canceled both the PNI-induced enhancement of spinal ATP levels and pain hypersensitivity. These phenotypes were recapitulated in mice with specific deletion of VNUT in dorsal horn neurons. Conversely, ectopic expression of VNUT in dorsal horn neurons produced pain hypersensitivity in VNUT-deficient mice. Together, these findings suggest that VNUT-expressing dorsal horn neurons are responsible for enhanced extracellular ATP levels within the spinal cord and neuropathic pain.

O2G-1-5 Spinal angiotensin system contributes to neuropathic pain in streptozotocin-induced diabetic mice

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The renin-angiotensin system (RAS) activity is increased under hyperglycemic states, and is responsible for the diabetic nephropathy and retinopathy. However, the role of RAS on diabetic neuropathic pain (DNP) remains unclear. Here, we examined whether spinal angiotensin (Ang) system is involved in neuropathic pain in streptozotocin (STZ)-induced diabetic mice. DNP was observed concurrently with increase of blood glucose levels from the next day after STZ injection, and lasted throughout 4 weeks. DNP was significantly inhibited by intrathecal administration of losartan, an Ang II type 1 (AT1) receptor antagonist, but not by PD123319, an AT2 receptor antagonist. The gene expression of angiotensinogen, Ang converting enzyme (ACE) and AT1A receptor, the major components of RAS, was significantly promoted on lumbar dorsal spinal cord in diabetic mice. Moreover, immunofluorescence intensity of ACE and Ang II was also increased in lumbar superficial dorsal horn of diabetic mice. These results suggest that the activation of AT1 receptors which accompanied the increase of spinal Ang II is involved in DNP.

O2G-2-1 A nonhuman primate model of knee osteoarthritis (OA) I: Medial meniscectomy (MMx)-induced OA

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Acute injury to the knee may lead to an OA characterized by pain and functional loss. Although total knee replacement restores function this procedure is not without risk, less invasive treatments are desirable. To further understanding of the pathophysiology of OA and to facilitate the development of effective treatments, a novel nonhuman primate (NHP) model of knee OA was induced by MMx. Following MMx, knee pressure threshold, weight bearing, and ipsilateral knee function were normal. However, following an exercise schedule, pain-related behavior, indicated by decreased pressure threshold and decreased weight bearing, and decreased functionality, indicated by increased gait disturbance, emerged. An analgesic dose of morphine decreased pain-related behavior and improved knee function. Cartilaginous erosion was measured with magnetic resonance imaging of the ipsilateral knee. Interestingly, this model reflects clinical OA in that pain and loss of function are seen following activity but not during inactivity. The current NHP OA model mirrors clinical symptoms of knee OA and could highly useful in further elaborating disease mechanism and testing treatments that both ameliorate symptoms and reverse disease.

O2G-2-2 A nonhuman primate model of knee osteoarthritis (OA) II: Monoiodoacetate-induced OA

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OA is the most prevalent joint disorder worldwide, characterized by joint disruption and pain. The lack of effective treatments for OA pathology as well as pain could be attributed in part to the reliance on preclinical rodent models that do not adequately reflect the human pathology. The phylogenetic closeness of non-human primates (NHP) to humans makes NHP an ideal species for model development. A state of OA was induced in the knee joint of NHP by intraarticular injection of monoiodoacetate (MIA). After MIA injection, the knee joint exhibited persistent inflammation and animals demonstrated pain-related behaviors, including significantly reduced weight bearing of the ipsilateral limb, increased sensitivity to pressure threshold ("hyperalgesia") and gait disturbance. An acute injection of morphine transiently reversed increased weight bearing, decreased hyperalgesia and ameliorated gait disturbance. Daily treatment with diclofenac ameliorated pain-related behaviors over time. The ipsilateral knee joint at the termination of the study showed signs of cartilage disruption. While OA pain in the current model is sensitive to commonly used analgesics, there is a need for treatments that reduce both symptoms as well as the disease state.

O2G-2-3 Preventive and therapeutic effects of polaprezinc, a zinc-containing drug, on cyclophosphamide-induced cystitis and/or bladder pain

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Zinc exhibits antioxidant activity and selectively inhibits $Ca_v3.2$ among three T-type calcium channel isoforms. Given our recent evidence for the pronociceptive role of $Ca_v3.2$ targeted by H_2S, a gasotransmitter, in the mouse with cyclophosphamide (CP)-induced cystitis, a model for interstitial cystitis, we asked if polaprezinc (Pola), a zinc-containing drug for treatment of gastric ulcer, exerts preventive or therapeutic effects on the CP-induced cystitis and/or bladder pain in mice. Oral preadministration of Pola at 400 mg/kg attenuated the CP-induced bladder edema accompanied by bladder pain/referred hyperalgesia. Oral Pola also prevented the CP-induced increases in levels of malondialdehyde, an indicator of lipid peroxidation, and protein expression of cystathionine-γ-lyase (CSE), an H_2S-generating enzyme, in the bladder tissue. On the other hand, Pola even at 30-100 mg/kg, when given orally after the development of CP-induced cystitis, suppressed the bladder pain/referred hyperalgesia without affecting the edema. Together, Pola appears to exhibit preventive and/or therapeutic effects on the CP-induced cystitis and related bladder pain probably through antioxidant activity or $Ca_v3.2$ inhibition.

O2G-2-4 Pharmacological profiling of μ opioid receptor agonists; analysis of "ligand-biased efficacy" using positive allosteric modulator

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"Ligand-biased efficacy" provides new opinions for differences among ligands that act on the same receptor. However, pharmacological differences depending on ligand biased signaling among several kind of μopioid receptor (MOR) agonists have not been yet to be elucidated fully. In the present study, we investigated whether pharmacological profiles of MOR agonist-induced internalization could be different by activating ligand-biased signaling of MOR. The treatment with a MOR agonist morphine to the HEK293 cells over-expressing Halo-fused MORs did not show the internalization of MORs. On the other hand, an another MOR agonist fentanyl dramatically induced MOR internalization. Then, we investigated the effect of the positive allosteric modulator (PAM), which has no intrinsic efficacy but enhances the efficacy of MOR agonists, on the MOR internalization induced by MOR opioids. Interestingly, morphine clearly induced internalization of MORs under the condition of co-treatment with PAM. These results suggest that each ligand acting at the MORs can differently MOR activate its signaling. This signaling could be modulated by PAM with possibly changing the receptor conformation.

O2G-2-5 Interaction between lysophosphatidic acid and endocannabinoid system in inflammatory and neuropathic pain

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Lysophosphatidic acid (LPA) and its receptor signaling plays key role of neuropathic pain. Recent study reveals that initial LPA production in the spinal dorsal horn is started by activations of cPLA2 and iPLA2 via NK1 and NMDA receptors. The LPA further amplifies the LPA production by activations of LPA1 and LPA3 receptor through the local circuit with glial cells and cytokines/chemokines. LC-MS/MS analysis revealed that the injury-induced elevation of LPA reached a plateau at 3-6 h, and its was abolished by pharmacological pretreatments with inhibitors of these signaling molecules, which block the neuropathic pain. On the other hand, endocannabinoids (ECs) such as anandamide or 2-arachidonoylglycerol (2-AG) are known to play roles in the suppression of pain transmitter signal through CB1 receptor on primary afferent sensory fibers as another type of reverse signals. In this study we discuss about the counter-balancing cross-talk system between LPA and ECs systems both in inflammatory and neuropathic pain.

O2G-3-1 Intracellular dopamine D2 receptor activation requires for dendritic spine formation

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The dopamine D2 receptor (D2R) is a target for antipsychotic drugs and its abnormality is associated with neuropsychiatric disorders. We here report that D2R alternatively spliced long isoform D2LR elicits persistent MAPK/ERK activation through Rabex5, which contributes for formation of dendritic spine structure in striatopallidal medium spiny neurons (MSNs). D2LR binds and activates Rabex5, thereby promoting early endosome formation. The endosomes containing D2LR and platelet-derived growth factor receptor-beta (PDGFRbeta) are transported to the Golgi complex, thereby activating Galphai3 protein and ERK signaling. Like phenotype of D2LR knockout (KO) mice, haloperidol-induced catalepsy and ERK activation in striatopallidal MSNs is absent in PDGFRbeta KO mice. Accordingly, D2LR deficiency causes decreases in dendritic spine density and neuronal activity in striatopallidal MSNs. Furthermore, Rab5 overexpression rescues these D2LR-related neuronal abnormalities. Taken together, the novel intracellular D2LR signaling is critical for activation and dendritic spine formation of striatopallidal MSNs in the behavioral responses of antipsychotic drugs.

O2G-3-2 Serotonin inhibits non-NMDA glutamatergic transmission onto rat basal forebrain cholinergic neurons via 5-HT_{1A} and 5-HT_{1B} receptors

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A whole-cell patch-clamp study was carried out to elucidate the modulatory roles of serotonin (5-HT) in non-NMDA glutamatergic transmission onto basal forebrain (BF) cholinergic neurons in P12-20 rat brain slices. Pharmacologically isolated non-NMDA receptor-madiated excitatory postsynaptic currents (EPSCs) were evoked by focal electrical stimulation. Bath application of 5-HT at 10 or 30 μM inhibited the amplitude of the evoked EPSCs. A 5-HT_{1A} receptor agonist, (R)-(+)-8-OH-DPAT (30 μμM), and a 5-HT_{1B} receptor agonist, CP93129 (30 μM), both inhibited the EPSCs. 5-HT-induced suppression of EPSCs is significantly larger in low (1.0 mM) Ca^{2+}-containing external solution than in normal (2.4 mM Ca^{2+}) Krebs solution. The amplitude of EPSCs was inhibited by bath application of ω-conotoxin GVIA (ω-CgTX; 3 μM) or ω-agatoxin-TK (200 nM). In the presence of ω-CgTX, 5-HT could still inhibit the EPSCs. These results suggest that 5-HT inhibits non-NMDA glutamatergic transmission onto BF cholinergic neurones via 5-HT_{1A} and 5-HT_{1B} receptors, and that the inhibitory effect is saturated in the normal external solution, also suggesting that multiple types of Ca^{2+} channel are involved in the transmission.

O2G-3-3 Involvement of G-coupled receptor-dependent cAMP/PKA signaling pathways on neural differentiation of mouse induced pluripotent stem cells

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The present study determined involvement of G-coupled receptor-dependent cAMP/ PKA signaling pathways on differentiation of mouse induced pluripotent stem (iPS) cells into neural progenitor cells. The differentiation initiated by embryoid formation was stimulated with all trans retinoic acid (ATRA; 1 or 3 μM), serotonin (0.01~0.3 μM) or carbachol (a muscarinic acetylcholine receptor agonist; 1~10 μM) for 4 days and then transferred to matrigel-coated dishes. The differentiation potential was evaluated by Nestin expression using immunofluorescence staining or western blot analysis. Although the treatment with serotonin (0.03 μM) significantly enhanced ATRA-induced Nestin expression and cAMP response element binding protein (CREB) phosphorylation, the treatment with carbachol (10 μM) significantly inhibited the effects of ATRA. Pretreatment with H89 (a PKA inhibitor; 1 μM) significantly inhibited the effect of serotonin. Pretreatment with forskolin (an activator of adenylate cyclase; 1 μM) significantly reversed the effect of carbachol. Thus, the differentiation of mouse iPS cells into neural progenitor cells may be modulated by the G-coupled receptor-dependent cAMP/ PKA signaling pathways.

O2G-3-4 Postsynaptic assembly of neuropeptide Y Y_5 receptor

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Precise cellular and subcellular localization of GPCR proteins has not been elucidated in the central nervous system *in vivo*. For the purpose of this question, testing specificity of anti-GPCR antibodies is required adequately. Scientific journals propound that the specificity of antibodies would be tested using knockout mice and/or cell lines devoid of targeted molecules. In this context, anti-Y_5 receptor antibody, which does not react to tissues from Y_5 knockout mice, was prepared and was subject to immunohistochemistry of Y_5 protein localization in wild-type brains. Y_5, a class A of the GPCR superfamily, functions orexigenically, but it is still unclear what types of neurons express Y_5 protein in the brain. In several nuclei and cortices, Y_5-immunoreactivity was observed as dot-like structures located at the neuronal surface, as expected for a membrane receptor. A higher magnification of each neuron showed clustering of Y_5-immunoreacticity (Φ 0.3 - 0.6 μm), suggesting assembly of Y_5. Co-localization of the Y_5 clusters and pre-/post synaptic markers showed postsynaptic localization of the Y_5 protein. To the best of our knowledge, this is the first evidence showing precise Y_5 localization using validated antibody *in vivo*.

O2G-3-5 Corticotropin-releasing factor enhances inhibitory synaptic transmission to type III neurons in the bed nucleus of the stria terminalis

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We previously reported that CRF-induced neuronal excitation in type II neurons of the dorsolateral part of the bed nucleus of the stria terminalis (dlBNST) is critical for pain-induced aversion. We have hypothesized that activation of type II neurons, which are considered to be GABAergic, would result in increased inhibitory inputs to type III neurons, which are considered to project to the ventral tegmental area (VTA) and positively regulate VTA dopaminergic neurons. To test this hypothesis, we examined the effect of CRF on type III neurons by using whole-cell voltage-clamp recordings in slice preparations obtained from rats. Bath application of CRF significantly increased the frequency of spontaneous IPSCs, indicating that CRF enhances inhibitory input to type III neurons. In the presence of TTX, CRF application failed to increase the frequency of miniature IPSCs, suggesting that CRF-induced increase in spontaneous IPSCs was dependent on action potentials. Combined with our previous finding that CRF specifically depolarizes type II dlBNST neurons, these results suggest that CRF attenuates type III neuron excitation by augmenting the inhibitory influence from type II neurons in the dlBNST.

O2G-4-1 Extracellular nucleotide-receptor signaling-mediated synchronization of the mammalian cellular clock

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The cell-autonomous circadian clocks synchronize in tissues, however, its mechanism is not fully understood. Extracellular nucleotides such as ATP are recently found to act as intercellular signaling molecules via stimulating purine P2 receptor family. In this study, we investigated the possibility that the extracellular nucleotide-receptor signaling mediates the synchronization of the mammalian central and peripheral clocks. We observed that the ATP or UTP caused a transient increase in the SCN slice culture, SCN-derived cell line, RS182, and the MEFs. These Ca2+ responses were mediated via Gq/11-coupled purine P2Y receptors (P2Y2, 4, 6). The treatment with UTP caused the phase shift of luminescent rhythms derived from Per1 promoter-luciferase trangenic RS182 cells and Per2-luciferase knock-in MEFs in a stimulation time-dependent manner. Also, UTP caused a transient induction of Per1 and Per2 mRNA in RS182 cells. Finally, the treatment with apyrase, an ATP/ADP degrading enzyme, facilitated the damping of Per2-luciferase bioluminescence rhythm in Per2-luciferase knock-in MEFs. Our observations suggest that the extracellular nucleotides might act as an intercellular synchronizer on the central and peripheral circadian clocks.

O2G-4-2 AMPK activation influences neuronal glutathione synthesis in the brain

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AMP-activated protein kinase (AMPK) plays a pivotal role in the regulation of energy homeostasis. AMPK is a serine/threonine protein kinase activated under some stressed conditions, such as fasting or prolonged exercise, leading to ATP depletion. A previous in vitro study demonstrated down-regulation of neuronal glutamate/cysteine transporter, excitatory amino acid carrier 1 (EAAC1), by AMPK activation. We have studied the regulation of neuronal glutathione (GSH) synthesis, especially focused on glutamate transporter-associated protein 3-18 (GTRAP3-18), which is an endoplasmic reticulum protein interacting with EAAC1 to inhibit its transport activity. Previously we showed that an increased interaction between EAAC1 and GTRAP3-18 decreased neuronal GSH levels in the brain. In this study, we report AMPK activation in the brains of fasted mice showing decreased GSH levels caused by an increased EAAC1/GTRAP3-18 interaction. These results indicate an involvement of AMPK activation in neuronal GSH synthesis through the regulatory mechanism of EAAC1/GTRAP3-18 interaction in the brain.

O2G-4-3 A role of p38 MAPK in neural stem cell activity

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Neural stem cells (NSCs) play a neuroprotective role under various pathophysiological conditions and are suggested to be a beneficial tool in cellular therapy for brain diseases. An efficient *in vitro* expansion of NSCs is crucial for the generation of a sufficient amount of transplantable cells. We confirmed that the kinate-induced proliferation of NSCs in the hippocampal dentate gyrus was more prominent in $p38\alpha^{+/-}$ mice than wild type (WT) mice. Then, hippocampal NSCs from $p38\alpha^{+/-}$ and WT mice were subjected to the neurosphere culture system, and NSCs activity was compared between the two genotypes. Unlike the case of WT-derived NSCs, $p38\alpha^{+/-}$-derived NSCs kept the capacity of self-renewal and neural differentiation in the long-term culture (over 50th passages). This phenomenon was recapitulated in WT-derived NSCs cultured in the presence of a p38 inhibitor, indicating that the inhibition of p38 activity can enable the successful *in vitro*> expansion of NSCs. To elucidate the mechanisms underlying the functional maintenance of NSCs under p38 inhibition, an array analysis for microRNA was performed, and we are now focusing several microRNAs. We will discuss the possible therapeutic application of NSCs expanded in the p38 inhibition culture.

O2G-4-4 Identification and characterization of a G protein-gated inward rectifier K^+ (K_G) channel blocker isolated by a cell growth-based screening system

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Down syndrome (DS) is the most frequently observed genetic abnormality and characterized by complicated neurological disorders including mental retardation. A major determinant of DS is an extra copy of chromosome 21, where *Kcnj6* gene is located. The gene encodes a neuronal K_G channel subunit Kir3.2, which is responsible for the slow IPSP formation. It is reported that, in DS-model mice, Kir3.2 is overexpressed and neuronal K^+ conductance is increased. Therefore, a Kir3.2 blocker may have a benefit to improve some neurological phenotypes for the DS patients. In this study, we tried to isolate Kir3.2 blockers using cell growth-based drug screening system. The host cell was a yeast strain lacking three K^+ transporters and became to grow in the low K^+ medium by expressing constitutively active Kir3.2 mutant. From a small chemical library, a single compound was isolated as a growth inhibitor of the transformant. The compound blocked Kir3.2 expressed in *Xenopus* oocytes in concentration- and voltage-dependent manners. Kir3.1/Kir3.4, but not Kir4.1, was sensitive to the compound. These results imply that this compound would be a lead for selective block of various K_G channels.

O2G-4-5 Polysulfides, an active form of H_2S, activate TRPA1 channels in rat brain

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Hydrogen sulfide (H_2S) is emerging as bioactive substance with various physiological functions. H_2S is produced from cysteine and oxidized to polysulfides of which the function is not well understood. We previously reported that polysulfides induce Ca^{2+} influx in astrocytes. However, the receptor that mediates the response has not been well understood. Here, we have shown that polysulfides induce Ca^{2+} influx by activating transient receptor potential ankyrin-1 (TRPA1) channels in rat astrocytes. The maximum response is induced by 0.5 μM polysulfide, which is about 1/300 of the concentration of H_2S required to achieve a response with a similar magnitude. TRPA1-selective agonists, allyl isothiocyanate and cinnamaldehyde, induced Ca^{2+} influx, and responses to polysulfides were suppressed by TRPA1-selective inhibitors, HC-030031 and AP-18, as well as by siRNAs selective to TRPA1.

The present study suggests that polysulfides are an active form of H_2S that stimulate TRPA1 channels in the brain. We propose polysulfides are potential endogenous ligands for TRPA1 channels. KAKENHI:26460352

O2G-5-1 Development and clinical evaluation of tau PET probe [^{18}F]THK-5351

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In our previous studies, [^{18}F]THK-5117 successfully visualized brain tau deposits in Alzheimer's disease (AD) patients. To reduce the non-specific binding of [^{18}F]THK-5117, we have developed a novel tau PET tracer [^{18}F]THK-5351. In vitro binding of THK-5351 to neurofibrillary tangles were firstly evaluated using the autoradiography of AD brain slices. Affinity and rate constants of THK-5351 against the hippocampal homogenates from AD patients and the subcortical white matter homogenates from healthy controls were compared with those of THK-5117. To evaluate the clinical utility of this tracer, first-in-man PET studies have been performed in healthy controls and AD patients. As a result, THK-5351 bound selectively to tau deposits on AD brain sections and showed high binding affinity to AD hippocampal homogenates. THK-5351 showed faster dissociation from the white matter homogenates than THK-5117. In clinical study, AD patients showed high THK-5351 retention in the lateral and medial temporal cortices. As expected, THK-5351 showed faster kinetics and lower white matter retention than THK-5117. From these findings, [^{18}F]THK-5351 is considered as a promising PET tracer for imaging tau pathology in humans.

O2G-5-2 From discovery of novel anti-microbial peptide to clinical trial for drug development in wound repair

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We developed a novel anti-microbial peptide, AG30/5C, which demonstrates angiogenic properties similar to those of LL-37 or PR39. To improve its stability and cost efficacy for clinical application, we examined the metabolites of AG30/5C, which provided the further optimized compound, SR-0379. SR-0379 enhanced the proliferation of human dermal fibroblast cells via the PI3 kinase-Akt-mTOR pathway. This compound displays antimicrobial activities against a number of bacteria, including drug-resistant microbes. We evaluated the effect of SR-0379 in would-healing models, an acutely infected wound with full-thickness defects and inoculation with S. aureus. Treatment with SR-0379 significantly accelerated wound healing when compared to fibroblast growth factor 2 which can be explained by enhanced angiogenesis, granulation tissue formation and antimicrobial activity. These results indicate that SR-0379 may have the potential for drug development in wound repair. Until now, we have almost finished the preclinical studies (pharmacological kinetics, immunotoxicity, toxicity, safety pharmacology) for physicians-initiated clinical trial and finished the peptide synthesis under GMP grade. We will start the clinical trial in 2015.

O2G-5-3 Difference of PPARα agonist-induced gene expression between in vivo and in vitro

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The Toxicogenomics Project produced a large transcriptome database of the liver of the rats received >150 drugs. Species difference in the toxicity study was expected to be overcome by using cultured hepatocytes, but few attempts have been done. Effects of PPARα agonists (PA) are directly related to gene expression. Our aim was to get a clue for the bridging between species using PA as a model. Reliable probe sets (Affymetrix), were obtained by the union of the up-regulated genes with three fibrates in the rat liver and hepatocytes. The probe sets responding in vitro were categorized into (a) equally increased in vivo and in vitro, (b) in vitro responses were smaller, and (c) in vitro responses were larger, than that in vivo. Group (a) appeared to be directly induced by PA, whereas the expression of (b) was suggested to involve indirect pathways. The cause of the difference in (c) was varied with the genes. Similar analysis of the human hepatocytes revealed that the numbers and the extent of the PA-induced gene expression was much less than those in rats, and there was a species difference in the feature genes. It was concluded that there might be some genes useful for bridging of species difference but considerable difficulties existed.

O2G-5-4 cDNA microarray screening of genes related to osteogenic feature of mesenchymal stem cells from patients with ossified spinal ligament

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Mesenchymal stem cells (MSCs) isolated from spinal ligaments with ectopic ossification were shown to have the propensity toward the osteogenic lineage. To explore the epigenetic control of the osteogenic feature of MSCs, MSCs obtained from the spinal ligaments of ossification of yellow ligament (OYL) patients and non-OYL patients with the DNA methyltransferase inhibitor, 5-Aza-2'-deoxycytidine (5AdC). A comparison was made of non-OYL groups to OYL groups (untreated and treated with 5AdC, respectively) by genome-wide microarray. To assess methylated and unmethylated state of genes, methylated DNA immunoprecipitation combined with quantitative real-time PCR was carried out. It is revealed that the expressions of 98 genes were significantly increased by the 5AdC treatment in MSCs from non-OYL patients, but not from OYL patients. Only two genes, *GDNF* and *WNT5A* showed significantly higher expression in OYL MSCs compared to non-OYL MSCs in normal culture condition without 5AdC treatment. Both genes were hyper methylated in non-OYL MSCs but not in OYL MSCs. These results suggest that osteogenic features of MSCs from OYL patients are promoted by unmethylated *GDNF* and *WNT5A* genes.

O2G-5-5 Screening of multidrug resistance-associated protein inhibitors with selective cytotoxicity toward neuroblastoma

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The current chemotherapeutic drugs cannot sufficiently treat high-risk neuroblastoma, and they sometimes cause side effects on neural cells. Therefore, the development of drugs, which possess selective cytotoxicity toward neuroblastoma via different mechanisms from the conventional, are desired. The aim of the present study was to clarify whether the inhibition of multidrug resistance-associated proteins (MRPs) exerts cytotoxicity to neuroblastoma, but not neural cells. Mouse primary cultured cortical neurons (MCN) and neuroblastoma Neuro2a cells (N2A) were used as neural and neuroblastoma model cells, respectively. The fluorescence intensity of MRP substrate Fluo-8 was detected in N2A treated with MRP inhibitor probenecid, but minimally detected in non-treated N2A. The fluorescence of Fluo-8 was detected in MCN regardless of the addition of probenecid. Probenecid significantly decreased MTT reduction activity in N2A whereas minimal effect of probenecid was observed in MCN, suggesting that cytotoxicity of probenecid is more remarkable in N2A, compared with MCN. These results suggest that inhibition of MRPs by traditional therapeutic drugs may exert selective cytotoxicity toward neuroblastoma with minimal neurotoxicity.

O2G-5-6 Suppression of cancer-type amino acid transporter LAT1 affects multiple cellular events in pancreatic cancer cells

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L-type amino acid transporter 1 (LAT1) is known as a cancer-type amino acid transporter and upregulated in various cancers including pancreatic cancer. Inhibition of LAT1 strongly arrested the growth of *Xenograft* tumor in animal models. Because intracellular events caused by LAT1 inhibition in cancer cells are not well studied, we have examined the effects of LAT1 inhibitors on phosphoproteome in this study. In MIA Paca-2 pancreatic cancer cells in which LAT1 was expressed on the plasma membrane, we confirmed that conventional LAT1 inhibitor BCH and newly developed inhibitors suppressed leucine transport and phosphorylation of p70S6K, a downstream effector of mTORC1 responsible for cell growth. The results from the comprehensive phosphoproteomics analysis addressing the effects of LAT1 inhibitors indicated that LAT1-mediated leucine transport affects multiple cellular events related to cellular metabolism, cell growth and cell motility. Signaling molecules which link LAT1 and many cellular events were also examined. In this study, based on the effects of LAT1 inhibitors on phosphoproteome, we confimred that LAT1 can be a therapeutic target for the treatment of LAT1-upregulating cancers.

O2H-1-1 Involvement of prostaglandin D_2 in exacerbation of pruritus induced by long-term topical treatment with glucocorticoids in allergic contact dermatitis mice

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Topical glucocorticoids (GCs) are the first-line therapy for chronic dermatitis. We previously reported that long-term topical GCs exacerbated pruritus in allergic contact dermatitis (ACD) mice. In the present study, we investigated the involvement of prostaglandin D_2 (PGD_2), known as a natural antipruritic agent, in the exacerbation of pruritus using the ACD mice.

Chronic ACD was induced in BALB/c or C57BL/6 mice by repeated application of TNCB, and the mice were treated with GC topically for 2 to 5 weeks after the elicitation of ACD. Topical betamethasone valerate (BMV), dexamethasone (DEX) and prednisolone (PSL) exacerbated the pruritus of ACD mice irrespectively of their GC potencies. All GCs reduced mRNA expression of hematopoietic PGD_2 synthase (H-PGDS) in the lesional mice skin and suppressed the level of PGD_2 release from RBL-2H3 mast cells. Additionally, topical BW 245C, an agonist of PGD_2 receptor (DP1), completely suppressed the augmentation of the scratching behavior induced by topical BMV.

These results suggest that the level of PGD_2 was decreased in the skin by topical GCs, and then degranulation of the mast cells would be accelerated via reduction of DP1 stimuli.

O2H-1-2 VEGFR1 signaling restores wound healing in diabetes through suppression of IL-1 from recruited VEGFR1-expressing macrophages

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Aims: Impaired diabetic wound healing is caused by persistent pro-inflammatory macrophages. Vascular endothelial growth factor receptor 1 (VEGFR1) is essential for tissue/organ repair through recruitment of macrophages. The present study examined the role of VEGFR1 signaling in diabetic wound healing. Methods: VEGFR1-tyrosine kinase knockout mice (KO) or their wild counterparts (WT) were treated with streptozotosin (STZ). Full-thickness skin wounds were created on the backs of mice. Results: Compared with vehicle-treated WT, wound healing and angiogenesis/lymphangiogenesis were suppressed in STZ-treated WT. STZ-treated WT exhibited increases in VEGFR1-macrophages expressing IL-1 (M1 marker) and decreases in VEGFR1-macrophages expressing mannose receptor (M2 marker). STZ-treated KO showed exaggerated delay in wound healing, and insufficient M1/M2 macrophage polarization. Treatment of STZ-treated KO with a neutralizing antibody against IL-1 restored wound healing and normalized M1/M2 macrophage polarization. Conclusions: These results indicate that VEGFR1 signaling plays a role in diabetic wound healing and angiogenesis/lymphangiogenesis through attenuation of IL-1 from recruited VEGFR1-macrophages.

O2H-1-3 Functional role of the dominant-negative splice variant of pH-sensitive, two-pore domain K^+ channel $K_{2P}5.1$

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The two-pore domain K^+ (K_{2P}) channel, $K_{2P}5.1$ is one of the background K^+ conductance, is activated by extra- and intracellular alkalization and contributes to the setting of the resting membrane potential in various types of cells. We recently identified the novel splice variants of $K_{2P}5.1$, $K_{2P}5.1B$ from mammalian spleens, which are lacking the N-terminal domains of the full-length $K_{2P}5.1A$ and inhibited the trafficking of $K_{2P}5.1A$ to the plasma membrane. Using a fluorescence imaging system, alkaline pH-induced hyperpolarization by the activation of native human $K_{2P}5.1A$ ($hK_{2P2P}5.1A$) was significantly suppressed and the influx of Ca^{2+} was simultaneously decreased in $hK_{2P}5.1B$-overexpressing human leukemia K562 cells. The potential roles of the two-pore domain K^+ channel $K_{2P}5.1$ are highlighted in the pathogenesis of autoimmune diseases, however, the potent and selective $K_{2P}5.1$ inhibitors has not yet been identified. The spliceosome inhibitors enhanced the expression of $hK_{2P}5.1B$ in K562 and thereby $K_{2P}5.1$ activity was significantly suppressed. The mRNA spicing mechanisms underlying the transcriptional regulation of $K_{2P}5.1B$ may implicate for a new therapeutic strategy in autoimmune and inflammatory diseases.

O2H-1-4 Effects of plasma glycosyltransferase on the ABO(H) blood group antigens of human von Willebrand factor

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The plasma von Willebrand factor (VWF) is known to have ABO(H) blood group antigens. Since the plasma concentration of VWF is influenced by blood groups, its glycosylation mechanism might be important. In the present study, we have investigated whether H-type glycan (blood group O) of VWF is changeable into A- or B-types by plasma or recombinant glycosyltransferase. (1) Bovine serum albumin conjugated with H-type glycan (H-BSA) or (2) VWF prepared from O-plasma (O-VWF) was incubated with either A-type plasma (AP) or recombinant A-glycosyltransferase (rATase), in the presence or absence of UDP-GalNAc (GALSERVE A/B; EIDIA). ABO(H) blood group antigens were detected using the enzyme-linked immunosorbent assay (ELISA) or western blotting analysis. The H-BSA or O-VWF were successfully glycosylated and transformed into A-type after incubation with rATase only in the presence of UDP-GalNAc. Blood group A antigen was detected on H-BSA but not on O-VWF after incubation with AP in the presence of UDP-GalNAc. These results suggest that plasma glycosyltransferase might have little effect on the construction of blood group antigens of VWF, and that VWF might be glycosylated within the endothelial cells before secretion into plasma.

O2H-1-5 Serpinopathy found in PAI-1

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Serpinopathy is characterized as abnormal accumulation of serine protease inhibitors (SERPINs) in their synthesizing cells and develops symptoms due either to lack of the SERPIN function or to excessive accumulation of the abnormal SERPIN molecule. Plasminogen activator inhibitor-1 (PAI-1) is a member of the SERPIN superfamily and is the primary physiological regulator of urokinase type plasminogen activator (uPA) and tissue type plasminogen activator (tPA). We recently identified a new complete PAI-1 deficiency. The patient possessed massive bleeding tendencies, which were also observed in the previous complete PAI-1deficient patients. The patient appeared to have single amino acid replacement in PAI-1 molecule at its very C-terminus (G397R). Though the mutant was full length and its reactive site is conserved, the activity and the antigen levels of PAI-1 were nearly undetectable in the patient's plasma. The mutant PAI-1 polymerized in the cells which interfered its efficient secretion from the producing cells. Collision between G397R with V56 was suggested to evoke large conformational change in PAI-1 molecule by disturbing the annealing between s5B and s6B.

O2H-2-1 Coordinate involvement of TRPV1 channels and integrin β4 in directional migration

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The directional migration of epithelial cells is crucial for wound healing. Integrin ß4(β4) has been assumed to be a promigratory factor, in addition to its role in adhesion. In turn, Ca^{2+} signal is also a key coordinator of migration. Keratinocytes reportedly express the TRPV1 channel; however, its function as a regulator of intracellular Ca^{2+} level in migration has remained uncharacterized. In this study, we investigated the role of TRPV1 in directional migration related to ß4 using a scratch wound assay on a monolayer sheet of keratinocytes. Double immunofluorescence staining revealed the de novo expression of ß4 and TRPV1 in migrating cells at the wound edge, and both expression were matched. Epidermal growth factor not only promoted the migration, but also caused the further up-regulation of both ß4 and TRPV1. In addition, the knockdown of the ß4 or TRPV1 gene significantly impeded wound closure. The TRPV1 agonist capsaicin significantly promoted migration, while a TRPV1 antagonist inhibited it. The gene knockdown of TRPV1 inhibited the expression of ß4 gene and that of ß4 protein in migrating cells. These findings suggest that TRPV1 may stimulate directional migration directly by eliciting a Ca^{2+} signal or indirectly via ß4 expression.

O2H-2-2 Up-regulation of the two-pore domain K^+ channel $K_{2P}5.1$ in splenic $CD4^+$ T-lymphocytes from chemically-induced murine inflammatory bowel disease model

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The alkaline pH-activated, two-pore domain K^+ channel $K_{2P}5.1$ plays an important role in maintenance of the resting membrane potential, and contributes to the control of Ca^{2+} signaling in various types of cells. Recent researches highlighted the potential role of $K_{2P}5.1$ in the pathogenesis of autoimmune diseases such as rheumatoid arthritis and multiple sclerosis. Utilizing real-time PCR and Western blot experiments, we found a significant increase in $K_{2P}5.1$ expression in the splenic $CD4^+$ T-lymphocytes from a mouse model of dextran sodium sulfate (DSS)-induced inflammatory bowel disease. The transcripts of the other alkaline pH-sensitive K_{2P} channels, $K_{2P}3.1$, $K_{2P}9.1$ and $K_{2P}16.1$ were less abundantly expressed in IBD model. Concomitant with an up-regulation of $K_{2P}5.1$, alkaline pH-induced hyperpolarization response is significantly larger in IBD model in the splenic $CD4^+$ T-lymphocytes of IBD model. Up-regulation of $K_{2P}5.1$ was not detected in the immature $CD4^+CD8^+$ thymocytes of IBD model. These suggest that $K_{2P}5.1$ is a potential therapeutic target and a biomarker for inflammatory bowel disease.

O2H-2-3 Effects of a steroidal anti-inflammatory drug on a culture model for murine cutaneous mast cells

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Steroidal anti-inflammatory drugs have been broadly used as the primary therapeutic drug for chronic inflammatory diseases, such as atopic dermatitis. Because a wide variety of cells are involved in the therapeutic process, the details of the actions remain to be clarified. We recently established a model culture system for murine cutaneous mast cells, in which IL-3-dependent bone marrow-derived mast cells are co-cultured with a fibroblastic cell line, Swiss 3T3, in the presence of stem cell factor. In this study, we investigated the effects of dexamethasone (Dex) on the cutaneous mast cell model. Prolonged treatment with Dex significantly decreased the enzymatic activity of chymase and tryptase and increased that of carboxypeptidase A and ß-hexosaminidase. Storage of histamine was drastically increased in the Dex-treated cells. Dex had no effects on degranulation induced by IgE-mediated antigen stimulation but significantly impaired that induced by compound 48/80 and substance P. Six days of treatment with Dex also significantly augmented the tissue histamine levels and suppressed degranulation of murine cutaneous mast cells induced by compound 48/80. We revealed the immediate effects of Dex on murine cutaneous mast cells.

O2H-2-4 Infl uence of inhaled carbon nanotubes (MWCNT) on mucociliary clearance in airway

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Airway epithelial cells play important roles in mucociliary clearance, a mechanism to exclude foreign body. Multi-walled carbon nanotubes (MWCNT) are newly developed material with potential application in many fields. However, some reports showed that the MWCNT might cause asbestos-like lung disease. We have previously shown that two different types of MWCNTs, small (MWCNT-S) and long (MWCNT-L), caused erosion and loss of ciliary cells in airway epithelium. But the degree of damage was different between these MWCNTs. Thus MWCNTs are supposed to decrease mucociliary clearance. In this study, we examined influence of these MWCNTs on mucociliary clearance using fluorescent beads. Wister ST male rats were treated with MWCNT-S, MWCNT-L or vehicle on day 1, 4, and 7. On day 8, rats were treated with 0.5μm fluorescent beads and sacrificed 2 h later. Trachea and lung were washed and the fluorescence in solution was measured. Fluorescence intensity was higher in MWCNTs groups than in vehicle group. Between MWCNT-S and MWCNT-L, the pattern of fluorescence intensity in trachea and lung was different. This different may be due to variation in damage of tissue. These results suggested that MWCNTs decrease mucociliary clearance, leading to retention of MWCNT in tissue and exacerbation of inflammations.

O2H-2-5 Effects of selective ET_B antagonist on the mouse septic brain

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We evaluated beneficial action selective ET_B antagonist hason the septic brain. In advance, we prepared the raw fecal fluid (RFF) of mice. Mice were randomly divided into three groups: pre-PBS/RFF group (Sepsis group), pre-BQ788/RFFgroup (BQ group), and pre-BQ788/PBS group (PBS group). According to each experimental condition, PBS or BQ788 was intravenously injected prior to intraperitoneal administration of RFF or PBS. All of them were sacrificed in 8h and their brain samples were prepared. In the Sepsis group, we observed an increase of apoptotic neuroblasts, an excess expression of *c-fos* in arginine-vasopressin-containing neurons, and an upregulation of various cytokines in the brain, compared with the PBS group. In the corresponding region, appreciable reactivation of microglia and astrocytes, and clear vascular leakage were observed. The expression of *c-fos*, neuroblasts apoptosis, upregulation of cytokines and reactivation of microglia, astrocytes were inhibited whereas vascular leakage was same level in the BQ group. Findings in the Sepsis group were supposed to relate to the Hans Selye's environmental stress reaction and inflammation by sepsis. We demonstrated that BQ788 could protect the brain from the sepsis-associated pathophysiological output.

O2H-3-1 Gene expression profile of Ca^{2+} and cAMP signaling proteins in diabetic model mouse

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Diabetes mellitus (DM) is one of high risk factors for cardiac diseases. In order to clarify the mechanism linking DM and cardiac dysfunction, we examined the age-dependent changes in expression levels of Ca^{2+} and cAMP signaling proteins in atria and ventricle of DM mice. DM was induced by streptozotocin (STZ) in male mice. Four weeks and 8 weeks (w) after injection of STZ (STZ-4W, STZ-8W), hearts were excised and mRNA levels, protein levels, and miRNA levels were quantified. In atria of STZ-4W mice, mRNA levels of $Ca_V1.2$ and $Ca_V1.3$ were significantly lower compared to control mice. In atria and ventricle of STZ-4W mice, mRNA levels of junctophilin-2, SERCA2 and RYR2 were lower than control mice. In contrast, mRNA levels of ß1-adrenergic receptor (ß1AR) was significantly higher in STZ-4W mice than control mice. These changes tend to be smaller in STZ-8W. Gene expression levels of ß2AR, ß3AR, adenylate cyclases, IP3R2 and muscarinic acetylcholine receptor M2 were not significantly different between DM and control mice. These results indicate that DM causes subtype-specific alteration in expression levels of Ca^{2+} and cAMP signaling proteins, which may raise the risk for cardiac diseases such as heart failure and atrial fibrillation.

O2H-3-2 Establishment of a primate model of intracranial aneurysm to assess the effect of drugs on aneurysms

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Intracranial aneurysm (IA) is a lesion characterized as a regional bulging of intracranial arterial wall. IA is a socially important disease as a cause of subarachnoid hemorrhage. Considered with the current situation that there is not any medical therapy available for patients and the poor outcome due to subarachnoid hemorrhage after rupture, the development of a novel therapeutic drug to prevent the enlargement or rupture of IAs should be established. Recently, we established the primate model of IA to assess the therapeutic effect of drugs. In this model, female Macaca fascicularis was used and aneurysms were induced through the ligation of one side of carotid artery and bilateral oophorectomy accompanied with the systemic hypertension by salt-overloading. In primate model, unlike rodent model, we can non-invasively keep track of IA formation by sequential magnetic resonance angiography (MRA) in same animals. Indeed, we could detect the induction of IAs in MRA in more than half of animals subjected to models and successfully followed up. In conclusion, primate models and sequential following up of IAs by MRA is a powerful tool to assess the formation of aneurysms and also the effect of drugs on aneurysms.

O2H-3-3 Endothelial dysfunction induces onset of aortic dissection

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Aortic dissection (AD) is life-threatening aortic disease, which is considered to be based on hypertension and degradation of media as well as aortic aneurysm progression. However, the pathological mechanism of AD is still unknown. Recently, it has been reported that endothelial dysfunction might be necessary for AD onset. Therefore, we examined the effect of N^{ω}-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase (NOS) inhibitor, on AD onset using angiotensin II (Ang II) and ß-aminopropionitrile (BAPN)-induced aneurysm model mice. L-NAME were administered in drinking water to C57BL/6 mice. Three weeks later, nitric oxide level and endothelial NOS expression were decreased in aorta of L-NAME-treated mice compared to control mice. After that, the infusion of Ang II (6 weeks) and BAPN (2 weeks), which induces the degradation of elastic fiber, were performed with the osmotic mini pumps. Incidence of AD, which was determined by the formation of false lumen, was increased in Ang II, BAPN and L-NAME (ABL) group compared with Ang II and BAPN group. MMP-2/9 activities and the expressions of inflammatory cytokines and VCAM-1 were significantly increased in ABL group. From these results, it was suggested that endothelial dysfunction is associated with AD onset.

O2H-3-4 A high signal-to-noise NO probe composed of a yellow fluorescent protein, Venus

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To visualize intracellular NO in living cells, various fluorescent-based NO probes have been developed. Here we report the development of a high-affinity NO probe composed of Venus fused to soluble guanylate cyclase beta1 subunit (sGC-Venus). To investigate the property of sGC-Venus, we measured the fluorescence intensity of sGC-Venus and EGFP fused to sGC beta1 subunit (sGC-EGFP), which was reported previously. As described previously, the fluorescence intensities of sGC-EGFP excited by all wavelengths were increased by NO-donor at 509 nm nm emission. On the other side, although the fluorescence intensity of sGC-Venus by 405 nm excitation was about 2.5 fold increased by NO-donor, the fluorescence intensities of sGC-Venus excited by other wavelengths showed much less increase. To measure the NO in living cells, the fluorescence intensity of sGC-Venus by 405 nm excitation normalized with that by 488 nm excitation, which showed no significant difference between with or without NO-donor, was adopted. In SH-SY5Y cells, the method using sGC-Venus showed higher sensitivity than the method using sGC-EGFP. These data suggested that sGC-Venus could be a useful tool for visualizing intracellular NO.

O2H-3-5 Analyses on new heterodimers of K_{2P} channels by single-molecule imaging technique

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Two pore domain K^+ channels (K_{2P}) are members of potassium channels. Most of K^+ channels is known to form tetramers on a plasma membrane, but K_{2P} family has been shown to form dimers. For instance, it has been reported that TWIK-1 forms homodimer via a disulfide bridge. However, it is unclear if K_{2P} channels form heterodimer among different K_{2P} subunits. In the present study, we focused on two K_{2P} channel subunits, TREK-1 and TALK-2, and examined if they form heterodimer by single-molecule imaging of fluorescent-labeled K_{2P} subunits in the plasma membrane using total internal reflection fluorescence (TIRF) microscopy. Fluorescence resonance energy transfer (FRET) analyses revealed that TREK-1 tightly interact with TALK-2 in plasma membrane. On the other hand, FRET signals were not detected in HEK293 cell expressing TASK-1 and TASK-5, both of which are known to have no interaction. Thus, we have shown a new pair of K_{2P} heterodimer, TREK-1 and TALK-2. Single-molecule GFP bleaching analyses supported the heterodimerization of them. These results suggest that our single molecular imaging systems are useful for the heterodimerization of K_{2P} and that K2P family has possibility of the further pairs of heterodimers to produce variable channel characters.

O2H-4-1 Propofol restores doxorubicin-induced disturbance of mitochondrial dynamisms

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Mitochondrial dynamisms are kept by fusion and fission processes, which are regulated by several mitochondrial proteins. Many drugs exhibit adverse effects by inhibiting the mitochondrial function resulting in causing myopathy or neuropathy. The mechanisms of drug-induced mitochondrial dysfunction, however, remain unknown. We have shown doxorubicin (Dox, an anthoracycline anticancer-drug) impairs the mitochondrial dynamisms revealed by decreased expressions of optic atrophy 1 and mitofusin 2 that promote mitochondrial fusion, indicating that Dox disrupts the balance between mitochondrial fission and fusion. In this study, we have examined whether the treatment with propofol (Pro, an intravenous general anesthetic) could protect the Dox-induced disturbance, since it has been reported to prevent Dox-induced oxidative stress. Addition of Dox to the culture of H9c2 rat cardiac muscle cells showed a significant decrease in area of mitochondria in cells (44.± 4.6% vs. control). Application of Pro reduced the Dox-induced mitochondrial fission and restored the area of mitochondria to the control level (101.9 ± 6.9% vs. control) indicating that Pro has a protective effect on Dox-induced mitochondrial disturbance and may ameliorate the Dox-induced cardiotoxity.

O2H-4-2 Pharmacological blockades in propofol-induced nausea in rats

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Propofol is known as a short-lasting hypnotic medications, and predominantly used for induction and maintenance of general anesthesia. Since propofol has antiemetic properties, nausea do not occur in patients receiving propofol. However, recent clinical reports demonstrated that some patients suffer from propofol-induced nausea. It is rather difficult to establish a standard therapy for propofol-induced nausea, because the exact etiology is still unclear. We reported that pica, kaolin ingestion behavior, could be used to evaluate nausea in rats. In this study, we investigated the effects of intravenous infusion of propofol on the pica in rats. Furthermore, the effects of antiemetics (granietron, prochlorperazine, diphenhydramine and aprepitant) on the pica were examined. Infusion of propofol (1%; 3h) significantly induced pica. This behavior was inhibited by pretreatment with granisetron (0.1 mg/kg) and prochlorperazine (5 mg/kg) but not diphenhydramine (10 mg/kg) and aprepitant (2 mg/kg) and the inhibitory effect of prochlorperazine on the pica is superior to that of granisetron. These results suggest that dopamine receptor antagonist may be useful to inhibit propofol-induced nausea and dopaminergic nervous system may contribute to the development of the symptom.

O2H-4-3 Glutathione, N-acetylcysteine, and cysteine have protective effects against cell injury induced by gas phase extract of cigarette smoke

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We have identified acrolein (ACR) and methyl vinyl ketone (MVK) as major cytotoxic factors in the gas phase extract of cigarette smoke (CSE) (Noya et al., 2013). ACR and MVK are unsaturated carbonyl compounds, and they can react with nucleophilic centers of proteins in the cells. We assumed that amino acids with a nucleophilic center as well as antioxidants can function as protective agents for cell injury induced by CSE. The purpose of this study is to establish a method for screening protective agents against the cell injury induced by CSE. We introduced a method based on PI uptake in combination with flow cytometry, and confirmed that this method was not affected by CSE and antioxidants. Using this method, we found that glutathione (GSH), N-acetylcysteine (NAC) and cysteine were protective against the cytotoxicity by CSE, whereas other nucleophilic amino acids as well as alanine and methionine were not cytoprotective. Because GSH is an endogenous antioxidant and because NAC and cysteine are precursors for GSH, these compounds might be cytoprotective by increasing the intracellular concentration of GSH. The cytoprotective mechanism will be discussed based on the data obtained with LC/MS.

O2H-4-4 Pharmacological mechanisms of nicotine-induced convulsive seizures in mice

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Nicotinic acetylcholine (nACh) receptors are implicated in seizure induction both in experimental animals and humans [e.g.: autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE)]; however, detailed mechanisms of nicotine-induced seizures remain unknown. Here, we performed behavioral studies and brain expressional analysis of Fos protein, a neural excitation marker, in mice to elucidate the mechanisms (e.g., causative nACh receptors and brain regions) of nicotine-induced convulsion. Nicotine elicited convulsive seizures at 3-4 mg/kg (i.p.). Nicotine (4 mg/kg)-induced seizures were fully blocked by mecamylamine (non-selective nAChR antagonist) and partly by methyllycaconitine (α7-nAChR antagonist), but not by dihydro-β-erythroidine (α4-nAChR antagonist). Fos expression was region-specifically increased with nicotine in the agranular insular cortex (AIC), piriform cortex (Pir), amygdala (AMG), medial habenular nucleus (MHb), thalamus, hypothalamus and solitary nucleus (Sol), which was mostly abolished by mecamylamine. Moreover, electrical lesion of AMG, but not Pir and MHb, significantly inhibited nicotine-induced seizures. These results suggest that nicotine causes seizures by activating AMG neurons partly through α7-nACh receptors.

O2H-4-5 Acute toxicity study of piroxicam in monogastric animals and the implication for secondary poisoning in puppies

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Because of increasing use in the treatment of carcinomas in dogs and cats, there is need for acute toxicity study of piroxicam in monogastrics. The LD50 of piroxicam in mouse (259.4±51.9 mg/kg), rat (259.4±69.7 mg/kg), rabbit (707.5±131.1 mg/kg), cat (468.8±69.7 mg/kg), guinea-pig (750.±112.0 mg/kg), monkey (750.±250 mg/kg), broiler (311.4±46.5 mg/kg), hen (728±56.0 mg/kg), turkey (707.5±131.1 mg/kg), pigeon (375±56.0 mg/kg) and duck (311.3±41.5 mg/kg) respectively suggest that piroxicam is toxic. But the human LD50 of piroxicam extrapolated from mice is 24+5mg/kg. The observed toxicity signs are torticollis, opistothonos, somnolence, lethargy, diarrhoea, gastroenteritis, generalized internal bleeding, anorexia, anaemia, effusions in thoracic and abdominal cavities, congestion of lung, liver, flaccid paralysis, cheesy lung, white flakes in the gastrointestinal contents, urinary incontinence, engorged urinary bladder, convulsive jerking of the limbs, lying in ventral recumbency, gasping for air, roaring and death at 207.5 mg/kg and above. Three died out of 6 puppies fed the carcases of poisoned turkey, duck and hen. Atropine (0.02 mg/kg) was administered to the three survivors that showed bradycardia, salivation and respiratory tract secretion.

O2H-5-1 PIH1D1 positively regulates mTOR complex 1-dependent rRNA transcription

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Cancer cells have many characteristics. Since abnormal proliferation goes through complex biological process, elucidation of the molecular mechanism is important. Ribosome biogenesis is a limiting factor for cell proliferation, so it is likely to be a critical process dysregulated in cancer. Several studies have reported that mTOR is an important regulator of protein synthesis. mTOR constructs two complexes, mTORC1 relates to cell proliferation and mTORC2 relates to cell death. R2TP complex is involved in mTOR regulation and PIH1D1 is major constituent of RT2P complex. It indicates PIH1D1 regulates mTOR function. However its mechanism is unsure.In this study, we found that PIH1D1 is highly expressed in various human breast cancer cell lines. The endogenous interaction between mTORC1 and PIH1D1 was also detected. Using siRNA mediated knockdown of PIH1D1, the phosphorylation of S6K was decreased. Furthermore, knockdown of PIH1D1 significantly decreased the amount of pre-rRNA. When rapamycin was added, no further inhibitory effect of PIH1D1 silencing on rRNA level was observed, suggesting that the effect of PIH1D1 is mTOR-dependent. These findings collectively suggest that PIH1D1 may have an important role in mTORC1 regulation in breast cancers.

O2H-5-2 Gemcitabine resistance in the PANC-1 cells tolerant to hypoxia or serum starvation

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Cancer cells are exposed to chronic hypoxia and nutrient deprivation, which are frequently associated with resistance to chemotherapy. We therefore hypothesized that the cells are acquired drug resistance in a process of the adaptation to stress environment. In order to confirm the linkage between resistance to stress environment and drugs, the cells tolerant to hypoxia or serum starvation were established from the parental cell line PANC-1, as follows: surviving cells were cultured for more than 3 months under 1% O_2 or 1% FBS. To examine gemcitabine sensitivity in the established cell lines, IC_{50} values were determined by the MTT assay. The IC_{50} on the serum-starvation and the hypoxia tolerance cells were, respectively, 142 and 37 times increased compared to that of the parental cells. We also evaluated apoptosis by Western blot analysis when these tolerant cells were treated with gemcitabine. Cleaved caspase-3 and PARP, apoptotic markers, were decreased in the tolerant cell lines, indicating that these cells acquired gemcitabine resistance through the suppression of apoptosis. These results concluded that PANC-1 cells tolerant to serum starvation or hypoxia acquired gemcitabine resistance.

O2H-5-3 Vasoactive intestinal peptide induces apoptosis and suppresses cell proliferation in human hepatocellular carcinoma cells

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Vasoactive intestinal peptide (VIP) modulates inflammatory responses. The VIP receptor is known to appear in several tumors such as colorectal and hepatocellular carcinoma (HCC). In our study, the existence of VIP and its receptors was verified in HCC tissues resected from patients and HCC cell line, Huh7 cells. The study was focused on the mechanisms of apoptosis because VIP treatment (10^{-10}M) significantly suppressed cell proliferation of Huh7 cells. Apoptotic-related proteins, caspase-3, Bcl-xL and CREB were changed in Huh7 cells cultured with VIP. VIP treatment significantly increased caspase-3 protein levels, decreased Bcl-xL protein levels and mRNA levels. Furthermore, cyclic AMP (cAMP) response element binding protein (CREB) protein level was also reduced. These effects were reversed by addition of the VIP receptor antagonist or cAMP Rp-cAMPS antagonist. These results suggest that VIP prevents the progression of HCC by apoptotic pathway.

O2H-5-4 Epigenetic regulation of Ca^{2+}-activated Cl^- channel TMEM16A in the breast cancer cell line YMB-1

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

O2H-5-5 Cellular uptake of 4-borono-L-phenylalanine, A ^{10}B carrier of boron neutron capture therapy, depends on the expression of cancer type amino acid transporter LAT1

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The 4-borono-L-phenylalanine (L-BPA), a tyrosine derivative, is one of the most commonly used boron carrier in Boron Neutron Capture Therapy (BNCT). BNCT is a powerful radiotherapy for cancer, especially in inoperative cancers such as brain, head and neck tumors. Accumulation of L-PBA in cancers is a key factor for the success of therapy. While L-BPA has been used in clinical investigations, mechanisms of uptake of L-BPA in cancers have not been clarified. Based on the structure of L-BPA, we examined whether L-BPA is transported by aromatic amino acid transporters expressed in *Xenopus* oocytes. We found that LAT2 and $ATB^{0,+}$as well as LAT1, a cancer type amino acid transporter, transported L-BPA. We further examined the expression of amino acid transporters in various cancer cell lines by proteomics and western blot analysis. LAT1 was expressed in most of tumor cell lines whereas LAT2 expression was not detected in tumor cell lines. A few cell lines showed the expression of $ATB^{0,+}$. We also found the strong correlation between LAT1 expression and L-BPA uptake in cancer cell lines. Kinetics analysis revealed that apparent affinity of L-BPA to LAT1 is higher than that to $ATB^{0,+}$. These results suggest that LAT1 is a major transporter mediating L-BPA uptake at least in tumor cell lines.

O2I-1-1 Daikenchuto facilitates gastric hyperemic responses through activation of TRPV1 and TRPA1 channels in rats: Role of capsaicin-sensitive sensory neurons

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Daikenchuto (DKT) is prescribed for patients with cold-related abdominal pain and bloating. It was shown that DKT increases colonic mucosal blood flow by adrenomedullin through activation of epithelial TRPA1 channels. In this study, we examined the role of TRPV1 and TRPA1 channels expressed on primary sensory neurons in mucosal hyperemic responses to DKT in rats. Male SD rats were used. Gastric mucosal blood flow (GMBF) was measured in the ex-vivo stomach by the laser Doppler flowmeter. DKT was topically applied to the stomach. Either the TRPA1 channel blocker HC-030031 or the TRPV1 channel blocker BCTC was applied topically to the mucosa before DKT treatment. Mucosal application of DKT increased GMBF in concentration-dependent manner. The increased GMBF responses induced by DKT were completely inhibited by the topical pretreatment of HC-030031, and were not completely but apparently attenuated by BCTC and sensory deafferentation. These results suggest that DKT increases GMBF through activation of TRPV1 and TRPA1 channels in rats. It is assumed that the increased GMBF in response to DKT was mediated by TRPV1 and TRPA1 expressed on capsaicin-sensitive sensory neurons.

O2I-1-2 Effects of phytosterol enriched refined extract of brassica campestris L. pollen on benign prostatic hyperplasia in a rat model

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In China the *Brassica campestris* L. pollen preparation QKPT is traditionally used for benign prostatic hyperplasia (BPH) therapy. However, in QKPT the content of supposedly active phytosterols is low at 2.6 %. Therefore, a phytosterol enriched (4.5 %) refined extract of the pollen (PE) was developed and effects on a BPH rat model was surveyed. Six groups of rats (n=8 each) as sham operated distilled water(DW) control, castrated DW control, castrated QKPT 2.0 g/kg, castrated PE 0.1 g/kg, castrated PE 0.2 g/kg, and castrated PE 0.4 g/kg were intragastrically treated with the respective daily doses. Testosterone (0.3 mg / day) was administered to all castrated rats. After 30 days, the animals were sacrificed and prostates as well as seminal vesicles (SV) were weighted and calculated prostate index (PI), prostate volume index (PVI), and SV index (SVI). After treatment with PE at 0.4 and 0.2 g/kg or QKPT, both indices were significantly reduced as compared to the castrated control. At the highest PE concentration both PI and SVI were also significantly reduced compared to the QKPT group. Both PE and QKPT demonstrated curative effects against BPH in the animal model.

O2I-1-3 Effects of phlorizin isolated from apple leaves and its aglycone phloretin on adrenal cortisol production

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Apple leaves are discarded in large amounts to facilitate the growth of apple fruits. To beneficially utilize the apple leaves, we tried to isolate some bioactive components and found a dihydrochalcone glycoside phlorizin. In response to ACTH, adrenal cortex secrets cortisol and maintains homeostasis or corps with stress. However, under the long-term or the excessive stress, the oversecreted cortisol reveals a lot of adverse effects and leads to many onsets of disorders. In the search of bioactivities for phlorizin, we have already reported that phloretin, an aglycone of phlorizin but not phlorizin inhibited the cortisol production in bovine adrenal cortical cells stimulated by ACTH. In this study, therefore, the inhibitory mechanism of phloretin in the cortisol production was investigated. Phloretin reduced the cortisol production induced by dibutyryl cAMP and drived by pregnenolne and 22-hydroxycholesterol. But phloretin did not change the cAMP elevation in the cells stimulated by ACTH. On the other hand, the aglycone blocked the ACTH-induced Ca^{2+} influx into the cells. These results strongly suggest that phloretin reduces the cortisol production due to the blockade of the ACTH-induced Ca^{2+} influx into the cells.

O2I-1-4 Cyclolepis genistoides D. Don (palo azul) increased UCP1 function in adipocytes

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Extract of Cyclolepis genistoides D. Don (vernacular name palo azul; palo) is a traditional medicine used in Paraguay and Japan for various diseases including obesity and diabetes. In this study, to elucidate anti-obese activity of palo, UCP1 function was examined since it uncouples the mitochondrial inner membrane to burn off excess calories.
3T3-L1 mouse pre-adipocytes (derived from white adipose tissue) or rat brown adipocytes were differentiated to mature adipocytes and following treatment of palo was performed for 7 days. Palo at 200 µg/mL significantly increased UCP1 mRNA by twice. Then UCP1 function was examined by using JC-1 staining which tells mitochondrial membrane potential in brown adipocytes. Palo at 200 µg/mL decreased the potential significantly compared with intact cells, which suggested that palo worked on UCP1 then mitochondrial membrane was uncoupled. UCP1 is known to be increased by stimulation of β_3-adrenergic receptor (β_3AR) signaling, however, combined treatment with palo and β_3-AR antagonist didn't alter the membrane potential.
Above all, our results suggest that palo promotes UCP1 function which could contribute anti-obese effect, through unknown mechanism rather than β_3-AR signaling.

O2I-1-5 Suppression of IL-33 gene expression by Sho-seiryu-to

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IL-33 is involved in the pathogenesis of chronic inflammations through the induction of Th2 cytokines and eosinophilla. We showed that the IL-33 gene expression level was correlated with the number of blood eosinophils in patients with pollinosis. We also showed that PKCδ involved in IL-33 gene up-regulation in Swiss 3T3 cells. Histamine H_1 receptor (H1R) gene expression level was correlated with the severity of allergic symptoms. As H1R gene up-regulation is also PKCδ-dependent, compounds targeted for PKCδ could alleviate symptoms of not only acute inflammations but also chronic inflammations. Here, we studied the effect of sho-seiryu-to (SST) on IL-33 and H1R gene up-regulation. Extracts from 5 commercially available SST were dose-dependently suppressed PMA-induced up-regulation of IL-33 gene expression in Swiss 3T3 cells. However, the IC_{50} values were varied from 0.22 to 3.2 mg/ml. The extracts also dose-dependently suppressed PMA-induced up-regulation of H1R gene expression in HeLa cells. In conclusion, SST suppressed the up-regulation of both IL-33 and H1R gene expression and could be a good therapeutics for both acute and chronic inflammations.

O2I-2-1 Differentiation-inducing factor-3 inhibits intestinal tumor growth *in vitro* and *in vivo*

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Differentiation-inducing factor-1 (DIF-1) produced by *Dictyostelium discoideum* strongly inhibits the proliferation of various cancer cells by suppressing the Wnt/β-catenin signal transduction pathway. In the present study, we examined the effect of differentiation-inducing factor-3 (DIF-3), a monochlorinated metabolite of DIF-1 also produced by *D. discoideum*, on the human colon cancer cell lines HCT-116 and DLD-1. DIF-3 strongly inhibited cell proliferation arresting the cell cycle in the G_0/G_1 phase. DIF-3 reduced the expression levels of cyclin D1 and c-Myc by facilitating their degradation in a time- and dose-dependent manner. DIF-3 also suppressed the expression of TCF7L2, a key transcription factor in the Wnt/β-catenin signaling pathway and thereby reduced the mRNA levels of cyclin D1 and c-Myc. Subsequently, we examined the *in vivo* effect of DIF-3 using Mutyh$^{-/-}$ mice with oxidative stress-induced intestinal cancers. Repeated oral administration of DIF-3 markedly reduced the number and size of cancers. These data suggest that DIF-3 inhibits intestinal tumor cells proliferation *in vitro* and *in vivo* probably by similar mechanisms to those identified in DIF-1 action, and that DIF-3 may have potential as a novel anti-cancer agent.

O2I-2-2 Spontaneous electrical activity recorded from myenteric interstitial cells of Cajal in the rat small intestine

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The nature of slow $waves_{ICC}$ recorded *in situ* from myenteric interstitial cells of Cajal (ICC-MY) in the rat small intestine were investigated using intracellular recording technique in the presence of nifedipine. Rat slow $waves_{ICC}$ consisted of upstroke and plateau components. Ni^{2+} inhibited the upstroke component of rat slow $waves_{ICC}$. The plateau component was inhibited by DIDS, a blocker of Cl^- channels, CPA, an inhibitor of the internal Ca^{2+} pump or bumetanide, an inhibitor of Na^+-K^+-$2Cl^-$ cotransporter (NKCC1). NKCC1-like immunoreactivity was detected in ICC-MY of the rat small intestine. Pinacidil, a K channel opener, hyperpolarized circular smooth muscle cells and attenuated the amplitude and dV/dt_{max} of slow $waves_{SMC}$. In contrast, pinacidil hyperpolarized ICC-MY and increased the amplitude and dV/dt_{max} of slow $waves_{ICC}$. These results suggest that the upstroke component of rat slow $waves_{ICC}$ is related to dihydropyridine-resistant voltage-dependent Ca^{2+} influx, whereas the plateau component is formed by Ca^{2+}-activated Cl^- efflux. NKCC1 is likely to be responsible for replenishment of intracellular Cl^- in ICC-MY. The results also suggest that slow $waves_{SMC}$ are electrotonic potentials driven by slow $waves_{ICC}$.

O2I-2-3 Vascular endothelial transient receptor potential vanilloid 4 links colonic inflammation in dextran sulfate sodium-induced colitis model mice

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Transient receptor potential vanilloid 4 (TRPV4) is a nonselective cation channel, and involved in physical sensing in various types of tissues. Few studies have addressed the role of the TRPV4 channel in colonic inflammation. In the present study, we investigated the alteration of TRPV4 distribution in the colon during DSS-induced inflammation. TRPV4 immunoreactivities were detected mostly in epithelial cells of normal distal colon. DSS treatment increased TRPV4 expression in vascular endothelial cells of mucosa and submucosal layer which colocalized with CD31, CD105, and VEGF receptor-2, but not in epithelial cells. Repeated intrarectal and intravenous administration of TRPV4 agonist GSK1016790A exacerbated the severity of DSS colitis. Increased vascular permeability and inflammatory cytokine expression were observed following single intravenous administration of GSK1016790A during colitis. These findings indicate that the alteration of TRPV4 in vascular endothelial cells may contribute to progression of colonic inflammation via increasing vascular permeability.

O2I-2-4 Functional role of transient receptor potential C6 channel in circular muscle contractility of mouse ileum

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Transient receptor potential (TRP) channels are a group of ion channels, which are relatively non-selectively permeable to cations. There is no direct evidence for functional TRPC6, a member of the TRPC subfamily, on gastrointestinal tract. Here, we examined electric field stimulation (EFS; 60 sec)-induced responses in the circular muscle of the ileum using two types of mice: smooth muscle-specific TRPC6 transgenic mice (Tg), and smooth muscle-specific dominant-negative TRPC6 transgenic mice (DN). In the ileum, EFS-induced contraction was showed during the stimulus. The amplitudes of EFS-induced contraction were greater in Tg than in wild-type mice (WT), and smaller in DN than in WT. In the experiment in which atropine was added, the amplitudes of EFS-induced contraction in Tg were similar in that of WT. Regarding of DN, the first phase of EFS (~15 sec)-induced contraction was similar in that of WT. In contrast, the second phase of EFS (15~60 sec)-induced contraction in DN was still smaller compared to WT. In this study, our results revealed that TRPC6 regulate the contractility in the ileum.

O2I-2-5 Japanese Kampo formula Daikenchuto inhibits gastric acid secretion through activation of transient receptor potential vanilloid 1 (TRPV1) channels in conscious mice

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Daikenchuto (DKT) is one of the most popular Japanese 'Kampo' formula used for gastrointestinal diseases in Japan. We have reported that DKT enhances gastric blood flow in anesthetized rats. DKT is getting lots of attention as the Kampo formula acting on thermosensitive TRPV1 channels that are activated by capsaicin and heat. The aim of the present study was to determine if DKT inhibits gastric acid secretion through activation of TRPV1 in conscious mice. Gastric acid secretion was determined by the mouse pylorus-ligation method. Intraduodenal administration of DKT dose-dependently reduced basal acid secretion in conscious mice. The pretreatment with the TRPV1 antagonist BCTC reversed the inhibition by DKT of basal acid secretion. The administration of its main constituent [6]-shogaol dose-dependently reduced basal acid secretion. The inhibition by [6]-shogaol of basal acid secretion was also reversed by BCTC. It is found that DKT and its main constituent [6]-shogaol inhibit gastric acid secretion through the activation of TRPV1 in conscious mice.

O2I-3-1 Contribution of Orai2 to store-operated Ca^{2+} entry and the cell cycle progression in bovine brain capillary endothelial cells

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Store-operated Ca^{2+} entry (SOCE) is mediated by the activation of Ca^{2+} release-activated Ca^{2+} (CRAC) channels, composed of the functional coupling between Orai and STIM. We have shown that Ca^{2+} influx facilitates proliferation in tBBEC117, a cell line derived from bovine brain capillary endothelial cells (BCECs). In this study, the contribution of CRAC channels to the cell cycle progression of BCECs was examined. The synchronization of specific cell cycle phase was performed in order to clarify the contribution of CRAC channel to the cell cycle progression in tBBEC117. The Orai2 expression level was up-regulated at the G2/M phase in comparison with the G0/G1 phase. It was found that this up-regulation caused the suppression of the SOCE activity based on the results from Orai2 knockdown experiments. Additionally, cell proliferation was significantly reduced by the Orai2 knockdown. FRET analyses revealed heteromeric interaction of Orai1 with Orai2. In conclusion, in BCECs, Orai2 was up-regulated at the G2/M phase to attenuate the SOCE activity and contribute to the proper progression of the cell cycle phases.

O2I-3-2 Aortic relaxation by acetylcholine is maintained against hyper dihydrobiopterinemia in *quinonoid* -dihydrobiopterin reductase knockout mice

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(6R)-L-erythro-5,6,7,8-Tetrahydrobiopterin (BH4) ,which is an essential cofactor for three types of nitric oxide synthases and also for aromatic amino acid hydroxylases. BH4 is converted to *quinonoid*-dihydrobiopterin coupling with the hydroxylation of aromatic amino acids, then reduced back to BH4 by *quinonoid*-dihydrobiopterin reductase (DHPR). Recently we established DHPR knockout (*Qdpr*$^{-/-}$) mice, and analyzed the function of their aortic rings. Dihydrobiopterin (BH2) content in the brain, liver and aorta of *Qdpr*$^{-/-}$ mice were 5.6 to 5.8 fold higher compared with those of wild types. BH4 content in the brain of *Qdpr*$^{-/-}$ mice was not significantly changed, and that in the liver was reduced to 73.4%. On the other hand, BH4 content in the aorta of *Qdpr*$^{-/-}$ mice was 3.3 fold higher compared to that of wild types. The plasma of *Qdpr*$^{-/-}$ mice contained similar amount of BH4 but 18.4 fold higher amount of BH2 than that of wild types. The aortic rings from *Qdpr*$^{-/-}$ mice were relaxed by acetylcholine and sodium nitroprusside as much as those from wild type ones. That the change of BH2/BH4 ratio in the aorta could be alleviated against hyperdihydrobiopterinemia so that aortic relaxation was maintained.

O2I-3-3 Effects of vildagliptin on endothelium-dependent relaxation in rabbit vein graft

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Vein bypass grafting is an effective and durable treatment for atherosclerotic occlusive diseases. However, intimal hyperplasia is a major obstacle to patency after vein grafting. Dipeptidyl-peptidase 4 (DPP-4) inhibitors control blood glucose level via an increase in the blood glucagon-like peptide-1 (GLP-1) concentration. These agents also inhibit vascular inflammation. We examined the effect of vildagliptin (a potent DPP-4 inhibitor) on intimal hyperplasia and endothelial functions in vein graft of rabbit. Vildagliptin administration was started 7 days before vein graft implantation and continued until harvest of the graft which was at 28 days after implantation. Vildagliptin increased the plasma GLP-1 concentration, without affecting plasma glucose or insulin level. Intimal hyperplasia was significantly less in the vildagliptin group than in the vildagliptin-non-treated group. Acetylcholine induced endothelium-dependent relaxation only in the vildagliptin group, and this was blocked by the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine. These results indicate that vildagliptin enhanced acetylcholine-induced endothelial NO release and reduced vein graft intimal hyperplasia, independently of any glycemic-control action.

O2I-3-4 Negative regulation of inflammatory responses by β-arrestin2 in human pulmonary microvascular endothelial cells

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ß-Arrestins, initially identified as being involved in G protein-coupled receptor (GPCR) desensitization, are now known to have diverse array of roles in GPCR-dependent and -independent signaling. Recently, ß-arrestins have shown to be important regulators of inflammation. The aim of this study was to determine the role of ß-arrestin2 in vascular endothelial cell inflammation. In human pulmonary microvascular endothelial cells, stimulation with LPS/interferon-γ (IFN-γ) resulted in a significant up-regulation of TNF-α, IL-1ß, IL-6, and ICAM-1 expression. When the knockdown of ß-arrestin2 was performed in endothelial cells using siRNAs, the ablation of ß-arrestin2 enhanced the pro-inflammatory cytokine responses to LPS/IFN-γ. Here, we demonstrate that ß-arrestin2 negatively regulates inflammatory responses of human pulmonary microvascular endothelial cells.

O2I-3-5 Role of TP signaling in enhancement of lymphangiogenesis in diaphragms of endotoxin induced petitonitis mice

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Lymphatic vessels in a diaphragm are essential for draining the inflammatory fluid during peritonitis. Recent studies suggest that lymphangiogenesis is also found during development of inflammation, and up-regulated by inducible COX-2. In the present study, we evaluated the role of TP signaling, during enhancement of the lymphangiogenesis in peritonitis mice. Male C57/BL6 mice were obtained. Peritonitis was induced by injections of lipopolysaccharide into peritoneal cavities of mice. As a parameter of lymphangiogesesis, we evaluated lymphatic microvessels using whole-mounted diaphragm tissues. Lymphatics were stained with antibodies against Lyve-1 and they were increased time dependently. A week after LPS application, gene expressions of COX-2 and VEGF-C in the diaphragm were up-regulated with the increased lymphangiogenesis in the diaphragm. In addition, thromboxane synthase (TXS) and CD3 were up-regulated in the diaphragm of peritonitis mice, and lymphangiogenesis were suppressed in the diaphragm of TP K/O mice. These results suggested that lymphangiogenesis is up-regulated by TXS and TP signaling possibly via induction of VEGF-C.

O2I-4-1 Downregulation of the proangiogenic prostaglandin E receptor EP3 and reduced angiogenesis in a mouse model of diabetes mellitus

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The healing process of diabetic foot ulcer is dependent on angiogenesis. We have previously shown that PGE_2-EP3 signaling enhance angiogenesis via increase of VEGFA expression inflammation using mouse model of sponge-implantation. We hypothesized that angiogenesis may be altered in Diabetes mellitus (DM) via modulation of PGE_2-EP3 signaling. DM was induced in male C57BL/6 mice by streptozotocin. Polyurethane sponge disks were implanted into subcutaneous tissues on the back of mice, and expression of related factors were analyzed in sponge granulation tissues. Blood vessel densities and PECAM-1 expression in sponge granulation tissues of DM mice significantly decreased compared with non-DM mice (Control mice). The reductions in DM mice were accompanied by reduced expression of inducible COX-2, mPGES-1, and PGE_2 receptor subtype EP3, whereas EP1, EP2, and EP4 were not. And, the expression of VEGFA and SDF-1 were both reduced in DM mice. Furthermore, treatment of DM mice with selective agonist of EP3 reversed suppression of angiogenesis, and reduction of expression of VEGFA and SDF-1. This suggests that topical application of an EP3 agonist could be a novel strategy to treat diabetic foot ulcers.

O2I-4-2 The relationship between uric acid and organ damage in hyperuricemia rat model

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<Bacground> Hyperuricemia is one of the complications in patients with chronic kidney disease (CKD). Recent investigations reported that uric acid is a risk factor leading to progression of CKD or cardiovascular disease (CVD), and suggest that uric acid itself causes CVD due to directly taking up to blood vessels or adipocytes as well as kidney. Therefore, we used hyperuricemia model rats and investigated whether uric acid directly affects functions of kidney or vessels. <Methods and Results> We used oxonic acid (OA) to induce hyperuricemia. Uninephrectmized rats were treated with or without OA (750 mg/kg/day) for 5 weeks. Non-treated rats were set as control group (Cont). Plasma uric acid level was significantly increased in OA group relative to the other 2 groups. Systolic blood pressure, body weight, urine output and hematocrit were comparable among all groups. Similarly, urinary protein excretion and CCr were equivalent levels among all groups. OA caused depression of vasodilatory response of thoracic aorta induced by Ach. Furthermore, OA exhibited significant thickening of aorta, infiltration of macrophages and increase of osteopontin expression compared with Cont. <Conclusion> Uric acid itself is a potential risk of vascular impairment.

O2I-4-3 Interleukin 27 induces endothelial differentiation in murine cardiac stem cells

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Background: Cardiac stem cells potentially differentiate into cardiac cells. Previously we demonstrated that Interleukin (IL)-6 family cytokines induce the endothelial differentiation of cardiac Sca-1+ cells through STAT3/Pim-1 pathway. Here, we examined whether IL-27, a member of IL-6/12 family cytokines that has both inflammatory and anti-inflammatory activity, induces the endothelial differentiation in cardiac Sca-1+ cells.
Methods and Results: Stimulation with IL-27 induces the endothelial cell marker genes, such as CD-31 and VE-cadherin, in cardiac Sca-1+ cells. RT-PCR analyses revealed IL-27 receptor(R) α (WSX-1) was expressed in cardiac Sca-1+ cells. We found that IL-27 induces the phosphorylation of STAT3 associated with the upregulation of Pim-1, suggesting IL-27 Rα transduces the signals as a functional receptor. Additionally, adenoviral transfection of dominant negative Pim-1 inhibited IL-27-induced endothelial cell differentiation of cardiac Sca-1+ cells, indicating that STAT3/Pim-1 axis is required for IL-27-induced endothelial cell differentiation.
Conclusion:IL-27 regulates the commitment of cardiac stem cells into the endothelial cell lineage, possibly contributing to neovascularization as a novel IL-27 induced biological function.

O2I-4-4 The effect of hypoxia on reactive oxygen species levels and the cell proliferation in human umbilical vein endothelial cells

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Endothelial cells play important roles in maintaining homeostasis of blood vessels. In order to clarify whether the insufficient oxygen supply to blood vessels affects endothelial functions, we investigated the effect of hypoxia on intracellular reactive oxygen species (ROS) levels and the cell proliferation in human umbilical vein endothelial cells (HUVECs). HUVECs were cultured in multi-gas incubator under normoxic (O_2, 20%) or hypoxic (O_2, 3%) condition. Nomoxia did not affect intracellular ROS levels evaluated by 2',7'-dichlorofluorescin diacetate (DCFH-DA) assay at any incubation time during 48 h. The hypoxia did not change intracellular ROS levels up to 9 h, but significantly increased them by the longer incubation time than 12 h, showing the maximal levels at 24 h. We found the linear proliferation of HUVECs under normoxia up to 72 h by the assay using WST-8 (cell counting kit-8TM, Takara). Hypoxia significantly enhanced the rate of proliferation of HUVECs during 72 h. The relation between the ROS levels and the cell proliferation in hypoxia is under investigation.

O2I-4-5 The role of GRK2 and β-arrestin2 in high glucose-induced ROS production in human vascular endothelial cells

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GPCR kinase (GRK) and ß-arrestin were initially identified as regulators of the GPCR desensitization. However, recent evidence suggests that GRK and ß-arrestin have a GPCR-independent role under various pathologic conditions. We reported that GRK2 promotes high glucose (HG)-induced reactive oxygen species (ROS) in human umbilical venous endothelial cells (HUVECs). This study examined the effect of GRK2 and ß-arrestin2 on the HG-induced ROS production and signaling. HUVECs were transfected with siRNAs against GRK2 or ß-arrestin2, and then treated with HG (22 mM glucose). ROS production was evaluated with DCF. The expression of ROS-generating NOX4 and other signaling molecules were assessed by real-time RT-PCR, Western blotting, and ELISA. NOX4 localization was detected by immunofluorescence staining. siRNA targeting GRK2 as well as ß-arrestin2 reduced HG-induced ROS production. The increase in NOX4 expression and IL-6 production under HG were up-regulated by ß-arrestin2 siRNA, but not affected by GRK2 siRNA. HG-induced NOX4 cytosolic translocation was prevented by ß-arrestin2 siRNA. We thus suggest that GRK2 and ß-arrestin2 are involved in diabetes-associated vascular endothelial dysfunction in a separate way.

O2I-5-1 BKγ subunit modulates function of BK_{Ca} channel in bronchial smooth muscle cells

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Large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel is activated by membrane depolarization and an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). BKCa channel is composed of pore-forming α (BKα) subunits and auxiliary subunits that control voltage dependence and Ca^{2+} sensitivity of BKα channels. Recently, BKγ1 subunit (BKγ1) was identified as a novel subunit in smooth muscles. BKγ1 subunit enhances voltage sensitivity of BKα channels independently of $[Ca^{2+}]_i$. However, the modulatory mechanism of BKγ1 subunit and its physiological function are still unknown. Here, we analyzed the expression and function of BKγ1 subunit by single-molecule imaging using a total internal reflection fluorescence (TIRF) microscope. Fluorescently-labeled BKα and BKγ1 subunits were transiently expressed in HEK293 cells. Fluorescence resonance energy transfer (FRET) and single-molecule GFP bleaching analyses suggested four BKγ1 subunits interacted with tetrameric BKα channels. Real-time PCR data revealed an abundant expression of BKγ1 subunit mRNA in murine airway smooth muscles. Immunocytochemical staining indicated BKγ1 subunits localized on the plasma membrane in murine bronchial smooth muscle cells (BSMCs). BK_{Ca} currents were largely activated at low $[Ca^{2+}]_i$ (pCa 8.0) in freshly isolated murine BSMCs. These results suggest that BKγ1 subunit enables tissue specific function of BK_{Ca} channels in BSMCs.

O2I-5-2 Vitamin C deficiency exacerbates pulmonary dysfunction and emphysema in a mouse model of chronic obstructive pulmonary diseases

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Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity worldwide, and most of the patients suffer from pulmonary dysfunction and emphysema. Since our microarray analysis using the lung tissue of COPD-like murine models suggests an imbalance between oxidants and antioxidants, and plasma concentration of Vitamin C (VC), one of the strongest anti-oxidants, is significantly decreased with age in COPD patients, we sought to investigate whether VC affects pulmonary phenotypes of βENaC-transgenic (Tg) mice, a mouse model of COPD. We first crossed βENaC-transgenic mice with senescence marker protein-30 (SMP30) knockout (KO) mice, which has been shown unable to synthesize VC endogenously, and further utilized βENaC-Tg-SMP30 KO mice deprived of VC for 8 weeks. Consistently, VC depletion increased the expression of oxidative stress markers in the lung tissue and plasma of βENaC-Tg-SMP30 KO mice. More importantly, VC depletion increased inflammatory status in lung tissue and exacerbated pulmonary dysfunction and emphysema, possibly due to an increased oxidative stress. Thus, our results demonstrate that VC plays an important role in the pathogenesis of COPD in murine models.

O2I-5-3 Superiority of pulmonary administration of mepenzolate bromide over other routes as treatment for chronic obstructive pulmonary disease

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We recently proposed that mepenzolate bromide (mepenzolate) would be therapeutically effective against chronic obstructive pulmonary disease (COPD) due to its both anti-inflammatory and bronchodilatory activities. In this study, we examined the benefits and adverse effects associated with different routes of mepenzolate administration in mice. Oral administration of mepenzolate caused not only bronchodilation but also decreased the severity of elastase-induced pulmonary emphysema; however, compared with the intratracheal route of administration, about 5000 times higher dose was required to achieve this effect. Intravenously or intrarectally administered mepenzolate also showed these pharmacological effects. The intratracheal route of mepenzolate administration, but not other routes, resulted in protective effects against elastase-induced pulmonary damage and bronchodilation at a much lower dose than that which affected defecation and heart rate. These results suggest that the pulmonary route of mepenzolate administration may be superior to other routes (oral, intravenous or intrarectal) to treat COPD patients.

O2I-5-4 Microsomal prostaglandin E synthase-1 enhances lung metastasis formation by accumulating MDSCs

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<Background>We have previously reported that host stromal microsomal prostaglandin E synthase-1 (mPGES-1) signaling appeared critical for tumor-associated angiogenesis and tumor growth. The accumulation of Myeloid-Derived Suppressor Cell (MDSC) to the tumor is known that related to prognosis. We estimated whether mPGES-1 induces metastasis formation by accumulating MDSCs to the metastasis organ or not.<Material and methods>Murine prostate cancer cells (RM9) were intravenously injected to Wild type mice (WT) and mPGES-1 knockout mice (mPGES-1KO). Lung metastasis was estimated by number of colonies in the lung and the lung weight. The expression of MDSCs was by fluorescence activated cell sorter (FACS) analysis and immunofluorecent analysis. <Results>Lung metastasis formation was significantly suppressed in mPGES-1 KO mice compared to WT mice. The number of CD11b+Gr1+ cells, specific marker for MDSC, in the lung tissue and peripheral blood, was suppressed in mPGES-1KO compared to WT by FACS. <Conclusions>These results suggested that mPGES-1 signaling induces tumor metastasis by accumulating MDSCs. The selective mPGES-1 inhibitors will be nice therapeutic tool for the inhibition of tumor metastasis.

O2I-5-5 Functional analysis of urate transporter URAT1 in renal hypouricemia with a compound heterozygote of URAT1

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Renal hypouricemia is an inherited disorder characterized by impaired tubular uric acid transport with severe complications, such as acute kidney injury. One of its responsible gene is the *SLC22A12* coding uric acid transporter 1 (URAT1). Here we report a functional analysis of URAT1 variants in Czech family with renal hypouricemia. The serum uric acid concentration in the proband was 1.1 mg/dL and the analysis of *SLC22A12* revealed compound heterozygous variants of G366R and R477H. The proband family members had heterozygous G366R or R477H and their serum uric acid concentrations remained within normal range. The uric acid uptake study with *Xenopus laevis* oocytes was performed. The R477H variant showed almost the same transport activity to URAT1 wild type. Co-expression of G366R and R477H (G366R/R477H) suppressed uric acid transport. The immunohistochemical staining of the G366R/R477H oocyte showed an accumulation of URAT1 in the endoplasmic reticulum. The findings suggest that the dominant-negative effect cause renal hypouricemia via loss of uric acid absorption, partly due to protein misfolding caused by accumulation of URAT1 protein in the endoplasmic reticulum.

O2I-5-6 High glucose-induced *N*-linked glycosylation of $Ca_v3.2$ T-type calcium channels facilitates secretory functions in neuroendocrine-like differentiated prostate cancer LNCaP cells

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Neuroendocrine (NE)-like differentiated prostate cancer cells secrete mitogenic factors, contributing to the androgen-independent pathology. $Ca_v3.2$ T-type Ca^{2+} channels are overexpressed and mediate Ca^{2+}-dependent secretion in NE-like differentiated prostate cancer LNCaP (NE-LNCaP) cells. Given evidence for the enhancement of $Ca_v3.2$ functions by high glucose (HG)-induced *N*-linked glycosylation, we examined the effect of HG on the T-channel activity and secretory functions in NE-LNCaP cells. NE-LNCaP cells cultured in an HG medium exhibited greater T-channel currents and showed more spontaneous secretion of prostatic acid phosphatase (PAP), known to be mediated by Ca^{2+} influx through $Ca_v3.2$, than the ones cultured in a low-glucose medium. The increases in T-channel currents and PAP secretion were suppressed by peptide-*N*-glycosydase F. The HG-induced enhancement of $Ca_v3.2$ activity through *N*-glycosylation thus appears to augment Ca^{2+}-dependent spontaneous secretory responses in NE-LNCaP cells, suggesting that hyperglycemia might aggravate androgen-independent pathology of prostate cancer through the increased secretion of mitogenic factors by NE-like cells.

O3G-1-1 Optogenetic activation of central serotonergic neurons suppress impulsivie-like action in mice

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Background: It has generally been thought that serotonin release in the forebrain attenuates impulsivity. However, there is so far no direct evidence proving this hypothesis because of technical limitations. Therefore, we aimed to obtaining direct evidence about the effects of acute serotonin release on impulsivity using recently developed optogenetic tools. Methods: We obtained transgenic mice expressing channelrhodopsin-2 (ChR2) mutant (C128S) in central serotonergic neurons only. We applied blue light to the dorsal or median raphe nucleus to open ChR2, and measured extracellular serotonin levels in the dorsal hippocampus or dorsal striatum using microdialysis. A 3-choice serial reaction time task (3-CSRTT) was used to assess impulsivity in mice.Results and Discussion: Blue light-induced serotonin release depended on stimulated and recorded brain regions. However, optogenetic activation of serotonergic neurons in the dorsal or median raphe nucleus reduced impulsive-like action in the 3-CSRTT, consistent with generally accepted hypothesis.

O3G-1-2 Mechanism of brain-derived insulin reduction in Alzheimer's disease

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Insulin plays diverse roles, such as inducing neuronal network formation that is implicated in the functions of learning and memory, in the central nervous system. A portion of the insulin in the central nervous system is locally produced in the brain. Recent studies show that produced insulin in the brain is decreased in hippocampal area in Alzheimer's patient. At the present time, however, little is known regarding the decreased mechanism of brain-derived insulin level in Alzheimer's patients. In the present study, we demonstrated the mechanism of brain-derived insulin expression using rat hippocampal neuron exposed by amyloid-$\beta_{1\text{-}42}$ ($A\beta_{1\text{-}42}$) which is one of the factors of Alzheimer's disease. Although $A\beta_{1\text{-}42}$ exposure for 48 h did not cause a significant reduction in cell viability, proinsulin level was decreased by 60%, and then a collapse of neurite outgrowth was detected. As is well known, neurotoxic $A\beta_{1\text{-}42}$ over-activates glycogen synthase kinase-3β (GSK-3β). Our study showed that lithium (a GSK-3 inhibitor) and GSK-3β knockdown prevented over-activation of GSK-3β and reduction of proinsulin. Taken together, these results suggest that over-activated GSK-3βin $A\beta_{1\text{-}42}$-induced Alzheimer's disease causes reduction of brain-derived insulin.

O3G-1-3 The roles of BDNF in the mesolimbic pathway in aversive memory

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The mesolimbic dopamine pathway is known to regulate different aspects of aversive memory; acquisition, retrieval, and extinction, but the molecular basis is yet to be fully understood. We thus investigated whether the mesolimbic dopaminergic BDNF signaling affected aversive memory encoding. Here, we utilized a transgenic mouse in which *BDNF* mRNA expression were time-restrictedly induced only in the mesolimbic dopamine neurons and its protein were overexpressed in their projecting regions. Passive avoidance test was conducted for assessing aversive memory processes. At day 1, the animals in a light compartment entered a dark compartment to escape from the brightness, where an aversive foot shock (0.4 mA, 5 sec) was delivered. The following day, BDNF overexpression started and lasted for 14 days. At day 15, animals were re-exposed to a light compartment and their ability to retrieve the aversive memory was assessed. Then animals received extinction training. At day 16, their ability to extinct the aversive memory was assessed. BDNF overexpression improved aversive memory extinction without affecting the retrieval process. These findings suggest that mesolimbic dopaminergic BDNF plays a role in promoting extinction of aversive memory.

O3G-1-4 Time-dependent changes of PFD5 expression during a long-term potentiation-like facilitation in adult mouse hippocampus induced by nicotine application

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Long-lasting synaptic plasticity requires changes of protein expression, although the mechanisms remain unclear. We previously reported that nicotine expressed a long-term potentiation-like facilitation, that is synaptic plasticity, in mouse hippocampus. In this study, we conducted to clarify the involvement of prefoldin subunit 5 (PFD5) in synaptic plasticity. After nicotine treatment (3 mg/kg, i.p.), mRNA and protein expression of PFD5 in hippocampus increased gradually during 2-24-hr period. This enhanced expression of PFD5 protein at 24-hr was inhibited by pretreatment of mecamylamine (0.5 mg/kg, i.p.), a nonselective nicotinic Ach receptor (nAChR) antagonist, although combined administration of ABT-418 (10 mg/kg, i.p.), an α4β2 nAChR agonist, and choline (30 mg/kg, i.p.), an α7 nAChR agonist, did not change the expression of PFD5 protein. Thus, the expression of PFD5 protein could be involved in the mechanism besides nAChRs. These findings demonstrated that PFD5 protein was enhanced during synaptic plasticity through coordinated regulation of gene expression and protein degradation. We propose that PFD5 protein plays an important role in long-lasting synaptic plasticity.

O3G-1-5 Genistein improves spatial learning and memory in male rats with elevated glucose level during memory consolidation

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Genistein (GEN) is one of the phytoestrogens, mainly containing in soy beans. Cognitive dysfunction due to diabetes has been reported previously. We investigated the effect of GEN on the central nervous system in glucose-loaded male rats. Rats were received administrations of GEN (1, 10 mg/kg, p.o.) and 20 % glucose solution (i.p.) at memory consolidation. We used a battery of behavioral tests including an appetite-motivated maze test (MAZE test), the open-field test, the elevated plus maze test and the step-through passive avoidance test. We also measured the blood glucose levels after the glucose load. In the MAZE test, both dosages of GEN improved the memory performance as mazes were advanced. In the open field test, exploratory behavior was increased by GEN treatment. Low dose of GEN prevented the significant elevation of blood glucose levels at 30 min after the glucose load. In this study, no significant differences were observed in emotionality, locomotor activity, and fear-motivated learning and memory. These results suggest that GEN treatment improved spatial learning and memory when glucose levels increased during memory consolidation.

O3G-2-1 Methamphetamine regulates Shati/Nat8l expression via activation of dopamine D1 receptor signaling

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A novel N-acetyltransferase, Shati/Nat8l, was identified in the nucleus accumbens (NAc) of mice with repeated methamphetamine (METH) treatment. Recently, we reported that Shati/Nat8l overexpression in the NAc of mice attenuated METH-induced hyperlocomotion, locomotor sensitization and conditioned place preference via activation of group II metabotrobic glutamate receptors (mGluRs) by N-acetylaspatylglutamate following N-acetylaspartate. However, the regulatory mechanism of Shati/Nat8l expression by METH is remained unclear. To investigate the mechanism, we performed acute slice experiment of the NAc, Shati/Nat8l promoter assay on PC12 cells and immunoblotting of some dopamine receptor signaling in vivo and ex vivo. We found that METH activated transcriptional potential of CREB for Shati/Nat8l, and also facilitated the pathways from PKA to CREB involving DARPP-32 and ERK1/2. Futhermore, dopamine D1 receptor antagonist SCH23390 inhibited the upregulations of Shati/Nat8l mRNA and the signaling activities. These results suggest that METH increased Shati/Nat8l mRNA by METH-induced transcriptional activity of CREB via dopamine D1 receptor signaling in mouse brains and that Shati/Nat8l might be a novel therapeutic target for drug addiction.

O3G-2-2 Intrinsic membrane plasticity in cholinergic neurons of the laterodorsal tegmental nucleus contributes to cocaine-induced addictive behavior

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The laterodorsal tegmental nucleus (LDT) contains cholinergic neurons, which project to dopamine (DA) neurons in the ventral tegmental area (VTA) and regulate their activity. Thus, neuroplasticity in LDT cholinergic neurons may affect the excitability of VTA DA neurons and mesocorticolimbic circuitry. In this study, we provide evidence that cocaine-induced intrinsic membrane plasticity in LDT cholinergic neurons is involved in addictive behaviors. *Ex vivo* whole-cell recordings revealed that firing activity of LDT cholinergic neurons after repeated cocaine exposure was higher than that after saline treatment. Bath application of riluzole reduced the increased firing in cocaine-treated neurons to a similar level of saline-treated neurons, suggesting that persistent sodium channels contribute to the increased activity. In addition, bilateral microinjection of riluzole into the LDT immediately before the test session in a cocaine-induced conditioned place preference (CPP) paradigm inhibited the expression of cocaine-induced CPP. These findings suggest that intrinsic membrane plasticity in LDT cholinergic neurons is causally related to the development of cocaine-induced addictive behaviors.

O3G-2-3 Implication of calmodulin in nucleus accumbens in the development of sensitization to ethanol-induced place preference after chronic ethanol treatment

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This study investigated the role of calmodulin (CaM) in mouse nucleus accumbens (NAcc) in the sensitization to ethanol (EtOH)-induced place preference using mice after 3 days of withdrawal from continuous EtOH vapor inhalation for 4 days. Mice after EtOH withdrawal significantly and dose-dependently showed place preference compared with control mice. Type 1 inositol 1,4,5-trisphosphate receptor (IP_3R-1) protein significantly increased in the NAcc of mice with EtOH vapor inhalation for 4 days and this increase reduced after EtOH withdrawal. In addition, the levels of IP_3R-1 protein in the NAcc of EtOH-conditioned mice after 3 days of EtOH withdrawal significantly increased. The i.c.v. administration of CaM inhibitor, W7, inhibited the sensitization of EtOH-induced place preference in a dose-dependent manner and completely abolished the increase of IP_3R-1 in the NAcc. These results indicate that upregulation of IP_3R-1 in the NAcc may contribute to the development of sensitization to EtOH-induced place preference and that CaM in the NAcc of mice showing EtOH-induced place preference may play possible regulatory roles in IP_3Rs-1 expression.

O3G-2-4 Studies of drug dependence (Rept. 500): Investigation of the rewarding effects of methamphetamine by the analysis of microendophenotypes using two inbred rat strains: Prologue for the next generation of "addiction research"

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Lewis (LEW) rats have been shown to be more sensitive to the behavioral effects of methamphetamine than Fischer 344 (F344) rats. In the present study, we demonstrated a difference in microendophenotypes in two inbred rat strains to further investigate the mechanism of the rewarding effects of methamphetamine. In a behavioral study, we found that methamphetamine produced a dose-dependent rewarding effect in LEW rats, but had no such effect in F344 rats. Interestingly, there was no difference in the discriminative stimulus effect of methamphetamine between LEW and F344 rats. Repeated treatment with methamphetamine significantly increased mRNA levels of D1-receptor, glutamic acid decarboxylase and dynorphin in this region of F344 rats, but not LEW rats. We are currently comparing multiplex gene expression with epigenetic modulation in response to methamphetamine between these two lines to further investigate the mechanism of the rewarding effects of methamphetamine. In the presentation, we will address the next generation of "addiction research".

O3G-3-1 Retrograde axonal delivery of ALS-related TDP-43 and FUS genes using AAV9 and lentivirus vectors to adult mouse motoneurons

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Formation of cytoplasmic aggregates in degenerating neuronal and glial cells is one of the pathological hallmarks of amyotrophic lateral sclerosis (ALS). Mutations in two genes encoding TAR DNA-binding protein 43 (TDP-43) and fused in sarcoma (FUS), both of which are main constituents of cytoplasmic aggregates, have been identified in patients with familial and sporadic ALS. Impairment of protein degradation machineries has also been recognized to participate in motoneuron degeneration in ALS. To establish ALS disease model in vivo, we produced recombinant adeno-associated virus type 9 (AAV9) and lentivirus (LxFuGB2) vectors encoding wild type and mutant TDP-43 or FUS, and those encoding shRNAs for proteasome (PSMC1) and autophagy (ATG5) systems. When these recombinant AAV9 or LxFuGB2 vectors were injected into the facial or sciatic nerves of adult mice, exogenous TDP-43 and FUS proteins or shRNAs for PSMC1 or ATG5 were successfully expressed in facial or lumbar motoneurons by a retrograde axonal transport of the viruses at 2-4 weeks postoperation as examined. We have so far demonstrated cytoplasmic aggregate formation of pathological TDP-43 and FUS in mouse spinal motoneurons.

O3G-3-2 Effects of narcotic analgesics on the multiple sclerosis-related pain

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Multiple sclerosis (MS) is the chronic inflammatory, demyelinating disease of the central nervous system with symptoms including spasticity and motor impairment. It has been reported that nearly 50% of patients with MS experience strong pain, which is hard to control with morphine. In the present study, the effects of narcotic analgesics on the MS-related pain were determined in mice. The animal model of MS-related pain is developed by immunization with myelin oligodendrocyte glycoprotein 35-55, complete Freund's adjuvant and pertussis toxin. The pain threshold on the hind-paw was measured by von Frey filament. After the immunization, the pain threshold was dramatically decreased, and this allodynia is prolonged for 4 weeks. On 6 days after immunization, the effects of narcotic analgesics morphine, oxycodone and methadone injected s.c. were determined. In the animal model of MS-related pain, the analgesic effects of morphine and methadone were markedly suppressed, whereas the analgesic effect of oxycodone was maintained. The analgesic effect of oxycodone maintained was suppressed by κ-opioid receptor antagonist nor-binaltorphimine. These results suggest that activating κ-opioid receptors oxycodone shows potent analgesic effect against MS-related pain.

O3G-3-3 Prostaglandin F_{2A} FP receptor inhibitor reduces demyelination and motor dysfunction in a cuprizone-induced multiple sclerosis mouse model

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Previously, we have demonstrated that prostamide/PGF synthase, catalyzes the reduction of prostaglandin (PG) H_2 to PG_{2A}, is constitutively expressed in myelin sheaths and cultured oligodendrocytes, suggesting that PG_{2A} has functional significance in myelin-forming oligodendrocytes. To investigate the effects of PGF_{2A}/FP receptor signaling on demyelination, we administrated FP receptor agonist and antagonist to cuprizone-exposed mice, a model of multiple sclerosis. Mice were fed a diet containing 0.2% cuprizone for 5 weeks, which induces severe demyelination, glial activation, proinflammatory cytokine expression, and motor dysfunction. Administration of the FP receptor antagonist AL-8810 attenuated cuprizone-induced demyelination, glial activation, and TNFα expression in the corpus callosum, and also improved the motor function. These data suggest that during cuprizone-induced demyelination, PGF_{2A}/FP receptor signaling contributes to glial activation, neuroinflammation, and demyelination, resulting in motor dysfunction. Thus, FP receptor inhibition may be a useful symptomatic treatment in multiple sclerosis.

O3G-3-4 The involvement of cerebral sodium-glucose transporter type 1 on focal cerebral ischemia

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We previously reported that cerebral sodium-glucose transporter (SGLT) involve in post-ischemic hyperglycemia-induced exacerbation of cerebral ischemic neuronal damage. In this study, we evaluated the effect of Na^+ influx into cells via SGLTs on neuronal cell death using α-methyl glucoside (α-MG, a nonmetabolizable analog of D-glucose). Additionally, to clarify involvement of the subtype of SGLTs, we focused on SGLT-1, which was identified in brain. Primary cultured cortical neurons were exposed to α-MG and cell survival rate was assessed by biochemical assay. The focal ischemia mouse model was generated by performing middle cerebral artery occlusion (MCAO). α-MG treatment induced a decline of neuronal survival rate, concentration-dependent manner in primary cultured cortical neurons. The developments of infarct volume and behavioral abnormality on day 3 after MCAO were improved in SGLT-1 knock down mice using SGLT-1 siRNA (i.c.v.), but not the increment of fasting blood glucose levels on day 1 after MCAO. Our date indicate that SGLT-1 may exacerbate cerebral ischemic neuronal damage. In addition, the exacerbation of neuronal cell death was induced by Na^+ influx into cells through neuronal SGLTs.

O3G-3-5 The role of glutamine transporter Slc38a1 in neurotoxicity

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Glutamine (Gln) is believed to play a dual role as a precursor for amino acid neurotransmitters such as glutamate and GABA, as well as a principal substrate for the Gln transporter Slc38a1 highly expressed by neurons in the brain. We generated mice carrying a conditional Slc38a1 allele with exon 2 flanked by loxP sites. These mice were crossed with *SynapsinI-Cre* transgenic mice to obtain mice defective of Slc38a1 from cells expressing synapsin-1. In brain coronal sections from male wild-type mice subjected to transient left middle cerebral artery occlusion (MCAO) for 2 h, drastic decreases were seen in TTC staining intensity and immunoreactivity for NeuN and MAP2 in the ipsilateral cerebral hemisphere 1 or 4 days after reperfusion compared with those in the contralateral hemisphere. Although these changes were significantly prevented in sections of male $Slc38a1_{synI}^{-/-}$ mice with MCAO for 2 h, no significant prevention was seen in ischemic mice deficient of Slc38a1 from cells expressing nestin. These results suggest that the Gln transporter Slc38a1 plays an important pathological role in the mechanism underlying neuronal survival after ischemia in neurons rather than neural progenitor cells.

O3G-4-1 Zinc-deferoxamine, a chelator of iron, reduced NMDA-induced neuronal damages in the rat retina

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It has been believed that excitotoxicity is involved in the neuronal cell death induced by glaucoma. Reactive oxygen spices are reported to be involved in excitotoxicity of neurons. Iron is necessary for generating hydroxyl radicals by Fenton's reaction. In this study, we examined the effect of zinc-deferoxamine, a cell-permeable iron chelator, on NMDA-induced neuronal damages in the rat retina. Male SD rats of 7 weeks old were subjected to intravitreous NMDA (200 nmol/eye). Eyes were enucleated 7 days later and horizontal sections of 5μm thickness through the optic nerve head were prepared. These specimens were subjected to morphometry. Interperitoneal administration of zinc-deferoxamine (1 ~ 30 mg/kg) was performed 15 min before NMDA injection, and 2, 4 and 6 days after NMDA injection. Zinc-deferoxamine significantly reduced neuronal cell death induced by NMDA in the retina. In addition, zinc-deferoxamine significantly reduced the number of TUNEL-positive cells and 8-OHdG-positive cells 24 h after NMDA injection. These results suggest that zinc-deferoxamine reduced excitotoxicity by reducing reactive oxygen species production in the rat retina.

O3G-4-2 Apelin protects retinal neuronal injury through the activation of Akt and ERK in glaucoma model mice

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Glaucoma is a progressive eye disease characterized by retinal ganglion cell death, which results in blindness. We have previously reported that apelin, the ligand of APJ receptor, protects retinal ganglion cell death in glaucoma model mice induced by intravitreal injection of NMDA. Here, we indicate the neuroprotective effect of apelin through the activation of Akt and ERK1/2 against retinal neuronal apoptosis in ganglion cell layer (GCL) and inner nuclear layer (INL). Western blotting analysis revealed that the intravitreal injection of apelin induced the activation of Akt and ERK1/2 in the retina. The phosphorylated-Akt and -ERK1/2 were expressed in retinal ganglion cells and Muller cells, respectively. NMDA-induced retinal neuronal apoptosis in GCL and INL were reduced by the intravitreal injection of apelin. The neuroprotective effect of apelin was inhibited by the intravitreal injection of LY294002, an inhibitor of PI3 kinase, or PD98059, an inhibitor of MEK1. Apelin-induced phosphorylation of Akt and ERK1/2 were also completely suppressed by LY294002 or PD98059. These results suggest that apelin protects retinal neuronal apoptosis through the activation of Akt and ERK.

O3G-4-3 Prothymosin alpha-induced protection of ischemic retina through microglial TLR4 signaling

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Reprogramming of TLR4 by brief ischemia or LPS leads to extensive tolerance against lethal ischemia in the CNS. Prothymosin α (ProTα), a nuclear protein, plays multiple functions including inhibition of ischemia-induced necrosis and apoptosis. However, the receptor-mediated beneficial signaling of ProTα under ischemia is unclear. In the present study, preconditioning treatment with ProTα 48 h prior to retinal ischemia prevents cellular damages estimated by HE staining and functional loss of retina by electroretinogram. ProTα preconditioning completely rescued ganglion cells with partial recovery of bipolar and photoreceptor cells, but no rescue of amacrine cells. ProTα treatment caused the proliferation and migration of retinal microglia and its preconditioning prevented the ischemia-induced microglial activation. The preventive action following ProTα preconditioning against retinal ischemia was abolished in TLR4 KO mice, and by pretreatments with anti-TLR4 antibody or minocycline, a microglial inhibitor, which themselves had no effects on the retinal ischemia-induced damages or microglia activation. Thus, the present study revealed that TLR4 mediates ProTα preconditioning-induced prevention through microglia in the retinal ischemia model.

O3G-4-4 Neuroprotective effects of conditioned medium of dental pulp cells stimulated by Chinese propolis

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The purpose of the present study is to clarify the effects of Chinese propolis on the expression level of neurotrophic factors in dental pulp cells (DPCs). We also investigated that the effects of conditioned medium (CM) of DPCs stimulated by the propolis against oxidative and endoplasmic reticulum (ER) stresses in human neuroblastoma SH-SY5Y cells, and on neurite extensions in rat adrenal pheochromocytoma PC12 cells. As results, the mRNA levels of NGF, but not BDNF and NT-3, in DPCs was significantly elevated by the propolis in a concentration-dependent manner. H2O2-induced cell death was significantly inhibited by the treatment with CM of DPCs. Moreover, the treatment with propolis-stimulated CM of DPCs had a more protective effect than that with CM of DPCs. In addition, the treatment with propolis-stimulated CM as well as CM of DPCs significantly inhibited tunicamycin-induced cell death. The treatment with propolis-stimulated CM of DPCs significantly induced the neurite outgrowth from PC12 cells than that with CM of DPCs in PC12 cells. These results suggest that CM of DPCs is an efficient source of new treatments for the neurodegenerative diseases and that propolis promotes the effects of CM of DPCs.

O3G-4-5 Nuclear TDP-43 causes neuronal toxicity by overcoming the intrinsic cell death-preventing mechanism mediated by hnRNPs

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Dysregulation of transactive response DNA-binding protein-43 (TDP-43) is thought to be linked to the pathogenesis of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). TDP-43 normally localizes in the nucleus but its main localization shifts to the cytoplasm in most affected cells of ALS and FTLD patients. It is not yet determined whether nuclear or cytoplasmic TDP-43 is responsible for TDP-43-induced neurotoxicity. In this study, we initially show that low-level overexpression of nuclear, but not cytoplasmic, TDP-43 is toxic to neurons. We also show that DNA/RNA-binding and dimerization of TDP-43 are both essential for TDP-43-induced neuronal cell death. Furthermore, we identify nuclear TDP-43-binding proteins including multiple heterogeneous nuclear ribonucleoproteins (hnRNPs). Knocking-down of endogenous hnRNP-U induces neuronal cell death whereas low-level overexpression of hnRNP-U or hnRNP-A2 inhibits TDP-43-induced neuronal cell death. In addition, hnRNP-U inhibits TDP-43-mediated increase in variant 2 of *POLDIP3* mRNA. These data together suggest that nuclear TDP-43 becomes neurotoxic by overcoming the intrinsic cell death-preventing mechanism mediated by hnRNPs

O3G-5-1 Attenuation of cold injury induced-brain edema formation by endothelin ETB receptor antagonists is caused by decreases in MMP9 and VEGF-A productions

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Brain edema is a fatal pathogenesis after brain insults. However, basal medications have not been established. Endothelins (ETs) are involved in several pathogenesis after brain insults. In this study, we examined the effects of ET receptor antagonists on brain edema in a mouse cold injury model. A copper rod cooled by dry ice was attached to cerebrum for 1 min. Brain edema was determined by water contents and permeability of microvessels was evaluated by extravasation of Evans blue. ET receptor antagonists were administrated into lateral cerebroventricle by two protocols. In Protocol 1, antagonists were administrated at 30 min prior to cold injury and the effects were evaluated in 12 h. In Protocol 2, antagonists were administrated at 24 h after cold injury and evaluated in 72 h. ETB antagonists, BQ788 and IRL-2500, attenuated cold injury induced-increase of water contents and extravasation of Evans blue in the Protocol 1 and 2. ETB antagonists reversed increases of matrixmetalloproteinases-9 (MMP-9) and vascular endothelial growth factor-A (VEGF-A) after cold injury. These results suggest that ETB antagonists ameliorate brain edema via decrease of MMP-9 and VEGF-A productions.

O3G-5-2 Minimally invasive method for intra-spinal dorsal horn microinjection enabling gene expression in cell type- and segment-specific manners

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To establish a non-invasive method for cell type- and segment-specific gene expression in the spinal dorsal horn (SDH) *in vivo* is a key challenge. Here we develop a new minimally-invasive method for gene delivery in the SDH in mice without laminectomy. Microinjection of recombinant adeno-associated virus (rAAV) 2/5 including a *gfap* promoter through a microcapillary which was inserted into the SDH through an interspace between Th13 and L1 vertebrae resulted in efficiently expressing genes specifically into L4 SDH astrocytes without any gliosis, neuronal loss and inflammatory responses. Using this method, we transduced L4 SDH astrocytes with a dominant-negative or constitute-active form of the transcription factor STAT3 and provided evidence that L4 SDH astrocytic STAT3 is necessary and sufficient for the maintenance of neuropathic pain. Our established method enables a manipulation of gene expression in cell type- and segment-specific manners without adverse effects and may be useful for basic researches in SDH physiology and pathology.

O3G-5-3 Effects of the anti-tumor immune response by activation of hypothalamic POMC neurons

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Suppression of anti-tumor immune responses was considered as the prime factor in the tumor growth. In addition, cancer inactivates the mechanism of host immune surveillance. On the other hand, ß-endorphin, which is a cleavage product of pro-opiomelanocortin (POMC), is known to have the ability to change innate immune function. In the present study, we evaluated the effect of controlling hypothalamic POMC neuron activity using optogenetic techniques on immune response and tumor growth. To perform the activation of POMC neurons, we incorporated the light-activated ion channel ChR2 into hypothalamus POMC neurons. The activation of POMC neurons in the hypothalamus by optical stimulation induced a significant decrease in the myeloid-derived suppressor cells (MDSCs) in the spleen. Furthermore, we also found a significant increase in NK cells in the spleen. Next, we implanted Lewis lung carcinoma (LLC) on counter leg and measured tumor size. At 6 days after tumor inoculation, the activation of POMC neurons in the hypothalamus by optical stimulation resulted in the decrease of tumor size. These findings suggest that activated POMC neurons in the hypothalamus may directly promote anti-tumor immunity and suppress tumor growth.

O3G-5-4 Immune activation promotes astrogliosis at presymptomatic stages in a mouse model of Sandhoff disease

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Sandhoff disease (SD) is a glycosphingolipid storage disease that arises from mutations in the *Hexb* gene and the resultant accumulation of the ganglioside GM2 and related glycolipids in the lysosomes, mainly of neuronal cells. The glycolipid storage causes severe neurodegeneration through a poorly understood pathogenic mechanism. The *Hexb*-/- mice, a mouse model of SD, are born and grow without obvious neurological defects until 12 weeks of age, at which time they develop a tremor, startle reaction and increased limb tone. *Hexb*-/- mice have been reported to exhibit neuronal loss in the brain in association with apoptotic signals, as well as extensive gliosis at the symptomatic stage (12-16 weeks of age). Our previous studies showed that astrogliosis began from 3-4 weeks of age in the presymptomatic stage. To determine whether the reactive astrogliosis is induced by the activation of immune system, we prepared *Hexb* and *FcR* gamma double-knockout mice. Interestingly, a significant decrease in GFAP signal was observed in the double-knockout mice. These results suggest that the activation of immune system at the presymptomatic stages lead to astrogliosis.

O3G-5-5 Clinical significance of CYLD downregulation in glioblastoma

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Introduction: Cylindromatosis (CYLD) regulates signaling pathways by acting as a deubiquitinating enzyme. Although we found loss of CYLD expression in hypoxic regions of human glioblastoma multiforme (GBM), the most aggressive brain tumor, biological roles of CYLD in GBM remain unknown. In this study, we elucidated the biological significance of CYLD down-regulation to GBM progression and therapy. Methods: We evaluated CYLD expression in tumor tissue specimens with GBM. To assess the biological roles of CYLD in GBM, we also investigated the effects of CYLD overexpression on GBM cancer cells in a xenograft mouse model. Results: CYLD expression was dramatically down-regulated in hypoxic GBM cells. Hypoxia enhanced both basal and tumor necrosis factor-α-induced expression of various proinflammatory cytokines, whereas CYLD overexpression strongly counteracted these responses. In the xenograft mouse model, CYLD overexpression, which had no impact on survival by itself, significantly improved the prosurvival effect of chronic anti-angiogenic therapy with bevacizumab, an anti-vascular endothelial growth factor antibody. Conclusion: CYLD down-regulation is crucial for hypoxia-mediated inflammation in GBM, which may affect the long-term efficacy of anti-VEGF therapy.

O3G-6-1 Chronic restraint stress-induced decrease of 5-HT_3 receptor activity in mouse prefrontal cortex

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Depression has been associated with alterations of serotonergic neuronal system, a part of which innervates the medial prefrontal cortex (mPFC). The mechanism underlying the action of anti-depressants has been proposed to involve changes in 5-HT_1, 5-HT_{2A} and other 5-HT receptor subtypes. To clarify 5-HT receptor subtypes involved in etiology of depression, we therefore aimed to examine the effects of 5-HT receptor agonists on field excitatory postsynaptic potentials (fEPSPs) evoked by electrical stimulation in the layer II/III and recorded in the layer V of mPFC slices obtained from chronic restraint stressed (CRS) mice, an animal model of depression. When applied by perfusion, 8-OH-DPAT, a 5-$HT_{1A, 6, 7}$ agonist, and sumatriptan, a 5-$HT_{1B/1D}$ agonist suppressed fEPSPs in both control and CRS mice groups to similar extents. mCPBG, a 5-HT_3 agonist, also suppressed the fEPSPs in control mice, but its effect was significantly attenuated in CRS mice. BW723C86, a 5-HT_{2B} agonist, was devoid of significant effects on fEPSPs in both groups. These results indicate that chronic restraint stress could result in a decrease in the activity of 5-HT_3 receptors in the mPFC.

O3G-6-2 The role of amygdalar glucocorticoid receptor system in depression

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Major depression is a life threatening psychiatric disorder, and hyperactivation of HPA axis is one of the pathophysiological changes of it. The hippocampus and the medial prefrontal cortex inhibit the HPA axis, besides the amygdala activates it. Binding of FKBP5 decreases glucocorticoid receptor (GR) sensitivity to its ligand and alters intra-nuclear translocation. Here we investigated the effect of single and repeated restraint stress (RS) (3 hr/day) on depression- and anxiety-like behaviors using forced swimming test (FST) and elevated plus maze test (EPM) in rats. Moreover, we assessed the effect of repeated RS on the serum corticosterone by EIA, and on the GR and FKBP5 in the medial prefrontal cortex, hippocampus and amygdala by Western blotting. We performed above these assessments 2 weeks after repeated stress. Seven times RS increased depression-like behaviors (immobility in FST) (P&lt0.017). Locomotor activity and anxiety assessed by EPM were no change. Serum corticosterone was increased by seven times RS (P&lt0.017). Protein level of GR was not changed, but that of FKBP5 was increased in the amygdala by seven times RS (P&lt0.05). Hyperactivation of HPA axis and increase of FKBP5 in the amygdala may be related to RS-induced depression-like behaviors.

O3G-6-3 Neuropathic pain-like stimuli change the expression of ribosomal proteins in the amygdala: genome-wide search for a pain-associated anxiety-related factor

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Neuropathic pain leads not only to increased pain sensation but also to emotional deficits. However, the molecular mechanism of such pain-induced emotional dysfunction has not yet been clarified. In a behavioral study, we observed the induction of pain-induced anxiogenic-like behavior as well as allodynia after 4 weeks of sciatic nerve ligation in mice, whereas sciatic nerve ligation for 1 week induced only allodynia without anxiogenic-like behavior. We next performed a genome-wide analysis of the changes in the expression of mRNA and microRNA (miRNA) in the amygdala of mice with sciatic nerve ligation. In this study, we found that sciatic nerve ligation for both 1 and 4 weeks changed the expression of many of mRNAs and miRNAs in the amygdala. Based on a pathway analysis, sciatic nerve ligation for 4 weeks changed the expression of mRNAs and miRNAs that are related to several ribosomal proteins in the amygdala, compared to 1 week. These results suggest that changes in the expression of ribosomal proteins in the amygdala may correspond to emotional deficits associated with pain sensation under a neuropathic pain-like state.

O3G-6-4 Identical blood biomarkers in late-onset major depressive disorder patients and model mice

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The absence of objective biomarkers is a crucial problem in psychiatric diagnosis and treatment of major depressive disorder (MDD). Objective tools to aid psychiatric examination have been explored, but no single molecular entity has been identified because MDD is heterogeneous, with different underlying biological etiologies. The lack of MDD biomarker also presents a substantial limitation to translational research in this field. Here we identified state-dependent biomarkers in blood cells of late-onset MDD patients and model mice of depression by cross-matching their gene expression profiles. Quantitation of mRNA levels for 4 molecules discriminated depressed and non-depressed states in patients from two independent hospitals. Of these, the mRNA level of 1 molecule in the blood cells was also an objective index for identifying the depressive state-like blood condition in model mice of depression. These blood biomarkers will be helpful for properly diagnosing late-onset MDD and bridging the gap between animal studies and human clinical trials.

O3G-6-5 Alzheimer disease therapeutic candidate, SAK3 restores mouse adult neurogenesis through T-type calcium channel

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In the adult brain, neurogenesis persistently occurs in the hippocampal dentate gyrus (DG), and decreased neurogenesis in Alzheimer disease model mice. Here, we investigated the effect of oral administration of SAK3, a novel T-type calcium channel agonist, on depressive-like behavior and adult neurogenesis in olfactory bulbectomized (OBX) mice as an AD model (Moriguchi and Sasaki et al., PLoS One 2013). Chronic SAK3 administration significantly reduced immobility times in tail suspension task and forced swim task, indicating its antidepressive-like effect. Furthermore, we assessed adult neurogenesis by double staining with bromodeoxyuridine (BrdU) and NeuN antibodies in the hippocampal DG. The reduced neurogenesis in OBX mice was significantly restored by the chronic SAK3 administration. Furthermore, the effect of chronic SAK3 administration was eliminated by pre-treatment with NNC55-0396, a specific T-type calcium channel antagonist. NNC55-0396 also inhibited the antidepressive-like effects of SAK3 in behavior tests and adult neurogenesis. Taken together, SAK3 ameliorates depressive-like behavior and reduced neurogenesis in OBX mice by stimulation of T-type calcium channel.

O3H-1-1 Red ginseng extract exerts an anti-allergic action through suppression of p70S6 kinase phosphorylation in basophils

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[Background] Basophils have been implicated as a source of histamine and eicosanoids in atopic dermatitis (AD) and are activated through p70 S6 kinase (p70S6K). We have recently shown that Korean red ginseng extract (RGE), a natural medicine, may have the potential for treatment of AD. Here, we examined whether RGE suppresses the signal pathway of p70S6K in basophils.
[Methods and Results] AD mice were caused by repeated application of 2,4-dinitrofluorobenzene to the ear. RGE (1%) were applied on affected area in mice. RGE significantly inhibited scratching behavior. p70S6K phosphorylation (p-p70S6K) level was increased in the ears of AD mice compared with control mice, but not in AD-RGE mice. Next, human basophils cell lines (KU812) were activated by antibodies for high-affinity immunoglobulin E receptor (FcεRI), for 24 hr after pre-incubation with RGE (100μ/ml). After activation for 15 min, p-p70S6K level was up-regulated in KU812 cells. The level was significantly attenuated by pretreatment of RGE.
[Conclusion] RGE in AD mice might play a role of anti-allergic agent by inactivation of p70S6K.

O3H-1-2 The effects of Juzentaiho-to and Hochuekki-to on myeloid-derived suppressor cell activity

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Myeloid-derived suppressor cells (MDSCs), which are defined as $CD11b^+$ and $Gr\text{-}1^+$ cells in mice, can directly suppress T cells and macrophages activation, and negatively regulate immune responses. Because MDSCs contribute to tumor immunity, chronic inflammation and autoimmune diseases, MDSCs may be a new therapeutic target for these diseases. However, pharmacological regulation of MDSCs activity has not been established. In the present studies, therefore, to find the drugs that regulate MDSCs activities, we examined the effects of Juzentaiho-to (TJ-48) and Hochuekki-to (TJ-41), traditional kampo medicines known as immunomodulatory drugs, on differentiation and gene expression in MDSCs. MDSCs were differentiated from BM cells by the treatment of IL-6 (40 ng/ml) and GM-CSF (40 ng/ml) for 4 days, and identified as $CD11b^+$ and $Gr\text{-}1^+$ cells using flow cytometry. TJ-48 decreased MDSCs population in concentration- (0.1-1 mg/ml) and time- (0-4 days) dependent manners. TJ-48 also decreased iNOS and argnase-1 mRNA in MDSCs. On the other hands, TJ-41 increased MDSCs in a concentration- (0.1-1 mg/ml) dependent manner. Taken together, TJ-48 and TJ-41 may be potent regulators for MDSCs and they may belong to a new category on immunomodulatory drugs.

O3H-1-3 Analysis for the effects of food additives on acquirement of oral tolerance

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Background: Because food allergy develops by an absence of oral tolerance, we tried to break the oral tolerance in the murine food allergy model. In this study, we estimated effects of food additives on an acquirement of oral tolerance in food allergy. Methods: Mice sensitized by injection of ovalbumin (OVA) were administered OVA orally to induce allergic symptoms, such as decreasing in body temperature and allergic diarrhea. Oral tolerance was established by administration of OVA before the sensitization. Additionally, we tried to lack the oral tolerance experimentally by administration of food additives in the induction of oral tolerance. We estimated a sweetener, a preservative, a food coloring and a mixture of them. Results: In this murine model, allergic diarrhea and hypothermia were induced by OVA administrations, and OVA specific IgE was elevated in the sera. Oral pretreatment with OVA inhibited the development of food allergy. The administrations of each food additive in the induction of oral tolerance broke the suppression of anaphylaxis and IgE production in some mice. The administration of the mixture induced remarkable anaphylaxis. Conclusion: We show a possibility of harmful effect of food additives on an acquirement of oral tolerance.

O3H-1-4 Betamethason treatment decreases demyelination and inflammation in experimental autoimmune encephalomyelitis model mice

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Multiple sclerosis (MS) afflicts more than two million people worldwide, over two thirds of which are woman. It is characterized by generating a chronic demyelinating autoimmune inflammation in the central nervous system. Experimental autoimmune encephalomyelitis (EAE) model mice as MS are appreciated as many models for investigating human diseases. C57Bl/6J (B6) mice were immunized with a myelin oligodendrocyte glycoprotein 35-55 peptide to induce EAE. We found that EAE model using B6 mice were treated with 0.1 or 0.5 mg/kg/day betamethason intraperitoneal administration beginning on post-immunization day 0 to 33 and monitored daily. In particular, clinical score was assessed throughout the disease course. Additionally, we assessed in histological analysis after final administration. We observed that decreased inflammation and demyelination at the C4-6 cervical level treated betamethason. Next, we assess in biochemical analysis after final administration.

O3H-1-5 Role of HMGB1 in the mechanism of anti-inflammatory action of levosimendan

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The calcium sensitizer levosimendan is used in treatment of decompensated heart failure and may also exhibit anti-inflammatory properties. However, the mechanisms underlying the anti-inflammatory action of levosimendan are not fully understood. This study was undertaken using the mouse macrophage cell line RAW264.7 to gain insight into its anti-inflammatory mechanisms. When macrophages were challenged with LPS, levosimendan inhibited overproduction of pro-inflammatory and chemotactic cytokines. While levosimendan had no effect on IκBA, ERK, JNK, p38 and Akt in macrophages, it greatly inhibited the release of high mobility group box 1(HMGB1) from the nucleus in LPS-stimulated cells. In mice with cecal ligation and puncture induced polymicrobial sepsis, serum HMGB1 levels were strikingly elevated and continued administration of levosimendan inhibited the elevation of serum HMGB1. From these results, the critical step for levosimendan anti-inflammatory action can be explained by that levosimendan is an effective inhibitor of HMGB1 release and its pro-inflammatory function.

O3H-2-1 Quantitative imaging analysis of p38 MAPK activity revealed dynamic regulation mechanism of stress signaling

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Signaling by the conserved stress-activated MAPK family is a major mechanism through which eukaryote cells respond properly to various extracellular stimuli, such as stress and cytokines and induce adaptive responses. Regulation of stress-MAPK signaling have been elucidated in detail, however, how cells induce appropriate cellular function according to the context of their surrounding environment remains unclear. To clarify this matter, we developed a FRET-based p38 kinase activity sensor and studied the dynamics of p38 activation in individual cells. Although previous biochemical studies suggested similar time course of p38 activation by pro-inflammatory cytokines and by translation inhibitors, we found that responses of individual cells were significantly different from that of population average depending on the type of stimulation applied to cells. Furthermore, we found that p38 activity fluctuated for more than 8 hrs after initial burst of activation when constant cytokine stimulation was given. In conclusion, visualization of single cell p38 activity revealed previously undescribed variability of p38 response depending on the stimulation type, which would explain how p38 induces right responses to the occasion.

O3H-2-2 Identification of compounds inhibiting NLRP3-inflammasome activation

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NLRP3, an innate immune sensor, forms the inflammasome with its adaptor protein ASC to induce inflammatory responses. Development of an anti-inflammatory drug targeting the NLRP3-inflammasome is urgently required because its aberrant activation causes production of interleukin (IL)-1beta and IL-18, resulting in development of inflammatory diseases, such as gout. Here we show identification of two chemical compounds capable of inhibiting NLRP3-inflammasome activation. Mitochondrial damage induces production of reactive oxygen species and assembly of the NLRP3-inflamamsome leading to maximal activation of the NLRP3-inflammasome. Compound A isolated from gram-positive bacteria potently inhibits monosodium urate (MSU) crystal-induced mitochondrial damage, thereby suppressing activation of the NLRP3-inflamamsome. Resveratrol, a natural polyphenol in grapes and melinjo, reduces the level of acetylated alpha-tubulin, thereby inhibiting microtubule-mediated assembly of the NLRP3-inflmmasome. Importantly, the administration of these compounds ameliorates symptoms of inflammation in C57BL/6 mice challenged with MSU crystals. Therefore, these compounds could be more effective medications than colchicine and NSAIDs for the treatment of NLRP3-related inflammatory diseases.

O3H-2-3 Mechanisms of transforming growth factor beta-induced c-kit down-regulation on mast cells

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TGF-ß plays a crucial role in the differentiation and proliferation of immune cells. TGF-ß stimulation causes decline in the surface expression of c-Kit which plays a central role in the differentiation of mast cells. However, the mechanisms of the surface c-Kit reduction are unknown. We studied the mechanisms of TGF-ß-induced reduction of c-Kit surface expression using bone marrow-derived mast cells (BMMC). TGF-ßR1 and R2 expressions and Smad 2/3 phosphorylations were measured by Western blot. The surface expression of c-Kit was analyzed by flow cytometry. The localization of c-kit in the cytosol was observed by confocal scanning microscopy. The soluble c-Kit in the supernatant was analyzed by immuno-precipitation. The treatment of TGF-ß decreased surface c-Kit expression, although the localization of c-Kit in BMMCs was unchanged. The soluble c-Kit in the supernatant was significantly increased. Both Akt inhibitors and Smad 3 phosphorylation inhibitors change TGF-ß-mediated surface c-Kit reduction. However, Smad 2 did not involved in the regulation of c-Kit expression. These findings indicate that phosphorylation of Akt and Smad 3, but not Smad 2 may be involved in a regulation of c-Kit expression on mast cells.

O3H-2-4 Adrenoceptor-mediated increase in transglutaminase 2 expression by macrophages

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Transglutaminase 2 (TG2) is a multifunctional protein that contributes to inflammatory disease when aberrantly expressed. Although macrophages express TG2, the factor stimulating TG2 expression remains poorly characterized in these cells. In the present study, we examined the effects of adrenaline on macrophage expression of TG2 in RAW264.7 murine macrophages and murine bone marrow-derived macrophages. Treatment with adrenaline markedly increased TG2 mRNA expression and increased TG2 protein levels. While the $ß^2$-adrenoceptor-selective antagonist ICI 118,551 completely blocked adrenaline-induced TG2 mRNA expression, the $ß^2$-adrenoceptor specific agonist salmeterol increased TG2 expression. The effect of adrenaline on TG2 mRNA expression was mimicked by treatment with the membrane-permeable cAMP analog 8-Br-cAMP. Thus, increased intracellular cAMP following stimulation of $ß^2$-adrenoceptors appeared to be responsible for adrenaline-induced TG2 expression. Because stress events activate the sympathetic nervous system and result in secretion of the catecholamines, adrenoceptor-mediated increase in macrophage TG2 expression might be associated with stress-related inflammatory disorders.

O3H-2-5 Signaling of LTB4 receptor type 1 (BLT1) inactivates hepatic neutrophils to restores acetaminophen-induced liver injury

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Aims: Leukotriene B4 (LTB4) is a potent chemoattractant for neutrophils, and is essential for promotion of inflammation. Following acetaminophen (APAP) over-dose, neutrophils accumulate into the injured liver. However, the role of neutrophils in APAP-induced liver injury is controversial. In this study, we investigated the role of LTB4 receptor type 1 (BLT1) in APAP hepatotoxicity. Methods: BLT1-knockout mice (BLT1-/-) or their wild-type counterparts (WT) were subjected to APAP over-dose (300 mg/kg) and neutrophil activation status was determined during liver injury. Results: BLT1-/- exhibited higher levels of ALT and necrotic area at 24h, and lower survival rate (WT;100% vs. BLT1-/-;40% survival rate). Recruited Gr1-positive neutrophils into the BLT1-/- livers were greater than WT livers, which was associated with enhanced CXCL2 expression. The mRNA expression of TNF, IL-1, IL-6, and MMP-9 in BLT-/- livers were enhanced. Neutrophils in WT livers showed up-regulation of Gr1 expression and priming for reactive oxygen during the injury. Conclusions: These results indicate that BLT1 signaling plays a role in liver injury elicited by APAP through inhibiting the accumulation and activation of hepatic neutrophils.

O3H-3-1 Evidences of the K^+-circulation current that regulates the electrochemical properties in the lateral cochlear wall

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Cochlear endolymph exhibits a high [K^+] of 150 mM and a highly positive potential of +80 mV. We previously revealed by electrophysiological assays and a computational model that these unique properties were maintained by K^+-current which circulated between the perilymph and the endolymph. The lateral cochlear wall consists of two epithelial layers; the inner and the outer layers. The latter expresses Na^+,K^+-ATPases on its perilymphatic surface, however, it remains uncertain whether they contribute to the circulation current. Recently, we showed that an inhibition of these ATPases decreased the intracellular [K^+] of the outer layer and consequently impaired the endolymphatic potential. Based on this experimental data, in this study we renewed the computational model, where the K^+-circulation current were set to flow through the ATPases in the outer layer. The model predicted that an inhibition of the ATPases reduced the circulation current, which leaded to a decrease of the extracellular [K^+] between the two layers. This alternation was indeed observed by <I>in vivo</I> electrophysiological experiments. These results support the concept of our model that the K^+-circulation current occurs across the lateral wall.

O3H-3-2 Novel function of β-arrestins (β-arrs) in the human adrenomedullin type 1 (AM_1) receptor internalization

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AM powerfully inhibits cardiovascular inflammation through the AM_1 receptor, which consists of the CLR receptor and accessory protein RAMP2. Receptor internalization is required for resensitization of the receptor. Intracellular ß-arrs inhibit the development of inflammation. Here, we examined the effects of cardiovascular ß-arrs 1 and 2 on the AM_1 receptor internalization. We constructed a chimera in which the C-terminal tail (C-tail) of CLR was replaced with that of the $ß_2$-adrenergic receptor and transiently transfected it into HEK-293 cells stably expressed RAMP2. The cell surface expression and internalization of the receptors was quantified by FACS analysis. The ^{125}I-AM binding and AM-induced cAMP production of the receptors were also determined. Co-expression of ß-arr 1 or -2 resulted in marked decreases in the AM_1 receptor internalization, without affecting AM binding and signaling. Surprisingly, dominant-negative (DN) ß-arr 1 or -2 decreased the AM_1 receptor internalization. The chimeric AM_1 receptor internalization was markedly augmented by co-transfection of each ß-arr and reduced by co-expression of each DN-ß-arr. Thus, both ß-arrs negatively control the AM_1 receptor internalization, which depends on the CLR C-tail.

O3H-3-3 Subtype specific direct interaction of adenylyl cyclase with membrane scaffold protein 4.1G

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Proteins of 4.1 family are subsets of subcortical cytoskeletal proteins and are known to stabilize cellular structures and proteins, including G protein-coupled receptor (GPCR), at the plasma membrane. We previously indentified that one of the 4.1 family proteins, 4.1G, regulates GPCR-mediated Gs signaling by suppressing adenylyl cyclase (AC)-mediated cyclic AMP (cAMP) production (Goto and Saito *et al.*, *Cell. Signal.*, *25*, 690-697, 2013). In the present study, we investigated role of 4.1G in suppression of the cAMP production with the central focus on direct interaction of 4.1G and AC. For this purpose, purified proteins of 4.1G (headpiece, FERM domain and C-terminus) and intercellular regions of AC6 (N-terminus, C1a loop, C1b loop and C2 loop) were prepared respectively, and protein interactions between the 4.1G proteins and the AC6 proteins were analyzed by *in vitro* pull-down assay. As a result, it was appered that 4.1G-FERM domain was strongly bound to N-terminus of AC6. Moreover, the FERM domain was also interacted with N-terminus of AC9, but not with AC 3 and 7. We are now underway to examine whether direct interactions of 4.1G with AC are responsible for suppression of the cAMP production by 4.1G.

O3H-3-4 Possible involvement of α1-adrenergic receptor signaling in bone metabolism

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Sympathetic nervous system regulates bone remodeling in part through the ß-adrenergic receptor (AR) in osteoblasts and osteoclasts, and our recent work demonstrated that the α1-AR signaling in osteoblasts are important for noradrenaline-mediated cell proliferation. To evaluate the functionality of α1-AR signaling on bone, we investigated the effects of pharmacological blockade of α1-AR signaling on bone mass by performed CT-based bone densitometry after the systemic administrations of prazosin, an α1-AR antagonist, at 10 and 30μg/kg for 2 weeks. The bone volume per trabecular volume of the distal end of the femur was significant lower in prazosin-administrated mice than in saline-administrated mice. Next, to examine the mechanism responsible for bone loss due to the prazosin treatment, we performed bone histomorphometric analysis of bone formation parameters and analyzed the mRNA expression of Runx2 and Osteocalcin (OC) in the distal femur. The administration of prazosin displayed decreased bone formation rates in the femur compared with saline-administrated mice. Moreover, the mRNA expression of Runx2 and OC in bone was significantly decreased in prazosin-administrated mice. These results indicate that α1-AR signaling regulates bone formation in mice.

O3H-3-5 Epigenetic regulation in osteoclasts

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The maintenance of bone homeostasis is dependent on the balance between bone resorbing osteoclasts and bone forming osteoblasts. Excessive bone resorption by osteoclasts is often associated with diseases accompanied by pathological bone loss, including osteoporosis and rheumatoid arthritis. Bisphosphonates are potent inhibitors of osteoclast-mediated bone resorption and are effective antiresorptive drugs for the treatment with osteoporosis. However, reports of osteonecrosis of the jaw have emerged with long-term use of bisphosphonates. Therefore, further development of antiresorptive drugs will be required to minimize its occurrence. Recently, we found that DNA methylation regulates osteoclastogenesis via epigenetic repression of the anti-osteoclastogenic gene. The importance of methyltransferase in bone homeostasis was underscored by the observation that mice with an osteoclast-specific deficiency in methyltransferase exhibit a high bone mass phenotype due to a decreased number of osteoclasts. Furthermore, inhibition of methyltransferase abrogated bone loss in models of osteoporosis. Thus, our study reveals the role of epigenetic processes in the regulation of osteoclast differentiation, which may provide the molecular basis for a new therapeutic strategy.

O3H-4-1 Mechanisms of biomagnetic waves in gut musculature

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Magnetometers to measure biomagnetic fields would thus provide non-invasive and aseptic estimations of how cellular organizations electrically communicate and affect function. In this study, we show measurements of magnetic vector fields induced by biological propagating current in gut musculature, using an improved magnetoimpedance (MI) gradio-sensor with a single amorphous metal wire and a pair of detector coils on both ends of the wire. Biomagnetic waves of up to several nT were recorded in the magneto sensor placed ~1 mm below the sample under control conditions. The direction of magnetic waves altered depending on the rotation of the musculature sample and magneto sensor, indicating the existence of propagating intercellular currents. Tetraethyl ammonium (TEA) facilitated and nifedipine suppressed magnetic waves, respectively, suggesting that L-type Ca^{2+} channels in smooth muscle layer are a major source for the propagating electric current. The magnitude of magnetic waves rapidly decreased to ~10% with only 2 mm separation between sample and sensor. The large distance effect is attributed to the feature of bioelectric circuits constructed by two reverse currents, i.e. intercellular propagating current and extracellular return current, separated by a small distance.

O3H-4-2 Image-based drug profiling reveals a dual inhibitor of EGF receptor tyrosine kinase and microtubules

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Small-molecule inhibitors are widely used as tools for biology and therapeutic drugs, and uncovering the target specificity provides a valuable insight into both basic and clinical studies. Here, we present image-based inhibitor profiling of EGF receptor pathway by quantitating their effect on both receptor trafficking and signal transduction. Unbiased multivariate analysis classified fourteen inhibitors into four clusters. Interestingly, EGFR inhibitor, a highly uni-specific tyrosine kinase inhibitor, was classified with a microtubule depolymerizer. Actually, EGFR inhibitor had microtubule depolymerizing activity both in vivo and in vitro, and also had an antimitotic activity. Our work indicates that image-based multivariate analysis is a powerful tool for discovering an unexpected drug properties, and EGFR inhibitor become as a novel seeds for multi-targeting cancer drug.

O3H-4-3 Coupling between cell edge protrusion, focal adhesions and actin retrograde flow visualized by new easy-to-use single-molecule speckle (eSiMS) microscopy

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Cells dynamically change morphology and direction of movement in response to physical stress imposed by outer environment. Direct viewing of individual molecules enables real-time monitoring of the mechanics linking cell adhesion and migration at the molecular level. We recently introduced new easy-to-use fluorescence Single-Molecule Speckle (eSiMS) microscopy (MBoC 25:1010, 2014). With electroporation of DyLight-labeled actin, this method enables labeling of cells with 100% efficiency at the very low density optimal for single-molecule observation and nanometer-scale displacement analysis of the actin network with a low localization error of ~8 nm. eSiMS expands application of single-molecule observation to diverse culture systems and cell physiology studies, including real-time monitoring of response to drugs and physical perturbations. Here we present our recent results revealing that (i) focal adhesions exhibits grabbing activity towards the surrounding actin network, especially in cell front, and (ii) actin polymerization at the cell leading edge functions as a sensor for mechanical force imposed by the extracellular environment.

O3H-4-4 The ligand binding ability of dopamine D1 receptors synthesized using a wheat germ cell-free protein synthesis system with liposomes

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G-protein coupled receptors (GPCRs) share a common seven-transmembrane topology and mediate cellular responses to a variety of extracellular signals. We synthesized human dopamine D1 receptors using a wheat cell-free protein synthesis system with liposomes and analyzed their receptor binding ability. From Scatchard plot analysis, the dissociation constant (Kd) and the maximum density (Bmax) of the synthesized receptors were 6.61 nM and 1.85 pmol/mg protein, respectively. The same analysis for rat striatal membrane gave a Kd of 2.67 nM and Bmax of 0.70 pmol/mg protein. Using a competition binding assay, Ki values of antagonists, SCH23390, LE300 and SKF83566, for the synthetic receptors were the same as those for rat striatal membranes, but Ki values of agonists, A68930, SKF38393 and dopamine, were 5-17 fold higher than those for rat striatal membrane. These results suggest that the dopamine D1 receptors synthesized in liposomes have a functional binding capacity. The different patterns of binding of antagonists and agonists to the synthetic receptors and rat striatal membranes indicates that G proteins are involved in agonist binding to dopamine D1 receptors.

O3H-4-5 TOR signaling pathway regulates transcription of Isp5, an amino acid permease, through GATA transcription factor Gaf1 in fission yeast

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In the fission yeast Schizosaccharomyces pombe, two TOR homologues, Tor1 and Tor2, oppositely regulate amino acid uptake. However, the underlying mechanism(s) remain to be determined. Here we carried out a promoter analysis of 38 amino acid permeases (AAP) by the cap analysis of gene expression (CAGE), a method of identifying transcription start sites (TSS). The CAGE results showed that Tor2 and Tor1, respectively, negatively and positively regulate the transcripts with the same TSS position of AAP Isp5. Consistent results were obtained by monitoring the promoter activity of Isp5. Systematic deletions using Renilla luciferase reporter showed that Isp5 promoter activity depends on the GATAAG motif. Genetic deletion of Gaf1, a zf-GATA type transcription factor bound to this motif, abolished its promoter activity, and three tandem copies of the GATAAG motif recapitulated Tor-mediated regulation of Isp5 promoter. Analysis of YFP-tagged Gaf1 showed that the trans-location of Gaf1 from cytosol to nucleus was regulated by Tor2, but not by Tor1. Altogether, the present results provide valuable insights into the role of TOR signaling in the regulation of amino acid uptake at the transcriptional level.

O3H-5-1 Assessment of the possible inhibitory effects of antidepressant drugs on guinea-pig urinary bladder (UB) contractile functions

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Recently new generations of antidepressant drugs with fewer side effects are clinically more available. However, there is little information on their effects on UB contractile functions. The present study was thus carried out to elucidate the extent of antidepressant drug-induced UB smooth muscle (UBSM) contractile dysfunctions that are attributed to their anticholinergic actions. Among the tested drugs in the present study, the most conspicuous competitive antagonistic actions against acetylcholine (ACh)-induced contractions of the isolated guinea-pig UBSM preparations were shown by tricyclics (imipramine, clomipramine, trimipramine, amitriptyline, nortriptyline) and the NaSSA, mirtazapine. Furthermore, these tricyclics and mirtazapine significantly diminished the intravesical pressure changes recorded by cystometry method in the anesthetized guinea-pig. On the other hand, sulpiride, trazodone, SSRIs (fluvoxamine, escitalopram), milnacipran (SNRI) and aripiprazole did not show any significant effects. These findings indicate that tricyclics and mirtazapine are likely to induce anticholinergic action-mediated dysuria, which should be considered in the pharmaceutical therapy of depression.

O3H-5-2 L-type Ca^{2+} channel sparklets revealed by TIRF microscopy in mouse urinary bladder smooth muscle

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Small discrete elevations of intracellular Ca^{2+}, referred to as Ca^{2+} sparklets have been detected in an intact urinary bladder smooth muscle (UBSM) electrical syncytium using a novel TIRF microscopy Ca^{2+} imaging approach. Sparklets were virtually abolished by the removal of extracellular Ca^{2+}. Co-loading of UBSM with the slow Ca^{2+} chelator EGTA-AM confirmed that Ca^{2+} sparklets are restricted to the cell membrane. Ca^{2+} sparklets were inhibited by Ca^{2+} channel blockers, but not by inhibition of P2X1 receptorsα, ß-meATP whilst sparklet frequencies were significantly reduced by atropine. Ca^{2+}sparklet frequency was significantly reduced by PKC inhibition with Go6976. In the presence of CPA, there was no apparent change in the overall frequency of Ca^{2+} sparklets. Under control conditions, inhibition of classical store operated Ca^{2+} entry using ML-9 had no significant effect. Amplitudes of Ca^{2+} sparklets were unaffected by any agonists or antagonists, suggesting that these signals are quantal events arising from activation of a single channel, or complex of channels. The effects of CPA and ML-9 suggest that Ca^{2+} sparklets regulate events in the cell membrane, and contribute to cytosolic and sarcoplasmic Ca^{2+} concentrations.

O3H-5-3 Effects of disease-associated mutations in the central region on the activity of RyR1 channels

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Type 1 ryanodine receptor (RyR1) is a Ca^{2+} release channel in the sarcoplasmic reticulum and the major target for muscle diseases, e.g., malignant hyperthermia (MH) and central core disease (CCD). It is widely believed that MH and CCD mutations cause hyperactivation of the Ca^{2+}-induced Ca^{2+} release (CICR), resulting in abnormal Ca^{2+} homeostasis in skeletal muscle. However, it remains unclear how the disease-associated mutations affect CICR. In this study, we investigated the CICR activity of RyR1 channel carrying 15 MH and MH/CCD mutations in the central region (1592-2508) by live-cell Ca^{2+} imaging and [^{3}H] ryanodine binding. These mutations divergently affect the gain (i.e., peak activity) and the sensitivity to activating Ca^{2+} of CICR in a site-dependent manner. The calculated CICR activity strongly correlated with the ER Ca^{2+} level, an index of Ca^{2+} leak. Notably, the accelerated sensitivity to activating Ca^{2+} was linked to pathogenesis of CCD. These effects were similar to those of the amino-terminal mutations. These results suggest that mutations in the amino-terminal and central regions have similar impacts on the CICR activity of RyR1 channel.

O3H-5-4 Molecular interaction between junctophilin-2 and caveolin in vascular smooth muscle cells

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Junctophilins (JPs) are scaffold proteins which form a junctional membrane complex (JMC) where the plasma membrane (PM) and the endoplasmic/sarcoplasmic reticulum (ER/SR) in excitable cells are juxtaposed at a distance of 10-15 nm. It has been reported that the JMC plays an important role in functional coupling between L-type Ca^{2+} channels on the PM and ryanodine receptors in the SR membrane. However, roles of JPs in smooth muscle cells (SMCs) are still unknown. In SMCs, caveola is known to form signal microdomains because caveolin-1 (cav1), a component of caveolae, binds to various signaling molecules. Therefore, we analyzed molecular coupling of JPs and cav1 in SMCs. In smooth muscles, real time PCR analysis revealed an abundant expression of JP2 that is an isoform identified in muscular types of cells. Double-immunocytochemical staining analysis showed JP2 was co-localized with cav1 in murine vascular SMCs. Single-molecule imaging by total internal reflection fluorescence microscopy showed JP2-GFP molecules were closely located with mCherry-cav1 in HEK293 cells. These results supposed that JP2 directly interacts with cav1 in vascular SMCs. JP2 may distribute in caveolae close to ER/SR and enable effective signal transduction between the PM and ER/SR.

O3H-5-5 Molecular mechanisms for endothelin-1-induced insulin resistance in rat skeletal muscle cells

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Endothelin-1 (ET-1) induces insulin resistance through a direct action on skeletal muscle. However, the signaling pathways of ET-1-induced insulin resistance in skeletal muscle are unknown. The purpose of this study was to determine molecular mechanisms underlying the inhibitory effect of ET-1 on insulin-stimulated Akt phosphorylation and glucose uptake in myotubes of rat L6 skeletal muscle cell line. mRNA expression levels of differentiation marker genes, MyoD and myogenin, were increased during myoblasts differentiation into myotubes. Some of myotubes possessed the ability to spontaneously constrict. In myotubes, insulin stimulated Akt phosphorylation at Thr^{380} and Ser^{473}, and [^{3}H]-labelled 2-deoxy-D-glucose ([^{3}H]2-DG) uptake. ET-1 inhibited insulin-stimulated Akt phosphorylation and [^{3}H]2-DG uptake. Blockade of ET type A receptor (ET_AR) and overexpression of a dominant negative G protein-coupled receptor kinase 2 (GRK2) construct counteracted the inhibitory effect of ET-1. Endogenous Akt directly bound to FLAG-GRK2 and their association was enhanced by ET-1. These findings suggest activation of ET_AR with ET-1 impairs insulin-induced Akt phosphorylation and [^{3}H]2-DG uptake in a GRK2-dependent manner in skeletal muscle.

O3H-6-1 ERK5 regulates catecholamine biosynthesis and homeostasis in neural cells and human adrenal medullas

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Extracellular signal-regulated kinases (ERKs) play important roles including proliferation and differentiation. Previously, we demonstrated that ERK5 were responsible for neurite outgrowth and tyrosine hydroxylase (TH) expression in pheochromocytoma cells (PC12). In the present study, we also show that ERK5 regulates TH levels in rat sympathetic neurons. Therefore, ERK5 signaling is responsible for catecholamine biosynthesis in TH-positive neural cells. Next, we attempted to examine a pathological role of ERK5 signaling in human adrenal pheochromocytomas which produce excess catecholamines. Although TH mRNA and protein levels were significantly elevated in pheochromocytomas, ERK5 levels were unexpectedly down-regulated. The protein levels of TH and ERK5 varied widely within individual samples, but TH levels were significantly correlated with ERK5 levels in normal adrenal medullas. In contrast, there is no significant correlation between ERK5 and TH levels in pheochromocytomas, indicating TH levels are regulated by alternative mechanisms in tumors. Taken together, ERK5 signaling is required for catecholamine biosynthesis to maintain appropriate TH levels. This pathway is disrupted in pathological conditions.

O3H-6-2 Cytoplasmic dynein light chain, Tctex-1, augments adenylyl cyclase activity in a dynein-independent manner

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Physiologic role of G protein-coupled receptors (GPCRs) is controlled by proteins interacting with carboxyl-terminus of the receptors. We previously reported that internalization of parathyroid hormone receptor (PTHR) was regulated by a cytoplasmic dynein light chain, Tctex-1 (Sugai and Saito et al., Biochem. Biophys. Res. Commun., 2003). However, the role of Tctex-1 on PTHR signaling has been unclear. In the present study, we showed that knockdown of Tctex-1 decreased PTH-(1-34)-induced cyclic AMP (cAMP) production in PTHR stably-expressing HEK293 cells. The reduction was also shown in cells expressing PTHR-KRVS mutant, which failed to interact with Tctex-1. Furthermore, forskolin-induced cAMP production was diminished by Tctex-1-knockdown, suggesting that Tctex-1 increased adenylyl cyclase activity. On the other hand, knockdown of another dynein component, dynein heavy chain, did not altered PTH-(1-34)- or forskolin-induced cAMP production. A microtubule depolymerization reagent, colchicine, did not suppressed forskoline-induced cAMP production. These data demonstrated that Tctex-1 was involved in augmentation of PTHR-mediated Gs/cAMP signaling by increasing adenylyl cyclase activity in a dynein-independent manner.

O3H-6-3 Identification of novel SENP1 inhibitors and their potential anti-tumor activities

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SUMOylation regulates multiple biological systems by changing the functions and fates of target proteins. Like ubiquitination, SUMOylation is reversible; SUMO can be cleaved from target proteins by SUMO specific proteases, SENPs. Among six human SENP isoforms, SENP1 is highly expressed in several types of cancers; therefore, SENP1 is believed to be a promising target for anti-cancer drug discovery. By use of an in situ-cell based screening system, we succeeded in identifying several small-molecule SENP1 inhibitors from the RIKEN NPDepo chemical library. Our compounds inhibited both in vitro and in vivo SENP1 activities while they did not inhibit other proteases. HIF-1α is a hypoxia-responsive transcriptional factor and important for cancer cell survival under hypoxic microenvironment. Consistent with a previous observation that SENP1 is indispensable for HIF-1α activation upon hypoxia, our SENP1 inhibitors reduced expression of HIF-1α induced by hypoxia. Importantly, our compounds suppressed tumor sphere formation for which HIF-1α is essential. Thus, our SENP1 inhibitors not only are useful tools for elucidating physiological roles of SENP1 in cells but also have a potential as tool compounds for anti-cancer drug development.

O3H-6-4 Loss of mDia1/3 in mice results in male infertility

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mDia, a Rho effector and actin nucleator, has 3 isoforms, including mDia1, 2 and 3 in mammals. In this study, we generated mice lacking mDia1 and 3 in combination (DKO). We found that although DKO mice were born at the Mendelian's expectation, DKO males were infertile. To analyze the underlying cause of male infertility of DKO mice, we performed in vitro fertilization experiment, and found that sperm from DKO mice could not fertilize with zona-intact eggs. Histological analysis revealed abnormal head morphology, thinning of annulus, reduced number and defect in polarity of DKO sperm. Therefore, impaired spermatogenesis is likely the cause of male infertility of DKO mice.Spermatogenesis is an intricate male germ cell developmental process facilitated by Sertoli cells. Both mDia1 and mDia3 were found abundantly expressed in Sertoli cells, but not in germ cells. We further confirmed the indispensable role of mDia1/3 in Sertoli cell for spermatogenesis by germ cell transplantation experiments. These results together suggested that mDia1/3 in Sertoli cell is required for normal spermatogenesis. In this presentation, we'll discuss about the possible molecular mechanisms of mDia action in spermatogenesis and the potential of mDia as a novel contraceptive target.

O3H-6-5 Proteomic analysis of membrane transport systems of the epithelial tissue in the mammalian cochlea

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The cochlear endolymph harbors 150 mM [K+] and a positive potential of +80 mV. These unique properties are essential for hearing and maintained by the K+-transport across the epithelial tissue of the cochlea. Major proteins involved in the K+-transport have been already identified. Although the epithelial tissue also carries a variety of other small molecules to function the cochlea, little is known about their molecular constituents. To clarify the proteins underlying these transport systems, we analyzed the membrane fractions of the epithelial tissue by a mass spectrometry. We identified 1,664 membrane proteins, which contained 25 ion channels and 79 transporters. 16 of the former and 65 of the latter have been for the first time detected in the epithelial tissue. We further identified 20 candidates for uncloned deafness genes. Our protein library is useful to elucidate not only molecular architecture of the membrane transport systems in the epithelial tissue but also pathological processes of deafness.

O3I-1-1 Oral administration of osteocalcin improves glucose utilization by stimulating glucgon-like peptide-1 secretion

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Uncarboxylated osteocalcin (GluOC), a bone-derived hormone, plays an important role in glucose metabolism by stimulating insulin secretion through its putative receptor GPRC6A. We previously reported that GluOC action on insulin secretion is largely mediated by glucagon-like peptide-1 (GLP-1). In the present study, we examined the effect of GluOC oral administration on glucose utilization as well as the fate of such administered GluOC in mice. Long-term intermittent oral administration of GluOC reduced the fasting blood glucose level and improved glucose tolerance in mice without affecting insulin sensitivity. A small portion of orally administered GluOC reached the small intestine and remained there for at least 24 h. GluOC also entered the general circulation, and the serum GLP-1 concentration was increased in association with the presence of GluOC in the intestine and systemic circulation. GPRC6A was detected in intestinal cells, and was colocalized with GLP-1 in some of these cells. These results indicate that orally administered GluOC improved glucose handling likely by acting from both the intestinal lumen and the general circulation, with this effect being mediated in part by stimulation of GLP-1 secretion.

O3I-1-2 EPRAP regulates gluconeogenesis in the liver

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EP4 receptor-associated protein (EPRAP) is a recently identified anti-inflammatory molecule in macrophages. Because metabolic disorders including obesity and type2 diabetes are linked to chronic inflammation, we investigated the role of EPRAP against metabolic disorders using EPRAP-deficient (EPRAP-/-) mice. Notably, in diet-induced obesity (DIO) model, EPRAP deficiency did not inhibit adipose tissue inflammation, but significantly improved glucose intolerance and insulin resistance. We hypothesized that EPRAP regulates gluconeogenesis in the liver, because EPRAP-/- mice exhibited markedly lower fasting blood glucose levels but the similar response in glucagon tolerance test compared with wild type (WT). To test the hypothesis, we isolated primary hepatocyte from EPRAP-/- and WT mice. Induction of gluconeogenic enzymes including G6pc and PEPCK by cAMP were suppressed in EPRAP-deficient hepatocytes. Consequently, EPRAP deficiency reduced cAMP-induced glucose production from hepatocytes. Our data indicated that EPRAP is involved pivotally in regulating hepatic glucose production through gluconeogenesis. Because excessive hepatic glucose production plays crucial roles in the pathogenesis of insulin resistance and diabetes, EPRAP could be a novel therapeutic target for diabetes.

O3I-1-3 Phenylalanine sensitive K562-D cells for the analysis of the biochemical impact of excess amino acid

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Although it is recognized that the abnormal accumulation of amino acid is a cause of the symptoms in metabolic disease such as phenylketonuria (PKU), the relationship between disease severity and serum amino acid levels is not well understood due to the lack of experimental model. Here, we present a novel in vitro cellular model using K562-D cells that proliferate slowly in the presence of excessive amount of phenylalanine within the clinically observed range, but not phenylpyruvate. The increased expression of the L-type amino acid transporter (LAT2) and its adapter protein 4F2 heavy chain appeared to be responsible for the higher sensitivity to phenylalanine in K562-D cells. Supplementation with valine over phenylalanine effectively restored cell proliferation, although other amino acids did not improve K562-D cell proliferation over phenylalanine. Biochemical analysis revealed mammalian target of rapamycin complex (mTORC) as a terminal target of phenylalanine in K562-D cell proliferation, and supplementation of valine restored mTORC1 activity. Our results show that K562-D cell can be a potent tool for the investigation of PKU at the molecular level and to explore new therapeutic approaches to the disease.

O3I-1-4 AICAR stimulation mimics metabolomic effects of muscle contraction

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Physical exercise has potent therapeutic effects on metabolic disorders. Many studies have suggested that 5'-AMP-activated kinase (AMPK) plays a pivotal role in regulating metabolism in contracting skeletal muscles, while several genetically manipulated animal models suggested the significance of AMPK-independent pathways. To elucidate significance of AMPK in contracting skeletal muscles, we conducted a metabolomic analysis comparing AICAR stimulation with the electrical contraction ex vivo in isolated rat epitrochlearis muscles, in which AMPK and glucose uptake were equally activated. CE-TOFMS analysis successfully annotated 132 molecules. AICAR stimulation exhibited high similarity to the electrical contraction in overall metabolites. Principal component analysis successfully characterized common effects and distinguished the difference. That also suggested a substantial change in redox status as a result of AMPK activation. The muscle contraction-evoked influences related to some amino acids metabolism are supposed to be independent of AMPK. Our results substantiate the significance of AMPK activation in contracting skeletal muscles and provide novel evidence that AICAR stimulation closely mimics the metabolomic changes in the contracting skeletal muscles.

O3I-1-5 Urocortin 1-induced anorexia involves peripheral α_2-adrenergic receptor-mediated inhibition of ghrelin in rats: Prevention by Rikkunshito

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The suppression of feeding behavior in stress conditions is involved in the activation of corticotropin-releasing factor receptors (CRFRs). We investigated the mechanisms of anorexia in rats induced by urocortin 1 (UCN), a CRFRs ligand, and the efficacy of its prevention by rikkunshito (RKT). Intracerebroventricular UCN decreases food intake and plasma levels of ghrelin, an orexigenic peptide, and these effects were prevented by a selective α_2-adrenergic receptor (AR) antagonist but not by a selective α_1-AR antagonist. The decrease in plasma ghrelin levels by UCN was not prevented by selective β_1- or β_2-AR antagonist. RKT prevented the decrease in food intake and ghrelin levels. The orexigenic effect of RKT was abolished by the co-administration of the ghrelin receptor antagonist. The components contained in RKT, namely glycycoumarin, 10-gingerol, 8- and 6-shogaol, and eudesmol, inhibited the binding to α_2-AR. Thus, these findings suggest that UCN decreases ghrelin secretion, which is mediated by the activation of peripheral α_2-AR, resulting in the inhibition of food intake. RKT may improve stress-induced anorexia by restoring ghrelin secretion involve in interaction with peripheral α_2-AR.

O3I-2-1 Tetrahydrobipterin suppresses platelet aggregation via enhancement of local nitric oxide production

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We have previously reported that orally administered tetrahydrobipterin (BH4) suppresses platelet aggregation in mouse whole blood (86th, 87th congress). Although BH4 is known as a cofactor of nitric oxide synthase (NOS), plasma NO_2/NO_3 level in our BH4-treated mouse was not increased and its mechanism remained to be clarified. In the present study, we measusured the intraplatelet cGMP concentration and investigated the concern of locally produced NO. Citrated blood was collected from male ICR mice (6 months old) after oral administration of sodium nitroprusside (SNP) 50 mg/kg or BH4 10mg/kg. The Blood was mixed with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX; 1 mM) for 30min, and centrifuged to obtain platelet pellet. The pellet was purificated by addiing 6% trichloroacetic acid, lyophilized and cGMP was quantified using an enzyme immunoassay kit (Amersham). Intraplatelet cGMP concentration of saline control mice (2.84 ± 0.42 pmol/10^8 platelets) was increased to 3.88 ± 0.14 by SNP, and to 3.98 ± 0.57 by BH4 administration, respectively. These results indicate that BH4 may suppress platelet activation by enhancing local NO production within platelet cells.

O3I-2-2 Cigarette smoke extract inhibits platelet aggregation

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Cigarette smoke contains numerous bioactive compounds, presenting a variety of effects to the body. Nicotine is known to promote platelet aggregation. However, the effects of other constituents of cigarette smoke on platelet function remain to be determined. Here we examined the effect of cigarette smoke extract (CSE) on platelet aggregation. In mouse platelets, CSE potently inhibited platelet aggregation induced by U-46619, an agonist for the thromboxane (TX) A_2 receptor TP, or collagen in a concentration-dependent manner. In contrast, the inhibitory effect of CSE on ADP-induced platelet aggregation was trivial. Among cytotoxic agents contained in CSE, acrolein inhibited U-46619- or collagen-induced platelet aggregation. Interestingly, the inhibitory effects of CSE and acrolein on collagen-induced aggregation were blunted in platelets lacking TP compared with those observed in wild-type platelets. In addition, CSE inhibited platelet TXA_2 synthesis induced by the addition of arachidonic acid. These results suggest that CSE inhibits TXA_2 synthesis, leading to an inhibition of platelet aggregation that depends on TXA_2 synthesis.

O3I-2-3 DPP-4 Inhibition improves dipping pattern of blood pressure in Dahl salt-sensitive rats

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We aimed to examine the effects of vildagliptin, a specific DPP-4 inhibitor, on blood pressure and its dipping pattern in Dahl salt-sensitive (DSS) rats. DSS rats were treated with a high salt (8% NaCl) diet and vehicle (0.5% carboxymethylcellulose) or vildagliptin (3 or 10 mg/kg, twice daily, p.o.) for 7 days (n = 7 per group). Mean arterial pressure (MAP) was measured by telemetry. High salt diet for 7 days significantly increased MAP with an extreme dipping pattern of blood pressure in DSS rats. Vildagliptin dose-dependently attenuated the development of salt-induced hypertension. Interestingly, vildagliptin significantly increased urine sodium excretion and normalized the dipping pattern. In anesthetized high salt-diet DSS rats, acute intra-cerebroventricular infusion of vildagliptin (50, 500 or 2500 μg in 10 μL solution) did not alter MAP or heart rate (n = 4). These data suggest that treatment with the DPP-4 inhibitor, vildagliptin, attenuates the extreme dipping pattern of blood pressure and development of salt sensitive hypertension by increasing urinary sodium excretion.

O3I-2-4 Physiological functions of mitofusin in the Ca^{2+} microdomain of smooth muscle cells

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Ca^{2+} is a key regulator of various biological responses. Ca^{2+} channels and its effectors are assembled in the limited region within the cells. They constitute a Ca^{2+} microdomain and mediate particular Ca^{2+} signaling by an efficient control of cytosolic Ca^{2+} level ($[Ca^{2+}]_{cyt}$). Intracellular organelle such as mitochondria and sarcoplasmic reticulum (SR) also constitute a Ca^{2+} microdomain. In this study, we investigated the functional significance of anchoring proteins, mitofusin (Mfn1 and 2) in Ca^{2+}microdomain in smooth muscle cells by using dominant-negative mutant of Mfn2 (Mfn2 R400Q). The expressions of Mfn1 and 2 wewe detected in mRNA level in aorta and mesenteric artery of the mice. When Mfn1 or 2 was transiently transfected into A10 cells, these were distributed in mitochondria and SR. The mitochondrial elongation was observed in Mfn1- or 2-transfected A10 cells but not in Mfn2 R400Q- transfected cells. The simultaneous measurement of $[Ca^{2+}]_{cyt}$ and mitochondrial Ca^{2+} level by confocal microscopy revealed Mfn2 involved in the mitochondrial Ca^{2+} uptake. These data suggested Mfn tether SR and mitochondria in smooth muscles and regulate Ca^{2+} movement between mitochondria and SR by forming the Ca^{2+} microdomain.

O3I-2-5 Adrenergic receptors β2 and β3 transduce differential signals in cardiac fibroblasts

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<Background> Cardiac fibroblasts (CFs) are the most prevalent cell types in heart. ß-adrenergic receptor (ßAR) signaling localized in caveolae plays central roles in the development of heart failure, and PKA and CaMKII are major effectors downstream ßAR. However, the role of caveolae AR in CFs remains unclear.<Method and result>To elucidate ßAR signaling of caveolae in CFs, we generated a fusion protein composed of phospholamban (PLN) and caveolin3 (Cav3) representing PKA activation as phosphorylation at S16 of PLN and CaMKII as that at T17 in caveolae. Thus, activation of PKA or CaMKII is detectable by anti-phospho-S16 or T17 antibody, respectively. In neonatal rat CFs (NRCFs) infected PLN-Cav3 adenovirus, stimulation by isoproterenol led to enhanced phosphorylation of both S16 and T17, suggesting PKA and CaMKII activation in caveolae of CFs. RT-PCR analyses showed ß2AR and ß3AR were present in NRCFs. Stimulation with ß2AR selective agonists activated both PKA and CaMKII, while ß3AR elicited solely PKA activation, analyzed by using ß3AR selective agonist/antagonist. <Conclusion> Both ß2 and ß3AR, expressed in NRCFs, transduce distinct signaling. Selective ßAR regulation could be potential novel anti-fibrotic therapeutics in heart failure.

O3I-3-1 Effect of TRPC6 deficiency on motor function disorder after hindlimb ischemia

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Peripheral circulatory disturbance is a major clinical outcome of peripheral artery disease (PAD). Recovery of peripheral blood flow via angiogenesis and arteriogenesis is believed to be an initial step to improve motor function disorder after hindlimb ischemia. In contrast, accumulating evidence has suggested that exercise is one of the beneficial therapies for PAD. Thus, we examined the mechanism underlying the improvement of peripheral circulatory disturbance after hindlimb ischemia in mice. Using femoral artery ligation (FAL) as a model of PAD, we demonstrated that voluntary exercise by free-wheel running almost completely recovered the peripheral blood flow after hindlimb ischemia. Among TRPC subfamilies, TRPC6 mRNA levels were significantly increased in ischemic gastrocnemius after FAL, and voluntary exercise completely suppressed this TRPC6 up-regulation. In addition, TRPC6 null knockout mice (TRPC6-KO) exhibited facilitation of blood flow recovery concomitant with increase of arteriogenesis. These results suggest that pathological increase of TRPC6 expression in ischemic gastrocnemius inhibits blood flow recovery after FAL and suppression of TRPC6 could be novel therapeutic target for PAD.

O3I-3-2 Effects of the SGLT2 inhibitor, empagliflozin, on blood pressure and urinary excretion of sodium in salt-treated obese OLETF rats

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We examined the effects of the sodium-glucose co-transporter 2 (SGLT2) inhibitor, empagliflozin, on blood pressure and urinary excretion of sodium in salt-treated obese OLETF rats. OLETF rats were treated with 1% NaCl (in drinking water, n = 10) and vehicle (n = 10) or empagliflozin (10 mg/kg/day, p.o., n = 10) for 5 weeks. Blood pressure was continuously measured by telemetry. High salt plus vehicle-treated obese OLETF rats developed non-dipper type hypertension (136+/-2 mmHg in waking time and 132+/-2 mmHg in sleeping time). Compared with high salt plus vehicle-treated animals, high salt plus empagliflozin-treated OLETF rats showed an approximately 1,000-fold increase in urinary glucose excretion and improved glucose metabolism. Furthermore, empagliflozin significantly decreased blood pressure and improved blood pressure circadian rhythm to a dipper profile, which were associated with increases in urinary sodium excretion of 30%. These data suggest that empagliflozin elicits beneficial effects on both glucose metabolism and hypertension in salt-treated obese rats.

O3I-3-3 Effects of various kinase inhibitors on the 5-HT-induced contraction in carotid arteries from type 2 diabetic Goto-Kakizaki rat

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5-hydroxytryptamine (serotonin, 5-HT) plays an important role in the vascular tone; however, the signal-transduction of 5-HT in smooth muscle under chronic type 2 diabetes remains unclear. Therefore, we investigated 5-HT-induced contraction and associated mechanisms, especially protein kinases, in carotid artery (CA) from chronic type 2 diabetic Goto-Kakizaki (GK) rats. To investigate the response mechanisms of arterial smooth muscle, we investigated 5-HT-induced contraction in the presence of various kinase inhibitors using endothelium-denuded preparations. Carotid arterial expressions of kinases (ERK1/2, p38 MAPK, PI3K, and Rho kinases) were detected by immunoblotting. 5-HT-induced contraction was increased in CA from GK compared to Wistar rats. In denuded preparations, we found that the inhibitors of p38 MAPK, PI3K, and Rho kinase abolished the differences of the contractions. Basal expressions of total ERK1/2, total and phosphorylated p38 MAPK, PI3K (p85 and p110α), Rho kinases (ROCKI and ROCKII) in CA were similar between two groups. These results suggest that 5-HT-induced contraction is augmented in GK rat CA through p38 MAPK, PI3K, and Rho kinases.

O3I-3-4 Stage-dependent benefits and risks of pimobendan in genetic dilated cardiomyopathy mice with progressive heart failure

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We examined therapeutic effect of pimobendan, a Ca^{2+} sensitizer, on a mouse model of human genetic dilated cardiomyopathy, which progressively develops end-stage heart failure (HF). Pimobendan prevented myocardial remodeling in compensated stage HF, but not in end-stage HF. Pimobendan significantly extended the life span at both stages of HF, but dose-dependently increased sudden cardiac death in end-stage HF. In cardiomyocytes from end-stage HF mice, pimobendan induced triggered activity probably due to early or delayed afterdepolarizations. Cilostazol, a specific inhibitor of phosphodiesterase 3 (PDE3), also induced triggered activity in these cardiomyocytes. Verapamil, a L-type Ca^{2+} channel blocker, decreased the incidence of triggered activity caused by pimobendan, which seemed to result from markedly up-regulated electrogenic sodium/calcium exchanger 1 activation by overly elevated cytoplasmic Ca^{2+} through PDE3 inhibition. These results indicate that pimobendan is beneficial irrespective of HF stage, but increases sudden cardiac death due to Ca^{2+} overload through PDE3 inhibition in end-stage HF, the risk of which could be decreased by pharmacological agents such as verapamil.

O3I-4-1 Altered autophagy in transgenic mice with cardiac specific expression of active histone deacetylase 6

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Histone deacetylase 6 (HDAC6) is known to act as a deacetylase, which can catalyze the deacetylation of lysine residues on alpha-tubulin. Although the HDAC6 may be associated with intracellular trafficking function as well as protein degradation system in cardiomyocytes, the functional role of the HDAC6 remains uncertain in the hearts. In the present study, we examined the effect of active HDAC6 overexpression on autophagy in the hearts. Cardiac LC3-II, a marker of autophagy, was decreased in the active HDAC6 TG mouse hearts. This reduction of LC3-II was also seen in the active HDAC6 mice treated with chloroquine, an inhibitor of autophagosome-lysosome fusion while the level of LC3-II was enhanced in non-transgenic (NTG) mice treated with chloroquine. These results suggest that the autophagy, particularly formation of autophagosome is inhibited in the active HDAC6 TG mouse hearts. In contrast to fed condition, LC3-II inductions were detected in both the active HDAC6 TG mouse and NTG mouse hearts 48hr after starvation. These results suggest that the autophagy induced by starvation is regulated by HDAC6-independent manner in the hearts.

O3I-4-2 Mechanisms underlying canstatin-induced migration of rat cardiac fibroblasts

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Canstatin, a fragment of type IV collagen α2 chain, is an anti-angiogenic factor. We investigated the underlying mechanisms of canstatin-induced migration of rat cardiac fibroblasts. Cardiac fibroblasts were isolated from male Wistar rats. Boyden chamber assay was performed to determine a cell migration. Protein expression and phosphorylation were detected by Western blotting. Canstatin increased extracellular signal-regulated kinase (ERK) phosphorylation, matrix metalloproteinase (MMP)-2 and -9 secretion, and migration. Both PD98059, an ERK inhibitor, and CTTHWGFTLC (CTT), an MMP inhibitor, inhibited the canstatin-induced migration. PD98059 inhibited the secretion of MMP-9 but not MMP-2. MMP-2 is known to activate epidermal growth factor (EGF) receptor (EGFR) through the release of EGF-like ligands. Gefitinib, an EGFR inhibitor, suppressed the canstatin-induced ERK phosphorylation and MMP-9 secretion. Secretory vesicles of MMP-2 were observed in the short-term canstatin-treated cells by an immunofluorescence staining. CTT inhibited the canstatin-induced EGFR phosphorylation. These results suggest that canstatin promotes cell migration through MMP-2-dependent transactivation of EGFR and subsequent MMP-9 secretion in rat cardiac fibroblasts.

O3I-4-3 Enhancement of Ca^{2+} transient and contractile function of cardiomyocytes by phospholamban specific RNA aptamer

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The sarco(endo)plasmic reticulum Ca^{2+}-ATPase 2a (SERCA2a)-phospholamban (PLN) system of the sarcoplasmic reticulum (SR) plays an important role in regulation of intracellular Ca^{2+} cycling in cardiomyocytes. In the failing heart, the Ca^{2+}-pumping activity of SERCA2a is reduced and the inhibitory effect of PLN on SERCA2a increases. PLN is, therefore, a potential target for the treatment of heart failure. Here, we selected PLN-specific phosphorothioate-substituted RNA aptamers from a library of RNA molecules containing a randomized 40-nucleotide sequence by SELEX with a fusion protein of the cytoplasmic region of human PLN. One of these aptamers was shortened to a 30-nucleotide oligomer (RNA-Apt30) without loss of function. RNA-Apt30 showed a high affinity for PLN (K_d = 11 nM), and increased the SERCA2a activity in isolated cardiac SR vesicles (EC_{50} = 18 nM). In addition, RNA-Apt30 conjugated with a cell-penetrating peptide enhanced both Ca^{2+} transients and contractile function of isolated adult rat cardiomyocytes. These results suggest that a PLN aptamer may be a new therapeutic agent for heart failure without the need for gene transfer or a change in endogenous protein expression.

O3I-4-4 Elucidation of molecular mechanism of cardiomyocyte necrosis induced by reactive oxygen species

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Background: Cardiomyocyte (CM) death is the major cause of heart failure (HF). While molecular mechanisms of apoptosis are well investigated, those of necrosis remain to be elucidated. Reactive oxygen species are one of well-known cell death inducers. However, its role in CM necrosis is not fully investigated.
Methods and Results: Hydrogen peroxide (H_2O_2) -induced CM death was not inhibited by a pan-caspase inhibitor, suggesting necrotic cell death. In contrast, H_2O_2-induced CM death was prevented by BAPTA-AM, a Ca^{2+} chelator (88.1±19.3 vs 4.0±2.7 in cell viability assay(%), n=3, $p<0.01$) and 2-APB, a multiple channel inhibitor (81.2±12.9% vs 23.4±8.6%, n=3, $p<0.01$). To examine the protective effect of 2-APB against CM death in vivo, C57BL/6 mice were subjected to transverse aortic constriction (TAC) and intraperitoneally injected with 2-APB (10mg/kg) or vehicle every other day. Treatment of 2-APB decreased fibrotic area in hearts 4 weeks after TAC (0.4±0.2% vs 2.1±0.7%, n=3-4, $p<0.01$), suggesting prevention of cell death.
Conclusion: Our present results indicate that H_2O_2 induces necrosis in a Ca^{2+}-dependent and 2-APB-sensitive manner in CM and prevention of necrotic cell death by 2-APB could be a promising therapeutic strategy for HF.

O3I-4-5 Dissecting the role of CCR4-NOT-associated ubiquitin converting enzyme in controlling heart functions

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CCR4-NOT complex is a global regulator of gene expression. NOT4 is known as an E3 ubiquitin ligase, associated with the CCR4-NOT complex. Cooperating with E2 ubiquitin conjugating enzyme (Ubc4/5), NOT4 regulates proteasome degradation of nascent polypeptide, chromatin modification through ubiquitination of histone demethylase Jhd2, and JAK-STAT signaling. However, the physiological and pathological significance of NOT4 in humans and animals are totally unknown. In this study, we aimed to dissect the role of NOT4 in mammalian species and generated NOT4 gene-deficient mice by homologous recombination in ES cells. Since our expression analysis revealed that NOT4 is highly expressed in heart, we analyzed heart functions in NOT4 heterozygous knockout mice. Interestingly, we observed the significant reduction of heart contractility in NOT4 heterozygous knockout mice, suggesting the significance of NOT4 in controlling heart functions. We currently investigate NOT4 heterozygous mice in transverse aortic constriction (TAC)-induced heart failure model and also are generating heart specific NOT4 knockout mice. We hope to discuss the physiological role of the multi-subunit complex in heart functions and homeostasis.

O3I-5-1 SGLT1 participates in cardiac remodeling in pressure-overload-induced cardiacmyopathy

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Introduction: It is known that the expression of a novel cardiac glucose transporter, sodium-glucose co-transporter 1 (SGLT1), exists in human heart. However, the role of SGLT1 in the induction of pressure-overload-induced cardiomyopathy is still uncertain. We examined the role of SGLT1 in the pathogenesis of pressure-overload-induced cardiomyopathy. Method and Results: Transverse aortic constriction (TAC) or sham procedure was undergone in SGLT1-dificient (SGLT-/-) and wild-type (SGLT+/+) mice. Six weeks after the procedure, all experiments were performed in mice. The reduction of left ventricular fractional shortening was observed in TAC-operated SGLT+/+ but not in TAC-operated SGLT-/- mice. Heart/body ratio was increased in TAC-operated SGLT+/+ compared with TAC-operated SGLT-/- mice. SGLT1 gene and protein expression was increased in TAC-operated SGLT+/+ compared with sham-operated SGLT+/+ mouse hearts. Phenylephrine significantly increased the size of cardiac myocytes in neonatal SGLT+/+ mouse hearts greater than in neonatal SGLT-/- mouse hearts. These results suggest that SGLT1 plays important roles in the induction of cardiac remodeling in pressure-overload induced-cardiomyopathy.

O3I-5-2 Roles of a proton-sensing receptor (TDAG8) in myocardial infarction

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Myocardial infarction, MI, is caused by occlusion of coronary artery, which occurs by disruption of plaque of atherosclerosis and aggregation of platelet. At MI, cardiomyocytes are died due to low oxygen supply, and they undergo apoptosis and necrosis. When MI is not properly treated, the heart develops heart failure. It is reported that pH of ischemic site quickly decreases after MI because anaerobic glycolysis produce a large amount of lactic acid. However the effects of acidosis are still largely unknown. We focused on T cell death-associated gene 8 (TDAG8), which is one of the pH-sensitive G protein-coupled receptors. We performed MI surgery for WT mice by ligation of left anterior descending coronary artery, and revealed that TDAG8 expression dramatically increased in infarct area 3 days after MI. Furthermore, we found that TDAG8 knockout (KO) mice had significantly higher mortality rate than WT mice. Consistent with this, the heart performances of TDAG8 KO mice were decreased after MI. Moreover, TDAG8 KO mice showed the significant increase of apoptotic cells numbers. These data suggested that TDAG8 has a cardioprotective role in cell survival at MI.

O3I-5-3 Development of an experimentally useful model of acute myocardial infarction: Subtotally nephrectomized triple nitric oxide synthases-deficient mouse

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Due to lack of an experimentally useful animal model that develops acute myocardial infarction (AMI), research and development of therapeutic strategies for preventing AMI have made little progress. To address this issue, we investigated the effect of subtotal nephrectomy on the incidence of AMI in mice deficient in all three nitric oxide synthases (NOSs). Two-thirds nephrectomy (NX) was performed on male NOSs$^{-/-}$ mice. The 2/3NX caused sudden cardiac death due to AMI in the NOSs$^{-/-}$ mice as early as 4 months after the surgery. The 2/3NX NOSs$^{-/-}$ mice exhibited fatal arrhythmias and accelerated coronary arteriosclerotic lesion formation. Cardiovascular risk factors (hypertension, hypercholesterolemia, and hyperglycemia) and an increased number of circulating bone marrow-derived vascular smooth muscle cell (VSMC) progenitor cells were noted in the 2/3NX NOSs$^{-/-}$ mice. Importantly, combined treatment with a clinical dosage of an angiotensin II type 1 receptor blocker, irbesartan, and a calcium channel antagonist, amlodipine, markedly prevented the incidence of AMI and improved the prognosis of those mice. The 2/3NX NOSs$^{-/-}$ mouse is the first experimentally useful model of AMI.

O3I-5-4 The properties of cardiac Sca-1+ resident stem cells are altered in response to myocardial inflammation

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Background: Despite limited regenerative activities of heart, histological recovery coincides with resolution of murine experimental autoimmune myocarditis (EAM). Herein, we explored the cellular dynamics of cardiac Sca-1+ resident stem cells (Sca-1+) in EAM.
Methods and results: Flow cytometry analyses showed that cardiac Sca-1+ contains Sca-1+CD31+ and Sca-1+CD31- cells. Sca-1+CD31- cells transdifferentiated into Sca-1+CD31+ in LIF containing media, suggesting those are endothelial progenitor cells (EPC). In EAM, expression of Sca-1 antigen in Sca-1+CD31- cells was upregulated 3w after induction (EAM 3w), inflammation phase (2.6 fold vs 0w, n=5, p<.01), and reduced to the basal level at EAM 5w (n=5-6). Moreover, the ratio of Sca-1+CD31- cells to Sca-1+ increased at EAM 3w (1.5 fold vs 0w, p<.05), indicating the population of EPC expands at EAM 3w. Consistently, capillary density, which was once declined at EAM 3w, replenished at EAM 5w (3927±350,1295±347,1798±153 capillaries/mm2 in 0w,3w,5w, n=5-7, p<.01, 3w vs others), indicating EPC expansion is accompanied by capillary increase.
Conclusion: Modulation of cellular dynamics of cardiac Sca-1+ stem cells could be a novel strategy for vascular regeneration in heart diseases.

O3I-5-5 IL-27 negatively regulates murine experimental autoimmune myocarditis (EAM)

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<Background> IL-27 is a heterodimeric cytokine composed of EBi3 and IL-27p28. IL-27, produced by antigen-presenting cells, such as macrophages (MΦ), exhibits pro- and anti-inflammatory properties. In EAM, Th17 or Treg cells infiltrate into myocardium and regulate inflammatory reactions positively or negatively, respectively. Here, we examined the functional significance of IL-27 in EAM, focusing on Th17 and Treg.<Methods and results> Real time RT-PCR analyses revealed that Ebi3 and IL-27p28 mRNA expression increased in EAM hearts (Ebi3 4.2±1.7, IL-27p28 4.5±3.6 fold increase vs unimmunized, n=7-8, p<0.05), suggesting that IL-27 is produced in EAM hearts. Consistently, the expression of F4/80, a MΦ marker, was upregulated in EAM hearts (F4/80 5.9±3.2 fold increase vs unimmunized, n=7, p<0.01). Then, Th17 and Treg cells were isolated from EAM spleen by flow cytometer and cultured them with or without IL-27 (20ng/mL). Intriguingly, IL-27 suppressed IL-17 production by Th17 cells (control 504.3±64.9, IL-27 246.5±27.5pg/mL, n=5-6, p<0.05) and increased IL-10 by Treg cells (control 167.3±21.1, IL-27 237.8±22.6pg/mL, n=5-6, p<0.01).<Conclusion> IL-27 plays an important role in limiting EAM and could be a novel therapeutic strategy against myocarditis.

O3I-6-1 Direct cardiotonic action of quercetin, a plant flavonoid, through mechanisms independent of its anti-oxidative action

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Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone, QCT) is a major flavonoid of plants, known to exhibit anti-oxidative, anti-inflammatory, and anti-cancer effects. In the present study, we found that QCT markedly enhanced the contractility of a single cardiomyocyte isolated from mouse hearts. Simultaneous measurement of Ca2+ transient in a Fura-2 loaded single cardiomyocyte revealed that QCT markedly increased the cytoplasmic Ca2+ both in diastole and systole under regular electrical stimulation. Echocardiography revealed that intravenous administration of QCT also increased the left ventricular function evaluated by ejection fraction in mice with reduced cardiac function due to a mutation causing genetic dilated cardiomyopathy (deltaK210 mutation in cardiac troponin T). QCT did not change the maximum force-generating capability and Ca2+ sensitivity of force generation in skinned (membrane-permeabilized) cardiac muscle fibers, indicating that QCT has no direct effects on the contractile machinery in cardiomyocytes. These findings indicates that QCT has a direct cardiotonic effect through enhancing the Ca2+ transient in cardiomyocytes independently of its anti-oxidative action.

O3I-6-2 Molecular mechanisms underlying membrane-trafficking defect of type I long QT syndrome (LQTS) mutations

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The LQTS is characterized on an ECG by prolongation of QT interval which increases the risks of ventricular tachycardia and sudden cardiac death. Type I LQTS (LQT1) is caused by loss-of-function mutations in KCNQ1, a pore-forming subunit of the repolarizing K^+ current in cardiac action potentials. It has been shown that some mutations in a transmembrane domain of KCNQ1 alter the electrophysiological properties and cell surface expression. On the other hand, although one third of LQT1 mutations are located within the cytoplasmic domain (CPD), little is known about the molecular mechanisms underlying the LQT1-induced channel dysfunction.In the expression system, the amount of LQT1 mutants in plasma membranes related to the current expression level. However, LQT1 mutants retained in the cytoplasm was escaped from the degradation. The surface expression level of the mutants was well correlated with a thermal stability of the KCNQ1 CPD carrying the mutations. The molecular architecture of the CPD was a tetramer of dimers with CaM. Therefore, the increase in thermal stability of the KCNQ1 CPD may facilitate the cell surface expression of LQT1 mutants and prevent the mutation-induced reduction in K^+ currents.

O3I-6-3 Effects of arrhythmogenic mutations in type-2 ryanodine receptor on Ca^{2+} induced Ca^{2+} release activity and Ca^{2+} homeostasis in HEK293 cells

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The type 2 ryanodine receptor (RyR2) is the Ca^{2+} release channel on cardiac sarcoplasmic reticulum and is the major target for genetic arrhythmogenic diseases, i.e., catecholaminergic polymorphic ventricular tachycardia (CPVT) and arrhythmogenic right ventricular cardiomyopathy (ARVC). The RyR2 channel is known to be regulated by both cytoplasmic Ca^{2+} (Ca^{2+} induced Ca^{2+} release: CICR) and luminal Ca^{2+} (store overload induced Ca^{2+}release: SOICR). The SOICR threshold and termination levels have been reported to be affected in the disease-associated mutations, whereas it remains unclear how these mutations affect CICR activity. CICR shows biphasic Ca^{2+} dependent activity consisting of 3 parameters: sensitivity to activating Ca^{2+}, sensitivity to inactivating Ca^{2+}, and gain. In this study, we expressed RyR2 channels carrying CPVT and ARVC mutations in HEK293 cells and monitored spontaneous Ca^{2+} oscillations by live-cell Ca^{2+} imaging using gene-encoded Ca^{2+} indicators for cytoplasm (GECOs) and ER (CEPIAs). In addition, the 3 parameters of CICR activity were determined with [^{3}H]ryanodine binding assay. Relation between CICR activity and Ca^{2+} oscillation pattern will be discussed.

O3I-6-4 Effect of late Na^+ current inhibitors on the automaticity of the guinea-pig pulmonary vein myocardium

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Background: The pulmonary vein (PV) myocardium has a reduced inwardly rectifying K^+ current density and shows spontaneous electrical activity, which probably plays a crucial role in the generation of atrial fibrillation (AF). We examined the involvement of the late Na^+ current in the PV automaticity.
Materials and methods: Standard glass microelectrode recordings were made from the myocardial layer of isolated guinea-pig PV tissue preparations.
Results: Application of tertiapin, a blocker of the inwardly rectifying K^+ current, induced a repetitive firing. Tetrodotoxin, a standard Na^+ channel blocker, and ranolazine, a selective late Na^+ current blocker, markedly suppressed the tertiapin-induced activity, but pilsicainide, a class I antiarrhythmic agent, did not. In action potentials evoked by 1Hz electrical stimulation, tetrodotoxin and ranolazine induced negative shift of the maximal diastolic potential and take off potential without affecting the rapid depolarization slope. In quiescent PV preparations, anemonia slucata toxin II, a potent late Na^+ current activator, induced a persistent electrical activity.
Conclusions: The late Na^+ current inhibitors suppress the PV automaticity, and may be useful for treatment of AF.

O3I-6-5 Azelnidipine reduces surface expression levels of $Ca_v1.2$ channel

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Azelnidipine, a third-generation calcium channel blocker, has characteristic hypotensive effect that persists even after its plasma concentration declines to zero. The prolonged effect of azelnidipine is considered to be due to its high hydrophobicity that causes retention of the drug in vascular tissues. On the other hand, prolonged inhibition of ion channels could also be achieved by reducing their surface expressions. It has been recently reported that various stimuli including drugs internalized several ion channels. Thus, in this study we examined possible internalization of $Ca_v1.2$ by azelnidipine. HEK293 cells were transfected with the pore-forming α_{1c} subunit ($Ca_v1.2$) and ancillaryβ_{2c} and $\alpha_2\delta$subunits, and treated with azelnidipine or amlodipine or nicardipine. Expression of $Ca_v1.2$ was detected with immunofluorescence staining and the cells were analyzed with confocal laser scanning microscopy. Surface expression levels of $Ca_v1.2$ were significantly reduced with treatment of 10^{-5} M azelnidipine for 6 h, while other treatments did not. The results suggested that azelnidipine reduced surface expression levels of $Ca_v1.2$ either by internalizing $Ca_v1.2$ from membrane or by inhibiting forward trafficking of $Ca_v1.2$.