2-P-001

Functional interaction and complex formation of metabotropic glutamate and GABA receptor

Hakushun Sakairi[1], Yuji Kamikubo[1], Masayoshi Abe[3], Toshihide Tabata[3], Takashi Sakurai[1,2]

[1]Department of Pharmacology, Juntendo University Graduate School of medicine, [2]Department of Pharmacology, Juntendo University School of Medicine, [3]Laboratory for Biological Information Processing, Graduate School of Science and Engineering, University of Toyama

G-protein-coupled receptors (GPCRs) may form homomeric or heteromeric complexes and cooperatively mediate intracellular responses. Previously, we showed modulation of type 1 metabotropic glutamate receptor (mGluR1) function by metabotropic gamma-aminobutyric acid receptor ($GABA_BR$) in cerebellar Purkinje cells. The activity of mGluR1 is mediated by a G_q protein, and has a crucial role in synaptic plasticity and motor learning. $GABA_BR$ inhibits neuronal activity through G_i protein, which regulates the release of neurotransmitters and the activity of ion channels. In this report, we investigated in greater detail the relationship of these GPCRs using non-neuronal cells. Using live cell imaging and biochemical analysis, we showed that mGluR1 and $GABA_BR$ form complexes at the cell surface. Moreover, using cAMP homogenous luminescence assay and calcium imaging, we found that mGluR1 and $GABA_BR$ regulate their signal transduction of each other. These findings provide a new insight into neuronal GPCR signaling and demonstrate a novel regulatory mechanism of synaptic transmission. This interaction would be involved in several important physiological and pathophysiological functions related to mGluR1 and $GABA_BR$, such as cerebellar motor learning and its dysfunction.

2-P-002

Effects of angiotensin II on excitatory synaptic transmission in rat nucleus tractus.

Yoshiaki Ohi[1], Daisuke Kodama[1], Akira Haji[1]

[1]Lab. Neuropharmacol., Sch. Pharm., Aichi Gakuin Univ.

Renin-angiotensin system is believed to have important roles in blood pressure regulation. Baro and chemoreceptor afferents project to the nucleus tractus solitarius (NTS) neurons. The expression of angiotensin II type 1 (AT_1) and 2 (AT_2) receptors in the NTS is confirmed. However the physiological roles of angiotensin II in NTS are not fully understood. We have previously reported that angiotensin II increased the frequency of spontaneous EPSCs in 33 % of the cells through the activation of AT_1 receptors and decreased it in 39 % of the cells through the activation of AT_2 receptors. In this report we aimed to reveal the effects of angiotensin II on tractus solitarius evoked EPSCs (eEPSCs) in the NTS by using a slice patch-clamp technique.

Angiotensin II decreased the amplitude of eEPSCs in 56 % of the neurons and had no effect in 44 % of the cells. Under the presence of AT_1 receptor blocker (ARB) losartan (10 μM), angiotensin II increased the eEPSCs in 37 % of the cells and decreased it in 15 % of the cells. The other neurons (48 %) showed no responses.

These results suggest that the activation of AT_1 receptors induces opposite effects on spontaneous and synchronous release of glutamate.

2-P-003

The effect of sex hormones on the interaction between synaptic adhesion proteins concerned with sociality

Nan Yagishita-Kyo[1], Minami Harada[2], Tomoko Uekita[3], Kei Maruyama[1], Yuki Ikai[4], Chihiro Koshimoto[4], Sosuke Yagishita[1,5]

[1]Dept. Pharm., Faculty of Med., Saitama Med. Univ., [2]Dept. Pharm., Faculty of Health and Med. Care, Saitama Med. Univ., [3]Dept. Psych., Kyoto Tachibana Univ., [4]Div. Bio-Resources, Dept. Biotech., Frontier Sci. Res. Ctr., Univ. Miyazaki, [5]Dept. Peripheral Nervous System Res., Natl. Inst. Neurosci., NCNP

The molecular basis with which we acquire and maintain sociality has been still unknown. Autism spectrum disorder (ASD) is defined by social communication deficits, indicating the molecular basis of sociality should be damaged in this condition. Many genes associated with ASD encode proteins involved in synapse formation or maintenance, especially synaptic adhesion molecules (SAMs). On the other hand, sex hormones may be involved in ASD onset, but its molecular basis remains uncertain. Here, we show a novel interaction between sex hormones and SAMs. We focused on *Octodon Degus* as a model animal due to their highly organized sociality. Degus are medium-sized diurnal rodents, and communicate with each other by using more than 20 vocal repertoires. We investigated amino-acid sequences of several SAMs expressed in degus brains and compared with those of humans. Interestingly, a particular SAM of degus shared more than 90% homology with human sequence. We analyzed the binding of the SAM pairs and revealed that a sex hormone disrupted their binding. We also found that one of these SAMs directly binds to the sex hormone. Therefore, we show a possible molecular mechanism of sex hormones affecting ASD and sociality formation.

2-P-004

Primary cilia shortening via neuronal ciliary GPCR signaling on hippocampal neuron

Yumiko Saito[1], Daisuke Miki[1], Tomoya Okada[1], Sakura Tomoshige[1], Yuko Sekino[3], Noriko Koganezawa[2], Tomoaki Shirao[2], Yuki Kobayashi[1]

[1]Grad. Sch. Integ Arts Science, Hiroshima Univ., [2]Dept.Neurobiol Behav., Grad. Sch.Med. Gunma Univ., [3]Grad. Sch. Pharmacol Sci. Tokyo Univ.

Primary cilia are microtubule-based organelles mediating sensory and neuroendocrine signaling. The importance of cilia function is underscored by ciliopathies often presenting clinical manifestations such as obesity. Many neurons possess primary cilia that are enriched for certain G protein-coupled receptors, including melanin-concentrating hormone (MCH) receptor 1 (MCHR1). The MCH system is known to mediate distinct aspects of energy balance and vital behavior. Although short cilia have been observed in genetic obese mice, a possible correlation between MCHR1-positive neuronal cilia length and energy metabolism has not been characterized. Here, we established a novel protocol to detect ciliary receptors in rat hippocampal slice culture. Ciliary MCHR1 were abundantly located in the CA1 and CA3 but not in DG. The features in each region were not uniform; the length of ciliary MCHR1 in CA1 were significantly longer than that in the CA3. Then, by using our culture system, we provide the first evidence that endogenous MCHR1-positive cilia length in neuron is significantly reduced by MCH at nanomolar order, and this appears to selectively occur in CA1 neurons. Future work will investigate the molecular mechanism of cilia shortening in responses to MCH.

2-P-005

Nanoscale molecular landscape of the synaptic vesicle release site in the hippocampus

Hirokazu Sakamoto[1], Taichi Onishi[1], Shigeyuki Namiki[1], Kenzo Hirose[1]

[1]Dept. Pharm., Grad. Sch. Med., Tokyo Univ.

Neurotransmitter release is confined to a specialized area of the presynaptic plasma membrane known as the active zone which consists of a large number of synaptic proteins including Munc13. The composition and in situ arrangement of the active zone proteins remain unclear. In this study, we developed an optimized immunostaining method to visualize active zone proteins at synapses in the hippocampus, and analyzed their nanoscale spatial distribution by multi-color and three-dimensional super-resolution imaging. We found that active zone proteins form discrete nanoscale supramolecular assemblies in an ordered arrangement as we have previously found for Munc13 proteins. The distance of individual supramolecular assemblies to Munc13 assembly, which marks the synaptic vesicle release site, varied among active zone proteins. Interestingly, the composition and distribution of active zone proteins varied among types of synapses, e.g. Schaffer collateral synapses, perforant path synapses, and mossy fiber synapses. Our results provide insight into supramolecular structure responsible for universality and diversity of synaptic functions.

2-P-006

The suppression effects of Ratanasampil on oxidative stress-induced neuronal damage and microglia-mediated neuroinflammation

Jie Meng[1], Aiqin Zhu[3], Junjun Ni[1], Yoshinori Hayashi[1], Hiroshi Nakanishi[4], Zhou Wu[1,2]

[1]Department of pharmacology and aging science, [2]OBT Research Center, Faculty of Dental Sciences, [3]Qinghai Provincial Hospital Institution of Geriatric, [4]Department of Pharmacology

Generation of reactive oxygen species (ROS) causes lipids, proteins and DNA damage, resulting in neuronal damage and neruoinflammation. Ratanasampil (RNSP), a traditional Tibetan medicine, clinically used for the mild-to moderate AD patients living at high altitude. In vivo, RNSP improved the learning and memory in an AD mouse model (Tg2576). However, mechanism underlying the effects of RNSP is unknown. In SH-SY5Y cells, RNSP significantly ameliorated the H_2O_2 –induced cytotoxicity. Furthermore, RNSP significantly reduced the H_2O_2-induced 8-oxo-2′-deoxyguanosine and attenuated the phosphorylation of p38 and ERK 1/2. In MG6 microglia, RNSP significantly ameliorated the cytotoxicity induced by hypoxia-reoxygenation. Furthermore, RNSP significantly suppressed the H6/R24-induced pro-inflammatory cytokines, ROS, DNA damage and phosphorylation of IκBα. These observations suggest that RNSP suppressed the H_2O_2-induced neuronal death through downregulation of p38-ERK activation and regulated the H/R-induced neuroinflammation through inhibition of oxidative stress and the activation of NFκB in MG6 cells. Therefore, RNSP may be beneficial for preventing oxidative stress-induced neuronal death and neuroinflammation.

2-P-007

The usefulness of KCC2 to analyze the effect of irradiation on rodent neuron.

Kento Igarashi[1], Kazuo Tomita[1], Tomoaki Sato[1]

[1]Dept. Applied pharm., Grad. Sch. Med. and Dent., Kagoshima Univ.

Radiation therapy (RT) is effective method to remove brain tumors. RT is also applied to pediatric patients, although comorbid adverse events, such as intellectual or cognitive disfunction, is severe problem. To understand the molecular events that occurs after RT will be promising to form pharmaceutical method to ameliorate these disfunctions. We examined the X-ray sensitivity of primary neuronal culture of embryonic rat (embryonic days of 16.5 – 18.5 days) cortex by employing trypan blue exclusion test. We found that the death fraction of cells after irradiation increased. We also performed immunofluorescence staining to detect a K^+-Cl^- co-transporter KCC2, which plays an important role in mediating intracellular Cl^- concentration ($[Cl^-]_i$), and found that perimembrane KCC2 signals declined, suggesting that decline of KCC2 would result in decrease of $[Cl^-]_i$, followed by hyperexcitability. We then testified whether afterward oxytocin administration restore declined KCC2 signals in X-ray irradiated cells and found that 10nM of oxytocin administration restored KCC2. In addition, we performed γ-ray irradiation to head of 10-week-old mice. It was found that KCC2 decreased in mice cortex which were irradiated with 3 Gy γ-ray irradiation by using western blot analysis. Collectively thought from these results, we assume that KCC2 is useful to analyze the effect of irradiation and to examine the afterward restoration by drug administration.

2-P-008

Investigation of membrane proteins interacting with food-derived antioxidant ergothioneine based on hippocampal proteomics analysis

Misa Nishiyama[1], Pornparn Kongpracha[2], Yusuke Masuo[1], Naoto Matsumura[1], Noritaka Nakamichi[1], Shushi Nagamori[2], Yukio Kato[1]

[1]Fac. Pharmacy, Kanazawa Univ., [2]Dep. Collaborative Research, Nara Medical Univ.

Hydrophilic antioxidant ergothioneine (ERGO) is not synthesized in mammals, but ingested from daily life in humans. Oral administration of ERGO exhibits several beneficial effects in the brain in experimental animals. ERGO promotes neuronal differentiation of neural progenitor cells in primary culture, and involvement of the activation of mTOR pathway has been proposed (Cell Signal 53, 269, 2018), although its directly interacting proteins have not yet been clarified. The aim of the present study is to perform comprehensive study to clarify the pathways involved in the pharmacological activity of ERGO. After repeated oral dose of ERGO or vehicle alone in normal mice, hippocampal dentate which is important in neurogenesis was isolated, and membrane proteome analysis using LC-MS/MS was conducted. Accordingly, 3,337 proteins were identified, and we found change in expression of proteins associated with mitochondria and synapse formation. Mouse neural stem cells were primarily cultured for 6 days, followed by incubation with ERGO. Gene product of neuronal marker b-III tubulin was increased by ERGO, confirming neuronal differentiation. Effect of ERGO on mitochondrial proteins is now under investigation.

2-P-009

The survival promoting effect of collagen peptides on differentiation of primary cultured cerebellar granule cells

Satomi Kogure[1], Hidetomo Kikuchi[1], Hiroshi Mano[1], Yosihumi Kimira[1], Naoki Inoue[2], Aya Matsushita[2], Yasuhide Hibino[1], Katsuyoshi Sunaga[1]

[1]Fac.Pharm.Pharmaceu.Sci.,Josai Univ., [2]Nitta Gelatin Inc.

Gelatin can be enzymatically hydrolyzed to yield collagen hydrolysates potentially applicable in the food industry. When collagen hydrolysates are ingested, several di- and tri-peptides (collagen peptide, CP) with various physiological activities are detected in human blood. We investigated whether CPs exert trophic effects on the differentiation of primary cultured cerebellar granule cells (CGC), using the MTT assay. Addition of specific tripeptides (TP-X) contained in the collagen hydrolysate in differentiating CGC cultures prevented extensive neuronal degeneration, which was observed in growth media containing low potassium (15 mM, K15). When CGC was cultured in K15, the cell viability was 64.4% relative to high potassium (25 mM) at 7 days *in vitro.* Under this condition, TP-X (10 μM) increased cell survival up to 76.6%. The effect was similar to the neuroprotective effect resulting from supplementation with 100 ng/mL brain-derived neurotrophic factor (76.5%) or 100 μM N-methyl-D-aspartate (97.2%). Currently, the mechanism underlying TP-X-mediated neuronal survival is unclear. Various functions of CP in skin, cartilage, and bone have been reported previously, and the present study further suggests a new possibility of improvement of cranial nerve function upon treatment with CP.

2-P-010

A possible mechanism of caffeine metabolites on cysteine uptake in hippocampal neurons

Nobuko Matsumura[1], Chisato Kinoshita[1], Kazue Kikuchi-Utsumi[1], Toshio Nakaki[2], Koji Aoyama[1]

[1]Dept. Pharm., Sch. Med., Teikyo Univ., [2]Fac. Pharma. Sci., Teikyo Univ.

Caffeine (1,3,7-trimethylxanthine) consumption reduces the incidence of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. We have previously shown that not only caffeine but also uric acid, which is a final metabolite of caffeine, increase the intracellular glutathione (GSH) levels in the hippocampus that is due to promotion of cysteine uptake into neurons (Neuroscience 2011). In our recent study, paraxanthine (1,7-dimethylxanthine), a major metabolite of caffeine also increased cysteine uptake in mouse hippocampus slices. In this presentation, we focused on the effect of caffeine metabolites on the cysteine uptake and the GSH levels in mouse hippocampal neurons. We analyzed the cysteine and GSH levels in hippocampal neurons after an intraperitoneal injection of 10 mg/kg caffeine, uric acid or paraxanthine into C57BL/6 mice. The cysteine and GSH contents of the tissues were quantitated by HPLC-fluorescence detection and the GSH levels in neuronal cells in hippocampal slices were detected by CMFDA staining. We show that caffeine and its metabolites promote cysteine uptake leading to the increased GSH levels in hippocampal neurons.

2-P-011

RAGE expression in Brain Endothelial Cells was increased by *Porphyromonas gingivalis* Infection

Fan Zeng[1], Junjun Ni[1], Zhou Wu[1,2]

[1]Department of Aging Science and Pharmacology, Faculty of Dental Sciences, Kyushu University, Fukuoka, Japan., [2]OBT Research Center, Faculty of Dental Sciences, Kyushu University

Accumulation of amyloid-β (Aβ) around cerebral blood vessels is found in more than 80% of Alzheimer's disease (AD) patients, and peripheral Aβ can accumulate in brain triggering degeneration. Recently, periodontitis has been reported positively link to AD, however, the mechanism of peripheral Aβ transport into brain is unclear. we hypothesized that periodontitis may involve in peripheral Aβ transport into brain. In the present study, we aim to examine the expression of Receptor for advanced glycation end products (RAGE) on brain endothelial cells after infection with *Porphyromonas gingivalis* (*P.g.*), the major pathogenic bacteria of periodontitis, because RAGE is mediated in transporting peripheral Aβ into brain. The mRNA level and immunofluorescent signal of of RAGE were significantly increased in hCMEC/D3 cells after *P.g* exposure. The expression of RAGE on CD31-positve endothelial cells were significantly increased in the *P.g* infected mice compared to control mice. Moreover, Aβ were detected on CD31-positve endothelial cells surrounding cerebral blood vessels in the *P.g* infected mice. These observations suggested that increased RAGE expression in endothelial cells is involved in Aβ influx into brain after *P.g* infection.

2-P-012

Systemic administration of an apelin receptor agonist protects against NMDA-induced retinal ganglion cell death

Yuki Ishimaru[1], Hiroko Konishi[1], Fumiya Shibagaki[1], Akiko Yamamuro[1], Yasuhiro Yoshioka[1], Sadaaki Maeda[1]

[1]Lab. Pharmacotherap., Faculty Pharmaceut. Sci., Setsunan Univ.

Glutamate excitotoxicity via NMDA receptors is associated with retinal ganglion cell (RGC) death in retinal diseases, such as glaucoma and diabetic retinopathy. We have previously reported that the apelin receptor is expressed in the RGCs and intravitreal injection of apelin inhibits RGC death induced by NMDA in mice. In the present study, we investigated whether systemic administration of an apelin receptor agonist protects the RGCs from NMDA-induced excitotoxicity. The apelin agonist ML233 (5 mg/kg) was administered intraperitoneally at 1 h before intravitreal injection of NMDA (10 nmol) in mice. The effect of ML233 on RGC death was assessed by immunohistochemistry with anti-Brn-3a and anti-calretinin antibodies. ML233 significantly prevented the decrease of the number of Brn-3a and calretinin-positive RGCs at 24 h after NMDA injection. Moreover, ML233 markedly suppressed NMDA-induced cell death of calretinin-positive amacrine cells, which are exquisitely sensitive to glutamate excitotoxicity in the retina. Our results suggest that systemic administration of apelin receptor agonists prevents retinal neuronal death induced by excitotoxicity via NMDA receptors.

2-P-013

Population dynamics of hippocampal CA1 pyramidal neurons in subthreshold membrane potentials *in vivo*

Asako Noguchi[1], Nobuyoshi Matsumoto[1], Yuji Ikegaya[1]

[1]Lab. Chem. Pharmacol., Grad. Sch. Pharmaceut. Sci., Univ. Tokyo

In the hippocampal CA1 area, characteristic oscillations in local field potentials (LFPs) have been related to important functions for memory encoding and consolidation. Synchronous or sequential activity of neurons during oscillations in LFPs is thought to encode information, but the relationships between LFPs and activity of multiple pyramidal neurons have not fully been investigated. In this study, we examined population dynamics of subthreshold membrane potentials, which underlie the firing activity, using multiple whole-cell recordings of up to four CA1 pyramidal neurons simultaneously with recordings of CA1 LFPs from anesthetized mice. In particular, we compared theta frequency-band (3-10 Hz) oscillations between LFPs (*i.e.*, type 2 theta) and membrane potentials of multiple neurons under urethane anesthesia. We found weak but significant correlations of event timings and frequencies of theta oscillations between LFPs and subthreshold membrane potentials. Our results provide an insight into our understanding of how subthreshold dynamics of each cell is incorporated in collective ensemble activity.

2-P-014
Region-specific regulation of dopamine signaling in the striatum

Keita Sugiyama[1], Mahomi Kuroiwa[1], Takahide Syuto[1], Takaichi Fukuda[2], Akinori Nishi[1]

[1]Department of Pharmacology, Kurume University School of Medicine, [2]Department of Anatomy and Neurobiology, Graduate School of Medical Sciences, Kumamoto University

In the striatum, dopamine modulates these functions via cAMP/PKA signal-mediated mechanisms. Recent studies revealed that structural organization and cortical innervation are different among subregions of the striatum. Therefore, we investigated dopamine signaling in subregions of the striatum. Mouse striatal slices were divided into seven subregions: (1) rostral part, (2-1) intermediate medial part, (2-2) intermediate lateral part, (2-3) intermediate most lateral part, (3) caudal part, (4) most caudal part, (5) nucleus accumbens. Each slice was treated with a D1 receptor agonist, SKF81297 (1 μM) and activity of cAMP/PKA signal was evaluated with the phosphorylation of DARPP-32, GluA1. The stimulatory effects of SKF81297 on the phosphorylation were the lowest in the subregion (3) in the rostrocaudal axis and in the subregion (2-3) in the mediolateral axis. Treatment of slices with a PDE10A inhibitor, papaverine, or SKF81297 plus a muscarinic receptor antagonist, atropine, increased the phosphorylation in subregions where the effect of SKF81297 on the phosphorylation was low. Thus, dopamine D1 receptor/cAMP/PKA signaling is differentially regulated in each subregion of the striatum, and the differences are mediated by PDE10 and/or muscarinic receptor.

2-P-015

Involvement of noradrenaline and dopamine systems in the anxiety-related behavior induced by long-term powdered food feeding

Fukie Yaoita[1], Masahiro Tsuchiya[2], Yuichiro Arai[3], Takeshi Tadano[4], Koichi Tan-No[1]

[1]Dept. Pharmacol., Fac. Pharmaceuticalsci., Tohoku Medical and Pharmaceutical Univ., [2]Dept. Nursing, Tohoku Fukushi Univ., [3]Tokyo Ariake Univ. Medical and Health Science, [4]Grad. Sch. Med. Sci., Kanazawa Univ.

Dietary habits are important factors affecting the development of emotion. We have shown that long-term powdered food (PF) feeding in mice increases locomotor activity and social interaction time (SI). Although the increased SI indicates low anxiety, the elevated plus maze test (EPM) shows not only anxiety-related behavior but also impulsive behavior. In this study, we investigated whether the PF feeding causes changes in anxiety-related behavior. Mice fed a PF for 17 weeks were compared with mice fed a standard food. The % of open arm time (OAT) and total number of arm entries were increased in PF-fed mice in the EPM. Moreover, we examined the effects of methylphenidate (MP), dopamine transporter (DAT) and noradrenaline transporter (NAT) inhibitor, atomoxetine (AT), selective NAT inhibitor, GBR12909 (GB), selective DAT inhibitor, and PD168077 (PD), selective D4 receptor agonist, on the changes of EPM in PF-fed mice. MP and AT are clinically used to treat ADHD symptoms. The OAT in PF-fed mice was decreased by MP, AT and PD, but not GB. These results suggest that the PF feeding may cause low anxiety or impulsivity, possibly via NA and DA systems and increase the risk for onset of ADHD-like behaviors.

2-P-016

The roles of serotonin 5-HT_{2C} receptor in locomotor activity, anxiety, and fear memory

Mao Nebuka[1], Yu Ohmura[1], Mitsuhiro Yoshioka[1]

[1]Dept. Neuropharmacol., Grad. Sch. Med., Hokkaido Univ.

Pharmacological studies have suggested that serotonin 5-HT_{2C} receptor is involved in locomotor activity, anxiety, and fear memory. However, the results of locomotor activity and anxiety in 5-HT_{2C} receptor knockout mice are mixed, and the effects of 5-HT_{2C} receptor knockout on fear memory have not yet been addressed. In the present study, we reconciled these inconsistent results by analyzing behavioral data in details. We revealed that the higher locomotor activity in 5-HT_{2C} receptor knockout mice is observed only in the late phase of the test. Moreover, we found that 5-HT_{2C} receptor knockout mice display a hesitating attitude, staying in the center area and risk assessment behavior, in the elevated plus maze test. This phenotype might explain the inconsistency of previous studies. In the contextual fear conditioning test, 5-HT_{2C} receptor knockout mice tended to show rapid within-session extinction of fear, but not between-session extinction, compared to the wild type mice.

2-P-017

Effects of 5-HT_{1A} agonist on levodopa-induced dyskinesia in unilateral 6-OHDA injection rat model

Hiroyasu Murasawa[1], Hiroyuki Kobayashi[1], Akiko Pawlak[1], Yasushi Hirasawa[1], Takahiko Nagase[1]

[1]Nihon Bioresearch Inc.

Establishment of dyskinesia, one of the symptoms (side effects) induced by levodopa was assessed in a unilateral 6-OHDA injection rat model. Since it has become clear that serotonin system has an important role in this rat model (levodopa-induced dyskinesia [LID] rats), a serotonergic agonist for inhibiting dyskinesia-like symptom was also assessed.

AIMs scores (locomotive, limb, axial, orolingual, and total) were high in the LID rats. Repeatedly administered tandospirone for 14 days also decreased AIMs scores (limb, axial, orolingual, and total) significantly; the reactivity was dose-dependent. The following findings were also noted in the LID rats: decreases in the contents of DA, DOPAC, HVA, 5-HT, and glutamate and DOPAC/DA and glutamate/GABA ratios in the striatum; decreases in DOPAC/DA and HVA/DA ratios, and an increase in glutamate/GABA ratio in the hypothalamus. Changes in the contents and ratios in the hypothalamus were improved by 14-day repeated administration of tandospirone.

As described above, tandospirone decreased AIMs scores without affecting rotational behavior induced by L-dopa administration in LID rats and inhibited development of side effects. The possibility is suggested that the inhibiting action occurs through at least the hypothalamus.

2-P-018

Optogenetic inhibition of central serotonergic neurons impairs model-based decision making

Kentaro Iwami[1], Yu Ohmura[1], Mitsuhiro Yoshioka[1]

[1]Dept. Neuropharmacol., Grad. Sch. Med., Hokkaido Univ.

It has been speculated that serotonin release in the forebrain is involved in model-based decision making. However, there is so far no direct evidence proving this hypothesis because there had been no method that selectively controls serotonergic activity. To resolve this problem, we developed transgenic mice expressing Archaerhodopsin T (ArchT) only in central serotonergic neurons. A lithium devaluation task was used to assess model-based decision making. In this paradigm, a mouse is first trained to poke its nose to illuminated holes to get a food pellet, and then the food is devalued by pairing it with lithium-induced illness. If the mouse associates the devaluation with nose-poking by mental simulation though the mouse has never experienced these two events simultaneously, the mice will refrain from poking its nose to holes (i.e. model based-decision making). Our results indicated that optogenetic silencing of serotonergic neurons in the dorsal raphe nucleus, but not the median raphe nucleus, impaired model-based decision making. Thus it is likely that serotonergic activity in the dorsal raphe nucleus has a pivotal role in model-based decision making.

2-P-019

Isoflurane-induced postoperative cognitive impairment and enhanced abnormal social interaction are associated with decrease in hippocampal dopamine D_2 receptor in mice

Takahiro Suda[1], Takeshi Iino[1], Tetsukazu Hamamoto[2], Kenjirou Seki[1]

[1]Dept. Pharmacol., Sch. Pharm., Ohu Univ., [2]LEAD CHEMICAL CO., LTD.

Social isolation is suggested to be a detrimental for the confusional states and abnormal interaction with cognitive impairment after the isoflurane plus surgery. However, the underlying mechanisms of these states remain unclear. After 2 hours exposure of isoflurane with abdominal surgery followed by social isolation for 24 h (ISO+SI-24h), the spontaneous alternations in Y-maze in male mice (7-10 weeks old) was significantly decreased, indicating that the spatial working memory was impaired by ISO+SI-24h. In general, only raring the 7-days of SI without the surgery, mice exhibit normal behaviors. However, the exposure of isoflurane with abdominal surgery in mice followed by raring 7 days of SI enhanced the mounting and sniffing behaviors against intruder mice in the home cage. In addition, the protein level of hippocampal dopamine D_2 receptors, but not prefrontal cortex was significantly decreased in mice with isoflurane plus surgery and SI. Since D_2 receptor is important for the cognitive function and psychosocial, these results imply the possibility that decrease in D_2 receptor contribute to the postoperative abnormal social interaction and cognitive dysfunction.

2-P-020

Methamphetamine-induced hyperlocomotion and cFos expression in specific brain regions involve T-type Ca^{2+} channels in mice

Nene Koike[1], Hiroki Yasui[1], Fumiko Sekiguchi[1], Atsushi Kawabata[1], Genzo Tanabe[2]

[1]Lab. Pharmacol. Pathophysiol., Fac. Pharm., Kindai Univ., 3-4-1 Kowakae, Higashi-Osaka 577-8502, Japan, [2]Lab. Org. Chem., Fac. Pharm., Kindai Univ., 3-4-1 Kowakae, Higashi-Osaka 577-8502, Japan

Among voltage-gated Ca^{2+} channels (VGCCs), low VGCCs/T-type Ca^{2+} channels (T-channels) regulate neuronal excitation and spontaneous neurotransmitter release, while high VGCCs are essential for evoked neurotransmitter release. It is still largely open to question how VGCCs contribute to CNS actions of psychostimulants, such as amphetamine and methamphetamine (MA). Interestingly, it has been reported that genetic deletion of $Ca_v3.2$ T-channels reduces amphetamine-induced hyperlocomotion (HL) in mice. Here, we examined effects of a selective T-channel blocker, TTA-A2, on MA-induced HL and brain Fos expression in C57BL/6J mice, as compared to pregabalin, a high VGCC α2δ inhibitor. TTA-A2, administered i.p. at 1 mg/kg, strongly suppressed MA-induced HL. In contrast, i.p. pregabalin at 1, 10 or 30 mg/kg had no such effect, although it exhibited slight suppressive effect at 3 mg/kg. MA caused cFos expression in specific brain areas including the prefrontal cortex, striatum, paraventricular hypothalamic nucleus, and hippocampal dentate gyrus and CA3 region, which were almost abolished by TTA-A2. Together, T-channels appear to play a critical role in MA-induced neuronal and behavioral excitation in C57BL/6J mice.

2-P-021

TRPM2 confers susceptibility to social stress but is essential for behavioral flexibility

Chihiro Andoh[1], Naoya Nishitani[1], Emina Hashimoto[1], Yuma Nagai[1], Keizo Takao[2], Tsuyoshi Miyakawa[3], Takayuki Nakagawa[4], Yasuo Mori[5], Kazuki Nagayasu[1], Hisashi Shirakawa[1], Shuji Kaneko[1]

[1]Department of Molecular Pharmacology, Graduate School of Pharmaceutical Sciences, Kyoto University, [2]Life Science Research Center, University of Toyama, [3]Division of Systems Medical Science, Institute for Comprehensive Medical Science, Fujita Health University, [4]Department of Clinical Pharmacology and Therapeutics, Kyoto University Hospital, [5]Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University

Transient receptor potential melastatin 2 (TRPM2) is a Ca^{2+}-permeable, nonselective cation channel and a member of the TRP channel superfamily that acts as a sensor of intracellular redox states. TRPM2 is highly expressed in the brain, but the physiological roles of TRPM2 in the central nervous system remain unclear. In this study, TRPM2-deficient mice were examined in a series of behavioral tests. In the Barnes circular maze, TRPM2-deficient mice learned the fixed escape box position at similar extent to wild-type littermates, suggesting normal reference memory. However, performance of the first reversal trial and probe test were significantly impaired in TRPM2-deficient mice. In the T-maze delayed alternation task, TRPM2 deficiency significantly reduced choice accuracy. These results indicate that TRPM2-deficient mice shows behavioral inflexibility. Meanwhile, social avoidance induced by repeated social defeat stress was significantly attenuated in TRPM2-deficient mice, suggesting that TRPM2 deficiency confers stress resiliency. Our findings indicate that TRPM2 plays an essential role in maintaining behavioral flexibility but it increases susceptibility to stress. (Andoh *et al.*, *Brain Research*, 2018)

2-P-022

Repeated social defeat stress impairs attentional set shifting irrespective of social avoidance and increases female preference associated with heightened anxiety

Hirotaka Nagai[1], Shu Higashida[1], Kazuki Nakayama[1], Ryota Shinohara[1], Masayuki Taniguchi[1], Midori Nagai[1], Takatoshi Hikida[2], Satoshi Yawata[3], Yukio Ago[4], Shiho Kitaoka[1], Shuh Narumiya[5], Tomoyuki Furuyashiki[1]

[1]Div. Pharmacol., Grad. Sch. Med., Kobe Univ., [2]Laboratory for Advanced Brain Functions, Institute for Protein Research, Osaka Univ., [3]Dep. Biol. Sci., Grad. Scho. Med., Kyoto Univ., [4]Lab. Biopharmaceutics, Grad. Scho. Pharm. Sci., Osaka Univ., [5]Medical Innovation Center, Grad. Sch. Med., Kyoto Univ.

Repeated social defeat stress (R-SDS) induces multiple behavioral changes in mice. However, the relationships between these behavioral changes were not fully understood. In the first experiment, to examine how the social avoidance is related to R-SDS-impaired behavioral flexibility, 10-week-old male C57BL/6N mice received R-SDS followed by the social interaction test and the attentional set shifting task. R-SDS impaired attentional set shifting irrespective of the development of social avoidance. In the second experiment, to examine whether R-SDS affects sexual preference and how this behavioral change is related to the social avoidance and R-SDS-heightened anxiety, another group of 10-week-old male C57BL/6N mice were subjected to R-SDS followed by the social interaction test, the female encounter test and the elevated plus maze test. The anxiety was heightened in the defeated mice without social avoidance, but not in those which showed social avoidance. Furthermore, female preference was increased specifically in the defeated mice which showed heightened anxiety, but was not related to the level of social avoidance. Together, these results showed that attentional set shifting is more sensitive to R-SDS than social interaction, and that female preference is affected by R-SDS in association with heightened anxiety rather than the social avoidance.

2-P-023

A role of innate immune molecules in behavioral changes induced by repeated social defeat stress in mice

Xiang Nie[1], Shiho Kitaoka[1], Kohei Tanaka[2], Atsubumi Ogawa[2], Fumitake Nakano[1], Yuki Imoto[3], Eri Segi-Nishida[4], Shuh Narumiya[2], Tomoyuki Furuyashiki[1]

[1]Division of Pharmacology, Kobe University Graduate School of Medicine, Kobe, Japan, [2]Medical Innovation Center, Kyoto University Graduate School of Medicine, Kyoto, Japan, [3]Department of Physiological Chemistry, Kyoto University Graduate School of Pharmaceutical Sciences, Kyoto, Japan, [4]Department of Biological Science and Technology, Faculty of Industrial Science and Technology, Tokyo University of Science, Tokyo, Japan

Repeated environmental stress induces neural inflammation along with depression and increased anxiety. Innate immune molecules such as toll-like receptors (TLR) recognize exogenous and endogenous ligands to provoke inflammatory response. In this study, we found that social avoidance and elevated anxiety induced by social defeat stress was abolished in TLR2 and TLR4 double knockout mice. These mice neither exhibited decreased neuronal response, neuronal dendrite retraction, nor changes in microglia activity observed in medial prefrontal cortex (mPFC) of wild-type mice. TLR2 and TLR4 knockdown specifically in mPFC microglia suppressed repeated social defeat stress induced-social avoidance.. Repeated social defeat stress increased interleukin-1α and TNF-α expression in mPFC microglia in TLR2 and TLR4 dependent manner. Inhibition of these inflammatory cytokines by neutralizing antibodis in mPFC reduced social avoidance induced by social defeat stress. These results demonstrated that repeated social defeat stress evoked microglial activation in mPFC through TLR2 and TLR4, and released inflammatory cytokines induced social avoidance presumably through neuronal changes.

2-P-024

Elucidating neuronal projections from the medial prefrontal cortex responsible for the resilience to social defeat stress in mice

Chisato Numa[1], Hirotaka Nagai[1], Tomoyuki Furuyashiki[1]

[1]Div. Pharmcol., Grad. Sch. Med. Kobe Univ.

Stress caused by aversive stimuli, if not excessive, is thought to provoke adaptive biological responses in rodents and primates. We have previously shown that single social defeat stress in mice activates dopamine D1 receptor in excitatory neurons of the medial prefrontal cortex (mPFC), leading to dendritic hypertrophy of these neurons and strengthening stress resilience. However, it remains elusive which brain regions mediate the action of the mPFC for stress resilience. In the present study, using c-Fos immunohistochemistry, we examined neuronal responses to single social defeat stress in multiple brain regions of adult male C57BL/6 mice. We found that the stress activated neurons in several subcortical brain regions, such as the bed nucleus of the stria terminalis (BNST), lateral septal nucleus and amygdala nuclei, which receive projections from the mPFC. We are currently exploring roles of mPFC projections to these brain areas in stress resilience by manipulating the activities of these projections using chemogenetics. Our preliminary finding points to the potential role of mPFC projection to some of these brain areas. Thus, our study paves the way for the notion that dopamine D1 receptor signaling in the mPFC coordinates its projections to subcortical areas upon short-term stress, thereby facilitating stress resilience.

2-P-025

Antidepressant effects of XJ-Et-8 in mice chronically exposed to corticosterone

Jiajing Shan[1,2], Akihiro Mouri[3,5], Yang Yang[1,2], Qiaohui Lu[2,3], Kazuo Kunisawa[4], Tomoaki Teshigawara[1], Mami Hirakawa[3], Yuko Mori[1], Yasuko Yamamoto[1], Zou Libo[2], Toshitaka Nabeshima[4,5], Kuniaki Saito[1,5]

[1]Dept. Dis. Cont.and Prevention, , Fujita Health Univ. Grad. Sch. of Health Sci., [2]Dept. Pharmacol., Life Sci. and Biopharm. Sch., Shenyang Pharm.l Univ., [3]Dept. Reg. Sci. , Fujita Health Univ. Grad. Sch. of Health Sci., [4]Adv. Diag. Sys. Res. Lab., Fujita Health Univ. Grad. Sch. of Health Sci., [5]Jpn. Drug Org. of Appropriate Use and Res.

High cortisol level in serum is one of the clinical features in depression. Exogenous administration of corticosterone (CORT) in rodents has been used as animal model of depression. Red resin of *Dracaena cochinchinensis S.C. Chen*, known as Chinese dragon's blood, has been used as a famous and precious traditional medicine since ancient times by many cultures. XJ-Et-8 is a compound extracted from Chinese dragon's blood. It has favorable effects on mouse models of Alzheimer' Diseases through the up regulating the BDNF level in the brain. The present study aimed to evaluate the XJ-Et-8 as antidepressant using a mouse model of CORT administration. CORT (20 mg/kg/day) was administered subcutaneously for 3 weeks, and XJ-Et-8 was given orally during the last 2 weeks. After corticosterone administration, mice were sequentially subjected behavioral tests: open field test, social interaction test, novelty suppressed feeding test, and forced swimming test. Corticosterone administration induced depressive and anxious behaviors and decrease of phosphorylation in AKT/mTOR/CREB signaling pathway and of BDNF contents in the prefrontal cortex. XJ-Et-8 reversed these behavioral changes, increased phosphorylation level in AKT/mTOR/CREB pathway and BDNF expression. These results suggest that the XJ-Et-8 could be a potential compound as an antidepressant via activating the AKT/mTOR/CREB pathway and BDNF expression.

2-P-026

Effect of Sertraline on decreased spontaneous activity of OVX mice

Megumi Furukawa[1], Nobuo Izumo[1], Masahiro Toho[2], Kosuke Hayamizu[1], Makoto Nakano[1], Takayuki Manabe[3], Yasuo Watanabe[1]

[1]Genar. Health Medi. Cen. Yokohama Univ. Pharm., [2]Lab. Pharmacotherapy, Yokohama Univ. Pharm., [3]Lab. Neuroanatomy and Neuropharmacol. Faculty of Nursing

We have already reported that ovariectomized (OVX) rats reduced spontaneous activity and serotonin release levels of the amygdala in the dark term (*B.B.R.* 227(1)1-6(2012)). In this study, we examined whether sertraline as a SSRI recovered a spontaneous activity on OVX induced despair-like behaviors mice. The female ICR mice of 9-week old were received ovariectomy or sham operation. Sertraline (10 mg/kg/day, s.c.) or saline were administered to each groups for 8 weeks (6 times/week) starting from 8 weeks after OVX. The spontaneous activity of the mice was evaluated by using an activity sensor at dark term (19:00-7:00). Moreover, RNA expression levels of tryptophan hydroxylase (TPH) and XBP-1 were measured in hippocampus and prefrontal cortex by Real-time PCR. In the result, we revealed that these OVX induced despair-like behaviors were improved by administration of sertraline. In result of RT-PCR, sertraline significantly suppressed down-regulation of TPH expression level in hippocampus induced by OVX. In addition, sertraline significantly suppressed up-regulation of XBP-1 expression level in hippocampus induced by OVX. These results suggested that sertraline could improve the decrease in spontaneous activity mediated by serotonin level in hippocampus by the OVX.

2-P-027

Chronic unpredictable mild stress-induced depressive behavioral changes are associated with dopaminergic hyperfunction in the nuclues accumbens and serotonergic hypofunction in the prefrontal cortex and hippocampus of mice

Akihiro Mouri[1,5], Lu Qiaohui[1,2], Yang Yang[2,3], Kazuo Kunisawa[4], Tomoaki Teshigawara[3], Mami Hirakawa[1], Yuko Mori[3], Yasuko Yamamoto[3], Li-Bo Zou[2], Toshitaka Nabeshima[4,5], Kuniaki Saito[3,5]

[1]Dept. Reg. Sci., Fujita Health Univ., Grad. Sch. of Health Sci., [2]Dept. Pharmacol., Life Sci. and Biopharm. Sch., Shenyang Pharm. Univ., [3]Dept. Dis. Cont. and Prevent., Fujita Health Univ. Grad. Sch. of Health Sci., [4]Adv. Diag. Sys. Res. Lab., Fujita Health Univ., Grad. Sch. of Health Sci., [5]Japanese Drug Org. of Appropriate Use and Res.

Augmenting evidences disclose that stressful events evoke molecular alteration in brain, considered as a pathology in major depressive disorder (MDD). Chronic unpredictable mild stress (CUMS) induced hyperactivity in novel environment, decrease of social interaction time in social interaction test, prolongation of feeding latency in novelty suppressed feeding test, and enhancement of immobility in forced swimming test. The contents of dopamine and its metabolites, Dopamine (DA) turnover and protein level of tyrosine hydroxylase (TH) were increased by CUMS in the nucleus accumbens. The contents of serotonin, and protein levels of tryptophan hydroxylase (TPH) and TH were decreased by CUMS in the hippocampus and prefrontal cortex. Accompanies with activation of dopaminergic function, phosphorylation levels of ERK, Akt, and CREB were increased by CUMS in the nucleus accumbens. Administration of fluoxetine and aripiprazole during CUMS prevented the abnormal behavioral changes. These data suggest that CUMS induced depressive behaviors are associated with dopaminergic hyperfunction and ERK/Akt/CREB pathway in the nuclues accumbens, and serotonergic hypofunction in the prefrontal cortex and hippocampus.

2-P-028

Analysis of epigenomic changes in prefrontal microglia induced by repeated social defeat stress.

Masayuki Taniguchi[1], Shiho Kitaoka[1], Shigehiro Kuraku[2], Mitsutaka Kadota[2], Tomoyuki Furuyashiki[1]

[1]Div. Pharmacol., Grad. Sch. Med., Kobe Univ., [2]Lab. Phyloinformatics, RIKEN BDR

Stress is caused by various adverse environments, and often causes emotional changes including depression and elevated anxiety. Using social defeat stress in mice, we previously reported that repeated social defeat stress activates microglia in the medial prefrontal cortex (mPFC), then decreases dendritic arborization of mPFC pyramidal neurons and leads to social avoidance. Recently, we found that microglial activation in the mPFC occurs more rapidly and strongly with repetition of social defeat stress. This finding led us to speculate that repetition of social defeat stress induced persistent epigenomic changes of microglia in the mPFC. However, due to the limited sensitivity of chromatin immune precipitation sequencing (ChIP-seq), a brain region- and cell type-specific epigenomic analysis has been challenging. Here we optimized the protocol of ChIP-seq for epigenomic analyses of mPFC microglia isolated by fluorescence-activated cell sorting. This protocol allows us to detect enrichment of active histone marks near microglia-specific genes in mPFC microglia. We are currently investigating repeated social defeat stress-induced epigenomic changes of mPFC microglia associated with emotional changes.

2-P-029

Infusion of resolvin E1 into the medial prefrontal cortex attenuates lipopolysaccharide-induced depression-like behaviors via BDNF/VEGF release and mTORC1 activation

Satoshi Deyama[1], Kohei Ishimura[2], Hayato Fukuda[2,3], Satoshi Shuto[2], Masabumi Minami[4], Katsuyuki Kaneda[1]

[1]Lab. Mol. Pharmacol., Inst. Med., Pharmaceut., Health Sci., Kanazawa Univ., [2]Lab. Org. Chem. for Drug Develop., Grad. Sch. Pharmaceut. Sci, Hokkaido Univ., [3]Pharmaceut. Org. Chem. Lab., Grad. Sch. Biomed. Sci., Nagasaki Univ., [4]Dept. Pharmacol., Grad. Sch. Phamaceut. Sci., Hokkaido Univ.

We have recently demonstrated that infusion of eicosapentaenoic acid-derived resolvin E1 (RvE1; 50 pg/side) into the medial prefrontal cortex (mPFC) exerts antidepressant effect in a murine lipopolysaccharide (LPS)-induced depression model. In the present study, we examined the roles of brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), and their downstream mTORC1 in the antidepressant actions of intra-mPFC RvE1 infusion in LPS-induced depression model mice using the tail suspension and forced swim tests. The results demonstrate that the antidepressant effects of intra-mPFC RvE1 infusion are completely blocked by co-infusion of a BDNF neutralizing antibody (nAb), a VEGF nAb or an mTORC1 inhibitor rapamycin. We also demonstrate that the antidepressant effects of intra-mPFC BDNF or VEGF infusion are blocked by co-infusion of rapamycin. Together, the current results indicate that BDNF/VEGF release and subsequent activation of mTORC1 in the mPFC are required for the antidepressant actions of RvE1.

2-P-030

Involvement of intracerebral hemorrhage-associated depression and increase of indoleamine 2,3-dioxygenase

Marina Akagi[1], Masatoshi Ohnishi[1], Atsuko Inoue[1]

[1]Grad. Sch. Pharm., Fukuyama Univ.

Five-hydroxytryptamine (5-HT) is made from L-tryptophan and known to play some roles in depressive states. Indoleamine 2, 3-dioxygenase (IDO) is the rate-limiting enzyme in the kynurenine pathway, converting tryptophan to kynurenine. We investigated the role of IDO in the intracerebral hemorrhage (ICH)-associated depression using an *in vivo* mouse model microinjected collagenase type VII into the striatum. IDO mRNA transiently increased at 3 days after ICH and was continuously high until day 21. IDO was expressed on 5-HTergic neurons and its protein level and activity increased 3 days after ICH. The 5-HT level decreased 3 days after ICH, which was reversed by the s.c. injection of 1-methyl tryptophan (1-MT), an IDO inhibitor. Taken together, the IDO increase was suggested to contribute to the down-regulation of 5-HT after ICH. Next, we investigated the mouse behavior relating to the depression. The immobility time of ICH mice was prolonged in the forced swim test, and the time was reversed by 1-MT at day 14. These results suggested that the down-regulation of 5-HT level due to the IDO increase after ICH is involved in the depression by the decrease of the stress tolerance.

2-P-031

Evaluation of spontaneous pain using grimace scale in the reserpine-induced fibromyalgia model rat

Reina Miura[1], Machiko Miwa[1], Miku Yoshida[1], Shigeharu Tanei[1], Yukinori Nagakura[1,2]

[1]Faculty of Pharmaceutical Sciences, Aomori University, [2]Center for Brain and Health Sciences, Aomori University

Fibromyalgia (FM) is a health burden due to its high prevalence, negative impact on patients' quality-of-life and lack of effective therapies. Given the primary symptom in FM patients is widespread spontaneous pain, the pain measurement using indicator reflecting patients' spontaneous pain should be implemented in the preclinical research for FM. The present study applied the rat grimace scale (RGS), coding of facial expressions, to the reserpine-induced myalgia (RIM) rat, a well-validated animal model of FM. Animals were videotaped by two high-resolution video cameras. Still images of the animal face were then captured. RGS scoring was conducted according to the method described by Sotocinal et al (2011). The RIM rat exhibited a long-lasting increase of RGS score. Time-course of the change of RGS score was compared to that of paw withdrawal threshold measured by von Frey hair filament, a conventional method for evoked pain. The elevated RGS score showed varied responses to clinically used analgesic drugs. The present study suggests that RGS score in the RIM rat simulates spontaneous pain in FM patients. This pain measurement scheme would contribute to the prediction of efficacies of analgesic therapies for FM patients.

2-P-032

The changes of c-Fos expression in each brain areas of early life stress mice after morphine injection

Shogo Tokuyama[1], Tsutomu Ichimizu[1], Asami Imanishi[1], Kazuo Nakamoto[1]

[1]Dept. Clin. Pharmacy, Sch. Pharmaceu. Sci., Kobe Gakuin Univ.

Previously, we have developed an early life stress model mice which subjected to maternal separation combined with social isolation (MSSI). These mice showed emotional dysfunction associated with early life stress, and exacerbated nerve injury-induced mechanical allodynia. In this study, to elucidate the mechanism underlying early life stress-induced increase of pain sensitivity, we investigated the changes of each opioid receptors mRNA expression in an each brain area of MSSI mice. Furthermore, we tested the changes of c-Fos induction in each brain area of MSSI mice after morphine injection by using immunohistochemical study. In the periaqueductal gray (PAG) area,a region that is implicated in the opioid control of nociception, m-, d-andk-opioid receptor (MOR, DOR and KOR) mRNAexpression were significantly decreased in MSSI model mice compared to control mice. A large number of c-Fos positive cells were observed in the PAG are of Control mice with morphine injection. On the other hand, in the PAG of MSSI model mice, c-Fos positive cells are hardly detectable after morphine injection. Finally, we conclude that MSSI induced decrease of MORmRNA expression and neuronal activity in the PAG area, suggesting that this phenomenon could be induced the increase of pain sensitivity in MSSI model mice.

2-P-033

Possible involvement of tachykinin neurokinin-1 receptors on nociceptive behaviors induced by intrathecally administered cholecystokinin-8

Takafumi Hayashi[1], Soh Katsuyama[2], Tsuneyoshi Suzuki[1], Shinobu Sakurada[3]

[1]Lab. Pharmaceu. Sci., Faculty Pharmaceu. Sci., Tohoku Med. Pharmaceu. Univ., [2]Center Experiential Pharm. Practice, Faculty Pharmaceu. Sci., Tokyo Univ. Pharmaceu. Life Sci., [3]Dept. Physiol. Anato., Faculty Pharmaceu. Sci., Tohoku Med. Pharmaceu. Univ.

Intrathecal (i.t.) injection of the sulfated octapeptide cholecystokinin (CCK-8) elicited a behavioral response consisting of scratching, biting and licking in mice. CCK-8-induced behavioral response was evoked significantly 5 - 10 min after i.t. injection and reached a maximum at 20 - 25 min. Dose-dependency of the induced response showed a bell-shaped pattern from 1 zmol to 25 pmol, and the maximum effect was observed at 10 amol and 10 pmol. The behavioral response elicited by CCK-8 (10 amol and 10 pmol) was dose-dependently inhibited by i.t. administration of CCK-B receptor antagonist, CI-988. The CCK-A receptor antagonist, SR-27897, had no effect on the response elicited by CCK-8. The tachykinin neurokinin-1 (NK_1) receptor antagonists, CP-99,994 and sendide, inhibited CCK-8 (10 pmol)-induced behavioral response in a dose-dependent manner. No significant reduction of CCK-8 (10 amol)-induced response was detected co-administration of NK_1 antagonists. These results suggest that the nociceptive behaviors induced by i.t. administration of CCK-8 are mediated through the spinal CCK-B receptors and NK_1 receptors.

2-P-034

Involvement of NMDA receptors in the development of mirror image pain

Chizuko Watanabe[1], Masaru Yoshizumi[1], Suzune Kawase[1], Shinobu Sakurada[1], Hirokazu Mizoguchi[1]

[1]Dept. Physiol. Anat., Tohoku Med. Pharm. Univ.

Damage on one side of the body evoked pain bilaterally on both the injured and uninjured sides. The abnormal phenomenon called mirror image pain, is generally observed in many clinical pain syndromes and in various animal pain models. However, its mechanism is not fully understood. In the present study, we investigated the mechanism of mirror image pain in the complete Freund's adjuvant (CFA)-induced inflammatory pain model measuring the revealed mechanical allodynia by von Frey filament test. After CFA injection, the paw withdrawal threshold to mechanical stimuli was significantly decreased not only in the ipsilateral paw (CFA injected paw) but also in the contralateral paw (CFA non-injected paw). The decrease in mechanical threshold of the contralateral paw, which is called mirror image pain, had delayed onset as compared to the ipsilateral mechanical allodynia. NMDA receptor antagonist treated after CFA injection did not attenuate bilaterally allodynia, however, pretreatment with NMDA receptor antagonist was significantly attenuated the development of mirror image pain, without affecting the ipsilateral mechanical allodynia. These results suggest that the mechanism of mirror image pain via NMDA receptor activation was different from ipsilateral mechanical allodynia.

2-P-035

Inhibitory effect of etidronate on partial sciatic nerve ligation-induced hyperalgesia in mice

Wataru Nemoto[1], Ryota Yamagata[1], Osamu Nakagawasai[1], Wan-Yi Hung[1], Kazuhiro Shima[2], Yasuo Endo[2], Koichi Tan-No[1]

[1]Dept. Pharmacol., Fac. Pharm. Sci., Tohoku Med. Pharm. Univ., [2]Div. Oral Mol. Regul., Grad. Sch. Dent., Tohoku Univ.

We have shown that intrathecal (i.t.) administration of etidronate (Eti) into mice produces an analgesic effect against the capsaicin-induced nociceptive behavior. However, the effect of Eti on neuropathic pain at the spinal level remains unknown. Therefore, we examined whether Eti attenuates pain after partial sciatic nerve ligation (PSNL). PSNL-induced tactile hyperalgesia observed on day 7 after the surgery was attenuated by oral and i.t. administration of Eti. The anti-hyperalgesic effect of i.t.-administered Eti was completely inhibited by an i.t. administration of ATP. The solute carrier family, SLC17, mediates the transport of pain transmitters, like ATP and glutamate. Indeed, we detected several members of the SLC17 family in the mouse dorsal lumbar spinal cord. Among the detected mRNAs, only Slc17a9, encoding for neuronal vesicular ATP transporter, was significantly increased upon PSNL. SLC17A9 protein levels were also significantly increased. In mice subjected to PSNL, SLC17A9 was present in neurons and microglia of the superficial dorsal horn. Collectively, our results suggest that Eti produces its anti-hyperalgesic effects by inhibiting SLC17A9-dependent exocytotic ATP release from the dorsal horn in mice subjected to PSNL.

2-P-036

Effects of 6-prenylnaringenin, a hop component, and its derivative, KTt45, on T-type Ca^{2+} channels, cannabinoid receptors and intractable pain

Yoshihito Kasanami[1], Reika Onishi[1], Takahiro Kino[1], Fumiko Sekiguchi[1], Maho Tsubota[1], Takaya Miyazaki[1], Shiori Hiramoto[1], Kyoko Okazaki[1], Huy Du Nguyen[2], Takuya Okada[2], Naoki Toyooka[2], Shigeru Yoshida[3], Tsuyako Ohkubo[4], Atsufumi Kawabata[1]

[1]Lab. Pharmacol. Pathophysiol., Fac. Pharm., Kindai Univ., 3-4-1 Kowakae, Higashi-Osaka 577-8502, Japan, [2]Grad. Sch. Sci. Technol., Univ. Toyama, 3190 Gofuku, Toyama 930-8555, Japan., [3]Dept. Life Sci., Fac. Sci. Engineer., Kindai Univ., 3-4-1 Kowakae, Higashi-Osaka 577-8502, Japan, [4]Div. Basic Med. Sci. Fundam. Nurs., Fac. Nurs., Fukuoka Nurs. Coll., 2-15-1 Tamura, Fukuoka 814-0193, Japan

T-type Ca^{2+} channels (T-channels) serve as targets for treatment of pain. We have reported that 6-prenylnaringenin (6-PNG), a hop component, and its derivative, KTt45, inhibit T-channels. Interestingly, many of cannabinoids block T-channels, while some of T-channel blockers stimulate cannabinoid CB_1 or CB_2 receptors. Thus, we compared the effects of 6-PNG and KTt45 as well as cannabinoids on $Ca_v3.2$ T-channels and CB_1/CB_2 receptors. In $Ca_v3.2$-expressing HEK293 cells, 6-PNG, KTt45, ACEA, a CB_1 agonist, and cannabidiol, a possible CB_1 antagonist/CB_2 inverse agonist, blocked T-currents, as assessed by whole-cell recordings; IC_{50} values (μM) were 0.69, 0.41, 0.57 and 0.64, respectively. Neither 6-PNG nor KTt45, exhibited agonistic activity in CB_1-expressing HEK293 cells, whereas 6-PNG at 0.3-1 μM, but not KTt45, agonistic activity in CB_2-expressing CHO cells. In distinct mouse models for neuropathic and bladder pain, i.p. administration of 6-PNG at 20-30 mg/kg and KTt45 at 10-30 mg/kg exhibited analgesic/anti-allodynic activity. Thus, 6-PNG and KTt45, T-channel blockers, are useful for treatment of intractable pain, and 6-PNG is considered a mixed T-channel blocker/CB_2 agonist.

2-P-037

Involvement of $Ca_v3.2$ T-type channel in inflammatory pain via an interaction with nociceptive TRPA1.

Minami Nakagawa[1], Kenji Takahashi[1], Toshio Ohta[1]

[1]Dept. Vet. Pharmacol., Fac. Agric., Tottori Univ.

Low voltage-activated Ca^{2+} channel ($Ca_v3.2$) and TRPA1 play a key role in inflammatory and neuropathic pain. We previously reported the functional interaction between $Ca_v3.2$ and TRPA1. However, little is known about the significance of this interaction in pathological conditions. Here, we investigated possible involvement of these channel interactions in inflammatory pain model constructed by intraplantar injection of CFA. At the inflammatory side of DRGs, the protein expression of $Ca_v3.2$, but not TRPA1 was increased. mRNA of both channels was unchanged by CFA. Depolarizing pulses evoked inward currents which were inhibited by NNC 55-0396, a T-type channel blocker, and were enlarged in the TRPA1-expressing DRG neurons at the inflammatory side. The $Ca_v3.2$-mediated $[Ca^{2+}]_i$ increases were enhanced and the inhibitory rate by HC030031, a TRPA1 blocker, was greater in the TRPA1-expressing DRG neurons at the inflammatory side. In the present study, we showed that the augmentation of $[Ca^{2+}]_i$ response to $Ca_v3.2$ activation may be mediated by the interactions of TRPA1 in addition to the increment of $Ca_v3.2$ expression under inflammatory conditions. These data suggest that both channels are promising therapeutic targets for inflammatory pain.

2-P-038

Formalin-induced nociceptive response is enhanced by serum exosomes isolated from partial sciatic nerve ligation (PSL) mice

Kengo Hamamura[1], Soh Katsuyama[2], Takaaki Komatsu[3], Tsukasa Sakurada[4], Kosuke Aritake[1]

[1]Chem. Pharm. Lab., Pharm. Sci., Daiichi Univ. of Pharm., [2]Ctr. Exp. Pharm. Edu., Pharm. Sci., Daiichi Univ. of Pharm., [3]Drug Anal. Lab., Pharm. Sci., Daiichi Univ. of Pharm., [4]Ctr. Sort. Pharm. Edu., Pharm. Sci., Daiichi Univ. of Pharm.

Exosomes are small (40-150 nm) membrane vesicles of endocytic origin that are found in bodying fluids, and supporting their role in intercellular communication. Although recent studies have demonstrated that various biomarkers involved in the extent of pain from the serum exosomes, the effects of exosomes on the onset and progression of pain have not been elucidated. The objective of this study was to examine the effects of serum exosomes in mice with PSL on nociceptive responses induced by 0.5% formalin.

We have confirmed that the i.t. injection of serum exosomes from PSL mice or sham-operated mice transferred into the normal mice did not show any spontaneous nociceptive responses. However, 0.5% formalin-induced nociceptive response was significantly enhanced by i.t. pretreatment with serum exosomes isolated from PSL mice but not from sham mice. In addition, we digested the exosomes isolated from PSL with trypsin to obtain the ‘‘surface protein shaved" exosomes. The surface protein shaved PSL exosomes were ineffective on formalin-induced response.

Our data indicate that the surface protein of exosomes in mice with PSL may play an important role in enhancing nociceptive responses.

2-P-039

The role of triply nitric oxide synthases in brain cerebral ischemia.

Kanako Kuniyoshi[1], Haruaki Kubota[1,2], Katsuhiko Noguchi[1], Mayuko Sakanashi[1], Toshihiro Matsuzaki[1], Jyunko Nakasone[1], Hiroaki Shimokawa[3], Kazuhiro Sugahara[3], Manabu Kakinohana[2], Masato Tsutsui[1]

[1]Department of Pharmacology and , [2]Department of Anesthesiology in Graduate School of Medicine at Ryukyu University., [3]Department of Cardiology in Tohoku University

There are some reports that neuronal and inducible NOSs (nNOS, iNOS) exacerbate cerebral infarction whereas endothelial NOS (eNOS) conversely alleviates cerebral infarction in a model of middle cerebral artery (MCA) occlusion. But the role of the whole NOSs system in cerebral infarction is not clarified yet. Although it has been examined in pharmacological studied with non-selective NOS inhibitors, the results are quite inconsistent, possibly because of non-specificity of the agents. In order to investigate the role of the whole NOSs system in the pathogenesis of cerebral infarction, in the study, we generated mice in which all 3 NOS isoforms are completely disrupted, and compared cerebral infarct size after middle cerebral artery occlusion between the triple NOSs$^{-/-}$ and wild-type.

2-P-040

The protective effect of a novel radical scavenger, NSP-116 on cerebral ischemia injury

Takahiko Imai[1], Sena Iwata[1], Miyo Daisuke[1], Shinsuke Nakamura[1], Masamitsu Shimazawa[1], Hideaki Hara[1]

[1]Molecular Pharmacology, Department of Biofunctional Evaluation, Gifu Pharmaceutical University, Gifu, Japan.

For patient outcome with stroke, recanalization of occlusion vessels is an effective therapy. However, recanalization induces oxidative stress via oxygen over-supply, which leads to neuronal damage as ischemia reperfusion injury (IRI). NSP-116 is a novel radical scavenger, and our previous study showed that NSP-116 suppressed oxidative stress. Therefore, we speculated that NSP-116 could ameliorate IRI damage. The purpose of this study is to investigate the effect of NSP-116 on IRI.

We established the IRI mouse model by using middle cerebral artery occlusion/ reperfusion (MCAO/R). NSP-116 (30 mg/kg) was orally administrated at 1 day before surgery and immediately after reperfusion. We evaluated the cerebral blood flow (CBF), neurological symptom and infarct volume after MCAO/R. In addition, using *in vitro* neural injury models, we assessed whether NSP -116 had the directly neuroprotective effect. NSP-116 administration improved neurological deficit and reduction of CBF. In infarct volume assay, NSP-116 had protective tendency. Moreover, NSP -116 pre-treatment suppressed the neuronal cell death in *in vitro* experiments.

Collectively, our findings suggested that NSP-116 could be useful for ischemic stroke therapy.

2-P-041

Effect of angiotensin II type 2 receptor on cerebral ischemic injury in mice with fetal growth restriction

Haruka Narumoto[1], Jun Iwanami[1], Bao-Shuai Shan[1], Masaki Mogi[2], Aoi Narumoto[1], Li-Juan Min[1], Masatsugu Horiuchi[1]

[1]Department of Molecular Cardiovascular Biology and Pharmacology, Ehime University, Graduate School of Medicine, [2]Pharmacology, Ehime University, Graduate School of Medicine

We previously observed that vascular remodeling in response to vascular injury is exaggerated in fetal growth restriction (FGR) mice. We reported that angiotensin II type 2 receptor (AT_2R) stimulation prevented cerebral ischemic damage. The AT_2R is highly expressed in fetal mice. However, the effects of AT_2R on ischemic brain damage in FGR mice is unclear. Therefore, we investigated the roles of AT_2R in brain damage in FGR mice using transgenic mice with overexpressed AT_2R in vascular smooth muscle cell ($smAT_2$-Tg) mice. Dams (wild-type and $smAT_2$-Tg mice) were fed an isocaloric diet containing 20% protein (NP) or 8% protein (LP) until delivery. On the day of delivery, all dams were returned to the NP diet. After weaning, offspring were fed the NP diet. When male offspring were 10 weeks of age, cerebral ischemic injury was induced by transient middle cerebral artery occlusion (MCAO). Systolic blood pressure did not differ among all groups at 10 week of age. Ischemic area 48 hours after MCAO in NP mice was smaller in $smAT_2$-Tg mice. Stroke size in WT-LP mice was significantly larger compared with WT-NP mice. This aggravation of stroke size by LP was weaker in $smAT_2$-Tg-LP mice. Cerebral blood flow in the whole brain in $smAT_2$-Tg mice was attenuated compared with that in WT mice at 48 hours after MCAO. These results suggested that AT_2R could enhance the cerebral protective effects in FGR at least in part due to the increase of CBF after ischemia.

2-P-042

A novel strategy for treatment of cancer cachexia targeting the altered purine metabolism in the brain

Miaki Uzu[1], Miki Nonaka[1], Kanako Miyano[1], Hiromi Sato[2], Nagomi Kurebayashi[3], Takashi Murayama[3], Takashi Sakurai[3], Akihiro Hisaka[2], Yasuhito Uezono[1,4]

[1]Div. Cancer Pathophysiology, National Cancer Center Research Institute, [2]Lab. Clinical Pharmacology & Pharmacometrics, Grad. Sch. Pharmaceut. Sci., Chiba Univ., [3]Dept. Pharmacology, Juntendo Univ. Sch. Med., [4]Div. Supportive Care Research, Exploratory Oncology Research & Clinical Trial Center, National Cancer Center

[Background] Cancer cachexia is a systemic wasting syndrome, which is characterized by anorexia and the loss of body weight. The central nervous system (CNS) plays a critical role in controlling an appetite. Therefore, this study describes the efficacy of targeting the altered metabolic pathway in the CNS in cachexic mice.

[Methods] Anesthetized 8-week-old male BALB/c nu/nu mice were subcutaneously inoculated with a human gastric cancer cell line, 85As2. Two weeks after inoculation, the brain was collected and quantitative alteration of 96 metabolites was determined using a CETOF-MS system. Xanthine oxidase (XO) activity was also measured by using a fluorescent XO substrate.

[Results] Subcutaneous implantation of 85As2 cells induced progressive tumor growth and significant body weight loss in two weeks, accompanied by gradual decrease of food consumption. Metabolome analysis using the brain showed the decrease of ATP accompanied by the increase of uric acid. Moreover, enzyme activity of XO was increased in the brain of cachexic mice.

[Conclusion] These results suggest that the purine metabolism is activated at the onset of cancer cachexia. Therefore, the effect of febuxostat, a XO inhibitor, is being investigated.

2-P-043

Propranolol prevents changes in cerebral blood flow and pain-related behaviors in migraine model mice

Yuki Kurauchi[1], Makito Haruta[3], Risako Tanaka[1], Kiyotaka Sasagawa[3], Jun Ohta[3], Akinori Hisatsune[2], Takahiro Seki[1], Hiroshi Katsuki[1]

[1]Department of Chemico-Pharmacological Sciences, Graduate School of Pharmaceutical Sciences, Kumamoto University, [2]Priority Organization for Innovation and Excellence, Kumamoto University, [3]Division of Materials Science, Graduate School of Science and Technology, Nara Institute of Science and Technology

Propranolol, a beta-adrenergic receptor blocker, is one of the most commonly used prophylactic drugs for migraines. Cortical spreading depression (CSD) is the propagation wave of neuronal excitation along with cerebral blood flow (CBF) changes over the cerebral cortex and has been implicated in the pathological process of migraine auras and its pain response. However, the effect of propranolol on CSD-related CBF changes and behavioral responses remains poorly understood. Here, we measured CSD-related CBF responses using a micro-device with a green light emitting diode (LED) and micro-complementary-metal-oxide-semiconductor (CMOS) image sensor and evaluated pain-related reduced locomotor activity in mice. An injection of KCl into the cortex caused CSD-related CBF changes; however, propranolol prevented the increase in CBF as well as delayed the propagation velocity in KCl-induced CSD. Furthermore, KCl injection reduced locomotor activity and induced freezing behavior in awake and freely moving mice, which were prevented by propranolol treatment. These results suggest that the modulation of CSD-related CBF responses by the blockade of b-adrenergic receptor contributes to its prophylactic effects on migraines.

2-P-044

Adenosine receptor-induced synaptic protection against ischemia under mild hypothermal conditions in the mouse hippocampus

Masahito Kawamura[1]

[1]Dept. Pharm, Jikei Univ. Sch. Med.

The therapeutic hypothermia for acute stroke might play an important role in neuroprotection. However, the mechanisms are complex and not yet fully understood. Here we investigated the role of adenosine A_1 receptors in mild hypothermia-mediated neuroprotection during the acute phase of ischemia. Severe ischemia-induced neurosynaptic impairment was mimicked by oxygen-glucose deprivation at normothermia (36°C) with extracellular recordings or whole-cell patch clamp recordings in acute hippocampal slices in mice. Mild hypothermia (32°C) induced the protection of synaptic transmission by activating adenosine A_1 receptors. Moderate hypothermia (28°C) caused additional neuroprotective effects by extending the onset time to the anoxic event; however, this effect was not associated with adenosine A_1 receptor. The response of adenosine-induced inhibition of hippocampal synaptic transmission was increased by lowering temperature to 32°C or 28°C. This study might reveal the involvement of adenosine in the therapeutic hypothermia (usually done at 32 -33 °C) for acute stroke.

2-P-045
Association with blood cholesterol and cerebral aneurysm.

Kazuya Hokamura[1], Hiroshi Makino[2], Tomo Suzuki[3], Takayuki Iwaki[4], Ryou Imai[2], Yoshiki Nakajima[2], Hiroki Namba[3], Kazuo Umemura[4]

[1]Dept. Med Edu., Hamamatsu Sch. of Med.,, [2]Dept. Anesth & Critical Care Med., Hamamatsu Sch of Med., [3]Dept. Neuro Surg., Hamamatsu Sch. of Med., [4]Dept. Pharmacol., Hamamatsu Sch. of Med.

[Introduction] Subarachnoid hemorrhage (SAH) is a life-threatening and can be frequently caused by a ruptured aneurysm of cerebrovascular blood vessels. Although one third of patients will survive with good recovery; one-third will survive with a disability; and one-third will die. It is well accepted that lowering blood cholesterol level is mandatory in prevention of cerebral circulatory disorder. However, the relationship between cholesterol and cerebral aneurysm is still controversial.In this study, we elucidate the above relationship by established aneurysm model in LDL receptor and Apobec 1 double knock out mice ($L^{-/-}$, $A^{-/-}$) and control mice.

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

[Results and conclusion] Lesser amount of cerebral aneurysm and subarachnoid hemorrhage were detected in $L^{-/-}$, $A^{-/-}$ mice compared to control mice. It is still needed to clarify whether the blood cholesterol is directly related to rupture of cerebral artery or through enhancement of blood coagulation system.

2-P-046

Donepezil decreases tau hyperphosphorylation induced by hypothermia *in vivo* and *in vitro*

Ryosuke Nakanishi[1], Yuki Takada-Takatori[2], Kayoko Takara[2], Konami Takashima[2], Katsuharu Tsuchida[2], Yasuhiko Izumi[3], Akinori Akaike[1], Tomohiro Miyasaka[4], Toshiaki Kume[1,5]

[1]Dept. Pharmacol., Grad. Sch. Pharm. Sci., Kyoto Univ., [2]Dept. Ratio. Med. Sci., Fac. Pharm. Sci., Doshisha Women's Col., [3]Lab. Pharmacol., Kobe Pharm. Univ., [4]Dept. Neuropathol., Fac. Life and Med. Sci., Doshisha Univ., [5]Dept. Appl. Pharmacol., Grad. Sch. Med. and Pharm. Sci., Univ. of Toyama.

Tau hyperphosphorylation is one hallmark of Alzheimer's disease (AD). Donepezil is a potent and selective acetylcholinesterase inhibitor developed for the treatment of AD. However, broad therapeutic effects of donepezil cannot be fully explained only by cholinergic hypothesis. Here, we investigated the effects of donepezil on tau hyperphosphorylation *in vivo* and *in vitro*. First, we examined whether donepezil reduces tau hyperphosphorylation in the brains of hypothermia mice model induced by anesthesia. Tau phosphorylations detected by anti-phospho tau antibodies, AT8 and PHF1, were significantly increased in anesthetized mice brains. Pretreatment with donepezil for 24 hr inhibited the tau phosphorylation. This was reproduced *in vitro* model of tau hyperphosphorylation induced by hypothermia using rat primary culture cortical neurons. We also found that the Glycogen synthase kinase-3b (GSK3b) inhibitor decreased in tau phosphorylation *in vitro* hypothermia model. *In vitro* kinase assay showed that donepezil suppressed the phosphorylation of purified recombinant tau by GSK3b. These results suggest that donepezil prevents tau hyperphosphorylation induced by hypothermia *in vivo* and *in vitro* through GSK3b inhibition.

2-P-047

Pharmacological effect of alkannin on amyloid β aggregation and neuronal cell death

Toru Hosoi[1], Michihiro Imada[2], Akari Tawara[2], Kyosuke Yazawa[2], Chihiro Tohda[3], Yasuyuki Nomura[4], Koichiro Ozawa[1]

[1]Dept. Pharmacotherapy, Grad. Sch. Biomed. Health Sci., Hiroshima Univ., [2]Dept. Pharmacotherapy, Sch. Pharm., Hiroshima Univ., [3]Div. Neuromedical Science, Ins. Natural Med., Univ. Toyama, [4]Dept. Pharmacol., Kurume Univ. Sch. Med.

Alzheimer's disease (AD) is a neurodegenerative disease, which accompanied with memory decline and cognitive dysfunction. Aggregated amyloid β formation and accumulation has been suggested to be one of the underlying mechanisms of the pathophysiology of AD. Based on this hypothesis of AD pathophysiology, we had done screening to identify compound, which can ameliorate amyloid β aggregation using library derived from plant compounds. Based on this screening, we found that alkannin has chemical chaperone activity, which may be able to inhibit amyloid β aggregation. In the present study, we investigated pharmacological action of alkannin on amyloid β aggregation and neuronal cell death. Using circular dichroism (CD) spectra analysis, we found that alkannin may be able to inhibit β-sheet structure formation. Electron microscope analysis indicate that alkannin may also inhibit amyloid β fibril formation. Furthermore, alkannin attenuated amyloid β-induced neuronal cell death in PC12 cell line. Finally, alkannin ameliorated chemotaxis of AD model of Caenorhabditis elegans (C. elegans), suggesting that alkannin may be able to inhibit neurodegeneration at the C. elegans of AD model. Overall, these results suggest that alkannin may be able to inhibit amyloid β aggregation and neuronal cell death in AD.

2-P-048
Long interspersed element 1 retrotransposition induced by $A\beta_{1-40}$ and $A\beta_{1-43}$

Noriyuki Okudaira[1]

[1]Div. Pharm., Meikai Univ. Sch. Dent.

Amyloid β protein (Aβ) is a peptide processed from amyloid precursor protein cleavage by β- and γ-secretases. Many evidences suggest that Aβ plays a central role in the development of Alzheimer's disease pathology. Aβ accumulation leads to a high-diversity neurotoxic mechanism, but little is known about the underlying influence of the chromatin and genome. The human genome consists of interspersed repeats, sequences that evidence the long-standing activities and high preservative quality of mobile DNAs. Long interspersed element-1 (*LINE-1* or *L1*), a highly active autonomous retrotransposon (RTP), is the most abundant endogenous retroelement in humans, and accounts for approximately 17% of the human genome, approximately 10% of which comprises "hot L1" copies, primed for "jumping" within the genome. This study aimed to identify the mechanism by which Aβ induces L1-RTP. We found that Aβ peptides, $A\beta_{1-40}$ and $A\beta_{1-43}$, induced L1-RTP, but not $A\beta_{1-42}$ wild type. Our results revealed that the Aβ peptides $A\beta_{1-40}$ and $A\beta_{1-43}$ induce L1-RTP in neuronal cell lines. This effect was found to be reverse transcriptase-dependent, but not accompanied by the induction of double-strand breaks. We studied this using an inhibitor (VO-OHpic) and siRNA against PTEN (phosphatase and tensin homolog deleted on chromosome 10), and analyzed it as the target factor of Aβ. Interestingly, biochemical analysis revealed that Aβ induced L1-RTP in a PTEN-dependent manner. Moreover, Aβ activated MAPK by phosphorylating p44/42 MAPK. Further, PD98059 as a MAPK inhibitor inhibited the Aβ-induced L1-RTP. Our investigations evaluated the dynamics of genome instability mediated by Aβ-induced L1-RTP from early-stage Alzheimer's disease to the advanced phases of the disease.

2-P-049

Aromatic-turmerone derivatives protect dopamine neurons in midbrain slice culture.

Yuria Hori[1], Takahiro Seki[1], Reiho Tutumi[1], Masahiro Sugiura[2,3], Yasuhiko Asikari[2], Makoto Nakasima[2], Yuki Kurauti[1], Akinori Hisatune[4,5], Hirosi Katuki[1]

[1]Dept. Chemico-Pharmcol. Sci., Grad. Sch. Pharm. Sci., Kumamoto Univ., [2]Dept. Organ. Chemi., Grad. Sch. Pharm. Sci., Kumamoto Univ., [3]Sci. Pharm. Sci., Sojo Univ., [4]Priority Organization for Innovation and Excellence, Kumamoto Univ., [5]Program for Leading Grad. Sch. HIGO Program, Kumamoto Univ.

Aromatic (ar)-turmerone is a major component of turmeric oil and naturally exists as an (S)-enantiomer Recent reports revealed that it has antitumor activity and anti-inflammatory activities. We have recently succeeded to synthesize derivatives of ar-turmerone in a short step. In the present study, we investigated the effects of these derivatives on microglial activation and survival of midbrain dopamine neurons. Lipopolysaccharide (LPS)-mediated elevation of inflammatory markers in microglial BV2 cells was significantly inhibited by the treatment of all derivatives. Especially, (R)-ar-turmerone and ar-atlantone showed more potent anti-inflammatory effect than (S)-ar-turmerone. Next, we examined the effect of (S)-, (R)-ar-turmerone and ar-atlantone on the degeneration of dopamine neurons, which was triggered by the microglial activation, in midbrain slice cultures. All three chemicals significantly reversed the loss of dopamine neurons triggered by the treatment of interferon-γ(IFN-γ) and LPS. However, they did not inhibit the inflammatory activation of microglia. These results indicate that derivatives of ar-turmerone protect dopamine neurons without inhibiting microglial activation in midbrain slice cultures.

2-P-050

Treatment with coffee ingredients protects central and myenteric neurons in parkinsonian model.

Ikuko Miyazaki[1], Nami Isooka[1], Kouichi Wada[1], Ryo Kikuoka[1,2], Yoshihisa Kitamura[2], Masato Asanuma[1]

[1]Dept. of Med. Neurobiol., Okayama Univ. Grad. Sch. of Med., Dent. and Pharmaceut. Sci., [2]Dept. of Clin. Pharm., Okayama Univ. Grad. Sch. of Med., Dent. and Pharmaceut. Sci.

Epidemiological studies showed that daily drinking coffee or teas decreases the risk of Parkinson's disease (PD) to 40-50%. Caffeic acid (CA) and chlorogenic acid (CGA) are coffee ingredients and exert antioxidative properties. Exposure to pesticides, such as rotenone, is an environmental factor that plays an important role in the pathogenesis of PD. In this study, we examined neuroprotective effects of CA and CGA against rotenone-induced neurodegeneration. Chronic subcutaneous injection of rotenone into C57BL/6J mice exhibited reduction of dopaminergic neurons in the substantia nigra and beta-tubulin III-positive neurons in the intestinal myenteric plexus. Daily oral administrations of CA or CGA inhibited rotenone-induced cell death of not only nigral dopaminergic neurons but also myenteric plexus. In addition, CA or CGA significantly increased expression of antioxidative molecule metallothionein in the striatal astrocytes. In coculture of neurons and astrocytes from the mesencephalon or intestine, CA and CGA inhibited rotenone-induced neuronal loss of mesencephalic dopaminergic and enteric neurons, respectively. These results suggest that daily intake of coffee ingredients prevents or delays the onset of PD.

2-P-051
Uptake and degradation of α-synuclein in brain pericytes

Shinya Dohgu[1], Mizuki Yano[1], Miki Yokoya[1], Fuyuko Takata[1], Junichi Matsumoto[1], Ikuya Kimura[1], Atsushi Yamauchi[1], Yasufumi Kataoka[1]

[1]Dept. Pharm. Care Health Sci., Fac. Pharm. Sci., Fukuoka Univ.

Parkinson disease (PD) is characterized by widespread distribution of aggregated α-synuclein (α-Syn) protein in inclusions known as Lewy bodies. α-Syn is secreted from neurons and transferred to neighboring cells including pericytes, one of the blood-brain barrier (BBB) constituent cells. This cell-to-cell transmission is thought to underlie the progress of PD. In addition, blood-borne α-Syn can penetrate into the brain across the BBB. Here, we investigated how α-Syn is taken up by pericytes. Uptake of α-Syn by pericytes was increased with time during a 120-min period. The α-Syn uptake by pericytes was decreased by an excess amount of α-Syn and showed a temperature-dependent manner, suggesting that α-Syn uptake by pericytes is mediated by a saturable transport system. This uptake was inhibited by cyclosporine, but not sertraline, a clathrin-mediated endocytosis inhibitor. Intracellular accumulation of α-Syn in pericytes during a 24-hr period was lower than that in brain endothelial cells and astrocytes. In the presence of a lysosome inhibitor bafilomycin A1, the intracellular accumulation of α-Syn in pericytes was increased. These results suggest that pericytes possess a specific transport and degradation system of α-Syn.

2-P-052

The role of fibroblast growth factor (FGF) 21 in the development of schwann cell line

Rieko Muramatsu[1], Tomohiro Ishi[1]

[1]Dept. Mol. Pharm., Nat. Inst. Neurosci., NCNP

Demyelination is a hallmark of the peripheral nerve injury and is associated with the neurological dysfunction after peripheral damage. Because remyelination is required for recovery from neurological dysfunction, the mechanism of peripheral remyelination is thought to contribute to restoration of neurological function. We previously reported that the central nervous system remyelination was promoted when the brain was exposed by the circulating molecules, such as FGF21. Because the receptor for FGF21 is not limited in the brain, we asked whether FGF21 also regulated remyelination in the peripheral nervous system. To test this, we first investigated FGF21 receptor expression in mouse schwann cells. We detected beta-klotho, which is a co-receptor for FGF receptors, in mice S100-positive cells in vivo and in vitro (cell line), suggesting that schwann cell can response to FGF21. Schwann cell proliferation is a process of schwann cell development, therefore, we evaluated whether FGF21 regulate schwann cell proliferation. By bromodeoxyuridine (BrdU) incorporation analysis, we found that FGF21 treatment prevented proliferation of schwann cells. These data provide the possibility that FGF21 is also involved remyelination in the peripheral nervous system.

2-P-053

Role of the cystathionine γ-lyase/H_2S pathway in paclitaxel-induced HMGB1 release from macrophages and its impact on the pathogenesis of peripheral neuropathy in mice

Itsuki Yamaguchi[1], Risa Domoto[1], Fumiko Sekiguchi[1], Maho Tsubota[1], Atsufumi Kawabata[1]

[1]Lab. Pharmacol. Pathophysiol., Fac. Pharm., Kindai Univ.

We have reported that inhibitors of cystathionine γ-lyase (CSE), an H_2S-generating enzyme, reverse paclitaxel (PCT)-induced peripheral neuropathy (PIPN) in rats (Neuroscience 2011;188:148-156), and that PIPN in rats and mice involves macrophage-derived HMGB1, a DAMP molecule (Neuropharmacology 2018;141:201-213). Thus, we investigated a possible crosstalk between CSE/H_2S and HMGB1 pathways in macrophages and its implication for PIPN in mice. Repetitive i.p. administration (days 0, 2, 4 and 6) of PCT caused mechanical allodynia, as assessed by von Frey test, which was prevented by repeated i.p. administration of DL-propargylglycine (PPG), a CSE inhibitor. A single administration of PPG as well as β-cyano-L-alanine (BCA), another CSE inhibitor, reversed the established PIPN. In macrophage-like RAW264.7 cells, PCT at 1 μM produced HMGB1 release, an effect abolished by PPG or BCA. Na_2S, an H_2S donor, at 30-100 μM also caused HMGB1 release from RAW264.7 cells, which was blocked by N-acetyl-L-cysteine, an antioxidant. Our data suggest that PCT-induced HMGB1 release from macrophages involves endogenous H_2S generated by CSE, contributing to PIPN in mice.

2-P-054

Chronic tear deficiency sensitizes TRPV1-mediated response in corneal cold-sensitive nerves

Yuka Yamashita[1], Takayoshi Masuoka[1], Narumi Hashikawa[2], Katsuya Nakano[1], Masashi Tawa[1], Matomo Nishio[1], Ikunobu Muramatsu[1], Takaharu Ishibashi[1]

[1]Dept. Pharmacol., Kanazawa Med. Univ., Sch. Med., [2]Dept. Life Sci., Grad Sci., Okayama Univ., Sci.,

Corneal cold-sensitive nerves expressing transient receptor potential melastatin 8 (TRPM8) contribute to the detection of ocular surface dryness. Chronic ocular dryness results in neuropathic firing in cold-sensitive nerves, which might be involved in the unpleasant sensation of dry eye. However, the impulse activities mediated by TRPV1, a polymodal nociceptive receptor, have not been well studied in cold-sensitive nerves in normal and tear-deficient guinea pigs. In the present study, we found that TRPV1 was expressed in some TRPM8-positive fibers in the corneal epithelial layer, and that capsaicin, a TRPV1 agonist, increased spontaneous impulse activities in these corneal cold-sensitive nerves in normal guinea pigs. The response latency to capsaicin was significantly shorter in chronically tear-deficient guinea pigs induced by bilateral excision of lacrimal glands, while the ratio of capsaicin-sensitive nerves and total impulse activity induced by capsaicin were not changed. These results suggest that corneal cold-sensitive nerves functionally express TRPV1 and chronic tear deficiency sensitizes TRPV1-mediated nerve activity in cold-sensitive nerves that may relate to hypersensitivity to nociceptive stimuli in dry eyes.

2-P-055

TRP channel responses in human iPSC derived sensory neurons using MEA system

Aoi Odawara[1,2,3], Naoki Matsuda[1], Ikuro Suzuki[1]

[1]Tohoku Institute of Technology, [2]Tohoku University, [3]Japan Society for the Promotion of Science

Functional evaluation assays using human induced pluripotent stem cell (hiPSC)-derived sensory neurons are expected to predict the pain-related toxicity of drugs and the pharmacological effects. However, evaluation assays in hiPSC-derived sensory neurons has not been established, and electrophysiological response to pain-related molecules are not known. In this study, we aimed to evaluate the physiological responses to pain-related molecules including anti-cancer drugs in cultured hiPSC-derived sensory neurons using high-throughput multi-electrode array (MEA) system. Evoked responses depending on TPRV1, TRPM8, and TRPA1 channel in capsaicin, menthol, and AITC administration were detected. We also confirmed that hiPSC-derived sensory neurons have the property of increasing spontaneous activity with increasing temperature, and that are heterogeneous cell populations against temperature change. Cold hypersensitivity responses were also detected in concentration dependent manner of oxaliplatin. These results indicated that this MEA evaluation method using human iPSC-derived sensory neurons is effective as a pain assessment for human peripheral neuropathy.

2-P-056

Possible involvement of cochlear macrophage in the onset of sensorineural hearing loss

Taro Yamaguchi[1], Yumi Hashimoto[1], Naoko Mitsuba[1], Masanori Yoneyama[1], Yusuke Onaka[1], Kiyokazu Ogita[1]

[1]Lab. Pharmacol., Setsunan univ.

Most of sensorineural hearing loss is caused by cochlear hair cell injury. Evidence for involvement of cochlear inflammation in hair cell damage came from the finding that activation of macrophages causes hair cell loss. In this study, we sought to determine the involvement of macrophages in the onset of hearing loss. The mice were exposed to noise at a 90-dB sound pressure level for 1 h per day for 5 days and measured the auditory threshold at the frequencies of 12, and 20 kHz. Cochlear macrophages were visualized by immunostaining used anti-F4/80 (macrophage marker) and anti-CD11b (microglia marker). Minocycline (microglia activation inhibitor) was intraperitoneally administered at a dose of 50 mg/kg once a day during noise exposure. The auditory threshold markedly increased at the frequencies of 12 and 20 kHz in the noise-exposure time-dependent manner. Noise exposure significantly increased macrophages positive to both F4/80 and CD11b closely localized (50 μm) to the inner hair cells. Treatment with minocycline significantly prevented the noise-induced elevation of the auditory threshold. Thease data suggest that activation of cochlear macrophages is involved in the onset of sensorineural hearing loss.

2-P-057

Alpha1B-adrenoceptor-mediated positive inotropic and positive chronotropic actions in the mouse atrium

Shuangyi Zhang[1], Takio Kitazawa[1], Hiroki Teraoka[1]

[1]Sch. Vet. Med. Rakuno Gakuen Univ.

Noradrenaline-β1 adrenoceptor regulates contraction of mammalian heart. However α-adrenoceptor-mediated regulation of heart contractility has been reported in several species. Aim of the present study is to determine function and expression of α-adrenoceptors in the mouse atrium using functional and molecular biological approaches. In the spontaneous beating right atrium, noradrenaline and phenylephrine caused positive inotropic and positive chronotropic actions. On the other hand, clonidine and xylazine caused positive inotropic actions and negative chronotropic actions at high concentrations. Phenylephrine-induced positive inotropic and chronotropic actions were partially decreased by propranolol, and both actions remained in the presence of propranolol were inhibited by prazosin. A low concentration of silodosin (< 100 nM) did not but a high concentration (1 μM) decreased the phenylephrine-induced chronotropic actions. Neither propranolol nor phentolamine decreased the negative chronotropic actions of clonidine and xylazine. Expression of α1B mRNA was the highest among α1-adrenoceptors (α1B＞α1A=α1D) in the atrium. In conclusion, α1B-adrenoceptors are dominant α1-adrenoceptor subtypes and regulate the contraction of mouse atrium.

2-P-058

Prostaglandin E_2 EP_3 receptor subtype in the paraventricular hypothalamic nucleus mediates the corticotropin-releasing factor-induced elevation of plasma noradrenaline level in rats

Naoko Yamaguchi[1], Kaoru Mimura[1], Shoshiro Okada[1]

[1]Dept. Pharmacol., Sch. Med., Aichi Med. Univ.

Brain prostaglandin E_2 (PGE_2) causes sympathetic activation such as pressor and tachycardiac effects. Brain PGE_2 is also known to elevate plasma level of noradrenaline, but not adrenaline. It is well elucidated that corticotropin-releasing factor (CRF) increases plasma catecholamine levels. We have reported that prostanoids other than thromboxane A_2 mediate the CRF-induced elevation of plasma noradrenaline level. In this study, we examined whether PGE_2 in the brain mediates the CRF-induced elevation of plasma noradrenaline level. Intracerebroventricular (ICV) administration of CRF increased PGE_2 level in PVN dialysates. ICV pretreatment with the antagonist of PGE_2 receptor EP_3 subtype suppressed the CRF-induced elevation of plasma noradrenaline level, while antagonists for other subtypes did not affect the elevation. Furthermore, we performed microinjection of EP_3 receptor antagonist into the paraventricular hypothalamic nucleus (PVN), the major integrative center for sympathetic regulation. Bilateral blockade of EP_3 receptors in the PVN suppressed the CRF-induced elevation. Our results suggest that PGE_2 mediates the CRF-induced elevation of plasma noradrenaline level via activation of EP_3 receptor in the PVN.

2-P-059

Apelin intensifies vascular relaxation through activation of the endothelial nitric oxide production pathway in rats with metabolic syndrome

Miho Shimari[1], Satomi Kagota[1], Kana Maruyama[1], Yayoi Shiokawa[1], Kazumasa Shinozuka[1]

[1]Dept. Pharmacol.II, Sch. Pharm. Pharmaceu. Sci., Mukogawa Women's Univ.

Perivascular adipose tissue (PVAT) modulates the vascular tone. We previously demonstrated that mesenteric arterial PVAT enhances vasodilation in SHRSP.Z-Leprfa/IzmDmcr (SHRSP.ZF) rats. However, the factors involved in the vasodilation effect of PVAT have not been identified. Therefore, this study aimed to determine whether apelin, an adipokine, is involved in mediating the beneficial effects of PVAT. We pre-treated mesenteric arterial segments from SHRSP.ZF rats with apelin before observing the vascular activities. Compared to controls without PVAT, apelin significantly enlarged vasodilation in response to acetylcholine; the result was similar to the response observed when PVAT is present. In contrast, the same trend in vasodilation was not observed in response to sodium nitroprusside. Additionally, when apelin was cumulatively administered to segments without PVAT from Wistar-Kyoto rats, vasorelaxation was not induced. These results suggest that apelin acts as a vasodilative enhancer under the condition of endothelial nitric oxide (NO) synthesis. Apelin may, therefore, be involved in mediating the beneficial effect of PVAT via a possible increase in NO production in metabolic syndrome.

2-P-060

Apelin attenuated hydrogen peroxide-induced cell death in endothelial cells by decreasing the intracellular ROS levels.

Yasuhiro Yoshioka[1], Ayaka Fujiwara[1], Akira Fujiwara[1], Manami Norimatsu[1], Haruku Yamamoto[1], Akiko Yamamuro[1], Yuki Ishimaru[1], Sadaaki Maeda[1]

[1]Lab. Pharmacotherap., Faculty Pharmaceut. Sci., Setsunan Univ.

Apelin plays an important role in the proliferation of vascular endothelial cells, and the expression of apelin is enhanced after ischemic stroke. Since the production of reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2) is elevated in the post-ischemic brain, the proliferated endothelial cells are subjected to oxidative stress. However, the effects of apelin on oxidative stress-induced injury in endothelial cells are poorly investigated. In this study we investigated the effect of apelin on H_2O_2-induced cell death in mouse endothelial cell line bEnd.3. Cell viability was determined by a colorimetric 3,4,5-dimethylthiazol-2-yl-2,5-diphenyl tetrazolium bromide (MTT) assay, and intracellular ROS levels were evaluated by dihydroethidium (DHE) staining. Treatment of bEnd.3 cells with H_2O_2 (150 μM) increased DHE-positive cells and induced cell death. Pretreatment with [Pyr1]-apelin-13 (10 μM) for 24 h reduced the increase in the number of DHE-positive cells and attenuated the cell death. These results suggest that apelin protects bEnd.3 cells from H_2O_2-induced cell death by decreasing the intracellular ROS levels.

2-P-061

Acanthopanax senticosus (Siberian ginseng) induces endothelium-dependent and -independent vasorelaxations

Yayoi Shiokawa[1], Yusa Watanabe[1], Satomi Kagota[1], Kana Maruyama[1], Shizuo Yamada[2], Kazumasa Shinozuka[1]

[1]Dept. Pharmacol. II., Sch. Pharm. Pharmaceu. Sci., Mukogawa Women's Univ., [2]Cent. Pharma-Food Res., Grad. Sch. Pharmaceu. Sci., Univ. Shizuoka.

Siberian ginseng (SG) has several biological properties including anti-fatigue, anti-stress, and sedative effects. Although SG has been reported to improve peripheral blood circulation in rats under healthy conditions, the underlying mechanism has not been well studied. Therefore, we investigated whether SG causes relaxation in isolated arteries in healthy control rats and determined the underlying mechanisms. We used a SG root powder extracted with hot water. In the thoracic aorta, isolated from Wistar rats, the vasodilator effects of SG using organ bath techniques were compared to that of acetylcholine (ACh). Similar to ACh, SG caused relaxations in a dose-dependent manner in arteries pre-contracted with phenylephrine. Unlike ACh, SG-induced relaxations were partially inhibited by treatment with antagonists of muscarinic receptor (atropine), nitric oxide (NO) synthase (L-NAME), and soluble guanylyl cyclase (ODQ), and by endothelium removal. These results demonstrate that SG-induced vasorelaxations occur via both NO production from the endothelium and NO-independent pathway in healthy rats; therefore, SG may improve peripheral circulation via vasorelaxation.

2-P-062

Effect of olanzapine, risperidone and quetiapine on the endothelium- and sympathetic nerve-dependent regulation of smooth muscle activities in rat mesenteric arteries

Han-Hsuan Yeh[1], Mei-Fang Chan[1], Tony Jer-Fu Lee[1]

[1]CVMRC, Department of Medical Research, Buddhist Tzu Chi Hospital, Hualien, Taiwan

Olanzapine, quetiapine and risperidone are antipsychotics used for schizophrenia, autism and bipolar disorder. Their effect is based on the blockade of receptors in the brain dopamine pathway. These receptors are also active in regulation of arterial tensions. Therefore, this study aims to examine how olanzapine, quetiapine and risperidone affect the rat mesenteric arterial tones by using the blood-vessel myography. The contractions induced by phenylephrine (an α-adrenoceptor agonist) were inhibited by olanzapine, quetiapine and risperidone in mesenteric arteries (MA) with IC_{50}=1, 3 and 0.03 μM, respectively. However, the U46619 (a thromboxane A2-receptor agonist)-induced contractions were little affected by them. On the other hand, the acetylcholine-elicited relaxations were concentration-dependently inhibited by olanzapine and quetiapine but not by risperidone in endothelium-intact MA. No inhibitory effect of the three agents on the sodium nitroprusside-induced vasodilations was observed. Furthermore, the nerve stimulation-provoked contractions of MA were also inhibited by these antipsychotics with a greater potency for risperidone. These results indicate that olanzapine, quetiapine and risperidone differently suppressed the receptors-mediated contractions and relaxations in MA, which may relate to the hypotensive condition observed in clinics.
Keywords:090, 534, 024

2-P-063
TRPV4 agonists dilate retinal arterioles in rats

Asami Mori[1], Kazuki Takeda[1], Daiki Asano[1], Akane Morita[1], Kenji Sakamoto[1], Tsutomu Nakahara[1]

[1]Dept. Mol. Pharmacol., Kitasato Univ. Sch. Pharm. Sci.

The impaired retinal circulation contributes to the onset and progression of glaucoma and diabetic retinopathy, leading to vision loss or blindness. In this study, we examined whether GSK1016790A, an activator of TRPV4, improves retinal circulation in rats. Ocular fundus images were captured and diameters of retinal blood vessels contained in the images were measured. Both systemic blood pressure and heart rate were continuously recorded. Intravenous infusion of GSK1016790A (0.2-2 μg/kg/min) increased retinal arteriolar diameter in a dose-dependent manner. The higher dose of GSK1016790A (2 μg/kg/min) slightly decreased blood pressure without changing heart rate. These responses to GSK1016790A were significantly attenuated by intravenous injection of GSK2193874 (0.3 mg/kg), an antagonist of TRPV4. These results suggest that agonists of TRPV4 dilate retinal arterioles more strongly than peripheral arteries. Thus, TRPV4 agonists may improve retinal circulation and become one candidate of therapeutics for preventing the development of ocular diseases, which are associated with impaired retinal circulation.

2-P-064

Influence of perivascular adipose tissue on vasodilation in the renal artery of metabolic syndrome rats

Satomi Kagota[1], Kana Maruyama[1], Rui Yamada[1], Miho Shimari[1], Yayoi Shiokawa[1], Kazumasa Shinozuka[1]

[1]Dept. Pharmacol II, Sch. Pharm. Pharmaceu. Sci., Mukogawa Women's Univ.

Perivascular adipose tissue (PVAT) is known to modulate vascular tone. We have demonstrated that impaired vasodilation is masked by the presence of PVAT at 20 weeks of age (wks) in the mesenteric arteries of SHRSP.Z-*Lepr*^{fa}/IzmDmcr rats (SHRSP.ZF) with metabolic syndrome (MetS); this enhanced vasodilation caused by PVAT disappears at 30 wks. Therefore, we investigated whether the compensatory effect of PVAT is observed at another vascular site in SHRSP.ZF and whether it differs from that in Zucker fatty rats, another animal model of MetS. In the renal arteries of SHRSP. ZF, acetylcholine-induced vasodilation was unchanged by the presence of PVAT at 20 wks, while an increase in the relaxation caused by PVAT was observed at 30 wks. In contrast, in Zucker fatty rats, PVAT did not affect vasodilation at 20 wks, while there was slightly increased relaxation at 30 wks. This study indicates that renal arterial PVAT has compensatory effects on impaired vasodilation in SHRSP.ZF, similar to that observed in the mesenteric artery, and this influence is consistent with that in Zucker fatty rats. PVAT may have an important role in retaining blood circulation, especially in the arteries supplying blood to organs such as the kidneys, in MetS.

2-P-065
Activation of TRPV4 channels prevents angiogenesis.

Akane Morita[1], Shiori Sugahara[1], Daiki Asano[1], Asami Mori[1], Kenji Sakamoto[1], Tsutomu Nakahara[1]

[1]Dept. Mol. Pharmacol., Kitasato Univ. Sch. Pharm. Sci.

Angiogenesis plays an important role in ischemic diseases, inflammation, wound healing, and tumor progression. Recent studies have demonstrated that transient receptor potential vanilloid 4 (TRPV4) channel, belonging to a Transient Receptor Potential (TRP) family of ion channels, regulates the cell survival and death in endothelial cells and tumor angiogenesis. In this study, we examined the effects of the TRPV4 channel agonist GSK1016790A on the cell survival and viability of human umbilical vein endothelial cells (HUVECs) and on retinal angiogenesis in neonatal mice. GSK1016790A (100 nM) increased the number of propidium iodide-positive cells and reduced the cell viability of HUVECs. Pre-treatment of HUVECs with the TRPV4 channel antagonist HC-067047 (1 μM) prevented GSK1016790A-induced reduction in the cell viability. Retinal angiogenesis was slightly delayed in mice treated with GSK1016790A (0.3 mg/kg, s.c.) from postnatal day (P) 2 to P5. These results suggest that an excessive activation of TRPV4 channels induces endothelial cell death and shows the anti-angiogenic effect. Thus, TRPV4 channels may be a target for anti-angiogenic intervention in ischemic diseases and tumor growth.

2-P-066

MMP-9 inhibitors attenuate retinal neurovascular degeneration in a neonatal rat model of NMDA-induced retinal neurotoxicity

Daiki Asano[1], Akane Morita[1], Asami Mori[1], Kenji Sakamoto[1], Tsutomu Nakahara[1]

[1]Dept. Mol. Pharmacol., Kitasato Univ. Sch. Pharm. Sci.

Retinal capillary degeneration occurs following retinal neuronal cell degeneration. In this study, we examined effects of inhibitors of matrix metalloproteinase (MMP)-9 on neuronal cell loss and capillary degeneration in a neonatal rat model of NMDA-induced retinal neurotoxicity. Intravitreal injection of NMDA (50 or 200 nmol) was performed on postnatal day (P) 7 and morphological changes in retinal neurons and vasculature were examined on P14. The MMP inhibitor CP101537 (100 nmol) or vehicle (DMSO) was intravitreally injected simultaneously with, or 2 days after NMDA injection. CP101537 protected against neurovascular degeneration in a time-dependent manner as follows: 1) simultaneous injection of CP101537 with NMDA prevented retinal neuronal loss induced by NMDA (50 nmol), and 2) capillary degeneration in NMDA (200 nmol)-treated retinas was suppressed when CP101537 was injected 2 days after NMDA treatment. Gelatin zymography and Western blot analyses indicated that the activity and protein levels of MMP-9 were enhanced in the retina from 4 h to 2 days after NMDA injection. These results suggest that MMP-9 inhibitors may have therapeutic potential in treatment of retinal neurovascular degenerative diseases.

2-P-067

The effect of anti-adiponectin antibody on experimental macular edema

Anri Nishinaka[1], Shinsuke Nakamura[1], Tomomi Masuda[1], Masamitsu Shimazawa[1], Hideaki Hara[1]

[1]Molecular Pharmacology, Department of Biofunctional Evaluation, Gifu Pharmaceutical University.

The macular edema is caused with the increase of vascular endothelial growth factor (VEGF) in diabetic macular edema (DME) and retinal vein occlusion (RVO) patients. However, there are some drawbacks after anti-VEGF therapy. In an earlier report, adiponectin which is an adipokine secreted from adipocytes was increased in the retina of streptozotocin-induced diabetic retinopathy murine model. The purpose of this study was to investigate the involvement of adiponectin in the pathophysiology of retinal vascular hyperpermeability.

We investigated in VEGF and adiponectin levels in vitreous humor of DME and proliferative diabetic retinopathy (PDR) patients by ELISA. Moreover, we performed adiponectin level in murine RVO model by RT-PCR and Western blotting. To evaluate the effect of anti-adiponectin antibody, retinal thickness was measured by HE staining.

The adiponectin level with the vitreous body of DME patients was higher than PDR patients. Interestingly, both mRNA and protein of adiponectin were increased in the retina of murine RVO model, and the increase of the retinal thickness was ameliorated by anti-adiponectin antibody therapy. These data indicate that adiponectin may represent one of potential therapeutic targets for retinal edema in DME and RVO patients.

2-P-068

Involvement of various membrane currents in guinea pig sinoatrial node automaticity revealed by measurement of action potential

Tomoki Igarashi[1], Shogo Hamaguchi[1], Iyuki Namekata[1], Hikaru Tanaka[1]

[1]Dept. Pharmacol., Fclt. Pharmaceut. Sci., Toho Univ.

Cardiac pacemaking is generated by the action potential from sinoatrial node which is consist with various membrane currents. In this study, we investigated the influence of membrane currents on the sinoatrial node automaticity. The action potential of sinoatrial node were measured with micro electrode method in guinea pig isolated right atrial tissue preparation. The beating rate of sinoatrial node was decreased by both 1 μM E-4031, a selective I_{Kr} blocker, and 30 μM Chromanol 293B, a selective I_{Ks} blocker. E-4031 prolonged APD_{50} and APD_{90}, while Chromanol 293B prolonged APD_{20}, APD_{50} and APD_{90}. The maximum diastolic potential was increased by E-4031 but not by Chromanol 293B. These results suggest that I_{Ks} and I_{Kr} have different roles in guinea pig sinoatrial node automaticity; I_{Kr} may contribute to the late repolarization phase and diastolic depolarization, whereas I_{Ks} may contribute to the early repolarization phase.

2-P-069

Metalloprotease nardilysin controls heart rate through the transcriptional regulation of ion channels critical for sinus automaticity

Mikiko Ohno[1], Hiroshi Matsuura[2], Takeru Makiyama[3], Hirotaka Iwasaki[1], Shintaro Matsuda[3], Eiichiro Nishi[1]

[1]Dept. Pharm., Grad. Sch. Med., Shiga Univ Med. Sci., [2]Dept. Physio., Grad.Sch.Med., Shiga Univ Med. Sci., [3]Dept. Cardiovasc. Med., Grad. Sch. Med., Kyoto Univ.

Nardilysin (NRDC; N-arginine dibasic convertase) is a metalloprotease of the M16 family. We reported that NRDC is a protease having localization-dependent multiple functions. NRDC-deficient mice (Nrdc-/-) showed wide range of phenotypes such as hypomyelination, hypothermia, and bradycardia. In this study, we have explored a role of NRDC in the regulation of heart rate. (1) Pharmacological blocking of autonomic nervous system revealed that an intrinsic heart rate of Nrdc-/- was significantly reduced compared with that of wild-type mice. (2) In Nrdc-/- hearts, mRNA levels of Cav3.1 and HCN1/4, ion channels responsible for sinus automaticity, were significantly reduced. (3) Funny (If) current and T-type Ca current measured in the sinus node cells were markedly reduced in Nrdc-/- cells, indicating that the functions of Cav3.1 and HCN4 are impaired. (4) Gene knockdown of NRDC in primary rat ventricular myocyte led to the reduction of mRNA level of HCN4. (5) Chromatin immunoprecipitation-PCR analysis showed that NRDC binds to the promoter region of Cav3.1 and HCN4, suggesting the direct involvement of NRDC in transcriptional regulation of these ion channels. (6) Atrium-specific Nrdc-/- (Sarcolipin-Cre) showed mild bradycardia and reduced Cav3.1 mRNA expression. Together, our results indicated that NRDC in cardiomyocyte controls heart rate through the transcriptional regulation of ion channels critical for sinus automaticity.

2-P-070
Modeling study of hERG facilitation effect by nifekalant

Kunichika Tsumoto[1], Kazuharu Furutani[2], Yoshihisa Kurachi[3]

[1]Dept. Physiol, Kanazawa Med Univ, [2]Dept. Physiol, Univ California, Davis, [3]Dept. Pharmacol, Grad. Sch. Med., Osaka Univ.

Some drugs that block the human ether-a-go-go-related gene (hERG) channel, the delayed rectifier potassium current (I_{Kr}), exert antiarrhythmic action by prolonging action potential (AP). However, the excessive AP prolongation increases the risk of lethal arrhythmias. We recently found that nifekalant not only inhibited the hERG current but also increased the current in a membrane voltage-dependent manner. How such a current increasing effect by hERG channel inhibitors, which referred to as a facilitation effect, affect the AP of cardiomyocytes is not yet fully understood. We constructed an I_{Kr} current model based on the voltage-clamp experiment of hERG channel expressed in HEK293 cells and macroscopic current recordings under the administration of nifekalant. Our constructed I_{Kr} current model was constrained by current recorded from HEK293 cells expressing hERG channels. Furthermore, we performed AP simulation with a human ventricular myocyte model replaced with our I_{Kr} model and found that the I_{Kr} facilitation prevents early afterdepolarization developments as compared to drugs without the facilitation effect. A specific hERG inhibitor with facilitation effect may contribute positively to suppress drug arrhythmogenicity.

2-P-071

Effects of L-type Ca^{2+} channel blockers on EAD in drug-induced arrhythmia

Akira Kimura[1], Shingo Murakami[1]

[1]Department of Electrical, Electronic, and Communication Engineering, Faculty of Science and Engineering, Chuo University

The conventional prediction of drug-induced arrhythmia has been conducted by testing effects of candidate drugs on the rapidly activating delayed rectifier K^+ current (I_{Kr}) because block of I_{Kr} may prolong action potential duration and cause arrhythmia. However, it turned out that a number of potentially useful drug candidates without the side effect were excluded in this approach. One of the reasons why this approach for arrhythmia risk prediction is not so accurate is that the occurrences of early afterdepolarization (EAD) can be different even under the same prolonged action potential duration. In the present study, we examined how various drug effects on properties of L-type Ca^{2+} channel can account for the different EAD occurrences in under the same prolonged action potential duration. By using O'Hara Rudy model, the *de facto* standard Human ventricular model, we examined simulated effects of various properties of L-type Ca^{2+} channel blockers on EAD. We found that the different EAD occurrences can be accounted for by the voltage dependent drug effects on L-type Ca^{2+} channel. These results suggest that the risk in drug-induced arrhythmia should be predicted not only by checking block of I_{Kr} and prolongation of action potential duration but also checking voltage dependent drug effects on L-type Ca^{2+} channel.

2-P-072

Monensin-induced Ca^{2+} overload suppresses mitochondrial ATP production in cardiac myocytes

Mizuki Yamaguchi[1], Saki Yamaguchi[1], Hibiki Kuramoto[1], Momoko Bonno[1], Iyuki Namekata[2], Hikaru Tanaka[2], Katsuharu Tsuchida[1]

[1]Dept. Ratio. Med. Sci., Fac. Pharm. Sci., Doshisha. Women's. Col., [2]Dept. Pharmacol., Toho Univ. Fclt. Pharmaceut. Sci.

[Background]
Monensin (Mo) has been reported to decrease the ATP content in various cells, however, its mechanisms are not fully understood in cardiac myocytes. In this study, the effects of Mo on ATP production was examined in guinea-pig ventricular myocytes.
[Methods]
The ATP production was evaluated from the time taken to open sarcolemmal K_{ATP} channel. Membrane potential and Ca^{2+} concentration of mitochondria (mito) was measured.
[Results]
When the extracellular solution containing 112 mM Na,o and the intracellular solution containing 10 mM Na,i and 0 mM ATP were used, Mo 10^{-5} M shortened time taken to open the K_{ATP} channel significantly. When 0 mM Na,o and 10 mM Na,i were used, Mo also shortened time. Next, when 0 mM Na,o and 0 mM Na,i were used, Mo shortened the time to a lesser extent. When EGTA 5 mM was replaced by BAPTA 40 mM in the patch pipette, the time taken to open the K_{ATP} channel was not shortened so much by Mo. Mo increased mito Ca^{2+} and depolarized the membrane potential in saponin-treated myocytes.
[Conclusion]
We conclude that the Mo-induced Na^{+} influx into mito alters Na^{+}/ Ca^{2+} exchange function, and the mito membrane depolarization may cause Ca^{2+} influx to mito matrix, leading to suppression of ATP production.

2-P-073

Uroguanylin affects the calcium current in a complicated manner

Saki Yamaguchi[1], Akari Okubo[1], Maki Kuraji[1], Natsuko Matsushita[1], Katsuharu Tsuchida[1]

[1]Dept. Ratio Med Sci, Fac. Pharm Sci, Doshisha Women's Col.

[Background] It is reported that natriuretic peptides affect the action potential and I_{CaL} in cardiomyocytes via an increase in cGMP by activation of particulate guanylate cyclase (pGC). Uroguanylin (uro) is known to increase cGMP through pGC. In this study, we examined the effects of uro on I_{CaL} in cardiomyocytes.

[Methods] Ventricular cells were isolated from guinea-pig hearts. The I_{CaL} was recorded by use of whole-cell patch clamp method.

[Results] When uro was applied to the myocytes while raising its concentration (3 nM〜0.3 μM) after stimulating I_{CaL} by 0.3 μM isoproterenol (iso), the stimulated- I_{CaL} was inhibited by uro on the whole. However, uro sometimes augmented the stimulated- I_{CaL}. The basal I_{CaL} was not affected by uro. ODQ, a selective soluble GC inhibitor, 0.1 mM did not exert the inhibitory effect on the action of uro. Rp-8-Br-PET-cGMPs (Rp), one of the most potent cGMP-PKG inhibitor, 10 μM in patch pipette did not affect either, however, the uro-evoked augmentation of the stimulated- I_{CaL} was not observed in the presence of Rp.

[Conclusion] Uroguanylin exerted the inhibitory effects on the iso-stimulated I_{CaL} in a lot of cases, but its effects were not consistent. The mechanism of the uro-induced activation of pGC and I_{CaL} remained to be elucidated

2-P-074

Assessment of the inhibitory effects of antipsychotics on acetylcholine (ACh)-induced contraction in rat urinary bladder smooth muscle (UBSM)

Fumiko Yamaki[1], Yukako Abe[1], Yohei Ikegami[1], Yume Hattori[1], Yume Hamamatsu[1], Keisuke Oabara[1], Kazuhiro Matsuo[2], Takashi Yoshio[2], Yoshio Tanaka[1]

[1]Dept. Chem. Pharmacol., Toho Univ. Sch. Pharmaceut. Sci., [2]Dept. Clin. Pharmacol. Toho Univ. Sch. Pharmaceut. Sci.

Schizophrenia is a psychiatric disorder that develops in relatively younger age groups. In Japan, the geriatric population continues to grow, and thus, the proportion of elderly patients with schizophrenia is increasing. Pharmacotherapy with antipsychotics is the primary treatment for schizophrenia. Additionally, antipsychotics are also effective against behavioral and psychological symptoms of dementia, which increases the chance of prescribing these drugs to elderly patients with dementia. However, in elderly people, drug-induced dysuria is likely to occur, and the most well-known triggers of this dysuria are drugs' anticholinergic effects. Thus, it is necessary to predict to what extent antipsychotics cause this type of dysuria. In this study, we examined the possible inhibitory effects of 26 commercially available and clinically used antipsychotics on ACh-induced contraction in isolated rat UBSM. Among them, pipamperone, sulpiride, sultopride, tiapride, nemonapride, risperidone, paliperidone, aripiprazole, and brexpiprazole did not significantly affect ACh-induced contraction, suggesting that these antipsychotics are unlikely to cause anticholinergic effects-associated dysuria.

2-P-075
Roles of brain nicotinic receptors in micturition of rats

Takahiro Shimizu[1], Yohei Shimizu[1], Hideaki Ono[1], Suo Zou[1], Masaki Yamamoto[1], Shogo Shimizu[1], Youichirou Higashi[1], Takaaki Aratake[1], Tomoya Hamada[1], Yoshiki Nagao[1], Yusuke Ueba[1], Motoaki Saito[1]

[1]Dept. of Pharmacol., Kochi Med. Sch., Kochi Univ.

Icv administered epibatidine (EP), a nicotinic receptor (nAChR) agonist, induced secretion of catecholamines (CAs, noradrenaline and adrenaline) from the rat adrenal medulla. Because CAs affect contractility of the bladder and urethra, we examined effects of EP on micturition and their dependence on CAs in urethane-anesthetized (0.8 g/kg, ip) male Wistar rats. Catheters were inserted into the bladder and the femoral artery to perform cystometrograms (CMG) and to collect blood samples, respectively. CMG was started 2 h after the surgery and 1 h after the start, EP (0.3 or 1 nmol) or vehicle was icv administered. Plasma CAs were measured at 5 min after the administration. In some rats, acute bilateral adrenalectomy (ADX) was performed before the insertion. Effects of icv pretreated mecamylamine (MEC, 100 or 300 nmol), a nAChR antagonist, on the EP-induced responses were also examined. Icv administered EP dose-dependently prolonged intercontraction intervals (ICI) and elevated plasma CAs. ADX abolished the EP-induced elevation of both CAs without affecting ICI prolongation. MEC dose-dependently attenuated the EP-induced ICI prolongation and elevation of CAs. These results suggest that activation of brain nAChRs might suppress micturition reflex, which is independent of CAs.

2-P-076

Evaluation of a rat model of functional urinary bladder outlet obstruction produced by chronic administration of L-NAME

Katsuhiko Noguchi[1], Kimio Sugaya[1], Saori Nishijima[1], Mayuko Sakanashi[2], Katsumi Kadekawa[1], Katsuhiro Ashitomi[1], Hideyuki Yamamoto[3]

[1]Southern Knights' Laboratory Co., Ltd., [2]Col. Pharmacy, Kinjo Gakuin Univ., [3]Dept. Biochemistry, Grad. Sch. Med., Univ. Ryukyus

Partial bladder outlet obstruction induced by partial ligation of the urethra has widely been used as a model of obstructed bladder, although bladder dysfunction is caused by not always mechanical but functional obstruction. Previous studies have demonstrated that long-term deficiency of nitric oxide (NO) can produce detrusor overactivity. However, the pathophysiologic features of this model have not been well defined. The aim of this study was to examine the characteristics of chronic NO deficiency-induced urinary bladder dysfunction in vivo and in vitro. Rats were divided into two groups. L-NAME was given p.o. for 4 w. In L-NAME group, blood pressure rose, and plasma nitrite/nitrate levels decreased compared to control group. Chronic L-NAME treatment caused frequent bladder contractions, increased residual volume and rises in urethral pressure. In addition, L-NAME group exhibited diminished carbachol-induced contraction of isolated detrusor strips and upregulation of an ischemic marker and a gap junction protein in the bladder. These data suggest that chronic L-NAME treatment produces bladder hyperactivity with residual urine, and may be a useful approach to simulating functionally obstructed bladder.

2-P-077

Therapeutic effects of alpha1-adrenoceptor antagonist silodosin or phosphodiesterase type 5 inhibitor tadalafil on detrusor overactivity in the spontaneously hypertensive rats

Yoshiki Nagao[1,2], Shogo Shimizu[1], Takahiro Shimizu[1], Tamaki Kataoka[1], Shiho Kamada[1], Youichirou Higashi[1], Takaaki Aratake[1], Suo Zou[1], Masayuki Tsuda[3], Mikiya Fujieda[2], Motoaki Saito[1]

[1]Dept. of Pharmacol., Kochi Med. Sch., Kochi Univ., [2]Dept. of Pediatrics, kochi Med. Sch., Kochi Univ., [3]Institute for Laboratory Animal Reserch, Kochi Univ.

Introduction: There is increasing evidence that a decrease in bladder blood flow (BBF) can cause the detrusor overactivity (DO) in human or animals. We investigated the effects of alpha1-adrenoceptor antagonist silodosin or phosphodiesterase type 5 inhibitor tadalafil on DO and BBF in the spontaneously hypertensive rats (SHR).

Methods: Twelve-week-old male SHRs were perorally administered with silodosin (100μg/kg), tadalafil (2 or 10 mg/kg) or vehicle once daily six weeks. Then, blood pressure (BP), urodynamic parameters and BBF were measured. Wistar rats were used as normotensive controls.

Results: SHRs showed significant increases in BP and micturition frequency (MF), and decreases in intercontraction interval (ICI) and BBF compare to the Wistar rats. Chronic treatment with silodosin or a higher dose of tadalafil (Tad10) ameliorated the MF, ICI and BBF in the SHRs. However, each drug failed to affect BP in the SHRs. And, there is no significant differences on these values between silodosin treated group and Tad10 treated group.

Conclusions: Silodosin or tadalafil ameliorated the hypertension related DO in the SHR via an increase in BBF.

2-P-078

Noradrenaline (NA)-induced relaxation is mainly elicited through the β_3-adrenoceptor (β_3-AR) in rat detrusor smooth muscle (DSM)

Keisuke Obara[1], Serena Suzuki[1], Hiroko Shibata[1], Naoki Yoneyama[1], Shoko Hamamatsu[1], Fumiko Yamaki[1], Koji Higai[2], Yoshio Tanaka[1]

[1]Dept. Chem. Pharmacol., Toho Univ. Sch. Pharmaceut. Sci., [2]Lab. Med. Biochem., Toho Univ. Sch. Pharmaceut. Sci.

β-ARs are sub-classified into three subtypes (β_1–β_3). Among these, β_3-ARs are present in various types of SM including DSM and are believed to play a role in relaxations of these muscles. To date, there has been little information available about the endogenous ligand that stimulates β_3-ARs to produce relaxations in DSM. In this study, to determine whether NA is an endogenous ligand of DSM β_3-ARs, NA-induced relaxation was pharmacologically analysed using rat DSM. In isolated rat urinary bladder tissues, mRNAs for β_1-, β_2-, and β_3-ARs were detected using RT-PCR. In DSM preparations contracted with methacholine (3×10^{-5} M), NA-induced relaxation was not inhibited by atenolol (10^{-6} M), ICI-118,551 (3×10^{-8} M), propranolol (10^{-7} M), and bupranolol (10^{-7} M). In the presence of propranolol (10^{-6} M), NA-induced relaxation was competitively inhibited by bupranolol (3×10^{-7}–3×10^{-6} M) or SR59230A (10^{-7}–10^{-6} M), with their pA_2 values being 6.64 and 7.27, respectively. None of the six NA metabolites showed significant relaxation in methacholine-contracted DSM. These findings suggest that NA, but not its metabolites, is an endogenous ligand for β_3-ARs to produce relaxations of DSM in rats.

2-P-079

The effect of oral administration of cloperastine on micturition reflex in mice

Ichiro Kimura[1], Fumio Soeda[1], Sumire Kudo[1], Aki Sato[1], Takayuki Koga[1], Shogo Misumi[2], Kazuo Takahama[2,3,4], Akihisa Toda[1]

[1]Daiichi Univ. Pharm., [2]Grad. Sch. Pharmaceut. Sci., Kumamoto Univ., [3]Academic-Industry Collaborative Researcher, Kumamoto Univ., [4]Kumamoto Health Sci. Univ.

We have previously reported that cloperastine (CP), a centrally acting non-narcotic antitussive, improved micturition dysfunctions in rodents. Although clinical application of CP is expected, it is unknown whether oral administration of CP affects micturition reflex. In this study, we measured micturition functions in awake and anesthetized mice chronic orally administered with CP. **Method** Female BALB/c mice were purchased. CP 20mg/kg was orally administered once a day for 14 days and used for the following two experiments. 1) Real-time micturition activity of freely moving mice was measured for 24 hours by using a sequential urine collection and recording system developed by us. 2) The intravesical pressure of the urethane-anesthetized mice was measured by conventional single cystometry. **Result** 1) In the awake mice, CP significantly increased voiding frequency, total voided volume and voiding duration in the dark period and also for 24 hours compared with the control group. 2) In the anesthetized mice, mean urine flow rate was significantly decreased, and voiding duration tended to increase in CP group compared with control. These results suggest that chronic oral administration of CP, at cough suppressant dose, may affect micturition reflex in mice.

2-P-080

A crosstalk between HMGB1/RAGE and CSE/H_2S/$Ca_v3.2$ pathways essential for cystitis-related bladder pain in mice: Impact of ATP-induced macrophage activation

Shiori Hiramoto[1], Yuki Toriyama[1], Aya Sakaegi[1], Kaoru Yamaguchi[1], Maho Tsubota[1], Junichi Tanaka[1,2], Fumiko Sekiguchi[1], Hiroyasu Ishikura[2], Masahiro Nishibori[3], Atsufumi Kawabata[1]

[1]Lab. Pharmacol. Pathophysiol., Fac. Pharm., Kindai Univ., [2]Div. Emergency and Critical Care Medicine, Fukuoka Univ., Hospital, [3]Department of Pharmacol., Okayama Univ. Graduate School of Medicine

Bladder pain accompanying cyclophosphamide (CPA)-induced cystitis involves two pathways: 1) upregulation of cystathionine-γ-lyase (CSE) that generates H_2S, which enhances $Ca_v3.2$ T-type Ca^{2+} channel activity (Br J Pharmacol 2012;167:917), and 2) RAGE activation by HMGB1, a DAMP protein (Neuropharmacology 2014;79:112). We thus analyzed molecular mechanisms underlying a possible crosstalk of those signaling cascades. The CPA-induced bladder pain and CSE upregulation in mice were suppressed by an anti-HMGB1-neutralizing antibody, RAGE antagonist or macrophage (Mφ) depletor. Mφ accumulation was detected in the bladder following CPA treatment. Acrolein, a hepatic metabolite of CPA, evoked prompt ATP release from cultured human urothelial T24 cells. ATP induced HMGB1 release from mouse Mφ-like RAW264.7 cells, an effect blocked by A438079, a $P2X_7$ antagonist, and by inhibitors of NF-κB or p38MAPK and an antioxidant. A438079 also attenuated CPA-induced bladder pain in mice. These data suggest that urothelium-derived ATP evokes ROS-dependent HMGB1 release from Mφ via $P2X_7$, which in turn causes RAGE-dependent CSE upregulation and then H_2S/$Ca_v3.2$ signaling essential for bladder pain.

2-P-081

Involvement of MARCKS in amylase release in GLP-1-stimulated pancreatic acini

Keitaro Satoh[1], Motoshi Ouchi[2], Asuka Morita[2], Yuta Ohno[1], Masanori Kashimata[1]

[1]Dept. Pharmacol., Sch. Dent., Asahi Univ., [2]Dept. Pharmacol. Toxicol., Sch. Med., Dokkyo Medical Univ.

It is well known that glucagon-like peptide 1 (GLP-1) can bind to the GLP-1 receptor of pancreatic islet to enhance insulin secretion through a cAMP-dependent pathway. However, little is known about the effects of GLP-1 on the pancreatic exocrine gland. In the gland, a signal transduction of amylase release is evoked mainly by an increase in intracellular Ca^{2+} levels and activation of PKC. Myristoylated alanine-rich C kinase substrate (MARCKS) is known as a major substrate for PKC. We previously demonstrated that MARCKS is involved in pancreatic amylase release through the Ca^{2+}-dependent pathway. Here, we studied the effects of GLP-1 on MARCKS phosphorylation and amylase release through the cAMP-dependent pathway in rat pancreatic acini. By the organ bath technique, GLP-1 did not induce amylase release in the intact pancreas. In contrast, it induced amylase release and MARCKS phosphorylation in isolated pancreatic acini. An inhibitor of PKA suppressed those effects. Furthermore, a MARCKS-related peptide inhibited the GLP-1-induced amylase release. These findings suggest that GLP-1 induces amylase release through MARCKS phosphorylation via activation of PKA in the isolated acini, but not in the intact pancreas.

2-P-082

Gastrin-releasing peptide modulates the pacemaker function of interstitial cells of Cajal

Noriyuki Kaji[1], Kazuhisa Kishi[1], Tamaki Kurosawa[1], Masatoshi Hori[1]

[1]Dept. of Vet. Pharmacol., Grad. Sch. of Agri. & Life Sci., The Univ. of Tokyo

Aim: Gastrin-releasing peptide (GRP) is a neuropeptide, which regulates wide range of biological processes. The aim of this study is to clarify the effect of GRP signaling on the interstitial cells of Cajal (ICC), an intestinal pacemaker cell.

Method: Immunofluorescence and RT-PCR was performed to identify the expression of GRP and GRPR. Maclurins spontaneous contraction was measured by using organ bath system. Ca^{2+} imaging was performed to assess the pacemaker function of ICC. Neuromedine C and [D-Phe6]-bombesin(6 -13)OMe was used as a GRPR agonist and antagonist, respectively.

Result: GRP was expressed in the enteric neuron at the level of myenteric plexus. GRPR mRNA was expressed in the FACS-sorted ICC. The frequency of spontaneous contraction in the antrum was increased by GRPR agonist. In contrast, the frequency of spontaneous contraction in the ileum was decreased by GRPR agonist. GRPR antagonist slightly increased the frequency of spontaneous contraction. The frequency of Ca^{2+} oscillation of ileal ICC was decreased by GRPR agonist and increased by GRPR antagonist.

Conclusion: GRP released from enteric neuron modulates the frequency of Ca^{2+} oscillation in ICC. GRP may have role in the regulation of basal rhythm of intestinal motility.

2-P-083

Functional roles of M_2 subtype of muscarinic receptors in the regulation of motor activity in mouse colon.

Naoshi Inaba[1], Horoshi Nagano[2], Alom Firoj[2], Hayato Matsuyama[1,2], Yasuyuki Tanahashi[3], Toshihiro Unno[1,2]

[1]Lab. Vet. Pharmacol., Dept. Vet. Med., Gifu Univ., [2]Dept. Pathogenetic Vet. Sci., United Grad. Sch. Vet. Sci., Gifu Univ., [3]Dept. Animal Med. Sci., Kyoto Sangyo Univ.

In gastrointestinal smooth muscles, both M_2 and M_3 subtypes of muscarinic receptors are co-expressed with a preponderance of the former subtype. Many previous studies have suggested that M_3 receptors exclusively contribute to the gastrointestinal smooth muscle contractions. However, the precise roles of the M_2 receptors in the regulation of gut motility remain to be elucidated. In the present study, we simultaneously recorded changes in the intraluminal pressure (IP), longitudinal tension (LT), and propelled volume (PV) in isolated colonic segments from M_2 receptor knockout (KO) and wild-type (WT) mice. In the WT preparations, luminal distension induced a continuous rhythmic contractile activity that was characterized by synchronous rises in IP and LT, occurring periodically at a constant interval. When atropine was applied, the frequency of rhythmic contractile activity was unchanged, but the amplitude of IP and PV were significantly reduced. Application of tetrodotoxin or hexamethonium abolished the rhythmic contractile activity. In the M_2KO preparations, the frequency of contractile activity elicited by luminal distension was comparable to that in WT preparations. However, the amplitude of IP and PV were significantly reduced in M_2KO preparations. These results suggest that M_2 muscarinic receptors participate in the regulation of colonic motor activity and have a significant role in propel of luminal contents.

2-P-084

Effects of HIV protease inhibitor on muscle contraction in ileal smooth muscle.

Hidenori Kanda[1], Akane Shimizu[1], Hisako Kaneda[1], Noriyasu Sasaki[2], Takeharu Kaneda[1]

[1]Lab. Vet. Med., Sch. Vet. Med., Nippon Veterinary and Life Science Univ., [2]Lab. Vet. Biochem., Sch. Vet. Med., Nippon Veterinary and Life Science Univ.

Anti-HIV therapeutic agents are divided into reverse transcriptase inhibitors, protease inhibitors (PI), integrase inhibitors, and entry inhibitors (CCR5 inhibitors). The combination therapy of HIV drugs with different mechanisms referred to as suppressing the proliferation of HIV in the long term. Unfortunately, HIV PI are accompanied by side effects in long-time treatment. The side effects are HIV PI-induced insulin-resistance, diarrhea and nausea. However, the effects of HIV PI on muscle contraction on gastrointestinal smooth muscle is still unknown. In the present study, we examined the effects of HIV PI on muscle contraction in the rat and guinea pig ileum. 1) In rat and guinea pig ileum, indinavir (3-100 μM) or ritonavir (0.1-10μM) inhibited high K^+- or histamine-induced contraction, dose-dependently, but the inhibition in high K^+-induced contraction more strongly than that in histamine-induced contraction. 2) In guinea pig ileum, ritonavir inhibited high K^+-induced increases of intracellular Ca^{2+} level and contraction. 3) In guinea pig ileum, ritonavir inhibited high K^+- or histamine-induced contraction of PSS including pyruvate instead of glucose. However, ritonavir -induced relaxation did not differ that of PSS including glucose. These results suggested that HIV PI-induced relaxation in ileum probably due to the inhibiting Ca^{2+} influx.

2-P-085

Study on measurement of internal anal sphincter movement in dog (application as evaluation method on defecation disorder)

Kazuaki Sasaki[1], Keiko Harada[1], Yasuo Nakamura[1], Masakazu Imaizumi[1], Seiichi Katayama[1], Katsuhide Nishi[1,2]

[1]Pharm.Dept.,LSImedience Corporation., [2]The Maruta Hospital

There are 3 types of fecal incontinence: leaky fecal incontinence, urgent fecal incontinence, and abdominal pressure-induced fecal incontinence. It is said that approximately 49% patients with fecal incontinence are leaky fecal incontinence. If anal sphincter is weakened, leaky fecal incontinence will occur. Since no effective therapies exist, new therapies and therapeutic drugs have being desired. Therefore, a measurement method of internal anal sphincter movement was developed by placing a force transducer used for gastrointestinal motility measurement in the internal anal sphincter muscle and each gastrointestinal tract, and measuring with a telemetry method under unanesthetized and unrestrained dogs. As a result, it is confirmed that adrenergic receptors existed in the internal anal sphincter, and noradrenaline α receptor and β receptor associated with contraction and relaxation of internal anal sphincter, respectively. However, this response under anesthesia was observed to be different from that under arousal, suggesting involvement of the autonomic nervous system; its mechanism of action and the involvement of other receptors were also investigated.

2-P-086

Measurement of cytokines expression and the effect of anti-mouse TNF-α antibody in collagen-induced arthritis mice

Takumi Yamazawa[1], Yoshiyuki Hayashida[1], Kenichi Yamamoto[1], Tomonari Miyazaki[1], Kinuko Zaizen[1], Kousuke Morizumi[1], Seiichi Katayama[1], Naoyuki Hironaka[1], Katsuhide Nishi[1]

[1]Pharm. Dept., Kumamoto Lab., LSI Medience Corp.

Rheumatoid arthritis (RA) is an autoimmune disease in which immune cells attack a bone, and finally joint functions are lost. In this study, we analyzed cytokine expression patterns which are relevant to RA (Experiment 1) and effects of anti-mouse TNF-α antibody (Experiment 2) in CIA (collagen-induced arthritis) mice. CIA was induced by immunizing DBA mice with type II collagen/Adjuvant (CFA or IFA) on day 0 (primary sensitization) and 21 (secondary sensitization). In experiment 1, the joint of tarsi of the hind limb were collected at 14 time points during day 0 and 49 and the expression of cytokines was measured with CBA assay and real time PCR. In experiment 2, the expression of cytokines at day 49 was measured following administration of anti-mouse TNF-α antibody intraperitoneally 3 times per week after the secondary immunization.

The results of the present experiment 1 showed that cytokines expression (IL-1β, TNF-α, IL-6) was peaked at day 31 to 38. Treatment with anti-mouse TNF-α antibody significantly decreased the expression of these cytokines compared with the vehicle group (Experiment 2). Thus, it is considered that our experiment system used in this study is useful for investigation of RA therapeutic drugs.

2-P-087

680C91, a small molecule inhibitor of TDO, suppresses production of IL-1β and IL-6 in LPS-stimulated murine macrophage cells

Ryusuke Sin[1], Naoki Sotogaku[1], Akinori Nishi[1]

[1]Department of Pharmacology, Kurume University School of Medicine

Tryptophan 2,3-dioxygenase (TDO), which converts tryptophan to kynurenine, is a rate-limiting enzyme of the kynurenine pathway. TDO has been reported to mediate immune responses in inflammatory diseases. Here, we report a novel pharmacological action of a well-known inhibitor of TDO, 680C91. The effects of 680C91 on the expression of LPS-stimulated pro-inflammatory cytokines were examined in Raw 264.7 cells and primary peritoneal macrophages prepared from wild-type and TDO knockout mice. In Raw264.7 cells and primary peritoneal macrophages prepared from wild-type mice, 680C91 significantly attenuated LPS-induced mRNA expression of IL-1β and IL-6, but not TNF-α. Interestingly, the inhibitory effects of 680C91 on LPS-induced mRNA expression of IL-1β and IL-6 were observed in primary peritoneal macrophages prepared from TDO knockout mice. In analyses of molecular mechanisms, we found that the phosphorylation of STAT3 and Akt induced by LPS was attenuated by 680C91. These observations suggest that 680C91 likely acts as an inhibitor of LPS-induced inflammatory responses in a TDO-independent manner via mechanisms involving STAT3 and Akt signaling.

2-P-088

α7 Nicotinic acetylcholine (ACh) receptors (α7 nAChRs) expressed on antigen-presenting cells (APCs) suppress the differentiation of CD4$^+$ T cells.

Mami Murase[1], Masato Mashimo[1], Masayo Komori[1], Takeshi Fujii[1], Shiro Ono[2], Yasuhiro Moriwaki[3], Hidemi Misawa[3], Koichiro Kawashima[4]

[1]Department of Pharmacology, Faculty of Pharmaceutical Sciences, Doshisha Women's College of Liberal Arts., [2]Laboratory of Immunology, Faculty of Pharmacy, Osaka Ohtani University., [3]Depertment of Pharmacology, Faculty of Pharmacy, Keio University., [4]Department of Molecular Pharmacology, Kitasato University School of Pharmaceutical Sciences.

All the immune cells such as T cells, macrophages and dendritic cells (DCs), have ACh-synthesizing ability and express α7 nAChRs involved in regulation of proliferation, and synthesis of antigen-specific antibodies and proinflammatory cytokines. We investigated role of α7 nAChRs on APCs in regulation of CD4$^+$ T cell differentiation. Spleen cells, including naïve CD4$^+$ T cells and APCs (macrophages and DCs), were isolated from ovalbumin (OVA)-specific TCR transgenic DO11.10 mice, and cultured with OVA in the presence of GTS-21, an α7 nAChR agonist. GTS-21 suppressed the OVA-activated CD4$^+$ T cell differentiation into regulatory T cells (Tregs), Th1, Th2 and Th17 cells. GTS-21 inhibited the production of Th cytokines (IFN-γ, IL-4 and IL-17). GTS-21 inhibited differentiation into Tregs of OVA-induced CD4$^+$ T cells co-cultured with APCs from the wild-type (WT) mice but did not affect differentiation of T cells co-cultured with APCs from α7 nAChR-deficient mice. These results suggest a critical role of α7 nAChRs on APCs in regulation of CD4$^+$ T cell differentiation, and that α7 nAChR agonists and antagonists could be potentially useful agents for immune response modulation and enhancement.

2-P-089

The role of serine, a non-essential amino acid, in the production of cytokines

Hiroya Ohta[1], Kento Kurita[1,2], Ibuki Shirakawa[3], Jun Okada[1], Yuka Ohno[1], Atsushi Ogino[1], Miyako Tanaka[1], Hiroshi Arima[2], Yoshihito Ogawa[4,5,6], Takayoshi Suganami[1]

[1]Department of Molecular Medicine and Metabolism, Research Institute of Environmental Medicine, Nagoya University, [2]Departments of Endocrinology and Diabetes, Nagoya University Graduate School of Medicine, [3]Department of Organ Network and Metabolism, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, [4]Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, [5]Department of Molecular Endocrinology and Metabolism, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, [6]Japan Agency for Medical Research and Development, Core Research for Evolutional Science and Technology (CREST)

Macrophages (MΦs) produce cytokines and control inflammatory responses. The production of cytokines by MΦs is regulated by various factors, including amino acids. The aim of this study was to elucidate the role of serine, a non-essential amino acid, in the production of cytokines. We first showed that cellular content of serine in MΦs was significantly decreased when cultured without serine, and this result indicates that MΦs are dependent on the serine present in extracellular spaces. We next showed that MΦs exhibited decreased production of interleukin (IL) 10, an anti-inflammatory cytokine, and increased production of IL6, a proinflammatory cytokine, in the absence of serine. Metabolome analysis showed that depletion of serine affected cellular metabolism of MΦs. We also found that cellular content of pyruvate, a crucial metabolite in the glycolytic pathway, was decreased. Administration of pyruvate to MΦs cultured without serine normalized the expression levels of IL10 and IL6. In conclusion, serine is crucial for the synthesis of pyruvate and contributes to adequate production of cytokines in MΦs. Thus, serine might be a novel target for anti-inflammatory drugs.

2-P-090

Critical role of formin-dependent cortical F-actin remodeling in TCR signaling

Yoshichika Katsura[1], Dean Thumkeo[1], Shuh Narumiya[1]

[1]Dept. Drug Discovery Medicine, Grad. Sch. Med., Kyoto Univ.

Acquired immunity is largely dependent on T cells. Upon the recognition of antigen by T cell, protein phosphorylation cascades downstream of T cell receptor (TCR) are triggered. It was previously shown that cytoskeletal F-actin is involved in TCR signaling. However, how such F-actin is regulated and exerts its function in TCR signaling remains unclear.

In this study, we investigated the role of the actin nucleating and polymerizing protein, formin, in TCR signaling by the using of a formin inhibitor, SMIFH2. We found that while phosphorylation of ZAP-70 remains intact, the phosphorylation of a ZAP-70 substrate, LAT, was strongly suppressed upon SMIFH2 treatment. In addition, we found that phosphorylated ZAP-70 colocalized with phosphorylated LAT on the cell membrane upon TCR stimulation in control cells, suggesting that LAT is phosphorylated by phosphorylated ZAP-70 on the cell membrane. Moreover, we employed superesolution microscopy and found that TCR stimulation-induced F-actin remodeling occurs in the cell cortex and requires formin's activity. These findings together therefore suggest that formin-dependent cortical F-actin remodeling is critical for the phosphorylation of LAT by ZAP-70 on the cell membrane.

2-P-091

Development of a steroid-resistant asthma model of mouse, and possible involvement of neutrophils in the pathogenesis

Naoki Takemoto[1], Miku Nomura[1], Maki Matsuo[1], Haruna Kanaya[1], Hiromu Takahashi[1], Masaya Matsuda[1], Kazuyuki Kitatani[1], Takeshi Nabe[1]

[1]Laboratory of Immunopharmacology, Faculty of Pharmaceutical Sciences, Setsunan University

Glucocorticoids have been extensively used for asthma therapy, whereas 5-10% of asthma patients are resistant to inhaled or oral glucocorticoids. Mechanisms underlying "steroid-resistant asthma" have been unclear. We aimed to develop a murine model for steroid-resistant asthma, and elucidated the mechanisms. Ovalbumin (OVA)-sensitized mice were intratracheally challenged with 0.02% or 2% OVA for 4 times. Dexamethasone was daily i.p. administered during the challenge period. Bronchoalveolar lavage and measurement of airway hyperresponsiveness(AHR) to methacholine were conducted after the 4th challenge. AHR and eosinophilia were induced in both 0.02% and 2% OVA-induced models with almost similar degree to each other. AHR and eosinophilia induced by 0.02% OVA were sensitive to dexamethasone, whereas those by 2% OVA were resistant to it. Interestingly, airway neutrophilia was also induced in both models, but the neutrophilia in 2% OVA model was more obvious than that of 0.02% OVA model. MIP-2, a murine IL-8 homologue was markedly increased in 2% OVA model. In conclusion, a steroid-resistant asthma model was successfully developed. Neutrophilic airway inflammation induced by MIP-2 may be involved in the pathogenesis.

2-P-092

Effect of QD on inflammatory response in DSS induced colitis mice.

Takaaki Shimizu[1], Kei Ozawa[1], Daichi Mori[1], Yuto Eizima[1], Chisa Takagi[1], Toshinori Sawano[1], Jin Nakatani[1], Hidekazu Tanaka[1]

[1]Lab. Pharm., Dept. Biomed Sci., Col. Life Sci., Ritsumeikan Univ.

Ulcerative colitis (UC) is an inflammatory bowel disease, and inflammation is its important therapeutic target. A wide variety of medications have been used to treat UC, including 5-aminosalicylates (5-ASA), salazosulfapyridine, and anti-tumor necrosis factor (TNF)-α antibodies. However, some patients fail to respond to these treatments. Several clinical trials indicate that Qing Dai/Indigo Naturalis (QD) powder derived from plant extracts ameliorate ulcerative colitis. However, the therapeutic mechanism of QD is unclear. In this study, we investigated the effect of QD on inflammatory response in the distal colon of UC model mice. UC was induced by administration of 1.25% dextran sulfate sodium (DSS) to 6 week-old male C57BL/6J mice. QD was distinguishingly more effective than 5-aminosalicylate and salazosulfapyridine in suppressing weight loss, diarrhea, and rectal bleeding. DSS induced the expressions of inflammatory genes on day 6 of DSS administration, and QD inhibited these gene expressions. These observations suggest that QD interferes with the early events of inflammatory pathway.

2-P-093

Role of prostaglandin receptor EP4 in lymphangiogenesis in dextran sulfate sodium (DSS)-induced colitis

Kanako Hosono[1,2], Yoshiya Itoh[1,2], Tomohiro Bettoh[2], Shuh Narumiya[3], Masataka Majima[1,2]

[1]Dept. Pharm., Sch. Med., Kitasato Univ., [2]Dept. Mol. Pharm., Grad. Sch. Med., Kitasato Univ., [3]Dept. Drug Discovery Med., Grad. Sch. Med., Kyoto Univ.

Prostaglandin E_2 regulates the colonic inflammation via PGE receptor EP4. EP4 facilitates wound healing through lymphangiogenesis. In this study, we examined whether EP4 is involved in the resolution of acute colitis by enhancement of inflammation-associated lymphangiogenesis. Experimental colitis was induced by administration of 2% DSS into C57BL/6 mice. DSS in drinking water was supplied for 7 days, followed by replacement of more 7 days-tap water. Mice were orally given EP4 antagonist (ONO-AE3-208; ONO) or vehicle for 7 days throughout the water-supply period. Compared with vehicle-treated mice, ONO-treated mice displayed increases in weight loss and clinical signs of colitis, and decreases in colon length. ONO increased lymphatic vessel density, which was associated with enhancement of lymphatic markers including LYVE-1 and VEGFR3. Pro-lymphatic vessel growth factors also were elevated. Extensive infiltration of macrophages into the colonic tissues was found, which was accompanied with up-regulated expression of TNFα and iNOS, and down-regulated expression of TGFβ. These results suggest that EP4 signaling promotes healing of DSS-induced colitis through attenuation of macrophage infiltration and lymphangiogenesis.

2-P-094

Role of the prostaglandin E_2-EP4 system in Concanavalin A-induced hepatitis

Yoshitaka Imamichi[1], Koh-Ichi Yuhki[1], Hitoshi Kashiwagi[1], Shima Kumei[1], Fumiaki Kojima[2], Katsura Nakanishi[1], Shuh Narumiya[3], Fumitaka Ushikubi[1]

[1]Dept. Pharmacol., Asahikawa Med. Univ., [2]Dept. Pharmacol., Kitasato Univ., [3]Dept. Drug Discov. Med., Kyoto Univ. Grad. Sch. Med.

Concanavalin A (Con A) induces hepatitis in mice, which is an established model of acute immune-mediated hepatitis. Real-time PCR analysis revealed that mRNAs for prostaglandin E_2 (PGE_2) synthetic enzymes and PGE_2 receptor subtype EP4 were up-regulated in the liver of wild-type mice after Con A administration. In this study, we attempted to clarify the role of the PGE_2-EP4 system in Con A-induced hepatitis. After Con A administration, serum transaminase (AST and ALT) levels in EP4-deficient mice were significantly higher than those in wild-type mice. Accordingly, histological examination of the liver revealed significant increase in the size of necrotic areas in EP4-deficient mice compared with that in wild-type mice. To evaluate whether EP4 agonists protect the liver, an EP4 agonist ONO-4819CD was administered into wild-type mice before Con A administration. ONO -4819CD prominently decreased serum transaminase levels after Con A administration, suggesting the liver protective effect of EP4 agonists in Con A-induced hepatitis. These results indicate that the PGE_2-EP4 system plays a liver protective role in Con A-induced hepatitis and suggests that EP4 agonists are promising therapeutic candidates for the treatment of immune-mediated hepatitis.

2-P-095

Memory Decline and Bone Loss in Middle-aged Mice are induced by LPS derived from *Porphyromonas gingivalis*

Yebo Gu[1], Junjun Ni[2], Muzhou Jiang[2], Zhou Wu[2,3], Ichiro Takahashi[1]

[1]Section of Orthodontics and Dentofacial Orthopedics, Faculty of Dental Science, Kyushu University, Fukuoka, Japan, [2]Department of Aging Science and Pharmacology, Faculty of Dental Sciences, Kyushu University, Fukuoka, Japan, [3]OBT Research Center, Faculty of Dental Sciences, Kyushu University

Emerging clinical studies suggest periodontitis is positively involved in cognitive decline in Alzheimer's disease (AD). In addition, periodontitis is also known related to osteoporosis with aging. We have found that chronic systemic exposure to Lipopolysaccharide from *Porphyromonas gingivalis* (*Pg*LPS) could induce AD-like phenotype in middle-aged mice in previous research. However, the evidence and mechanism bridging bone loss and memory decline during periodontitis is still poor. The present study aimed to explore bone loss and hippocampus-dependent memory decline in middle-aged mice after chronic systemic exposure of *Pg*LPS. Significant decrease of bone volume/tissue volume ratios as well as trabecular numbers, and significant increase of structure model indexes in the proximal tibia were determined in middle-aged mice at week 3 after chronic systemic exposure to *Pg*LPS compared to control group. Moreover, significant memory decline and significant upregulated expression of IL-17 and IL-6 in microglia were detected at week 3 after systemic exposure to *Pg*LPS. These findings provide the evidence that bone loss was parallel to hippocampus-dependent memory decline in middle-aged mice during chronic systemic exposure to *Pg*LPS.

2-P-096

Examination focusing on intestinal microbiota composition of patients with sarcopenia

Miki Doi[1], Rikako Inoue[1], Aki Ogawa[2], Yukihiro Yoshimura[3], Satoko Hiramatsu[1], Makoto Ayabe[4], Yasuyuki Irie[1]

[1]Dept. Nutri. Scie., Okayama Pre. Univ., [2]Dept. Clini. Nutri. and Diet., Konan Women's Univ., [3]Fac. Nutri., Kobe Gaku. Univ., [4]Dept. Huma. Info. Engineer., Okayama Pre. Univ.

Sarcopenia is defined as a decrease in muscle mass with aging, which causes a decrease in physical function, high risk of falls, and bedridden state, resulting in a decrease in QOL and an increase in medical and caring burden. On the other hand, as the prevalence of constipation increases in the eldery, the intestinal environment is presumed to deteriorate. However, the relationship between intestinal environment and sarcopenia is to be elucidated. The aim of current study is to clarify the relationship between sarcopenia and intestinal environment in humans.

We examined and analyzed dietary habits (BDHQ), body composition, exercise habits, past history, intestinal microflora-constituting bacteria, etc. for 42 subjects over 65 years old. In the sarcopenia group (n = 8), body weight, limb skeletal muscle mass, ingested nutrient content and the like tended to be low. For intestinal bacterial flora, the ratio of Firmicutes / Bacteroidetes was significantly reduced in the sarcopenia group.

Accordingly, it is suggested that maintenance of physical function, improvement of the quality of meal, and amelioration of the gut microbiota may be necessary to prevent sarcopenia.

2-P-097

Calmodulin-like skin protein suppresses hydrogen peroxide- or ultraviolet-induced increase in senescence associated beta-galactosidase in keratinocytes

Yusuke Takahara[1,2,3], Nobuyuki Miyachi[1,3], Mikirou Nawa[2], Ryosuke Ota[3], Kenta Shingaki[3], Masaaki Matsuoka[1,2]

[1]Department of Dermatological Neuroscience , Tokyo Medical University, [2]Department of Pharmacology , Tokyo Medical University, [3]Noevir Co., Ltd

Calmodulin-like skin protein (CLSP) is a secreted peptide that is restrictedly produced in skin keratinocytes and some related epithelial cells. It has been previously shown that CLSP is recruited via blood stream into the central nervous system where it likely exerts neuroprotective effect against toxicity related to Alzheimer's disease (AD) by binding to the heterotrimeric humanin receptor and activates intracellular survival signaling. However, it remains to be elucidated whether secreted CLSP has some biological activities outside the central nervous system. In the current study, using hydrogen peroxide (H_2O_2) - and ultraviolet (UV) -induced senescence models of primarily cultured skin keratinocytes, we have addressed to the question as to whether CLSP is involved in senescence of skin keratinocytes. We first found that CLSP expression was potentiated by the treatment with H_2O_2 and the exposure to UV in keratinocytes. Furthermore, the co-incubation with recombinant CLSP reduces Senescence-associated beta-galactosidase-positivity in keratinocytes that is induced by the treatment with H_2O_2 and the exposure to UV. These results suggest that CLSP may function as a senescence-suppressing factor for keratinocytes.

2-P-098

Characterization of inductions of neurite outgrowth and neuronal markers in NSC-34 cells during differentiation with prostaglandin E_2

Hiroshi Nango[1], Yasuhiro Kosuge[1], Yuri Aono[2], Tadashi Saigusa[2], Yoshihisa Ito[1], Kumiko Ishige[1]

[1]Lab. Pharmacol., Sch. Pharm., Nihon Univ., [2]Dept. Pharmacol., Nihon Univ. Sch. Dent. at Matsudo

NSC-34 cells differentiated into motor neurons (MN) by exposure to retinoic acid (RA) are widely used as an experimental model of MN. We reported previously that prostaglandin E_2 (PGE_2) also differentiates these cells and promotes neurite outgrowth, a morphological marker of neuronal differentiation. However, it still remains unclear whether PGE_2-treated cells possess characteristics of mature MN. In this study, we compared the biochemical and functional properties of PGE_2-treated NSC-34 cells with those of RA-treated cells. PGE_2 (30 μM) induced neurite outgrowth which reached a peak at day 2, whereas RA (10 μM) -induced neurite outgrowth reached the same level at day 7. Immunoblotting showed that expression levels of a neuronal marker (Synaptophysin) and MN markers (HB9 and Islet-1) in PGE_2-treated cells at day 2 were comparable to those in RA-treated cells at day 7. The level of choline acetyltransferase protein and the basal acetylcholine release in PGE_2-treated cells were higher than those in RA-treated cells. These results suggest that both PGE_2 and RA promote neuronal maturation of NSC-34 cells, but PGE_2 is more effective to promote cholinergic function in these cells than RA.

2-P-099
Anti-stress effect of Humanin, a biologically active peptide

Natsumi Ikegawa[1], Minetaka Murakami[1], Takako Niikura[1]

[1]Dept. Inf Comm Sci., Fac Sci Tech., Sophia Univ.

Humanin (HN) is a secretory 24-residue peptide. HN was first identified as a cytoprotective factor that suppresses neuronal death in Alzheimer's disease and exerts the activity through cell surface receptors. Subsequent studies revealed that HN can reverse disease-associated change in cellular functions of various types of tissues including brain, muscle, and pancreas. It is also reported that HN increases mitochondrial ATP production in some cell types. The level of HN in circulation decreases age-dependently in rodents and human. However physiological roles of HN is largely unknown. In this study, we assessed the effect of HN against stress in mice. We gave immobilization stress to young male mice and measured their blood glucose levels over time. The immobilization stress caused increase in the glucose level after 30 min to 90 min of the treatment. Intraperitoneal injection of S14G-HN, a highly potent HN derivative, attenuated the stress-induced increase in the glucose level. S14G-HN alone showed no significant change in the glucose level which was similar to that of non-stressed mice. A neuroprotection-defective HN mutant did not affect the stress-induced increase in the blood glucose level, suggesting that this anti-stress effect of HN is mediated by a receptor.

2-P-100
Effect of AGEs-Lf interaction on macrophage TNF-α expression

Shuji Mori[1], Masahiro Watanabe[1], Hidenori Wake[2], Keyue Liu[2], Kiyoshi Teshigawara[2], Hideo Takahashi[3], Masahiro Nishibori[2], Takao Toyomura[1]

[1]Dept. Pharmacol., Sch Pharm., Shujitsu Univ., [2]Dept. Pharmacol., Okayama Univ. Grad. Sch. Med. Dent. Pharm. Sci., [3]Dept. Pharmacol., Fac. Med., Kindai Univ.

Advanced glycation end products (AGEs) are the potential inflammatory molecules produced by non-enzymatic glycation reaction between reducing sugar and amine residues on biomolecules, and have been suggested to be involved in various age-related diseases. AGEs stimulate the pattern recognition receptors (TLR4, TLR2, RAGE) on immune cells such as macrophages and vascular endothelial cells, and are involved in chronic inflammation and tissue remodeling, Therefore, it is thought that regulation of AGEs-induced inflammatory responses can be an important therapeutic target for prevention and treatment of age-related diseases. In previous studies, we found that AGEs and lactoferrin (Lf) can interact with high affinity. Based on this finding, it was examined the effect of AGEs-Lf interaction on TNF-α mRNA expression in macrophage in this study. For this purpose, we initially made the triple KO macrophage cell line which lacked three receptors (TLR4, TLR2 and RAGE) by genome editing. When this triple KO strain was stimulated with Lf (10 μg/mL), it was shown that the increase in expression of TNF-α mRNA expression. On the other hand, this stimulation was significantly suppressed by co-addition of AGEs (1,000 μg/mL). From these findings, it was suggested that AGEs interact with Lf with high affinity, and they are involved in the pathogenesis of tissue remodeling.

2-P-101

Polyphosphate modulates the STAT1 pathway and suppresses the expression of CXCL10 and iNOS in macrophages

Kana Harada[1,2], Nao Abe[2], Koyo Nakashima[2], Megumi Kusumoto[2], Kazuaki Nakatomi[2], Miho Hirayama[2], Momoko Okamoto[2], Mizuki Kimura[2], Izumi Hide[1], Shigeru Tanaka[1], Norio Sakai[1], Kumatoshi Ishihara[2]

[1]Dept. Mol. Pharmacol. Neurosci., Inst. Biomed. Health Sci., Hiroshima Univ., [2]Lab. Neuropharmacol., Fac. Pharmaceut. Sci., Hiroshima Int'l Univ.

Although polyphosphate [poly(P)], a linear polymer of orthophosphates, is found in various tissues, its function remains largely unknown. We previously reported that the treatment with poly(P) reduced the expression of inducible nitric oxide synthase (iNOS), an inflammatory mediator, in macrophages activated by bacterial lipopolysaccharide (LPS). In this study, we show that LPS-induced expression of chemokine (CXC motif) ligand (CXCL) 10 is also decreased by poly(P). Poly(P) consistently inhibited the phosphorylation of signal transducer and activator of transcription (STAT) 1, a transcription factor for CXCL10 and iNOS, from 2 to 24 h after LPS treatment. The activation of Janus kinase (JAK) 1 and tyrosine kinase (TYK) 2, which phosphorylate STAT1, was suppressed by poly(P) at 2 h but not 12 h. At 12 h, poly(P) enhanced the activation of Src homology-2 domain containing protein tyrosine phosphatase (SHP) 2, which dephosphorylates STAT1. These results suggest that poly(P) suppresses LPS-induced STAT1 activation through at least two different mechanisms, the inhibition of JAK1/TYK2 and the activation of SHP2, resulting in decreased production of CXCL10 and iNOS. This may affect inflammation and host defense against infection.

2-P-102

The production and role of hydrogen sulfide and hydrogen polysulfides in mammalian cells

Norihiro Shibuya[1], Shin Koike[2], Yuka Kimura[1], Ryo Miyamoto[1], Yuki Ogasawara[2], Hideo Kimura[1]

[1]Natl. Inst. Neurosci., NCNP, [2]Analyt. Chem., Meiji Pharm. Univ.

Accumulating evidence shows that hydrogen sulfide (H_2S) has physiological functions in various tissues and organs. It includes regulation of neuronal activity, vascular tension, a release of insulin, and protection of the heart, kidney, and brain from ischemic insult. H_2S is produced from L-cysteine by pyridoxal 5'-phosphate (PLP)-dependent enzymes, cystathionine beta-synthase (CBS) and cystathionine gamma-lyase (CSE). 3-Mercaptopyruvate sulfurtransferase (3MST) is the third H_2S-producing enzyme. 3-Mercaptopyruvate (3MP) is provided from L-cysteine and α-ketoglutarate by a PLP-dependent cysteine aminotransferase (CAT). An additional pathway for the production of H_2S from D-cysteine involving 3MST and D-amino acid oxidase (DAO) has been identified. Recently, hydrogen polysulfides (H_2S_n), which are oxidized forms of H_2S, have been found to stimulate transient receptor potential ankyrin1 (TRPA1) channel, much more potently than H_2S. H_2S_n is produced from 3MP by 3MST. In addition to the enzymatic production, the interaction between H_2S and nitric oxide (NO), another gaseous signaling molecule, also generates H_2S_n. These observations provide new insight into the production of these molecules and their mechanisms of physiological functions.

2-P-103

The inhibitory effect of escitalopram on $Na_v1.2$ voltage-gated sodium channels

Yoshihiko Nakatani[1], Taku Amano[1]

[1]Department of Pharmacotherapeutics, School of Pharmacy, International University of Health and Welfare

Escitalopram was developed as an antidepressant of the selective serotonin reuptake inhibitors (SSRIs) from citalopram as its (S)-stereoisomer. It has been known that escitalopram showed the high selectivity to serotonin transporters compared to other SSRIs. However, it has been also considered that escitalopram, including other antidepressants, have a risk to induce seizures, such as epileptic patients. In this study, we examined the effect of escitalopram in $Na_v1.2$ voltage-gated sodium channels (VGSCs) transfected HEK293 cells. $Na_v1.2$ VGSCs current decreased by approximately 50.7±8.3 % under treatment with 100 μM escitalopram. The IC_{50} of escitalopram against $Na_v1.2$ VGSCs current was 114.17 μM. Moreover, the treatment with 100 μM escitalopram could shift the activation curve toward hyperpolarization side and the voltage at half-maximal activation shifted from -13.8 ± 4.6 mV to -21.5 ± 3.9 mV toward hyperpolarization. In addition, the treatment with 100 μM escitalopram also could shift the inactivation curve toward hyperpolarization side and the voltage at half-maximal inactivation shifted from -50.3 ± 3.7 mV to -56.7 ± 6.0 mV toward hyperpolarization. These findings suggested that escitalopram might be able to inhibit $Na_v1.2$ VGSCs current and changes the kinetics of both activation and inactivation.

2-P-104

Regulatory mechanism underlying up-regulation of Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ in inflammatory $CD4^+$ T cells of IBD model mice

Susumu Ohya[1], Miki Matsui[1,2], Junko Kajikuri[1], Hiroaki Kito[1], Kyoko Endo[1,2], Takayoshi Suzuki[3]

[1]Dept. Pharmacol., Grad. Sch. Med. Sci., Nagoya City Univ., [2]Dept. Pharmacol. Div. Pathol. Sci., Kyoto Pharmaceut. Univ., [3]Grad. Sch. Med. Sci., Kyoto Prefect. Univ. Med.

Previous study showed the up-regulated expression of the Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ in inflammatory $CD4^+$ T cells has been implicated in the pathogenesis of inflammatory bowel disease (IBD). Histone deacetylases (HDACs) are involved in intestinal inflammation, and HDAC inhibitors such as vorinostat ameliorate autoimmune colitis. In the present study, we examined the involvement of HDACs in the up-regulation of $K_{Ca}3.1$ in the $CD4^+$ T cells of IBD model mice. The expression levels of $K_{Ca}3.1$ and its transcription regulators were quantitated using a real-time PCR assay, Western blotting, and depolarization responses induced by the selective $K_{Ca}3.1$ blocker TRAM-34 (1 μM) were measured using a voltage-sensitive fluorescent dye imaging system. The treatment with 1 μM vorinostat, a pan-HDAC inhibitor, for 24 hr repressed the transcriptional expression of $K_{Ca}3.1$ in the splenic $CD4^+$ T cells of IBD model mice. Accordingly, TRAM-34-induced depolarization responses were significantly reduced. HDAC2 and HDAC3 were significantly up-regulated in the $CD4^+$ T cells of IBD model mice. The down-regulated expression of $K_{Ca}3.1$ was observed following treatments with the selective inhibitors of HDAC2 and HDAC3. Taken together, The $K_{Ca}3.1$ K^+ channel regulates inflammatory cytokine production in $CD4^+$ T cells, mediating epigenetic modifications by HDAC2 and HDAC3.

2-P-105

A slit diaphragm mechanosensor protein podocin may regulate the mechanical potentiation of receptor-activated TRPC6 channel

Jun Ichikawa[1], Midori Nakagawa[1], Ryuji Inoue[1]

[1]Dept. Physiol., Fukuoka Univ. Sch. Med.

It has been suggested that excessive activities or focal segmental glomerulosclerosis (FSGS)-associated gain-of-function mutations of the canonical transient receptor potential 6 (TRPC6) channel may result in a proteinuria and progressive kidney failure. In this study, we investigated the impact of podocin, a mechanosensor at the slit diaphragm of glomerulus, on these enhanced channel activities by Ca^{2+} imaging and patch clamp techniques. Co-expression of podocin suppressed mechanically-enhanced responses of receptor-activated wild-type and 131T-FSGS mutant of TRPC6 channels overexpressed in HEK293 cells. In differentiated cultured podocytes (MPCs) stably overexpressing TRPC6, its mechanical potentiation after receptor activation was found to be decreased as compared with that observed in the heterologous system. However, this decrease was reversed by siRNA knockdown of endogenous podocin expression. These results suggest that the mechanosensitivity of receptor-activated TRPC6 channel may be negatively regulated by interaction with podocin, the mechanism being presumably important to normalize otherwise exaggerated TRPC6-mediated Ca^{2+} responses.

2-P-106

TRPM2 channel-Stat3 complex regulates the polarity of tumor-associated macrophage and tumor angiogenesis.

Yamada Yuji[1], Yoshifumi Ueda[1], Ryuhei Kurogi[1], Yoshiaki Hasegawa[1], Tarek Mohamed Abd El-Aziz[1], Masayuki Mori[1], Yasuo Mori[1]

[1]Dept. Synth. Chem. and Bio. Chem., Grad. Sch. Eng., Kyoto Univ.

The tumor microenvironment is a complex tissue composed of various stromal cells including immune cells. Especially, tumor-associated macrophages (TAM) are one of the major components of tumor tissues, and they play a pivotal role in prompting the various tumor growths by producing growth factors and reactive oxygen species (ROS). Previously, we have reported that TRPM2 channel, a ROS-sensitive Ca^{2+} channel, is abundantly expressed in macrophages and is tuning gene expression via transcription factor NF-kB. Here, we report the significance of TRPM2 channel in the regulation of pro-inflammatory M1 and pro-angiogenic M2 phenotype of TAM. In TRPM2 knockout mice, TAMs around s.c.-injected B16F10 melanoma tumor showed strong expression of M2 phenotypic markers and proangiogenic factor VEGF according to the enhanced activity of Stat3. Interestingly, blood vessels in TRPM2 knockout mice tumor were so-called non-productive, which is characterized by an increase in vascular density and decrease in tissue perfusion and thus the tumor progression was suppressed. We also found that the activation of TRPM2 channel induced by H_2O_2 suppress the activity of Stat3 in vitro. Importantly, TRPM2 protein showed physical interaction with Stat3 protein, and their complex was degraded gradually in the presence of H_2O_2. Therefore, our data suggest that TRPM2-Stat3 complex is critical for handling the phenotypes of macrophages depending on the environmental oxygen/redox conditions.

2-P-107
Role of TRPM7 channel in pancreatic stellate cells

Tatsuya Hirano[1], Takashi Kuwamura[1], Reiko Sakaguchi[1,2], Tomohiro Numata[1], Yasuo Mori[1]

[1]Dept. Synthetic Chemistry and Biological Chemistry. Grad. Sch. Eng, Kyoto Univ., [2]iCeMS., KUIAS., Kyoto Univ.

Pancreatic cancer is the 5th leading cause of death among various cancers and is characterized by poor prognosis. Recently, pancreatic stellate cells (PSCs) have been identified as one of the causes to increase the malignancy of pancreatic cancer. In this study, we focused on transient receptor potential melastatin 7 (TRPM7) channel. TRPM7 is ubiquitously expressed in various tissues and cells and reported to be involved in cell proliferation ability, gene expression, and differentiation.

To investigate the function of TRPM7 in PSCs, we constructed TRPM7 conditional knock out (cKO) mouse using the Tamoxifen (Tam)-inducible Cre / loxP system. The expression of TRPM7 was confirmed both in vivo and in vitro, which was abolished by Tam administration. We investigated the condition of Tam administration to PSCs isolated from TRPM7 cKO mouse and found that it takes one week for TRPM7 to be completely down-regulated after Tam induction.

PSCs activate the surrounding pancreatic cancer cells by inflammatory cytokines and growth factors such as Platelet-Derived Growth Factor (PDGF). When PSCs were treated with PDGF, enhanced expression of TRPM7 was confirmed.

In conclusion, our data suggest that TRPM7 is very stable in PSC and is involved in the activation of PSCs.

2-P-108

Three amino-acid sequences regulate the expression level of aquaporin-4

Abe Yoichiro[1], Ryosuke Suzuki[1], Wakami Goda[1], Masato Yasui[1]

[1]Dept. Pharm., Sch. Med., Keio Univ.

Aquaporin-4 (AQP4) is a water cannel playing a role in water transport and homeostasis of the brain. Previously, we observed that deletion of the C-terminal domain causes degradation of AQP4. In this report, we identified three amino-acid sequences that regulate the expression level of AQP4. First, deleting C-terminal 10 amino acids (Asp^{314}-Val^{323}) greatly reduced the level of AQP4. Substitutions of Ala for $Asp^{314}$and/or Glu^{318}mimicked this effect, suggesting that two acidic amino acids in this region is important to prevent AQP4 from degradation. Second, this reduction of AQP4 was rescued when the C-terminal domain was deleted more than 43 amino acids, suggesting that the region between $Val^{280}$and Lys^{313}contains a signal for the degradation. Substitution of Phe for Tyr^{277}or Arg for Val^{280}increased the level of AQP4 lacking C-terminal 42 amino acids (Δ282-323), suggesting that a tyrosine-based endocytic motif (YXXΦ) is involved in the degradation of AQP4. Finally, deletion between $Lys^{259}$and Ala^{270} increased the level of AQP4. In contrast to the disruption of the putative YXXΦmotif, the 12-amino-acid deletion could not rescue AQP4 Δ282-323 from degradation, indicating that the deletion increased the level of AQP4 with a different mechanism from YXXΦmotif mutants.

2-P-109

Investigating Lysosomal Regulation of mTOR Signaling Pathways via Vacuolar-type H^+-ATPase

Chisa Hiraoka[1], Hirofumi Morihara[2], Yasunori Okabe[2], Marina Watanabe[2], Kiichiro Tomoda[2], Michio Asahi[2]

[1]Osaka Medical College, [2]Dept. Pharm., Osaka Medical College

Vacuolar-type H^+- ATPase (v-ATPase), a multi-subunit protein complex, has two distinct functions on lysosomes: acidifying the lysosomal lumen and controlling mTOR-S6K (mTORC1) signaling, both of which are crucial for several biological processes. However, little is known about how both functions of v-ATPase are coordinated and whether lysosomes are also involved in mTOR-AKT (mTORC2) signaling. We found that knocking down (KD) of a subunit of v-ATPase impairs cell proliferation of undifferentiated induced pluripotent stem cells (iPSCs) although all cells do not die. As expected, lysosomal pH increased and mTORC1 signaling was attenuated in the KD cells. Unexpectedly, mTORC2 signaling was also impaired. Treatment of iPSCs with bafilomycin A1, a specific inhibitor of v-ATPase proton pump, increased lysosomal pH, and impaired both mTORC1 and mTORC2 signaling pathways. When treating Hek293, a cancer cell line, with the inhibitor, attenuation of mTORC2 activity was observed. Therefore, in addition to mTORC1, v-ATPase regulates the mTORC2 activity. We are now investigating how the proton pump affects the mTOR signaling using deletion mutants of the subunit and some chemicals that affect pH in lysosomes. We will discuss our results in this meeting.

2-P-110

Fundamental study of novel chemotherapy for malignant glioma targeting mTOR signaling

Takeyoshi Eda[1,2], Yu Kanemaru[2], Masayasu Okada[2], Manabu Natsumeda[2], Makoto Oishi[2], Yukihiko Fujii[2]

[1]Division of Pharmacy, Medical and Dental Hospital, Niigata Univ., [2]Dept. of Neurosurgery, Brain Reseach Institute, Niigata Univ.

mTOR (mammalian target of rapamycin) is an enzyme protein involved in intracellular signal transduction. It is known to constitute the mTOR pathway with many molecules and control important functions related to cell survival such as cell division, growth, metabolism and autophagy. Rapamycin and its derivatives are immunosuppressor macrolides that inhibit mTOR function and yield anti-proliferative activity in various malignancies. We screened for antibiotics that inhibit mTOR as therapeutic drug candidates and examined the effect of the drug. Clindamycin (CLDM), belonging to macrolides, inhibited survival and proliferation in human-derived glioma cell lines (U251, T98G, LN-229). CLDM also sensitized the antitumor effects of temozolomide. CLDM suppressed the phosphorylation of S6 protein and p70S6 Kinase in a dose-dependent manner. In NGT -41, a cell line derived from autopsy of an epithelioid glioblastoma patient, CLDM induced G1-S cell cycle delay and apoptosis. These results suggest that CLDM regulates the mTOR signaling as an intracellular communicator in glioma and controls tumor growth. We discuss the current and future applications of CLDM and related translational research possibly leading to novel therapeutic strategies against malignant glioma.

2-P-111

Effect of serotonin on phosphorylation of ribosomal p70 S6 kinase in primary cultures of adult rat hepatocytes

Kota Naito[1], Kazuki Kurihara[1], Hajime Moteki[1], Mitsutoshi Kimura[1], Masahiko Ogihara[1]

[1]Dept. Clin. Pharmacol., Pharmace. Sci. Josai Univ.

Previously, we reported that serotonin (5-hydoxytryptamine; 5-HT) induced DNA synthesis and proliferation in primary cultures of adult rat hepatocytes. The 5-HT effect was due to transforming growth factor (TGF)-α secreted by activation of 5-HT_{2B} receptor/phospholipase C (PLC)/Ca^{2+} pathway. In this study, we investigated whether 5-HT would stimulate phosphorylation of ribosomal p70 S6 kinase (p70S6K) in the cultured cells. Phosphorylated p70S6K was identified by Western blotting analysis using anti-phospho-p70S6K monoclonal antibody. The phosphorylated p70S6K was increased at 5 min, and reached a peak at 30 min after 5-HT addition. On the other hand, the phosphorylation of p70S6K induced by 5-HT was completely abolished the 5-HT_{2B} receptor antagonist, LY272015, U-73122, a PLC inhibitor, BAPTA/AM, a membrane-permeable Ca^{2+} chelator, verapamil, L-type Ca^{2+} channel blocker, and somatostatin. Moreover, specific inhibitors of growth-related signal transducers (e.g., LY294002, PD98059, and rapamycin) blocked phosphorylation of p70S6K induced by 5-HT. These results suggest that secretion of TGF-α accelerates hepatocyte proliferation through the mitogen-activated protein kinase (MAPK)/p70S6K pathway.

2-P-112

Differentiation-Inducing Factors modulates Hippo signaling pathway through YAP in HeLa cells

Fumi Takahashi[1], Shin Ishikane[1], Yumiko Toyohira[1]

[1]Dept. Pharm., Univ. Occup. & Env. Health

Differentiation-inducing factors, produced by *Dictyostelium discoideum*, show anti-tumor activity by suppressing the Wnt/β-catenin signaling pathway via activation of GSK-3 in several human tumor cells. We tried to clarify the all signaling pathways affected by DIF using DNA microarray analysis and found that Hippo signaling pathway is one of pathways which activities were modified by DIFs. The Hippo signaling pathway controls organ size in animals through the regulation of cell proliferation and apoptosis. Further, Hippo signaling pathway is known to be related with cancer development and metastasis formation. DIF-1 and DIF-3 significantly elevated expression of Hippo signaling pathway target genes in a time-dependent manner. We next analyzed the effect of DIF-1 on Yes-associated protein (YAP), a key transcriptional coactivator in the Hippo signaling pathway. We found that DIF-1 significantly reduced phosphorylation level of YAP, thereby activating YAP to translocate to nucleus in HeLa cells. We now try to clarify the mechanism by which DIF reduces YAP phosphorylation.

2-P-113

Contraction of hepatic stellate cells by Endothelin-1 is mediated by calcium- myosin light chain kinase system and Rho-kinase system.

Reina Hase[1], Ryosuke Suzuki[1], Naoki Dohi[1], Yumeto Wakabayashi[1], Ryota Nishiyama[1], Momoka Yamaguchi[1], Shin-Ya Saito[1,2], Tomohisa Ishikawa[1]

[1]Dept. Pharmacol., Sch. Pharm. Sci., Univ. Shizuoka, [2]Sch. Vet. Med., Okayama Univ. Sci.

Quiescent hepatic stellate cells (qHSCs) possess processes and surround the sinusoids, the capillaries of the liver. We have suggested that HSCs regulate hepatic blood flow under physiological conditions. However, there is no direct evidence whether qHSCs contract. In this study, we established a new method to evaluate the contraction of cells and used this method for studying an effect of receptor agonist on the contractile function of qHSCs. qHSCs isolated from mice were seeded on collagen gel with fluorescent beads, so that beads were attracted to contracting qHSCs. By measuring the distance of the beads movement, we evaluated the contraction. 10 nM endothelin-1 (ET-1) induced slow contraction of qHSC and 1μM bosentan inhibited this contraction. This suggested ET-1 make qHSCs contract via endothelin receptor. Myosin light chain kinase (MLCK) inhibitor, ML-9 (1 μM) inhibited this contraction. Calcium free condition reduced ET-1-induced contraction but still sustained contraction was remained. This was diminished by combination use with 1 μM H-1152, the Rho kinase inhibitor. These results suggest that ET-1 induces contraction in qHSCs which is mediated by calcium-MLCK system and Rho-kinase system.

2-P-114
Palmitoylation of 67kDa laminin receptor

Seika Okamoto[1], Naoko Adachi[2], Daiki Hayashi[3], Shuji Ueda[3], Minoru Yamanoue[3], Naoaki Saito[2], Yasuhito Shirai[3]

[1]Kobe Univ., [2]Mol. Pharmacol., Biosignal Res. Ctr., Kobe Univ., [3]Dept. of Agrobioscience, Grad. Sch. of Agricultural. Sci., Kobe.Univ.

Diacylglycerol kinase (DGK) is a lipid kinase to convert DG to phosphatidic acid (PA). Both DG and PA are important lipid messengers. Thereby, DGK is thought to have important roles in lipid signaling. In addition, the enzyme recently has been gotten attention as a pharmaceutical target because of its involvement in cancer, neuronal disease and diabetes etc. We have reported that Epigallocatechin gallate (EGCg) and α-tocopherol (VtE) activate DGKα via 67kDa laminin receptor (67LR), resulting in improvement of diabetic nephropathy. The 67LR is believed to be concentrated in lipid raft as homo dimer. However, it is still unknown whether 67LR is palmitoylated although many proteins localized in raft have the modification. Therefore, we investigated palmitoylation of 67LR and its physiological meaning in the activation of DGKα.

67LR was slightly palmitoylated in resting state. Both EGCg and VtE treatment induced palmitoylation. The VtE-induced palmitoylation was peaked at 5 min and then decreased gradually. The time course of the palmitoylation of 67LR was fit to a time course of translocation (activation) of DGKα by VtE. The VtE-induced translocation of DGKα was abolished by treatment of a palmitoyltransferase inhibitor. These results indicate the palmitoylation of 67LR is necessary for the activation of DGKα by VtE or EGCg. The physiological meaning of the palmitoylation of 67LR in localization and dimerization are under investigation.

2-P-115

Sir2D, a Sirtuin family protein, regulates the adenylate cyclase A expression through the interaction with a transcription factor MybB

Hideo Taniura[1], Shuhei Soeda[1], Himeka Uemura[1], Risa Kinoshita[1], Atsuki Takeshita[1], Katsuichiro Fukuta[1], Saki Arita[1]

[1]Laboratory of Neurochemistry, Department of Pharmaceutical Sciences, Ritsumeikan University

Sirtuin interacts with many regulatory proteins involved in energy homeostasis. We investigated the functional roles of Sir2D during the early *Dictyostelium*development upon starvation. We found that the ectopic expression of Sir2D accelerated the development and upregulated the adenylate cyclase A (aca) mRNA expression at 2, 4 and 6 h after starvation. RNAi-mediated Sir2D knockdown cells was generated and found that the development was delayed, and aca expression was decreased at 4 h after starvation. Sir2D expression restored the developmental impairment of Sir2D knockdown cells. The induction of aca upon starvation starts with the transcriptional activation of MybB. The ectopic expression of MybB accelerated the development and increased the expression of aca at 2 and 4 h after starvation but could not restore the phenotype of Sir2D knockdown cells. Thus, Sir2D is necessary for the full induction of aca at 4 h by MybB. MybB was co-immunoprecipitated with Sir2D, suggesting the interaction between MybB and Sir2D. The result suggests that Sir2D regulates the aca expression through the interaction with a transcription factor MybB at the early *Dictyostelium*development upon starvation.

2-P-116

Analysis of affinity and binding property of fatty acid-binding protein inhibitors

Yasuharu Shinoda[1], Tetsunori Yamamoto[1], Kohji Fukunaga[1]

[1]Dept Pharmacol, Grad Sch Pharm Sci, Tohoku Univ

[Background] We previously revealed α-synuclein aggregation is promoted by fatty acid-binding protein 3 (FABP3). Development of their ligands has been performed for FABP4. In this report, we analyzed the affinity and binding property of ligands for FABP3.
[Methods] Recombinant FABPs was purified from *E. Coli*. We used arachidonic acid (AA), FABP4 ligand (BMS309403) and its ten derivatives in ANS assay and investigated their affinity to FABPs. Also, we analyzed binding properties of PA-FABP4 and BMS-FABP3 complex from their crystal structures using PLIP. We further performed prediction analysis of BMS derivative-FABP3 complex structure with docking simulation and PLIP.
[Results and Discussion] AA showed high affinity to both FABP3 and 4 (K_d = 133 and 674 nM respectively). BMS showed higher affinity to FABP4 (K_d = 593 nM) rather than FABP3 (K_d = 34,920 nM) Among ten derivatives, ligand #1 showed the highest affinity to FABP3 (K_d = 261 nM), however identical to FABP4. PLIP study revealed AA and BMS bind with FABP4 by similar interactions. Prediction analysis indicated 104Ile in FABP3 caused steric obstruction with BMS, which might explain its high selectivity to FABP4. These results will lead to further research for FABP3-selective ligand.

2-P-117

Molecular mechanism of substrate recognition by Leucine-Specific Binding Protein

Yu Ma[1], Wiriyasermkul Pattama[2], Suguru Okuda[1], Ryuichi Ohgaki[1], Shushi Nagamori[2], Yoshikatsu Kanai[1]

[1]Dept. Bio-sys. Parm., Grad. Sch. Med., Osaka Univ., Osaka, [2]Lab. Bio-Mol. Dynamics, Dept. Collab. Res., Nara Med. Univ., Nara

In *Escherichia coli*, the active transport of branched-chain amino acids was performed by three different kinetically system: Leucine-isoleucine-valine (LIV)- I, II and Leucine-specific (LS) system. The transport capacity of LS system depends on a periplasmic protein, leucine-specific binding protein (LS-BP). In previous studies, the substrate specificity of LS-BP was revealed *in vivo*, but the mechanism of substrate recognition remains unclear. In this study, we purified LS-BP and measured the affinity for leucine and its derivatives by BIACORE. Since the affinity of LS-BP for leucine and its derivatives exceeded the maximum range of BIACORE, the Km value could not be determined. Then we developed an *in vitro* assay to investigate the substrate recognition of LS-BP by using radiolabeled leucine. In this assay, the derivatives modified with NH_2, COO, or Cγ did not show an obvious binding to LS-BP, while no significant differences were observed between leucine and its derivatives modified with OH, Cα or Cβ. Those results suggest that LS-BP recognizes NH_2, COO, and Cγ of leucine.

2-P-118

Mitochondrial nonspecific channel is crucial for the maintenance of their function

Takeya Sato[1], Ryosuke Nomura[2], Masaki Saito[1], Jun Sukegawa[3], Shigeki Kushimoto[2], Teruyuki Yanagisawa[1]

[1]Dept. Mol. Pharm. Grad. Sch. Med.Tohoku. Univ., [2]Dept. Emerg. Crit. Care, Grad. Sch. Med. Tohoku Univ,, [3]Dept. Human Health Nutri, Shokei Gakuin Univ.

Mitochondria regulate various cellular processes. Though highly active anti-retrovirus therapy (HAART) is effective cure for HIV, the therapy also causes life-threatening clinical manifestations resulting from mitochondrial toxicity caused by nucleoside reverse transcriptase inhibitors (NRTIs) involving 3'-azido 3'-deoxythymidine (AZT), which are a key component of HAART. The mechanism underlying the mitochondrial toxicity of NRTIs, however, remains uncertain. An active metabolite of AZT (AZT triphosphate, AZT-TP) is responsible for AZT toxicity. Mitochondrial permeability transition pore (mPTP) is a nonspecific channel permeable to any molecules < 1.5 kDa that penetrates the mitochondrial inner and outer membranes by forming a complex with cyclophilin D (CypD) locating in the matrix, ATP/ADP translocator lying at the inner membrane, and the voltage dependent anion channel positioned at the outer membrane. The mPTP-opening causes mitochondrial dysfunction. In this study, we examined the role of CypD on AZT-induced mitochondrial dysfunction. CypD expression was inhibited by RNAi. In the control cells, cyclosporin A (CsA) which binds to CypD blocks the mPTP-opening caused by AZT and restores the mitochondrial function impaired by AZT. Knockdown of the CypD abolished the effects of CsA on the inhibition of mPTP-opening caused by AZT and made worse the mitochondrial function. These results suggest that CypD is prerequisite for the inhibition of mPTP-opening by CsA.

2-P-119

Role of ceramide kinase on lamellipodia formation and cell migration

Hiroyuki Nakamura[1], Satoshi Tomizawa[1], Toshihiko Murayama[1]

[1]Lab. Chem. Pharmacol., Grad. Sch. Pharmaceu. Sci., Chiba Univ.

Ceramide kinase (CerK) produces the bioactive lipid ceramide-1-phospahte and appears as a key enzyme for regulating cell growth and arachidonic acid metabolism. Although CerK is known to regulate cell migration, the precise mechanism is not fully understood. Lamellipodia is dynamic surface extensions of the cell which plays a pivotal role in cell migration. In this study, we focused the role of CerK on lamellipodia formation and cell migration. EGF is known to enhance the formation of lamellipodia. When A549 or MCF-7 cells were treated with EGF, CerK was colocalized with actin in lamellipodia. Knock-down of CerK enhanced the formation of lamellipodia in A549 and MCF-7 cells. Same results were shown in CerK knock-out mouse embryonic fibroblast cells. Transfection of CerK inhibited the formation of lamellipodia. Rac1 is known to drive the formation of lamellipodia. In A549 cells, knock-down of CerK enhanced the activity of Rac1 and translocation of Rac1 to the plasma membrane. Enhanced formation of lamellipodia by inhibition of CerK was attenuated by inhibition of Rac1 in A549 cells. Migration of A549 cells was also enhanced by knock-down of CerK, which was attenuated by inhibition of Rac1. These results suggest that CerK/C1P negatively regulates lamellipodia formation and cell migration via inhibition of Rac1 activity.

2-P-120

The actin-organizing formin protein fhod3 is essential for neural tube closure

Hikmawan Wahyu Sulistomo[1], Yohko Kage[1], Takayuki Nemoto[1], Ryu Takeya[1]

[1]Department of Pharmacology, University of Miyazaki, Japan

Neural tube closure is a morphogenetic process that transforms the neural plate into a neural tube. Throughout this process, contraction of the actin-myosin network is required for apical constriction of the neural plate and maintenance of cell-cell junctions. Fhod3, a member of formin family proteins that mediate nucleation and polymerization of the actin filament, is expressed in the neural tube and heart. In our previous study, we showed that *Fhod3* null mouse embryo exhibits cardiac defects and exencephaly, a type of neural tube closure defect wherein the brain is located outside of the skull. However, the mechanism of Fhod3 to regulate neural tube closure is still lacking. Here, we show that *Fhod3* is expressed at the lateral wall of the neural tube in the hindbrain. Closure of the neural tube is normally completed by the E9.5, but *Fhod3* null embryo shows a persistently open neural tube from the hindbrain/cervical boundary towards the rostral portion. On the apical side of the closing neural plate, loss of *Fhod3* disrupts actin-myosin network as well as the cell-cell junction. Taken together, Fhod3 regulates neural tube closure by mediating contraction of actin-myosin network at the cell-cell junction.

2-P-121
Regulation of apoptosis by PDZRN3 protein in myoblasts

Takeshi Honda[1], Ukyo Shinagawa[1], Yu Mizuno[1], Yuki Yokosuka[1], Makoto Inui[1]

[1]Dept. Pharmacol., Grad. Sch. Med., Yamaguchi Univ.

We previously demonstrated that PDZRN3, an E3 ubiquitin ligase, is essential for myogenic differentiation from myoblasts to myotubes. In regeneration of injured skeletal muscle *in vivo*, the expression of MyoD is induced in stem cells and these differentiated cells (myoblasts) expand through proliferation. We reported that PDZRN3 is upregulated along with MyoD during regeneration of injured muscle. In this study, we aimed to clarify a role of PDZRN3 in proliferation of myoblasts. When exposed to starvation stress, PDZRN3-depleted C2C12 myoblasts by RNAi showed higher levels of apoptotic markers as compared with those of control cells. On the other hand, PDZRN3-depletion suppressed the activation of anti-apoptotic Akt, indicating the involvement of PDZRN3 in apoptotic regulation. In addition, we found that the expression of cyclin A2, a direct activator of Akt, was reduced in PDZRN3-depleted cells. Cyclin A2 directly activates the translation of DNA repair factor Mre11. In fact, the expression of Mre11 was decreased in the PDZRN3-depleted cells, and the activation of p53 was enhanced in these cells probably due to the DNA damage accumulation. These results indicate that PDZRN3 plays an important role in apoptotic regulation of myoblasts, modulating the expression of cyclin A2.

2-P-122

Analysis of hydrogen peroxide resistance mechanism on hydrogen peroxide resistant cancer cells.

Taisuke Nagasawa[1], Kazuo Tomita[1,4], Yoshikazu Kuwahara[1,2], Kento Igarashi[1], Yuko Takashi[1,3], Koh-Ichi Tanaka[4,5], Junichi Kitanaka[5], Nobue Kitanaka[5], Motohiko Takemura[5], Nobuyoshi Nishiyama[4], Akihiro Kunimasa[2], Tomoaki Sato[1]

[1]Dept. Applied Pharmacol., Kagoshima Univ. Grad. Sch. Med. & Dent. Sci., [2]Radiat. Biol. and Med, Faculty of Med, Tohoku Medical and Pharmaceutical Univ., [3]Restorat Dent and Endodontol., Grad. Sch. Med. and Dental Sci., Kagoshima Univ., [4]Div. Pharmacol., Dept. Pharm., Sch. Pharm., Hyogo Univ. Health Sci., [5]Dept. Pharmacol., Hyogo Col. Med.

<purpose>
Hydrogen peroxide is known as one of ROS which gives oxidative stress to cells and induces apoptosis. However, details of the mechanism for cancer cells by hydrogen peroxide is still unknown. We have established "hydrogen peroxide resistant (HR) cancer cells" that are resistant to high concentration hydrogen peroxide. The mechanism of resistance to hydrogen peroxide acquired by HR cancer cells has not yet been elucidated, however if the mechanism becomes clear, it could be applied to cancer treatment. In this study, therefore, we aimed to elucidate its hydrogen peroxide resistance mechanism and carried out the following experiment.
<method>
Cell lines that continued to survive against graded hydrogen peroxide treatment of HeLa (up to 70 μM) and SAS (up to 35 μM) were subjected to hydrogen peroxide at the concentrations of 0, 25, 50, 75 and 100 μM respectively, and the cell viability was examined by WST assay. Subsequently, the endogenous catalase enzymatic activity of HR cancer cells was measured using Catalase Assey Kit (SIGMA). Furthermore, lipid peroxidation of HR cancer cells was analyzed by immunofluorescence using 4-hydroxynonenal (HNE) and 5-lipoxygenase (5-LOX) antibody. HNE is typical lipid peroxidation marker and 5-LOX is known as lipid peroxidase.
<results and discussion>
In HeLa and SAS parental cells, they survived to the extent of 25 μM by hydrogen peroxide treatment. On the other hand, stepwise hydrogen peroxide-treated cells survived up to100 μM (HeLa) and 50 μM (SAS), showingresistance to hydrogen peroxide. Analysis of catalase enzyme activity showed significant increase in HeLa HR cells compared with the HeLa parent, but there were no

2-P-123

Increased DNA methylation of *SHATI/NAT8L* promotor sites in the blood from unmedicated patients with depression

Hajime Miyanishi[1], Kyosuke Uno[1], Mina Iwata[1], Yu Kikuchi[1], Hidenaga Yamamori[2], Yuka Yasuda[2], Kazutaka Ohio[2], Ryota Hashimoto[2,3], Tomiki Sumiyoshi[4,5], Atsumi Nitta[1]

[1]Dept. Pharm. Thera. & Neuropharma., Fac Pharm. Sci., Grad. Sch. Med. & Pharma. Sci., Univ of Toyama, [2]Dept. Psychi., Grad. Sch. Med. Osaka Univ., [3]Department of Pathology of Mental Diseases, National Institute of Mental Health, National Center of Neurology and Psychiatry, Tokyo, Japan, [4]Department of Preventive Intervention, National Institute of Mental Health, National Center of Neurology and Psychiatry, Tokyo, Japan, [5]Bio Bank of National Center of Neurology and Psychiatry, Tokyo, Japan

Depression is one of the most common psychiatric diseases, and is often resistant to treatment. Its diagnose is based on clinical interview, but not objective examinations. Since there is no biomarkers for depression, we evaluated DNA methylation in *SHATI/NAT8L* promotor regions in patients with the illness, and examined the effect of medication. Methylation rates of *SHATI/NAT8L* promotor regions at the CpG site in peripheral blood from unmedicated patients were significantly greater compared to those of healthy subjects. By contrast, medicated patients revealed significantly less rates compared to healthy subjects for the same measure of methylation. Since previous studies of DNA methylation were performed using samples from treated patients, the present results from untreated subjects provide valuable information on the role for methylation of *SHATI/NAT8L* promotor regions in the diagnosis of depression.

2-P-124
miR-96-5p modulates RNA-binding proteins that down-regulate GTRAP3-18.

Chisato Kinoshita[1], Toshio Nakaki[2], Koji Aoyama[1]

[1]Dept. Pharm., Sch. Med., Teikyo Univ., [2]Fac. Pharm-Sci., Teikyo Univ.

Glutathione (GSH) is a key antioxidant that plays an important neuroprotective role in the brain. Decreased GSH levels are associated with neurodegenerative diseases. We previously reported that one of the important microRNA, contributed the neuroprotection against oxidative stress through regulating the expression of excitatory amino acid carrier 1 (EAAC1) and GSH levels. In this study, we focused on GTRAP3-18, the negative factor of EAAC1, as a new target of miR-96-5p.
First, we investigated whether the expression of GTRAP3-18 is affected by manipulation of miR-96 -5p level using western blot analysis and luciferase reporter gene assay in human neuroblastoma SHSY-5Y cells. Next, we identified the candidates of GTRAP3-18 regulators using mass spectrometry analysis since we found out GTRAP3-18 is indirectly regulated by miR-96-5p. Then, we have tested whether these candidate proteins could be a direct regulator of GTRAP3-18.
The result shows that GTRAP3-18 is up-regulated by miR-96-5p at transcriptional and translational levels. Furthermore, we identified several miR-96-5p regulating RNA binding proteins that negatively regulate GTRAP3-18.
In conclusion, miR-96-5p could reduce RNA-binding proteins that down-regulate GTRAP3-18 to decrease neuronal GSH levels.

2-P-125

HIF-1a and c-Myc oppositely regulate human EP4 receptor promoter activity in human colon cancer HCA-7 cells

Kazuyuki Yamagata[1], Naofumi Seira[1], Keijo Fukushima[2], Yumi Araki[1,2], Naoki Kurata[1,2], Naoki Yanagisawa[1], Masato Mashimo[3], Hiroyuki Nakamura[1], John Regan W.[4], Toshihiko Murayama[1], Hiromichi Fujino[2]

[1]Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Chiba University, [2]Dept of Pharmacology for Life Sciences, Graduate School of Pharmaceutical Sciences & Graduate School of Biomedical Sciences, Tokushima University, [3]Laboratory of Pharmacology, Faculty of Pharmaceutical Sciences, Doshisha Women's College of Liberal Arts, [4]Department of Pharmacology & Toxicology, College of Pharmacy, The University of Arizona, Tucson, Arizona

Although the up-regulated expression of E-type prostanoid (EP) 4 receptors has been demonstrated during colorectal development, another study showed that the expression levels of EP4 receptors were higher in normal colon tissues than in cancer tissues. To examine the underlying mechanisms/reasons for why inconsistent findings have been reported regarding EP4 receptor expression levels in homeostasis and carcinogenesis by focusing on cellular densities, we here demonstrate that the expression of EP4 receptors is tightly regulated by c-Myc and hypoxia-inducible factor (HIF)-1a by binding to Sp-1 as cellular density-dependently in HCA-7 cells. This tight regulation of EP4 receptor expression by c-Myc and HIF-1a may be an essential system for maintaining homeostasis in normal colorectal epithelial cells. Therefore, once the system is altered, it may cause aberrant cellular proliferation, the transformation from normal to cancerous phenotypes, which represents the trigger for the early stage of colorectal carcinogenesis. The present results provide one plausible reason for why conflicting findings exist for the roles of the expression levels of EP4 receptors in carcinogenesis.

2-P-126

Renoprotective mechanism analysis of agmatine on ischemic acute kidney injury with high coverage expression profiling

Takahiro Sugiura[1], Miki Sugiyama[1], Takahito Imaizumi[1], Yasushi Hirasawa[1]

[1]Nihon Bioresearch Inc.

Acute kidney injury (AKI) represents a major clinical problem with high mortality in kidney transplantation and nephron-sparing surgery. The ischemic AKI model by the interception of the bloodstream is the superior model that can evaluate an efficacy evaluation and condition of a patient elucidation in a short term for AKI without the therapeutic drug. We reported that agmatine has the preventive effect on ischemic AKI. But we do not find the detailed mechanism about the renoprotective effect of agmatine. Therefore, we examined the detailed renoprotective mechanism of agmatine with the high coverage expression profiling (HiCEP). Male Crl:CD1 mice's right kidney was removed. After a 2-week, to induce ischemic AKI, the left renal artery and vein were occluded with a clamp for 50 min. Agmatine was injected 5 min before the ischemia. At 6 h after reperfusion, we excised the left kidney and detected the gene change with HiCEP. As a result, we obtained the gene changes of TRXR, synbindin, ADAMTS1 and PEA15.

2-P-127
Effect of lactoferrin on neurite outgrowth of PC12 cells

Rina Iwasaki[1], Nobuo Izumo[1], Shinji Kagaya[2], Akira Tabuchi[1], Yurina Mima[1], Kosuke Hayamizu[1], Makoto Nakano[1], Tatsuo Hoshino[2], Yasuo Watanabe[1]

[1]General Health Medical Center, Yokohama University of Pharmacy, [2]NRL Pharma, Inc.

Lactoferrin (LF) is a protein that is rich in breast milk. Recently, it is reported that LF associated with memory and affection to improve cognitive function. Thus, we examined the effect of LF on neurite outgrowth of rat adrenal pheochromocytoma PC12 cells, in this study. Moreover, cAMP response element-binding protein (CREB) inhibitor, KG-501 (KG;5mM) was added onto the PC12cells treated with LF. In addition, RNA expression level of Neurofilament light(NF-L) was measured by Real-time PCR. PC12 cells seeded onto 12-well plate (1.2×10^4cells/well) were cultured in 10% FBS DMEM. After 24h,the cells were incubated for 3 days in serum free DMEM containing LF (250mg/mL) with/without KG. On day1 and 3, morphometric analysis of the neurites and length was performed by Neurocyte Image Analyzer software (KURABO). LF (250mg/mL) significantly enhanced these neuritic parameters. Furthermore, KG inhibited the effects of LF. In result of RT-PCR, the NF-L expression level was significantly incresed by adding of LF. These results suggested that neurite outgrowth should be facilitated by LF and be associated with cAMP. Near future, we will investigate the association of other potential pathways to enhance neurite outgrowth except cAMP pathway, because cAMP inhibition by KG could not suppress neurite outgrowth completely.

2-P-128

Comparison of the rat brain activity with fMRI by the taste stimulation of the sweeteners

Yukiko Kondo[1], Satomi Higuchi[2], Fumio Yamashita[2], Masamichi Hirose[3], Makoto Sasaki[2], Eiichi Taira[1]

[1]Signal Transduction Dept. Pharmacol., Iwate Med. Univ., [2]Ultrahigh Field MRI, Institute for Biomedical Science, Iwate Med. Univ., [3]Molecular and Cellular Pharmacol., Dept. Pharm., Iwate Med. Univ.

The artificial sweeteners are added in much food and drink as a low-calorie sweetener. These sweeteners were originally developed for the purpose of treatment for adiposity, diabetes and the metabolic syndrome. These sweeteners include saccharin, sucralose, aspartame and acesulfame K. However, it was reported recently that the excessive intake of the artificial sweetener causes an onset risk of diabetes by the enterobacterial flora change and excessive eating. By the rat experiment, the rat got to eat lots of the sweet diet and gained weight by intake of the artificial sweetener. But at all events I have not yet understood the detailed mechanism well. The functional MRI (fMRI) is laboratory procedure using the blood oxygenation-level dependent (BOLD) signal by MRI as change of the brain activity by noninvasive procedure. Therefore, fMRI might be useful tool for the examination of the brain function including brain activity of a taste stimulation. In this study, we compared the rats brain activity by the taste stimulation of acesulfame K against sucrose using fMRI. And then, the rat brain activity by acesulfame K stimulation was different from by sucrose. In addition, the brain activity of the rats given sweeteners before for two weeks was different from the rats not given sweeteners.

2-P-129

Effect of Lactoferrin on decreased calcification by DEX of osteoblast-like MC3T3-E1 cells

Ayako Inaba[1], Nobuo Izumo[1], Shinji Kagaya[2], Maki Yamazoe[1], Yuki Kurihara[1], Kosuke Hayamizu[1], Makoto Nakano[1], Tatsuo Hoshino[2], Yasuo Watanabe[1]

[1]Genar. Health Medi. Cen. Yokohama Univ., [2]NRL Pharma, Inc.

Lactoferrin (LF) is a protein contained in milk of mammalians including human. We have already reported that LF prevented dexamethasone (DEX)-induced osteopenia in mice (Global Drugs and Therapeutics 2018). We investigated the effect of LF on decreased calcification by DEX with mouse osteoblastic cell line (MC3T3-E1), in this study. In addition, RNA expression levels of Alkaline phosphatase (ALP) and osteocalcin were measured in MC3T3-E1 cells by Real-time PCR. MC3T3-E1 cells were cultured in α-MEM containing 10%FBS. On day 4, medium was replaced by α-MEM containing 2% FBS, ascorbic acid (AA; 50μg/ml), beta-glycerophosphate (b-GP; 5mM) and DEX (3.3μg/mL) with/without LF (100μg/mL). On day11, hydroxyapatite was added into the culture medium. After 3hrs, the cells were stained with Alizarin red to evaluate calcification levels. In RT-PCR, sampling for RT-PCR was collected without exposing hydroxyapatite. In result of Alizarin red staining, decreased calcification by DEX was significantly suppressed by adding of LF. In result of RT-PCR, osteocalcin as a bone formation maker significantly decreased with exposure of DEX. The decreased osteocalcin expression level was significantly suppressed by adding of LF. These results suggested that LF suppressed reduction of calcification by DEX.

2-P-130
Kaempferia parviflora (KP) reduced the visceral fat in mice

Masaya Miyazaki[2], Nobuo Izumo[1,2], Yu Kuwahara[2], Jun Sakurai[2], Kazuto Honma[2], Kohsuke Hayamizu[2], Makoto Nakano[1,2], Yasuo Watanabe[1,2]

[1]Yokohama University of Pharmacy General Health Medical Center, [2]Food chemistry/Functional food laboratory

Kaempferia parviflora (KP) is a plant of the ginger family and has been used as a folk remedy in Thailand. Its rhizomes have been used to improve several diseases. However, the influence on visceral fat hasn't become clear. In this study, we searched for the mechanism of anti-obesity actions of KP. **Method** C57BL/6J male mice were used and the test feed was administered for 8 weeks. The dose of feed was limited (3g/day). Experimental groups were divided as follows, ①normal diet ②high fat diet (HF) ③HF + KP extraction 0.5% ④HF + KP extraction 1.0%. After 8 weeks of administration, visceral fat of mice was collected and weighed. In addition, expression levels of adiponectin, leptin, IL-6, IL-1β were measured by RT-PCR. **Result** The weight and the expression levels of leptin and IL-6 of visceral fat were significantly decreased in ③ and ④ groups compared with ②group, although the adiponectin levels did not show any changes. **Conclusion** Our results suggest that the mechanism of anti-obesity effects of KP is due to the induction of leptin resistance in a adipose tissues.

2-P-131

Effects of butyrate-producing probiotics administration on obesity progression in ob/ob mice

Yukiko Naito[1], Hiroyuki Ohnishi[1]

[1]Dept. Health Sci., Sch. Allied Health Sci., Kitasato Univ.

It has been reported that the administration of butyrate, a short-chain fatty acid produced by microbiota in the intestine, induces expression of the genes involved in peroxisomal fatty acid b-oxidation. In the present study, we investigated whether administration of the butyrate-producing probiotics (*Clostridium butyricum* MIYARI 588, MYR) inhibit the progression of obesity and studied the effect of added dietary fiber and the mechanism in lipid metabolism. Male C57BL6J-ob/ob mice were divided into 3 groups, Control, MYR and inulin (INU) groups. Animals in MYR and INU groups were fed diet containing 3% MIYARI 588 *ad libitum*. Drinking water for INU group were added 1% inulin. Last week of the 7-week administration period, oral glucose-tolerance test was performed. The relative weights of liver and white adipose tissues (WAT) in MYR and INU groups were lower than Control group. Compared with Control group, *Adiponectin* expression in WAT of MYR group was tended to be higher, and that of MYR group was higher. In conclusion, it is suggested that the 7-week administration of MYR induces the changes in lipid metabolism involved in *adiponectin* expression in WAT accompanied by the decreases in liver and WAT weight.

2-P-132

Siraitia grosvenori suppresses glycemic rise after loading carbohydrate in human with hyperglycemia

Aki Ogawa[1,2], Sayaka Yoshida[3], Takahito Ichi[4], Rikako Inoue[1], Maya Tsumagari[5], Miho Asai[5], Satoko Hiramatsu[1], Kazuko Sumiyoshi[5], Yasuyuki Irie[1]

[1]Dept. Nutr. Sci., Fac. Health & Welfare Sci., Okayama Pref. Univ., [2]Dept. Clin. Nutr. Diet., Fac. Clin. Nutr. Diet., Konan Women's Univ., [3]Yokohama Oils & Fats Industry Co. Ltd, [4]FONTEC R&D, [5]Dept. Nursing Sci., Fac. Health & Welfare Sci., Okayama Pref. Univ.

Siraitia grosvenori (SG) is a traditional Chinese fruit. Its extract (SG-ex) contains potent sweet elements with a sweetness several hundred times higher than table sugar. SG-ex has been found to inhibit α-glucosidase and reduce hyperglycemia in rats. The present study was performed to examine the effect of SG-ex supplementation in the diet on preventing postprandial hyperglycemia in human.
Healthy male and female volunteers between 50 and 78 years of age (n=39) were given pastries with and without SG-ex in a cross-over manner. The subjects ingested bean-jam bun as a loading carbohydrate together with water. Blood glucose levels were measured at 0, 15, 30, 60, and 120 min after the loading.
SG-ex significantly prevented postprandial hyperglycemia, and the mean area under the curve (AUC) of plasma glucose over 2 h was significantly reduced in subjects with blood glucose level of >200 mg/dL at 120 min. In subjects with second-degree family history of diabetes, the administration of SG-ex significantly lowered the mean blood glucose level 60 min after the meal.
The ingestion of SG-ex suppressed the meal-induced hyper glycemic response. SG-ex may be useful for patient with diabetes or prediabetes.

2-P-133
Canola oil toxicity in SHRSP — possible involvement of RAS —

Naoki Ohara[1], Mai Nishikawa[1], Yukiko Naito[2], Kenjiro Tatematsu[3], Daisuke Miyazawa[1], Harumi Okuyama[1]

[1]Biol. Pharm., Coll. Pharm., Kinjogakuin Univ., [2]Health Sci., Sci. Allied Health Sci., Kitasato Univ, [3]Lab. Radiochem., Gifu Pharm. Univ.

In the present study, we examined if renin-angiotensin system (RAS) is involved in the Canola oil (C)-induced toxicity in male SHRSP.

Methods: Male SHRSPs were given AIN-93G diets containing 10w/w% soybean oil (S, control) or C and tap water ad lbitum for 8 weeks, sacrificed, and plasma renin and angiotensin II concentrations, and ACE activities in the kidney and lung were determined. In addition, mRNA expressions for renin in the kidney, angiotensinogen in the liver, ACE in the lung and ACE2 in the testis were examined.

Results and Discussion: Both renin and angiotensin II concentrations in C group, 753±97 and 66.9 ±6.5 pg/mL were higher than respective those in S group, 459±23 and 46.4±5.5 pg/mL ($p<0.01$, $N=12$ and $p<0.05$, $N=10$). Expressions of mRNA for renin in the kidney was significantly high and ACE2 in the testis was low in C group comparing with S group, while ACE in the lung and angiotensinogen in the liver were comparable. Thus the enhanced production of renin in the kidney and increased plasma angiotensin II level lead to the acceleration of blood pressure elevation and vascular injuries, and may be involved in the C toxicity. ACE2 may also be concerned via steroid hormone metabolism.

2-P-134
Effect of malted-rice amazake on intestinal environment

Rikako Inoue[1], Aki Ogawa[2], Yukihiro Yoshimura[3], Yasuyuki Irie[1]

[1]Dept. Nutri. Scie., Okayama Pre. Univ., [2]Dept. Clini. Nutri. and Diet., Konan Women's Univ., [3]Fac. Nutri., Kobe Gaku. Univ.

There are two types of amazake: malted-rice amazake and Sake lees amazake. It has been reported that Sake lees amazake improves human intestinal flora and relieves constipation. But there is no report on malted-rice amazake for improving constipation. The purpose of this study is to conduct a long-term ingestion test on humans and consider whether intake of malted-rice amazake will improve constipation.

The subject is adult females with periodic menstrual cycles. Feces were collected in the follicular phase to minimize the influence of premenstrual syndrome. The DNA extracts from feces were analyzed using quantitative PCR using specific primers. The defecation status was investigated by self-report questionnaire.

In the defecation state, soft stool of the stool was found significantly by intake of malted-rice amazake in hard stool group. Symptoms of constipation improved in 83% of constipation group. Analysis of intestinal bacterial flora showed a significant decrease the ratio of Firmiscutes / Bacteroidetes in constipation group due to intake of malted-rice amazake.

It was suggested that intake of malted-rice amazake changed the construction of gut microbiota as well as the intestinal environment, resulting in improvement in constipation.