1-LBS-03 Late-Breaking Session

Role of astrocytic histamine *N*-methyltransferase in brain functions

Yuna Takahashi, Takeo Yoshikawa, Rina Otsuka, Kazuhiko Yanai

Dept. Pharmarcol., Tohoku Univ. Grad. Sch. Med.

Histamine *N*-methyltransferase (HNMT), which inactivates histamine to 1-methylhistamine, plays an important role in the regulation of histamine concentration and brain functions. Although previous studies indicated the possible involvement of neurons and astrocytes in brain histamine inactivation, the contribution of each cell type to the histamine inactivation was not fully elucidated. In the present study, we phenotyped astrocytes-specific *Hnmt* knockout mice (cKO) to reveal the importance of astrocytic histamine inactivation for brain functions. First, we generated cKO mice by crossing *Hnmt flox* mice and Gfap-Cre mice which expressed Cre recombinase specifically in astrocytes. Increase in brain histamine concentration of cKO mice was modest compared to that of conventional Hnmt knockout mice, indicating the limited contribution of astrocytes to histamine metabolism. Behavioral test battery showed the lower locomotor activity of cKO mice in novel environment and home cages, although anxiety-like behaviors and depression-like behavior were not changed by *Hnmt* deletion in astrocytes. These results demonstrated that astrocytic Hnmt maintained normal locomotor activity despite of its minor role in histamine clearance.

1-LBS-04 Late-Breaking Session

The development of novel anti-depressant targeting AMPA receptor

Hara Megumi

Yokohamacity university

Depression is the major mental disorder characterized by the decrease of motivation, interest and activity and over one million patients are suffering from this disease. It was also reported that the number of patients showing resistance toward anti-depressant, i.g. SSRI and SNRI, got increase nowadays. We have already known that molecular mechanism underlying depression is heterogeneous so that it is hard to estimate the efficacy of anti-depressant depends without molecular rationale. Postmortem human brain analysis indicated that the number of AMPA receptors (AMPARs), major molecule controlling synaptic functions, varied among depression patients compared to healthy subjects and these results were not consistent. To clarify the dynamics of AMPARs in depression patients, we developed the PET (positron emission tomography) imaging drug to measure the density of AMPARs in depression patients. This result showed that depression patient decreased AMPARs expression broadly throughout the brain. This fact motivated us to develop novel AMPARs potentiator in order to cure the depression. To find the compound showing high affinity to AMPARs and high BBB penetratability, we modified the compound A, previously known to bind specifically to and activate AMPARs, and finally succeeded in synthesizing the seed compound B, showing higher BBB penetratability compared with compound A. This compound B could exert the anti-depressant effect quickly and sustained for a week after withdrawal from repetitive one-week administration. Furthermore, this anti-depressant effect was significantly stronger that another AMPARs potentiators already under development in clinical trials.

1-LBS-05 Late-Breaking Session

QPRT Deficit Leads Motor and Cognitive Dysfunction through Increase of Oxidative Stress in the Dopaminergic Neuronal System by Quinolinic Acid

Akihiro Mouri[1], Moe Niijima[1], Tomoaki Teshigawara[2], Kazuo Kunisawa[1], Hisayoshi Kubota[1], Yasuko Yamamoto[2], Kuniaki Saito[2], Toshitaka Nabeshima[3]

[1]Department of Regulatory Science for Evaluation & Development of Pharmaceuticals & Devices, Fujita Health University Graduate School of Health Science, [2]Department of Disease Control and Prevention, Fujita Health University Graduate School of Health Science, [3]Advanced Diagnostic System Research Laboratory, Fujita Health University Graduate School of Health Science

Quinolinic acid phosphoribosyltransferase (QPRT) metabolizes quinolinic acid (QA) to nicotinamide adenine nucleotide (NAD^+) via kynurenine pathway. QA is a excitotoxic substance that activate N-methyl-D-aspertate (NMDA) receptors and NAD^+ is essential for cell survival. In this study, we evaluated QPRT knock out (KO) mice to explore the physiological role of QPRT in central nervous system. QPRT KO mice demonstrated motor deficits (decrease of locomotor activity, decrease of duration time to maintain balance on the rotarod, wide stance in footprint pattern test) and cognitive deficits (decrease of spontaneous alternation behavior in Y-maze test, and prolongation of latency to enter the target hole in the Barnes-maze test). But emotional change was not observed except for decrease in number of buried marbles in marble burying test. Dopaminergic dysfunction was observed in prefrontal cortex, nucleus accumbens and striatum of QPRT KO mice. Dopamine D_1 receptor agonist (SKF81297)-induced hyperactivity is not observed in QPRT KO mice. Dopamine D_2 receptor antagonist (raclopride)-induced catalepsy is more sensitive in QPRT KO mice. The activation of dopaminergic function by methylphenidate attenuated the impairment of short-term memory and hypoactivity of QPRT KO mice. QPRT KO mice showed increased level of QA in serum but normal level of NAD+ in brain. QA-mediated NMDA receptor signaling (phosphorylation of CaMK2 and activation of calpain) and oxidative stress were enhanced in prefrontal cortex, nucleus accumbens and striatum of QPRT KO mice. These results suggested that deficiency of QPRT lead motor and cognitive deficits associated with dopaminergic dysfunction via QA-induced calpain activation and oxidative stress.

1-LBS-06 Late-Breaking Session

Oxytocin recovers Aβ-induced impairment of hippocampal synaptic plasticity in mice

Takahshi Junpei[1], Daisuke Yamada[1], Yudai Ueta[1], Takashi Iwai[2], Eri Koga[1], Mitsuo Tanabe[2], Jun-Ichiro Oka[1], Akiyoshi Saitoh[1]

[1]Lab Pharmacol, Fac Pharm Sci, Tokyo Univ of Science., [2]Lab Pharmacol, Fac Pharm , Kitasato Univ

Oxytocin (OXT) is a peptide hormone synthesized in the hypothalamic paraventricular nucleus. OXT has been reported to be involved in regulation of learning and memory performance. However, there is no report that shows the effect of OXT on the amyloid-beta (Aβ)-induced impairment of synaptic plasticity. Here, we examined whether OXT have effects on the Ab-induced impairment of synaptic plasticity in mice.

Methods: Male ddY mice were used. To investigate the effect of OXT on synaptic plasticity, we prepared acute hippocampal slice for extracellular recording, and assessed long-term potentiation (LTP) with A$\beta_{25\text{-}35}$ perfusion in the absence and presence of OXT.

Results: In the present study, we found that OXT recovered the LTP impaired by perfusion of A$\beta_{25\text{-}35}$ in the mouse hippocampus. These effects were blocked by the pretreatment with a selective OXT receptor antagonist L-368,899. Further, the pretreatment with an ERK inhibitor U0126 and a selective Ca^{2+}-permeable AMPA receptor antagonist NASPM were completely antagonized the effects of OXT, respectively.

Conclusion: These results suggested that OXT recovered Aβ–induced impairment of hippocampal synaptic plasticity through the OXT receptors in the mice. We proposed that ERK phosphorylation and Ca^{2+}-permeable AMPA receptors are involved in these effects of OXT.

1-LBS-07 Late-Breaking Session

NOX1/NADPH in the hypothalamus regulate anxiety-like behaviors in mice

Masakazu Ibi, Xueqing Zhang, Chihiro Yabe-Nishimura

Kyoto Prefectural University of Medicine

The involvement of reactive oxygen species (ROS) in psychiatric disorders has been reported. However, the source of ROS has not been identified yet. NADPH oxidase is a superoxide-generating enzyme composed of multiple subunits including a membrane-spanning catalytic subunit, NOX. We investigated the role of NOX1/NADPH oxidase in the anxiety-like behavior using mice deficient in *Nox1*(NOX1-KO).

When anxiety-like behaviors were evaluated in elevated plus-maze and open field tests, no difference in anxiety levels was observed between wild-type mice (WT) and NOX1-KO. Increased anxiety-like behavior was demonstrated in WT subjected to two hour-restraint stress, but it was markedly ameliorated in NOX1-KO. Delivery of miRNA against NOX1 to the hypothalamus suppressed the anxiety-like behavior in WT. An increase in oxidative stress induced by restraint stress was blunted in the hypothalamus of NOX1-KO. Concomitantly, elevated levels of plasma ACTH as well as corticotropin-releasing hormone (CRH) and c-fos mRNA in the hypothalamus were significantly attenuated in NOX1-KO subjected to restraint stress. In hypothalamic slice cultures, the increase in CRH mRNA induced by a protein kinase A activator, forskolin, was suppressed in NOX1-KO. Moreover, the levels of phosphorylated CREB in the hypothalamus caused by stress were ameliorated in NOX1-KO.

Taken together, NOX1/NADPH oxidase appear to play a key role in stress-induced anxiety,possibly by regulating activation of the PKA-CREB pathway in the hypothalamus.

1-LBS-08 Late-Breaking Session

New animal behavioral pharmacology using wireless power supply and implantable sensors

Kazuhiko Yanai[1], Takeo Yoshikawa[1], Kenichi Sagara[2], Shu Sasaki[2]

[1]Tohoku Univ. Sch. Med. Dept. Pharmacol., [2]HIKARIDENSHI, CO., LTD

Current animal behavioral studies have much room for improvement. Data-driven drug discovery research and AI research using big data have become hot topics in human clinical research. Radiotelemetry provides an alternative means of obtaining physiological measurements from awake and freely moving laboratory animals, without introducing stress artifacts. For researchers, especially those in the fields of pharmacology and toxicology, the technique may provide a valuable tool for predicting the effectiveness and safety of new compounds in humans. The current embedded type sensor has a built-in battery and is therefore large, and cannot be individually identified, so that simultaneous measurements cannot be made in multiple animals at the same time. We developed a compact telemetry system using a new electromagnetic power supply system. The new system can continuously measure long-term biometric data such as locomotor activity and body temperature in a plurality of individually identified mice. The excellent feature of this device is that biometric data can be measured by five individuals over a long period without changing the sensor embedded in the body. The newly-developed technology is an important tool for the stress-free collection of these physiologic data in small rodents, including mice.

1-LBS-09 Late-Breaking Session

Effect of the herbicide glufosinate-ammonium exposure on central nervous system

Hironori Izumi[1], Maina Demura[1], Ayako Imai[1], Tomoyuki Yoshida[1], Ryohei Ogawa[2], Hisashi Mori[1]

[1]Dept. of Mol. Neurosci., Grad. Sch. of Med. & Pharmaceut. Sci., Univ. of Toyama, [2]Dept. Radiol., Grad. Sch. Med. & Pharmaceut. Sci., Univ. of Toyama

Exposure to pesticides can induce neurobehavioral effects in humans, as well as in other mammals, including rodents. However, the effects of the toxicity of pesticides on the central nervous system (CNS) remain largely unclear. We previously developed *Arc*-promoter-driven luciferase transgenic (Tg) mouse strains for non-invasive monitoring of the neuronal-activity-dependent gene expression in mouse brain under physiological and pathological conditions. In this study, we examined the effects of glufosinate-ammonium (GLA), one of herbicides used in a variety of crops, on neuronal activity using *Arc-Luc* Tg mice and detected a decrease in bioluminescence signal at juvenile stage after chronic treatment with GLA. Next, we performed transcriptome analysis of primary cultured neurons and identified differentially expressed genes related to axonal guidance signaling between GLA-treated and saline-control neurons. Linked to these results, we further found disturbance of synapse formation after low dose exposure to GLA. Our results provide valuable evidence to understand the mechanistic basis for the effect of GLA on the CNS.

1-LBS-10 Late-Breaking Session

Novel Reporter System Monitoring IL-18 Specific Signaling can be Applied to High-Throughput Screening

Yonezawa Tomo[1], Kurata Riho[2], Xiaofeng Cui[3], Masamitsu Harada[1], Jun Nagai[1], Ysuhiro Yoshida[4]

[1]Center for Therapeutic Innovation, Gene Research Center for Fronties Life Sciences, Nagasaki University, Graduate School of Biomedical Sciences, [2]Education and Research Center For Fundamental Pharmaceutical Sciences, Osaka University of Pharmaceutical Sciences, [3]School of Chemistry, Chemical Engineering and Life Sciences, School of Materials and Engineering, Wuhan University of Technology, [4]Department of Immunology and Parasitology, University of Occupational and Environmental Health

Very recently, the immunotherapies against cancer, autoimmune diseases, and infection have been feasible and promising. Thus, we have examined the possibility whether or not human gamma delta T cells can be applied for the novel immunotherapies. We previously established the cells stably maintaining NFkB-driven human secreted embryonic alkaline phosphatase (SEAP) expression. The cells can be used to determine the transcription activity of NFkB with high-standard dynamic range and accuracy. Because IL-18 is a kind of cytokines that enhances cytotoxicity and activity of human gamma delta T cells through NFkB activation, we have focused on the activity and signaling of IL-18. In this study, we modified the previous reporter cell that can determine the transcription activity of NFkB to express two subunits consisted of human IL-18 receptor. The modified cells secreted SEAP in response to treatment with human recombinant IL-18 in a concentration-dependent manner. We also observed the concentration-dependently enhancement of NFkB activity in the cells treated with mouse recombinant IL-18 although the affinity was lower compared to human recombinant IL-18. We also previously established the cells stably expressing and secreting human recombinant IL-18 and then validated whether or not the conditioned medium from the cells activate NFkB transcription activity using this assay. We demonstrated drug screening using number of extracts derived from marine bacteria and synthetic compounds.

Ca^{2+}-sensing receptor-$G_{q/11}$ protein signaling pathway is involved in nitric oxide release from human vascular endothelial cells

Takahiro Horinouchi, Yuichi Mazaki, Soichi Miwa

Dept. Cell. Pharmacol., Grad. Sch. Med., Hokkaido Univ. Sapporo 060-8638, Japan.

Ca^{2+}-sensing receptor (CaSR) belongs to family C of G protein-coupled receptors and is activated by the endogenous agonists such as Ca^{2+}. Stimulation of CaSR expressed in vascular endothelial cells through the increase in extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) is reported to induce vasorelaxation via the production of nitric oxide (NO). The purpose of the present study is to characterize the CaSR-mediated NO production in human vascular endothelial cells. In human endothelial EA.hy926 cells, the increase in $[Ca^{2+}]_o$ from 0.2 to 2 mM induced a concentration-dependent increase in intracellular Ca^{2+} concentration, which was significantly inhibited by NPS 2143 (a CaSR antagonist) and YM-254890 (a $G_{q/11}$ protein inhibitor). Stimulation with 2 mM Ca^{2+} for 4 h elicited an increase in the phosphorylation level of eNOS at Ser^{1177}, which was significantly depressed by NPS 2143, YM-254890, and removal of Ca^{2+} from the medium. Ca^{2+} (2 mM) induced an increase in NO production, which was inhibited by NPS 2143, YM-254890, removal of Ca^{2+} from the medium, and L-NAME (a competitive eNOS inhibitor). These results provide evidence that activation of CaSR with extracellular Ca^{2+} facilitates NO release from human vascular endothelial cells via a $G_{q/11}$ protein-eNOS-dependent pathway.

1-LBS-12 Late-Breaking Session

Pimaric acid inhibits contraction of pulmonary artery via BKCa channel activation

Ishida Masashi, Yoshiaki Suzuki, Hisao Yamamura

Dept. Mol. Cell. Pharmacol., Grad. Sch. Pharmaceut. Sci., Nagoya City Univ.

Large-conductance Ca2+-activated K+ (BKCa) channels are expressed in vascular smooth muscle cells and regulate the membrane excitability. Activation of BKCa channels following membrane depolarization and/or cytosolic [Ca2+] increase causes membrane hyperpolarization and subsequent cytosolic [Ca2+] decrease. Therefore, BKCa channels are recognized as a key factor for the negative feedback regulation of vascular tone. Pimaric acid is a common resin acid naturally contained in pine rosin. We previous reported that pimaric acid activated BKCa channels and slightly blocked voltage-dependent Ca2+ channels. However, the effects of pimaric acid on contractile response of vascular smooth muscles are still unclear. In the present study, we examined the effects of pimaric acid on contraction of pulmonary artery. Pulmonary arteries were isolated from male Sprague-Dawley rats and contracted with high K+ solution. The high K+-induced contraction was reduced by pimaric acid in a concentration-dependent manner (1-100 mM). Quantitative real-time PCR data revealed that the a and b1 subunits of BKCa channel were highly expressed in human pulmonary arterial smooth muscle cells. These results indicate that pimaric acid enhances the activity of BKCa channels and results in the relaxation of pulmonary arterial smooth muscles.

1-LBS-13 Late-Breaking Session

Inhibitory effect of melatonin on voltage-dependent potassium (Kv4.2) channels

Mishima Hiroki, Yoshiaki Suzuki, Hisao Yamamura

Dept. Mol. Cell. Pharmacol., Grad. Sch. Pharmaceut. Sci., Nagoya City Univ.

Melatonin is synthesized in and secreted from the pineal gland as a neurohormone. Secreted melatonin regulates the circadian rhythm. It has been reported that melatonin acts on ion channels directly, or indirectly via the melatonin receptors. The voltage-dependent potassium channel family is expressed in the most types of tissues and contributed to the physiological functions including the regulation of resting membrane potential and the formation of action potential. In this study, the effects of melatonin on voltage-dependent potassium (Kv4.2) channels were analyzed by whole-cell patch clamp techniques. In HEK293 cells stably expressed with Kv4.2 channels, outward currents with fast activation and inactivation were observed by membrane depolarization to +100 mV from the resting potential of -80 mV for 500 ms. The outward currents were clearly reduced by the application of 1 mM melatonin and partly recovered by wash-out. Quantitative real-time PCR data revealed that Kv4.2 channels were highly expressed in pineal glands from the rats. These results suggest that melatonin regulates the activity of Kv4.2 channels in pineal glands, potentially contributing to the regulation of circadian rhythm.

Adverse Outcome Pathway (AOP) development on Wnt/beta-catenin signaling pathway related to cancer

Shihori Tanabe[1], Kazuhiko Aoyagi[2], Sabina Quader[3], Horacio Cabral[4], Ryuichi Ono[5], Akihiko Hirose[1], Hiroshi Yokozaki[6], Hiroki Sasaki[7]

[1]Div. Risk Assessment, Biol. Safety Res. Center, Natl. Inst. Health Sci., [2]Dept. Clin. Genomics, Natl. Cancer Center Res. Inst., [3]iCONM, [4]Grad. Sch. Engineer., Tokyo Univ., [5]Div. Cel. Mol. Toxicol., Biol. Safety Res. Center, Natl. Inst. Health Sci., [6]Dept. Pathol., Grad. Sch. Med., Kobe Univ., [7]Dept. Transl. Oncol., Natl. Cancer Center Res. Inst.

Adverse Outcome Pathway (AOP) is developed for the prediction of the adverse effects. Epithelial-mesenchymal transition (EMT) plays an important role in the acquisition of cancer stem cell (CSC) feature and drug resistance, which are main hallmarks of cancer malignancy. Although previous findings have shown that Wnt/beta-catenin signaling pathway is activated in the cancer progression, the precise mechanism of Wnt/beta-catenin signaling in EMT and CSCs are not fully understood. To reveal the network pathways in EMT, gene expression in mesenchymal stem cells (MSCs) and diffuse-type gastric cancer (GC) as well as intestinal-type GC have been analyzed and compared. The network pathways in MSCs and GC were analyzed with Ingenuity Pathway Analysis (IPA). The gene expression profiling demonstrated that gene expression of cadherin 1 (*CDH1*), Wnt family member 9A (*WNT9A*) and catenin beta 1 (*CTNNB1*) were up-regulated in diffuse-type GC compared to MSCs. The gene expression of growth factor receptor bound protein 7 (*GRB7*) and erb-b2 receptor tyrosine kinase 2 (*ERBB2*) were up-regulated in intestinal-type GC compared to diffuse-type GC. Wnt/beta-catenin signaling, as well as ERBB signaling networks, involved in EMT, CSCs and drug resistance, have been investigated and profiled in bioinformatics. In conclusion, the Wnt/beta-catenin signaling pathway was included in EMT-related molecular network pathways in MSCs and GC, which may contribute into the elucidation of mechanism in the drug resistance of CSC population. AOP related to Wnt/beta-catenin signaling pathway is discussed.

1-LBS-15 Late-Breaking Session

Early postnatal lethality of mice lacking mitochondrial protein p13

Norihito Shintani, Hiroki Ueno, Satomi Hara, Sae Ogura, Naoki Inoue, Yohei Morota, Hitoshi Hashimoto

Grad. Sch. Pharmaceut. Sci., Osaka Univ.

p13 is mitochondrial protein *widely expressed in central* and peripheral tissues. Recently, we generated mice lacking p13 (p13-/- mice), and found that p13-/- genotype was smaller than the expected Mendelian ratio at 3 weeks of age (approx. 40% of the expected ratio). Here, we investigated the possible mechanisms underlying the loss of p13-/- mice. At postnatal day 0 (P0), Mendelian segregation of pup genotypes from heterozygous breeding was observed (n = 294, $P = 0.25$, χ^2 analysis), suggesting a significant loss of p13-/- pups specifically during the postnatal period. Kaplan-Meier survival analysis demonstrated that more than half of p13-/- mice died during the first 2 postnatal days. At P0, we observed the presence of milk in p13-/- pups stomach, however, their blood glucose levels were significantly lower than that of wild-type littermates. Taken together, the present results suggest that p13 contributes to early postnatal survival and maintenance of the normal blood glucose levels.

Effects of different sweetener charge-methods on blood glucose level and insulin concentration of mice

Sachiko Sato, Yukiko Kondo, Eiichi Taira

Dept. of Pharmacol., Iwate Med. Univ., Morioka, Japan

Excessive intake of sugar (sucrose) has been considered to be related to obesity, diabetes mellitus and lipid metabolism disorder, but there are not many reports on blood glucose level and insulin secretion affected by the way of sucrose charge. In this study, we investigated the effects of different types of sweeteners and their charge methods. Male ddY mice were divided into three groups: water-group, sucrose-group, and glucose-group. Furthermore, they were divided into three groups: intraperitoneal administration (IP)-group, oral administration (PO)-group, and free intake-group. The free intake-group was divided into those with and without food, and each drink and food were freely taken for 1 hour. Blood glucose level and insulin concentration were measured at 0, 30, and 60 minutes after the start of the experiment. Blood glucose levels and insulin levels of IP-group, PO-group, and free-intake-group (without food) increased more in the glucose-group than in the sucrose-group. The blood glucose level in the free intake-group (with food) did not differ depending on the type of sweetener, but insulin was secreted in large amounts, and the insulin secretion in the glucose-group was particularly high. Therefore, taking sweet drinks with food promoted insulin secretion as compared to taking sweet drinks alone.

1-LBS-17 Late-Breaking Session

Age-dependent change in size distribution of plasma sEV from Wistar rat

Yusei Fujioka, Kosuke Otani, Muneyoshi Okada, Hideyuki Yamawaki

Lab. Vet. Pharmacol., Sch. Vet. Med., Kitasato Univ.

Small extracellular vesicles (sEV) are lipid-bilayer-capsuled particles with a 50-150 nm diameter. They contain various molecules, such as proteins, lipids, nucleic acids, and metabolites. sEV affect cellular function via signal transduction through binding to cell surface receptors or delivering contents by phagocytosis, pinocytosis or membrane fusion. Therefore, sEV are recognized mediating cell-to-cell communication. Recent studies suggest that sEV play a key role during various disease states. However, little is known about changes in sEVs characters by aging. We then aimed to compare the size distribution of plasma sEV from young and aged Wistar rats. We isolated sEV from plasma of male Wistar rats (6- and 15-week-old; 6w-sEV and 15w-sEV) by polyethylene glycol precipitation and ultracentrifuge method. sEV particle distribution was measured by a tunable resistive pulse sensing method. Mean diameter in 15w-sEV was higher than 6w-sEV. Particles with less than 150 nm of diameter in 15w-sEV were lower than 6w-sEV. The present study for the first time revealed that the size of plasma sEV in Wistar rat increases by aging. Further studies are needed in order to clarify physiological significance of the increase in particle diameter by aging.

1-LBS-18 Late-Breaking Session

Pharmacological profile of fingolimod for pulmonary arterial hypertension

Moe Fujiwara[1], Aya Yamamura[2], Yoshiaki Suzuki[1], Hisao Yamamura[1]

[1]Dept. Mol. Cell. Pharmacol., Grad. Sch. Pharmaceut. Sci., Nagoya City Univ., [2]Dept. Physiol., Aichi Med. Univ.

Pulmonary arterial hypertension (PAH) is pathophysiologically characterized by vasoconstriction and vascular remodeling of the pulmonary artery. Pulmonary vascular remodeling is mainly mediated by the enhanced cell proliferation of pulmonary arterial smooth muscle cells (PASMCs). In this study, we examined the pharmacological effects of fingolimod on the development of PAH. The proliferation rate of PASMCs from idiopathic PAH (IPAH) patients was much higher than that of PASMCs from normal subjects. In normal-PASMCs, fingolimod at low concentrations did not affect the cell proliferation, whereas higher concentrations partly reduced the cell proliferation. On the other hand, the application of fingolimod clearly inhibited the proliferation of IPAH-PASMCs and the inhibitory effect was in a concentration-dependent manner. In monocrotaline-induced pulmonary hypertensive rats, intraperitoneal administration of fingolimod ameliorated both pulmonary vascular remodeling and right ventricular hypertrophy. In addition, fingolimod improved the mortality rate. Our results suggest that fingolimod blocks the development of PAH through inhibiting the excessive proliferation of PASMCs. Fingolimod may be a novel option for the treatment of PAH.

1-LBS-19 Late-Breaking Session

BK_{Ca} channel inhibition decreases the proliferation of human hepatic stellate cells

Kondo Rubii, Yoshiaki Suzuki, Hisao Yamamura

Dept. Mol. & Cell. Pharmacol., Grad. Sch. Pharmaceut., Nagoya City Univ.

Hepatic stellate cells are liver-specific pericytes that play central roles in the development of liver fibrosis. During liver injury, hepatic stellate cells transdifferentiate from the quiescent phenotype into the myofibroblast-like phenotype, resulting in high proliferation and extracellular matrix production. Large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels are expressed in many types of tissues and involved in the regulation of membrane potential, intracellular Ca^{2+} concentration, and cell proliferation. However, the involvement of BK_{Ca} channels on liver fibrosis remains unclear. In this study, we investigated the pathophysiological roles of BK_{Ca} channels in a human hepatic stellate cell line, LX-2. The mRNA expression analysis revealed that LX-2 cells highly expressed the α subunit of BK_{Ca} channels. In LX-2 cells, extracellular Ca^{2+} restoration in the presence of thapsigargin induced store-operated Ca^{2+} (SOC) entry, which potentially mediated by Orai/STIM channels. The SOC entry was significantly reduced by a specific inhibitor of BK_{Ca} channels, paxilline. In addition, the proliferation of LX-2 cells was clearly attenuated by paxilline. These results suggest that BK_{Ca} channels are functionally expressed in LX-2 cells and contribute to cell proliferation by regulating intracellular Ca^{2+} signaling.

2-LBS-20 Late-Breaking Session

Specificities in dendritic branching pattern and spine density along the dorso-ventral axis of the hippocampus

Hojo Mika, Mai Deguchi, Yutaka Nomura, Chiaki Takeuchi, Miho Ishikawa, Toshinori Sawano, Jin Nakatani, Hidekazu Tanaka

Lab. Pharm., Dept. Biomed Sci., Col. Life Sci., Ritsumeikan Univ.

The hippocampus is functionally segregated along the dorso-ventral axis in rodents. The dorsal hippocampus is involved in spatial memory whereas the ventral is in emotional responses. Consistently, each region receives afferents from distinct brain regions. Furthermore, within the dentate gyrus (DG), the proximal and distal portions of granule cell (GC) dendrites receive medial and lateral perforant pathway axons, respectively, which are originating from different regions of the entorhinal cortex. Differences in dendritic morphology and spine density presumably reflect differences in activities of their partner axon termini. Here, we investigate the morphological features of the GC in the dorsal and the ventral DG, and their responses to social defeat stress as an example of emotional inputs. Visualization of single neurons by microinjection of Lucifer Yellow revealed that the branching patterns of GC dendrites are distinct between dorsal and ventral DGs; the peak of the number of intersections in Sholl analyses localized more proximal in the dorsal than in the ventral DG. Spine density of ventral DG was higher than dorsal DG. Social defeat stress was found to suppress the dendritic branching and spine densities of GCs both in dorsal and ventral DGs to similar extent. Mushroom and thin spines decreased significantly whereas stubby spines did not. The data suggest that the dendritic branching pattern and spine density of GCs are distinct between the dorsal and ventral hippocampus, but undergo uniform morphological remodeling in response to an emotional stress.

2-LBS-21 Late-Breaking Session

Involvement of central L-lactate and AMP-activated protein kinase in fear memory in diabetic mice

Aimi Yamagishi[1], Daiki Ueda[1], Naomi Yonemochi[1], Junzo Kamei[2], Hiroko Ikeda[1]

[1]Dept. Pathophysiol. Ther., Hoshi Univ Sch. Pharm. Pharmaceut. Sci., [2]Dept. Biomol. Pharmacol., Hoshi Univ Sch. Pharm. Pharmaceut. Sci.

The prevalence of mental disorders in diabetes mellitus is reported to be higher than that in general population. However, its mechanism is unclear. Since glucose is metabolized to L-lactate in the astrocytes of the brain and L-lactate suppresses AMP-activated protein kinase (AMPK), we investigated whether L-lactate and AMPK in the brain are involved in fear memory in streptozotocin (STZ)-induced diabetic mice. In the conditioned fear test, L-lactate injection increased freezing. In addition, injection of the AMPK inhibitor compound C also increased freezing. Freezing induced by both L-lactate and compound C was inhibited by the AMPK activator AICAR. We next examined the levels of L-lactate and AMPK in the amygdala and the hippocampus, which are known to play important roles in fear memory. L-lactate was increased in the amygdala and the hippocampus in STZ-induced diabetic mice. In contrast, phosphorylated AMPK, which is an active form of AMPK, was reduced in the amygdala and the hippocampus in STZ-induced diabetic mice. In addition, the increase of freezing in STZ-induced diabetic mice was suppressed by AICAR. These results suggest that L-lactate production is increased in the amygdala and the hippocampus in diabetes, which enhances fear memory through inhibition of AMPK.

2-LBS-22 Late-Breaking Session

The relationships of pharmacological effects and radical scavenging abilities on BBB permeable Ca^{2+}/Calmodulin antagonist CV-159.

Masato Kamibayashi[1], Ikuo Nakanishi[2], Sumire Ikuno[3], Hisashi Matsuda[3], Shigeru Oowada[4]

[1]Chiba Uiv., QST/NIRS, Kyoto Pharmaceut.Univ., [2]QST/NIRS, [3]Kyoto Pharmaceut.Univ., [4]Asao Clinic

Redox imbalances by overproduction of reactive oxygen species (ROS) are known to play an essential role in the pathological events of cerebral and cardiac ischemic injury, hypertension, inflammation and cancer. Widely used calcium channel antagonists such as nifedipine, nicardipine, amlodipine are not effective for ischemia-reperfusion (I/R) injuries at clinical used low dose on animal models.

BBB permeable Ca^{2+} /calmodulin antagonist CV-159, 1,4- Dihydro -2,6-dimethyl- 4-(3-nitrophenyl) -3,5-pyridinedicarboxylic acid methyl 6-(5-phenyl-3-pyrazolyloxy)hexyl ester characterized by markedly inhibitory effects for infarct size and edema on cerebral I/R injury. In cyclic voltammetry and ESR studies, hydroxyl radical scavenging ability of CV-159 was detected 100 times stronger than that of nicardipine, and it also suppressed mitochondrial superoxide and iNOS generation. Radical spin trapper G-CYPMPO (CAS No.1350616-52-2) and CV-159 markedly relaxed the high concentration of K^+-induced contractions in isolated endothelium-denuded rat aortic strips, suggesting the existence of a novel role of oxygen radical in smooth muscle signal transduction.

2-LBS-23 Late-Breaking Session

Bladder sensation evaluation of a carrageenan-induced chronic prostatitis model using a direct measurement of the bladder mechanosensitive single-unit afferent nerve activity

Naoki Aizawa, Tomoe Fujita

Dept. Pharmacol. Toxicol., Dokkyo Medical Univ.

Aims: Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) causes chronic pain and/or storage symptoms. This study aimed to evaluate whether bladder sensation is deteriorated in a carrageenan induced CP/CPPS model by a direct measurement of the bladder mechanosensitive single-unit afferent nerve activity.

Methods: Male adult Sprague-Dawley rats were used. Fifty μL of 3% λ-carrageenan was injected into both lobes of the ventral prostate and for the control rats, fifty μL of saline was used. Seven days after injection, histology was examined along with cystometry and mechanosensitive single-unit afferent nerve activity. Statistical significance was determined using an unpaired Student's t-test with a two-sided significance level of 0.05.

Results: In the carrageenan group, weight increase and inflammatory cell infiltrations in the prostate were confirmed, basal and threshold-pressures of the bladder were remarkably increased, when compared to the sham group. Regarding Aδ- or C-fibers, the mechanosensitive afferent nerve activities revealed no differences in either group.

Conclusions: The carrageenan-induced CP/CPPS rat model showed edema and inflammation in the prostate, whereas little change was detected in bladder sensation. These findings, which were evaluated using a direct measurement of the mechanosensitive single-unit afferent nerve activity, suggest that the bladder sensation is unlikely deteriorated in this model.

2-LBS-24 Late-Breaking Session

Oral glutathione administration rescues neurons by reduction of neuroinflammation in Alzheimer's disease mice

Kohji Fukunaga, Reiko Fukunaga, Hisanao Izumi, Keita Sato

Tohoku University. Pharmacology

[Background] Glutamate-cysteine ligase modifier subunit (GCLM) null mice display a 70-80% reduction in total glutathione (GSH) level (Killoy et al. Exp Neurol 2018 Apr;302:129-135). The GCLM null mice causes motor neuron degeneration in mice like SOD1 mutant mice. We here investigated whether oral GSH administration can rescue neurons form neuroinflammation in Alzheimer's disease model mice.
[Methods] After 3 weeks administration of glutathione (100 or 500 mg/kg/day, p.o.) in 12 month-old wild and *APP-NL-GF* knock-in mice, oxidative stress, neuroinflammation and cognition were investigated. We also assessed the GSH levels in mouse brain.
[Results] *APP-NL-GF* knock-in mouse brain display a 50% reduction in total GSH as compare to wild mouse brain. The lipid oxidation assessed by 4-hydroxy-2-nonenal (4-HNE) was also markedly increased in *APP-NL-GF* knock-in mouse brain. The GSH administration dose-dependently diseased the oxidative stress and suppressed microglial activation in the hippocampus. Likewise, the GSH administration improved cognitive impairment observed in *APP-NL-GF* knock-in mice.
[Conclusion] Taken together, the oral GSH administration rescues neurons from oxidative stress and neuroinflammation in neurodegenerative disorders and should be try in Alzheimer disease patients.

2-LBS-25 Late-Breaking Session

Effect of histamine H_3 receptor agonist on the chemotherapy-induced fatigue in mice

Kouichi Yamamoto

Dept. Med. Sci. Tech., Div. Health Sci. Grad. Sch. Med., Osaka Univ.,

We report that tumor necrosis factor-alpha (TNF-α) prodction via histamine H_4 receptors contributes to cisplatin-induced fatigue. Previous studies reported the activation of histamine H_3 receptors also have the potential to reduce the inflammatory peptides. In this study, we investigated the effects of the H_3 receptor agonist on the development of chemotherapy-induced fatigue in mice. Cisplatin (7.5 mg/kg, i.p.) induced anorexia and decrease of voluntary wheel running within 24 hours of its administration and they continued for 3 days, and daily administration of a selective H_3 receptor agonist (immethtridine, 10 mg/kg, s.c.) significantly inhibited the development of anorexia and decrease of voluntary wheel running. Cisplatin significantly increased TNF-α mRNA expression in the hypothalamus and spleen, and the period of expression increase paralleled the onset period of anorexia and decrease of voluntary wheel running. Pretreatment with immethridine inhibited splenic TNF-α mRNA expression. These results suggest that peripheral TNF-α mRNA expression via H_3 receptors may contribute to the development of cisplatin-induced fatigue.

2-LBS-26 Late-Breaking Session

The effect of tumor suppressor Pdcd4 knockdown on gene expression on mouse fibroblast cells and mouse melanoma cells

Eriko Iwata[1], Noriko Yoshikawa[1], Kana Nishikaze[1], Satomi Kagota[2], Kazumasa Shinozuka[2], Kazuki Nakamura[1]

[1]Dept.Pharmacol.I, Mukogawa Women's Univ., [2]Dept.Pharmacol.II, Mukogawa Women's Univ.

Purpose: Programmed cell death 4 (Pdcd4) is a novel tumor suppressor gene which is known to act as a negative regulator of protein translation and malignant transformation. However, the tumor suppressor mechanism of Pdcd4 remain unclear. In order to elucidate the mechanism of inhibition of tumor malignancy by Pdcd4, we conducted the knockdown of Pdcd4 in cells.

Methods: In this study, we used three mouse cell lines. The C57BL/6J-emb and the NIH3T3 are normal fibroblast cells, the B16-F0 are low-metastatic melanoma cells. We established the Pdcd4 knockdown cells using siRNA systems. mRNA was extracted from Pdcd4 knockdown C57BL/6J-emb and B16-F0, we performed DNA microarray analysis. To confirm the results of microarray analysis, we performed real-time PCR analysis.

Results: The mRNA levels of Pdcd4 in each cell line were significantly decreased after 24 hr or 48 hr exposure to siPdcd4. Among 23,474 mouse genes, microarray analysis identified 3 significantly up-regulated and 10 significantly down-regulated genes in both Pdcd4 knockdown C57BL6J-emb and B16-F0 (ratio>=2 [up and down]). Real-time PCR showed that the mRNA levels of Skp2 which was identified down-regulated gene, significantly decreased in Pdcd4 knockdown C57BL/6J-emb and NIH3T3 compared with the control cells.

Summary/Conclusion: These results suggest that Skp2 might be one of genes involved in the tumor suppression by Pdcd4.

2-LBS-29 Late-Breaking Session

Effects of oyster extract on 5-Fluorouracil (5-FU) induced toxicity in rat

Noriyuki Yamazaki[1], Kohtaro Suzuki[1], Keita Saito[1], Kohei Araki[1], Yuki Ishida[1], Hiroki Morikawa[1], Tatuya Ishida[2], Hiroyuki Matsui[2], Yoshikazu Matsuda[2]

[1]New Drug Research Center, Inc, [2]Central Research Institute, Japan Clinic Co, Ltd.

Oyster (*Crassostrea gigas*) contains abundant nutritional elements, including glycogen, vitamine, zinc and taurine. It is reported that oyster extract exhibited several physiological activities. In this study, the symptom relieving effects of oyster extracts on 5-fluorouracil (5-FU) induced toxicity in rats was examined mainly on gastrointestinal toxicity and myelotoxicity.

Male SD rats were used in this study. Test groups were as follow, control, 5-FU, 5-FU and oyster extract (200 and 500 mg/kg/days). Oyster extracts were administrated for 21 days in rats. 5-FU was administrated for 5 days after 14 days of oyster extracts administrated. After termination of administration of oyster extracts, recovery period was established for 3 days, and autopsy was performed.

No deaths were observed throughout the study period. Regarding body weight and food intake, significant reduction suppression and dose-dependent reduction tendency was observed. In the hematological examination, influence was observed on the white blood cell count, red blood cell count, hemoglobin amount and hematocrit value due to administration of 5-FU.

Evaluation of intestinal mucosa by histopathological examination, mucosal thickness, villous height and crypt thickness were dose - dependent or high trend without dose - dependence. In addition, a suppression tendency was also observed for mucosal atrophy of the duodenum due to administration of 5-FU. From the above results, it was suggested that oyster extract is effective in alleviating gastrointestinal toxicity by 5-FU.

2-LBS-30 Late-Breaking Session

Pharmacology of Baloxavir (Xofluza®); a First-in-Class Cap-dependent Endonuclease Inhibitor for Treatment of Influenza

Shishido Takao

Shionogi & Co., Ltd.

Baloxavir marboxil is an oral prodrug that is rapidly converted to its active form baloxavir acid, a potent inhibitor of influenza cap-dependent endonuclease function of influenza A and B viruses. Baloxavir was approved for treatment of uncomplicated influenza A and B virus infections in 2018 in Japan (for those weighing >10 kg) and the United States (for those aged 12 years and older)

Key nonclinical characteristics of baloxavir include broad spectrum activity against various types and subtypes of influenza virus strains *in vitro* as well as rapid and profound reduction in viral load *in vivo*. The phase III study (CAPSTONE-1) was a multicenter, randomised, double-blind, placebo- and oseltamivir-controlled study of otherwise-healthy patients in Japan and US (n=1436). The primary endpoint was time to alleviation of influenza symptoms (TTAS). TTAS was shorter with baloxavir than placebo (median 53.7 hr vs 80.2 hr, $p<0.0001$). Median time to cessation of viral shedding was 24 hr in baloxavir-treated patients, compared with 72 hr for oseltamivir ($p<0.0001$) and 96 hr for placebo ($p<0.0001$). Baloxavir was well tolerated and appeared to have no significant safety issues identified. Testing of laboratory isolates passage or clinical isolates identified isoleucine-to-threonine substitution at amino acid position 38 in N-terminal domain (PA/I38T). PA/I38 substitutions conferred reduced susceptibility to baloxavir and reduced fitness in variants. In the poster session, we report the key nonclinical and clinical profiles of baloxavir.

2-LBS-31 Late-Breaking Session

Drug discovery screening based on epigenetic control of COPD – Benserazide inhibits the prothymosin α-H1 histone interaction

Keigo Tanaka, Naoki Dozono, Shuji Kaneko, Hiroshi Ueda

Department of Molecular Pharmacology, Kyoto University Graduate School of Pharmaceutical Sciences

Anti-cholinergic inhibitors have been used for the treatment of chronic obstructive pulmonary disease (COPD). Because of their side effects, there is a big demand for new type of drugs. Su et al., (Nature Commun, 2013) has proposed a hypothesis that prothymosin α (ProTα) upregulated in emphysema patients binds to histone H1 and eliminates the histone deacetylase (HDAC) bound to H1, leading to an epigenetic upregulation of matrix metalloprotease (MMP) gene expression, which may cause pulmonary cell damage. In addition, Borge et al., (Nature, 2018) demonstrated that ProTα binds to H1 at a picomolar level of Kd value. Based on these reports we attempted to find inhibitors of ProTα-H1 interaction by use of homogenous time-resolved fluorescence (HTRF). Using an existing drug compound library (～2300 compound), we obtained benserazide, which inhibits the interaction by 70% at 30 μM. Although it is under investigation whether benserazide has beneficial actions against the toxicity of cigarette smoking extract (CSE) or its constituents, here we will present following findings, as follows; 1) ProTα gene expression is very high in A549 lung cancer cells, 2) the treatment with siRNA ProTα gene down-regulated the expression of MMP2 gene as well as ProTα gene in A549 cells, 3) benserazide alone has no action, but it deteriorated the CSE-induced damage of survival activity of A549 cells, 4) from the RNAseq analysis of lung, which has been treated with CSE (i.v,) for 6 weeks, it was found that some candidate genes involved in CSE-induced toxicity and its reversibility by benserazide.