1-S01-1 Symposium1

Regulation of anterograde transcytosis of neurotrophin receptors and its role in health and diseases

Naoya Yamashita

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Neurotrophins are one of the best-known examples of target-derived instructive cues that regulate neuronal development. Upon nerve growth factor (NGF) binds to its receptor TrkA at axon terminals, these complexes are internalized and retrogradely transported back to cell bodies. However, how neurons replenish TrkA in nerve terminals remains unknown. We show that retrograde signaling by NGF is necessary for soma-to-axon transcytosis of TrkA. Activated TrkA receptors are retrogradely transported to cell bodies, where they are inserted on soma surfaces and promote phosphorylation of resident naive receptors, resulting in their internalization. Prior to axonal transport, endocytosed TrkA is dephosphorylated by PTP1B to ensure targeting of inactive receptors to axons to engage with ligand. These results identify phospho-regulatory mechanisms of anterograde transcytosis of TrkA, which regulate neuronal sensitivity to NGF. We are now asking whether other membrane proteins are co-transcytosed with TrkA and we identify amyloid-beta precursor protein (APP) as a candidate. The TrkA-APP co-transcytosis regulates NGF functioning and APP metabolism. Since APP and its proteolytic products play an important role in pathogenesis of Alzheimer's disease (AD), NGF-dependent transcytosis might be also related to the onset of AD.

1-S01-2 Symposium1

Neuronal Signaling in Islet Development and Function

Rejji Kuruvilla

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Pancreatic islets, the functional units in regulating blood glucose levels, are richly innervated by sympathetic nerves. Employing genetic mouse models and neuron-islet co-cultures, we discovered that sympathetic innervation is essential for organizing islet structure during development (Borden et al., Cell Rep. 2013). Further, we demonstrated that this inductive interaction at early stages is critical for mature islet function in regulating glucose metabolism, suggesting that developmental perturbations in sympathetic innervation might underlie metabolic dysfunction in humans. We are building on these findings to identify the molecular pathways by which sympathetic nerves instruct islet organization and the acquisition of functional maturity. To identify how sympathetic nerves influence islets, we recently asked whether neuronal activity is required for islet formation and function. Sympathetic nerves innervating the pancreas secrete the neurotransmitter, norepinephrine, which acts through pancreatic adrenergic receptors. We addressed the effects of blockade of sympathetic neurotransmission on islet morphology and function in mice via expression of tetanus toxin. Silencing of sympathetic activity resulted in defects in islet cyto-architecture and impaired expression of islet cell adhesion markers, similar to observations in sympathectomized mice. Surprisingly, "silenced" animals displayed improved glucose tolerance, enhanced glucose-stimulated insulin secretion, and higher insulin responsiveness, in contrast to findings in sympathectomized mice. These findings are consistent with previous studies describing an acute role for sympathetic activity in inhibiting islet insulin secretion and augmenting glucagon secretion. These results suggest that the presence of sympathetic nerves and neural activity exert similar effects on islet morphology during development but have distinct effects on islet function later in life.

1-S01-3 Symposium1

InSyn1 regulates GABAergic inhibition via the dystroglycan complex and is required for cognitive behaviors in mice

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Human mutations in the dystroglycan complex (DGC) result in not only muscular dystrophy, but also cognitive impairments. However, the molecular architecture critical for the synaptic organization of the DGC in neurons remains elusive. Here we report Inhibitory Synaptic protein 1 (InSyn1) is a critical component of the DGC whose loss alters the composition of the GABAergic synapses, excitatory/inhibitory balance *in vitro* and *in vivo*, and cognitive behavior. Association of InSyn1 with DGC subunits is required for InSyn1 synaptic localization. InSyn1 null neurons also show a significant reduction in DGC and GABA receptor distribution as well as abnormal neuronal network activity. Moreover, InSyn1 null mice exhibit elevated neuronal firing patterns in the hippocampus and deficits in fear conditioning memory. Our results support the dysregulation of the DGC at inhibitory synapses as a driver of altered neuronal network activity and specific cognitive tasks via a novel component, InSyn1.

1-S01-4 Symposium1

Chemico-genetic discovery of molecules underlying tripartite-synaptic function in vivo

Tetsuya Takano

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Neuronal synapses are intimately ensheathed by abundant astroctytic perisynaptic processes, which is critical for synapse formation and function. In contrast to well-studied neuronal synaptic compartments, however, the molecular mechanisms of how astrocytic perisynaptic structures govern neuronal synapses remain ill-defined. Here, we develop a new in vivo chemico-genetic approach, Split-TurboID-GRAPHIC, that uses a cell surface fragment complementation strategy combined with informatics to identify the molecules at astrocyte-synapse junctions in vivo. We identify more than 100 proteins enriched at astrocyte-neuronal junctions. We find novel adhesion molecules highly expressed in cortical astrocytes whose deletion dramatically alters excitatory/inhibitory synaptic balance and also impairs spatial learning. Using Split-TurboID-GRAPHIC we thus establish a new mechanism by which astrocytes coordinate inhibitory synaptic balance with excitation via a chemo-affinity code of the tripartite synapse.

1-S02-1 Symposium2

Activation of the nursing education through utilizing revision of Rules for Designation of Public Health Nurse, Midwife and Nurse Schools and Training Schools among nursing universities in Japan

Yukari Sugita

Ministry of Education, Culture, Sports, Science and Technology

In 2019, the number of nursing universities increased to 272 schools in Japan. One out of three universities have a nursing school, and nursing universities continue to increase.
Ministry of Education, Culture, Sports, Science and Technology introduced the "Model Core Curriculum for Nursing Science Education in Japan" (MCCNSE) in 2017. MCCNSE aims at the acquirement of necessary and indispensable nursing competencies in the undergraduate course, which enumerates learning targets to be useful for making the curriculum.
MCCNSE has 7 areas to develop qualities and abilities of a nurse for a lifetime. A is basic qualities/abilities required of nursing professional. B is society and nursing science. C is basic knowledge necessary for understanding objects of nursing, includes pharmacological science. D is basic knowledge of specialty underlying nursing practice. E is basic knowledge necessary for nursing practice in various settings. F is clinical and regional training practice, and G is research of nursing science.
Nursing universities are required to comply with both the School Education Act and the Act on Public Health Nurses, Midwives, and Nurses. Nursing universities are expected to formulate the more complete and original curriculum through revision of Rules for Designation of Public Health Nurse, Midwife and Nurse Schools and Training Schools.

1-S02-2 Symposium2

A message from clinical nursing : What to ask for pharmacology education for safe medication

Chieko Komiyama

Dept.Nurs, Hosp, Tokyo Univ.

Most patients admitted to the hospital receive some form of medication. The nurse prepares the medicine, and checks as the final performer of pharmacotherapy, the "right patient" "right drug" "right purpose" "right dose" " right route" and the "right time", and give the patient medication. Furthermore, it confirms that the medicine is surely carried into the patient's body, observes the patient's reaction, and plays a role in quickly responding to any abnormalities. The medication by nurses is an extremely important and responsible task.

Will new nurses just graduate start working in hospital with the knowledge, skills, and judgment necessary to safely perform this important task? Their knowledge about drugs is fragmented, they do not integrate knowledge about diseases and conditions, drug actions and mechanisms, treatment guidelines and protocols. And they have not been acquired techniques of the medication intravenous injections, gastric catheter injection etc. They need to learn a lot after graduating from nurse school.

In the current acute ward, patients with different clinical conditions and treatment methods are admitted to the hospital, and the patient condition and drugs involved by nurses are diversified. It has become a difficult environment for nurses to safely carry out medication operations. Incidents related to nurse medications have not diminished, and nurses are concerned that they may become parties to the incident.

In hospital, we are exploring how to build work environments and educational systems for safety medication. In order for nurses to administer medicines that are safe for both patients and themselves, they must understand the dangerous environment around them and the knowledge and skills that are lacking in themselves. It is important that they can properly seek assistance from the surrounding staff.

In this symposium, I would like to think about what can be strengthened by basic education at school and what should be strengthened by on-the job education in order for nurses to administer medication safely.

1-S02-3 Symposium2

The latest midwifery education on pharmacology during pregnancy, childbirth, postpartum and breastfeeding period

Masumi Imura

Global Health and Midwifery, Grad.Sch.Nurs., Japanese Red Cross College of Nursing

Midwife is recognized as a responsible and accountable professional who contributes to the sexual and reproductive health/rights and welfare of individuals, families, and communities. In particular, midwife work in partnership with women to give the necessary support, care and advice during pregnancy, childbirth and postpartum period, to conduct normal births on her own responsibility with fully bringing out women's natural body functions, to support breastfeeding, and to provide care for newborns and infants. This care includes preventative measures, the promotion of normal birth and breastfeeding, the detection of complications in mothers and children, accessing of medical care or other appropriate assistance and the carrying out of emergency measures.

In light of this, midwife should firstly learn biological functions of women's body, as well as acquire knowledge on the in-vivo mechanisms of substances such as hormones, neurotransmitters and enzymes during pregnancy, childbirth, postpartum and breastfeeding period. Subsequently, midwife need to learn drug treatments to complement and support biological functions in case of disorders or impairments of women's body and mind.

In this revision of the midwifery curriculum, we intend to include the basics of drug treatment (pharmacological action, pharmacodynamics and drug interactions), individual drug treatments, and a wide range of knowledge from alternative drug treatments including herbal medicine, well as health food products and common food items. We hope to build a midwifery educational program on personalized, client-centered pharmacology to support mothers, newborns, infants, and childrearing families.

1-S02-4 Symposium2

Pharmacology education at university：To train nurses to gain expertise in drug treatment

Tomoko Akase

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The 2017 model core curriculum and the 2019 proposed revisions to the designated rules call for the enhancement of pharmacology education necessary to exercise holistic assessment and clinical judgment skills.
The doctor prescribes according to the standard protocol considering the patient's condition and symptoms. The pharmacist confirms the details prescribed by the doctor and explains the efficacy and side effects of the drug to the patient or gives instruction on general precautions. The nurse's role is to watch the patient, check the effectiveness of the administered drug and adverse events, and report them to the doctor. To gain the expected efficacy from the drug and prevent an unwanted episode, however, the nurse should pay attention to the use of the drug.
In one case, the patient wearing a patch died after using an electric blanket, which caused a high blood concentration. Also, if a patient is dissatisfied with the prescribed medication and has difficulty maintaining medication adherence, the nurse needs to understand the reason why the patient has become unable to take medicine.
In other words, the nurse must be able to manage drug treatment and support medication after comprehensively understanding the patient's body, life, and psychological situation. To that end, the nurse needs to know the drug works, how to manage the treatment effect and safety, and how to use the drug on justified grounds. The author advocates the need for pharmacology education that integrates the knowledge of medicinal drugs and principles of nursing science so that the nurse can figure out the optimal medication method.

1-S03-1 Symposium3

Trans-layer omics analysis, disease biology, and drug discovery

Yukinori Okada

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Statistical genetics is a research field that evaluates causality of human genetic variations on diseases, using statistical and bioinformatics approaches. Recent developments of sequencing technologies have provided human disease genome data of hundreds of thousands of the subjects, and successfully identified comprehensive catalogues of genetic susceptible loci. However, little is known regarding how to develop methodology to integrate large-scale human omics data with diverse biological resources, to which statistical genetics should contribute. We propose trans-layer omics analysis as a key to solve this challenging task. We have developed such methods and applied to a pioneering example of large-scale genetic association studies on a variety of human complex traits, including immune-related diseases. We demonstrated that the disease risk genes were significantly enriched in overlap with the target genes of the drugs currently used for treatment of the diseases, and that network analysis between the disease risk genes and the drug target genes could identify candidates of drug repositioning (e.g. CDK4/6 inhibitors for rheumatoid arthritis). These results should empirically show the value of statistical genetics to dissect disease biology and novel drug discovery.

1-S03-2 Symposium3

Revolutionary therapeutic peptides

Hiroaki Suga

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Macrocyclic peptides possess a number of pharmacological characteristics distinct from other well-established therapeutic molecular classes, resulting in a versatile drug modality with a unique profile of advantages. Macrocyclic peptides are accessible by not only chemical synthesis but also ribosomal synthesis. Particularly, recent inventions of the genetic code reprogramming integrated with an in vitro display format, referred to as RaPID (Random non-standard Peptides Integrated Discovery) system, have enabled us to screen mass libraries (>1 trillion members) of non-standard peptides containing multiple non-proteinogenic amino acids, giving unique properties of peptides distinct from conventional peptides, e.g. greater proteolytic stability, higher affinity (low nM to sub nM dissociation constants similar to antibodies), and superior pharmacokinetics. The field is rapidly growing evidenced by increasing interests from industrial sectors, including small start-ups as well as mega-pharmas, toward drug development efforts on macrocyclic peptides, which has led to several *de novo* discovered peptides entering clinical trials. This lecture discusses the aforementioned screening technology, the RaPID system, and several showcases of therapeutic potentials of macrocyclic peptides.

1-S03-3 Symposium3

Sterile inflammation and inflammasome in cardiovascular medicine: current status and prospects of therapy

Masafumi Takahashi

Div. Inflammation Research, Jichi Medical University

Increasing evidence indicates that NLRP3 inflammasome plays a crucial role in the pathophysiology of cardiovascular diseases associated with sterile inflammation, including atherosclerosis and acute myocardial infarction (MI). NLRP3 inflammasome is a multimeric protein complex that leads to activation of caspase-1, which further induces maturation of interleukin (IL)-1β and IL-18. Activated caspase-1 also induces a particular form of cell death called pyroptosis via the cleavage of gasdermin D. We have shown that inhibition of the NLRP3 inflammasome attenuates the inflammatory response and ameliorates the severity of disease in murine models of cardiovascular diseases, such as atherosclerosis, MI, and aortic aneurysm. Moreover, the recent CANTOS trial showed that inhibition of IL-1β was efficacious in secondary prevention for cardiovascular events in patients with previous MI. These findings suggest that NLRP3 inflammasome may be a potential target for the prevention and therapy of cardiovascular disease. In this session, I summarize the current status of knowledge regarding the role of NLRP3 inflammasome in cardiovascular disease and discuss the prospects of NLRP3 inflammasome-targeted therapy.

Evaluation of emotional behavior characteristics of stress-adaptive and -maladaptive mice

Kazuya Miyagawa, Minoru Tsuji, Kazuhiro Kurokawa, Atsumi Mochida-Saito, Kohei Takahashi, Hiroshi Takeda

Dept. Pharmacol., Sch. Pharm., Int. Univ. Health and Welfare

In order to evaluate the emotional characteristics of mice, it is necessary to create an appropriate model and to adopt a useful behavior experiment device. The automatic hole-board test is an experimental method that can serve as a useful tool for evaluating the changes in various emotional states of mice. Especially, because the treatment with anxiolytics or anxiogenics and exposure to acute restraint stress affect head-dipping behavior, these behavioral changes may reflect the anxiogenic and/or anxiolytic state of mice. In addition, we created stress-adaptive and -maladaptive models in mice. A single exposure to restraint stress for 60 min produced a decrease in head-dipping behaviors of mice in the hole-board test, and these acute emotional responses were recovered by exposure to repeated restraint stress for 60 min/day for 7 or 14 days. However, mice that had been exposed to repeated restraint stress for 240 min/day for 7 or 14 days continued to show a decrease in head-dipping behavior in the hole-board test. Applying these models, we have recently found that prenatal stress exposure induces stress vulnerability and that histone deacetylase inhibitors can develop stress adaptation. In this symposium, we will introduce and discuss our recent findings.

Animal models in addiction research

Soichiro Ide, Kazutaka Ikeda

Addictive Substance Project, TMiMS.

Recently, various addiction problems have spread in the world, and the situation is growing serious. Addiction is a condition that results when individuals ingest an addictive substance or perform a specific action that can be pleasurable but the continuous use or act of which becomes compulsive and interferes with ordinary life responsibilities. It is very important to clarify the mechanisms underlying addiction, but there are still many unclear points. Animal studies have been crucial in understanding the biology and pathophysiology of drug addiction.
In recent years, although self-administration tests and conditioned place preference tests have been widely used as drug-dependence evaluation methods, intracranial self-stimulation (ICSS) method that electrically stimulate the medial forebrain bundle (lateral hypothalamic region) of the mesolimbic dopamine system could be also useful tools that can provide different insights in drug dependency assessment. In this symposium, we would like to discuss the advantage and disadvantage of ICSS method in drug dependence research and reward-system neural network analysis, in light of previous research and recent trends using the ICSS method.

Examples of animal behavior assessment tools developed through academic-industrial collaborations.

Hitoshi Takahashi

MUROMACHI KIKAI CO.,LTD

We, MUROMACHI KIKAI, established in October 1959, are a manufacturer and distributor of animal research apparatus. In addition to our own products, we also sell imported products.
"Animal research apparatus" has a huge variety of types in terms evaluation, methods and etc.
To give some familiar examples, there are anxiolytic evaluation, antidepressant evaluation, and evaluation of antidementia, which is in other words learning and memory. Moreover, there are analgesia, dependence, motor function, regenerative medicine, and…, so there is no end.
A wide variety of evaluation exist. This means that there are considerable types of devices to do the evaluation, over the world. For example, some devices still carry out experimental methods that have been used for more than 30 years.
We have been involved in animal research instruments for a long time, and in the ever-advancing science technology, we are not only dealing with new products, but also continuing instruments that already have a long history, with some minor updates.
Looking back the highlights of our developments, there are several products that have been jointly developed according to the requests and wishes from researchers.
In this symposium, I would like to some examples of industry-academia collaboration in behavioral experiment apparatus such as automatic Hole-board test and Aggression response meter.

Development of animal behavior evaluation method by image analysis

Takahisa Murata, Koji Kobayashi, Seiji Matsushita, Naoyuki Shimizu

Animal Radiology, Tokyo Univ.

Spontaneous movement of the mouse is an important parameter that reflects the health and mental state of the mouse. In the present study, we tried to develop a new method that can measure the spontaneous movement of mice for 24 hours in a normal breeding environment using a simple and versatile video camera. Mice were housed in a standard cage in a 12 hour: 12 hour light / dark environment. The infrared lamp was used during the dark period. Mice were continuously photographed from above using a video camera. The position of the gravity center of the mouse in each frame of the captured video was calculated, and the amount of movement of the gravity center per second was expressed as the amount of exercise. We first confirmed that the momentum of mouse in the dark period was larger than that in the light period as is reported. When caffeine, a central nervous stimulant, was administered to mice, spontaneous motor activity increased until 3 hours after the administration, and then it returned to normal. When chlorpromazine, a sedative was administered to mice, the spontaneous movement of the mice almost disappeared, and the effect continued until 8 hours after the administration. In the present study, we succeeded in establishing a method that can analyze the spontaneous movement of mouse using a versatile video camera in a state close to a normal breeding environment. This system can analyze the spontaneous movement after drug administration.

1-S05-1 Symposium5

Lymphatic vessels in health and disease

Timothy Padera

E.L Steele Laboratories for Tumor Biology, Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA USA

Lymphatic vessels are responsible for draining interstitial fluid from tissues and for transporting immune cells to lymph nodes to maintain the body's immune surveillance. Thus, lymphatics are important in maintaining both tissue fluid balance and proper function of the immune system. Predictably, disruptions of the lymphatic system lead to lymphedema and the conditions for chronic infections. Lymphatic vessels also facilitate the dissemination of cancer cells from a primary tumor to regional lymph nodes. Here we will discuss how normal lymphatic function is controlled during normal physiological conditions using insights from intravital microscopy and mathematical modeling. We will then explore how different pathological settings, including bacterial infections, can disrupt lymphatic pumping. We will show how bacterial infections can cause permanent damage to collecting lymphatic vessels and long-term disruption of lymphatic function.

1-S05-2 Symposium5

Neuropeptide CGRP regulates inflammation by increasing lymphangiogenesis

Kanako Hosono[1,2], Yoshiya Ito[1,2], Hideki Amano[1,2], Masataka Majima[1,2]

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Lymphatic vessels play an essential role in maintaining tissue fluid homeostasis. In inflamed tissue, the lymphatic vasculature undergoes extensive remodeling and expansion, including lymphangiogenesis, which is the formation of new lymphatic vessels. Accumulating evidence suggests that inflammation-associated lymphangiogenesis is not an endpoint phenotype of inflammation, but rather a dynamic and active reaction that regulates resolution of inflammation and tissue repair. Calcitonin gene-related peptide (CGRP) regulates inflammation through signaling for receptor activity-modifying protein (RAMP) 1, a subunit of CGRP receptor. We have demonstrated that RAMP1 signaling in immune cells, is important for suppression of inflammation. In addition, the recruited immune cells in the inflamed tissue produce pro-lymphangiogenic factors, VEGF-C and VEGF-D to increase lymphangiogenesis as indicated by increased LYVE-1$^+$ vessels during healing of wounds and peritoneal inflammation. Indeed, RAMP1 in macrophages promotes skin wound healing and lymphangiogenesis in the granulation tissues. In the peritonitis, RAMP1 in macrophages and T cells facilitates lymphangiogenesis in the diaphragm tissue and enhances peritoneal drainage function of lymphatics. These findings suggest that RAMP1 signaling in immune cells plays a critical role in enhancement of lymphangiogenesis; therefore, a specific agonist for RAMP1 may be a therapeutic option for wound tissue healing or peritoneal inflammation.

Regulation of dermal lymphatic vascular formation by blood vessel-related factors

Masanori Hirashima

Div. Pharmacol., Grad. Sch. Med. Dent. Sci., Niigata Univ.

Development of lymphatic endothelial cells (LECs) and subsequent formation of lymphatic vasculature are highly related to blood vessels. Previous studies indicated that lymphatic vascular patterning is regulated, at least in part, by blood vessel-related factors. LECs are differentiated from venous endothelial cells and migrate adjacent to arteries toward peripheral tissues, such as the skin. Following migration along arteries, LECs should eventually be repelled from blood vessels to form a random lymphatic vascular network. We previously showed that this repulsive migration is regulated by the artery-derived guidance factor Semaphorin 3G. Lymphatic vasculature in adults is separate from blood vessels except for physiological lymph-venous connection sites at the venous angle and is free of blood cells, such as erythrocytes and platelets. Here I will discuss a possible role and molecular mechanism of platelets in partitioning blood and lymphatic vascular compartments by promoting LEC retraction in mouse embryonic skin.

1-S06-1 Symposium6

Neuroprotective properties of the excitatory amino acid carrier 1 (EAAC1).

Koji Aoyama

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Glutathione (GSH) is produced intracellularly from glutamate, cysteine, and glycine. In neurons, cysteine □□uptake is the rate-limiting step of GSH production mediated by excitatory amino acid carrier 1 (EAAC1). The dysfunction of EAAC1 reduces intracellular cysteine □□uptake and suppresses neuronal GSH production, causing neurodegeneration. EAAC1 dysfuction has been reported in the brain of patients with neurodegenerative diseases, suggesting that promoting the function of EAAC1 may lead to suppression of neurodegeneration.

Our studies have suggested that both glutamate transporter–associated protein 3-18 (GTRAP3-18), which is an endoplasmic reticulum protein, and miR-96-5p, which is one of microRNAs, are involved in the regulation of EAAC1 functions. Since GTRAP3-18 directly binds to EAAC1 to suppress the expression of EAAC1 on the cell membrane, suppression of GTRAP3-18 promotes the function of EAAC1. MiR-96-5p acts directly on the 3'-UTR of EAAC1 mRNA and suppresses the expression of EAAC1 protein. Recent results of our studies indicate that treatment of anti-miR-96-5p can promote the function of EAAC1 to increase neuronal GSH levels in vitro and in vivo.

In this symposium, we will focus on neuroprotective effects of EAAC1 by regulating GTRAP3-18 or miR-96-5p.

1-S06-2 Symposium6

The involvement of EAAC1 in diurnal variation of ischemic Zn^{2+} toxicity

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Temporal changes in the severity of ischemic brain injury have been demonstrated, but the mechanism is not fully understood. In the post-ischemic hippocampus, massive amounts of Zn^{2+} accumulates in neurons, resulting in neuron death. On the other hand, excitatory amino acid carrier 1 (EAAC1), a cysteine transporter that plays an important role in GSH synthesis, reduces ischemia-induced hippocampal Zn^{2+} toxicity. Recently, it was reported that EAAC1 protein expression exhibits a diurnal change with a peak in the dark period in mesencephalon. In this symposium, we will introduce the involvement of EAAC1 in diurnal variation of post-ischemic injury. Male C57BL/6 mice were subjected transient global ischemia by clamping bilateral common carotid arteries at 09:00 (ZT4, in the light period) or 23:00 (ZT18, in the dark period), and Zn^{2+} accumulation was assessed by the Zn^{2+}-specific probe, TSQ. Compared to ZT4, the number of TSQ-positive cells were significantly decreased at ZT18. The protein levels of EAAC1 was found to be high at ZT18 than ZT14. Furthermore, pretreatment with TBOA, an EEAC1 inhibitor, increased the number of TSQ-positive cells at ZT18. These findings indicate that ischemia in the dark period reduces Zn^{2+} accumulation, and that this reduction might be mediated by temporal changes of EAAC1 protein expression.

EAAC1 gene deletion reduces adult hippocampal neurogenesis after transient cerebral ischemia

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Several studies have demonstrated that excitatory amino acid carrier-1 (EAAC1) gene deletion exacerbates hippocampal and cortical neuronal death after ischemia. However, presently there are no studies investigating the role of EAAC1 in hippocampal neurogenesis. In this study, we tested the hypothesis that reduced cysteine transport into neurons by EAAC1 knockout negatively affects adult hippocampal neurogenesis under physiological or pathological states. This study used young mice (aged 3–5 months) and aged mice (aged 11–15 months) of either the wild-type (WT) or EAAC1-/- genotype. Ischemia was induced through the occlusion of bilateral common carotid arteries for 30 minutes. Histological analysis was performed at 7 or 30 days after ischemia. We found that both young and aged mice with loss of the EAAC1 displayed unaltered cell proliferation and neuronal differentiation, as compared to age-matched WT mice under ischemia-free conditions. However, neurons generated from EAAC1-/- mice showed poor survival outcomes in both young and aged mice. In addition, deletion of EAAC1 reduced the overall level of neurogenesis, including cell proliferation, differentiation, and survival after ischemia. The present study demonstrates that EAAC1 is important for the survival of newly generated neurons in the adult brain under physiological and pathological conditions. Therefore, this study suggests that EAAC1 plays an essential role in modulating hippocampal neurogenesis.

1-S06-4 Symposium6

Glutathione in astrocytes as a target of neuroprotection

Masato Asanuma, Ikuko Miyazaki

Dept. of Medical Neurobiology, Okayama Univ. Grad. Sch. of Med., Dent. and Pharmaceut. Sci.

Recent studies showed that dysfunction of astrocytes is involved in susceptibility of neuronal cells in several neurological disorders. Glutathione (GSH) is the most abundant intrinsic antioxidant in the central nervous system, and its substrate cysteine is easily oxidized to cystine. Since neurons lack the transport system for cystine, GSH synthesis in neurons is dependent on the cystine up-take via cystine/glutamate exchange transporter (xCT), synthesis and release of GSH in/from surrounding astrocytes. We previously demonstrated that zonisamide and levetiracetam increased the expression of xCT and GSH levels and release of S100b in/from the striatal astrocytes and showed neuroprotective effects against dopaminergic neurodegeneration in parkinsonian models. Also, we studied on neuroprotective properties of astrocytes, and found three target astroglial systems for neuroprotection: (1) cystine transporter xCT-GSH synthesis system, (2) serotonin 5-HT1A receptor-transcription factor Nrf2-strong anti-oxidant zinc-binding protein metallothionein system, and (3) glutamate transporter GLT1 system (Miyazaki and Asanuma, 2016, 2017), and vulnerability of neurons depend on the region-specific profiles of astrocytes. In this symposium, possible neuroprotective strategy targeting antioxidative molecules in astrocytes will be reviewed.

Double-Nanodomain Coupling of P/Q-type Ca^{2+} Channels, Ryanodine Receptors, and BK Channels Controls the Generation of Burst Firing

Tomohiko Irie

Div. Pharm., National Institute of Health Sciences

Action potentials clustered into high-frequency bursts play distinct roles in neural computations. However, little is known about ionic currents that control the duration and probability of these bursts. We found that, in cartwheel inhibitory interneurons of the dorsal cochlear nucleus, the likelihood of bursts and the interval between their spikelets were controlled by Ca^{2+} acting across two Ca^{2+} nanodomains, one between plasma membrane P/Q-type Ca^{2+} channels and endoplasmic reticulum (ER) ryanodine receptors, and another between ryanodine receptors and large conductance, voltage- and Ca^{2+}-activated K^{+} (BK) channels. Each spike triggered Ca^{2+}-induced Ca^{2+} release from the ER immediately beneath somatic, but not axonal or dendritic, plasma membrane. Moreover, immunolabeling demonstrated close apposition of ryanodine receptors and BK channels. Double-nanodomain coupling between somatic plasma membrane and hypolemmal ER cisterns provides a unique mechanism for rapid control of action potentials on the millisecond timescale.

Multiscale Ca^{2+} imaging for brain function analysis

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Central nervous system has a hierarchical organization from neurons to huge complex network, and each hierarchy can process and can hold the information. However, analysis of the neuronal signal from every hierarchy of the brain is not easy. To resolve this issue, we are developing the multiscale Ca^{2+} imaging methods that enable the multi-scale analysis of the brain functions. For in vivo whole brain activity analysis, we selected the quantitative activation-induced manganese-enhanced MRI (qAIM-MRI) method. qAIM-MRI is based on the use of Mn^{2+} as a surrogate marker of Ca^{2+} influx. Mn^{2+} shortens the longitudinal relaxation time (T_1) of H^+. Therefore, qAIM-MRI can measure the history of the neuronal activities non-invasively. For in vivo local circuit imaging with single cell resolution, we have developed ultra-thin florescence endoscope imaging system. This endoscope can record the multicellular neuronal activities from deep brain region. To reveal the cellular and molecular mechanisms for exhibiting brain function, in vitro experiments is needed. Thus, we conduct fluorescent imaging study on the brain slice preparations. These three imaging methods can be applied to the same individual and can be combined with behavioral and biochemical studies. At present, we apply individual techniques of multiscale imaging to the some model mice. I will show the concept of the multi-scale imaging and some data at the conference.

1-S07-3 Symposium7

Regulation of calcium signal pathway in fear-related memory

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Na^+/Ca^{2+}exchangers (NCXs) are mainly expressed in the plasma membrane and exchange one $Ca^{2+}$for three Na^+, depending on the electrochemical gradients across the plasma membrane. NCXs have three isoforms, NCX1–3, encoded by distinct genes in mammals. Here, we report that heterozygous mice lacking NCX1 ($NCX1^{+/-}$) exhibit impaired amygdala-dependent cued fear memory. $NCX1^{+/-}$mice showed significant impairment in fear-related behaviors measured with the elevated-plus maze, light-dark, open-field, and marble-burying tasks. In addition, $NCX1^{+/-}$mice showed abnormality in cued fear memory but not in contextual fear memory in a fear-conditioning task. In immunohistochemical analyses, $NCX1^{+/-}$mice had significantly increased number of c-Fos positive cells in the lateral amygdala (LA) but not in the central amygdala following fear-related tone stimuli. c-Fos expression peaked at 1 h. In concordance with the aberrant fear-related behaviors in $NCX1^{+/-}$mice, enhanced long-term potentiation was also observed in the LA of these mice. Furthermore, enhancement of CaMKII or CaMKIV activity in the LA was observed in $NCX1^{+/-}$mice by immunoblot analyses. In contrast, $CaMKII^{+/-}$but not $CaMKIV^{-/-}$mice insufficiently exhibited tone-induced cued fear memory and there was no increase in the number of c-Fos positive cells in the LA. Altogether, the increased CaMKII activity and consequent c-Fos expression likely account for the dysregulation of amygdala-dependent cued fear memory in $NCX1^{+/-}$mice.

Involvement of Redox Modification of Ca^{2+}-Release Channels in Cerebellar Learning and Aging

Sho Kakizawa

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Ca^{2+} release from intracellular store, as well as Ca^{2+} influx from extracellular fluid, contributes elevation of cytosolic Ca^{2+} revels. However, regulatory mechanisms of Ca^{2+}-release channels and their functional roles in relation to learning and memory have not been fully understood. Recently, accumulating data suggest that redox molecules such as reactive oxygen species (ROS) and nitric oxide (NO) act as signaling molecules through redox modification of proteins, although these molecules have been considered as damaging oxidizers of the macromolecules, for a long time. In this symposium, I will introduce our current studies indicating involvement of redox modulation of type 1 ryanodine receptor (RyR1) in regulation of brain functions.

Novel Ca^{2+} release mechanism, NO-induced Ca^{2+} release (NICR), was recently demonstrated in cerebellar Purkinje cells. S-nitrosylation of thiol group in RyR1 was revealed to be essential for NICR. Furthermore, using knock-in mice defect in NICR, involvements of NICR in cerebellar long-term potentiation (LTP) and motor learning were indicated. On the other hand, because thiol group is also the target of ROS, effects of ROS on cerebellar synaptic plasticity through redox modification of RyR1 are also expected. Involvements of ROS in inhibition of cerebellar LTP and induction of cerebellar long-term depression will be also discussed.

1-S08-1 Symposium8

Projection-identified large-scale recording reveals pathway-specific information outflow from the subiculum

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The hippocampus processes multimodal information, including place, time, speed, reward, and memory. However, how such information is distributed to multiple downstream areas remains poorly understood. The subiculum is the major hippocampal output structure that receives the hippocampal CA1 output and projects to multiple cortical/subcortical areas. Despite its anatomical importance, the nature of information distribution from the subiculum is unknown. We investigated this issue by optogenetically identifying the projection targets of subicular neurons during large-scale extracellular recordings in freely behaving rats. We introduced channelrhodopsin-2 and a 256-channel silicon probe into the subiculum, and implanted four optical fibers above subicular projection targets. Then, axonal projections of the recorded subicular neurons were determined by detecting antidromic spikes generated by blue-light irradiation to the projection targets. During multiple behavioral tasks and sleep, the subicular projection neurons were entrained to neural oscillations differentially and conveyed distinct information depending on the projection targets. These results suggest the prominent role of the subiculum in distributing hippocampal information to multiple downstream targets in a pathway-specific manner.

A novel ligand selectively visualizes and activates chemogenetic receptors in non-human primates

Yuji Nagai

Dept. Functional Brain Imaging, NIRS, QST

A chemogenetic technology, Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) affords a means to temporally and remotely control the activity of a target neural population expressing a "designer receptor" by an agonist compound. The combination muscarinic-based DREADDs, hM_3Dq (excitatory) and hM_4Di (inhibitory), and biologically inert compound, clozapine-N-oxide (CNO) has been successfully applied in a variety of *in vitro* and *in vivo* contexts, extending to non-human primate studies to modify behavior. For the application of DREADDs to non-human primates, it is desirable to monitor the DREADD expression *in vivo* because in non-human primates, 1) viral vector-based transgene delivery into target neurons has not been fully established, and 2) the behavioral study is performed for relatively the long-term. In addition, CNO has poor brain permeability and can be metabolized to clozapine, which has potential for causing unwanted off-target actions. We solved these issues by developing a novel ligand, deschloroclozapine (DCZ), which serves a dual purpose in chemogenetics: (1) as a selective compound for visualization of DREADD expression *in vivo* by positron emission tomography (PET) imaging and (2) as a selective agonist for muscarinic-based DREADDs. In this talk, I will introduce the property of DCZ and the role of PET imaging in neuroscience research using non-human primates.

Novel optogenetic and chemogenetic tools for understanding of molecular mechanisms which underlie learning and memory.

Wataru Kakegaea, Michisuke Yuzaki

Dept. Physiol., Keio Univ. Sch. of Med.

In our brain, approximately 100 billion neurons connect to each other through "synapses" to make neuronal circuits essential for higher brain functions, such as learning & memory. So, elucidation of molecular mechanisms which underlie synapse formation and functions is one of the important issues in understanding learning & memory (Kakegawa and Yuzaki, *BRAIN & NERVE*, 2018). However, because of a complex environment in the brain, technologies that can selectively intervene at specific synapses are still developing.

Recently, we studied on cerebellar neuronal circuits essential for motor coordination and motor learning, and we developed novel optogenetic and chemogenetic tools to regulate the localization and functions of glutamate receptors, which are key players for synaptic transmission and plasticity (Kiyonaka et al., *Nat Chemistry*, 2016; Kakegawa et al., *Neuron*, 2018). In particular, a novel optogenetic tool, PhotonSABER, controlled not only synaptic plasticity but also cerebellum-dependent motor learning in a light-dependent manner (Kakegawa et al., *Neuron*, 2018). These novel tools are used as a powerful tool that can artificially manipulate the functions of specific synapses in the brain. Using these technologies, we will know the causal relationship between synaptic functions and the behaviors in more detail.

CRMP2 Binding Compound Accelerates Recovery from Central Nervous System damage

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Damage to the central nervous system (CNS) causes severe neurological condition, such as sustained sensory, motor, cognitive dysfunction and compromise work capacity and self-care. No pharmacological intervention that could foster recovery and complement current rehabilitation has yet been established as effective. Restoration of motor impairment after CNS damage is considered to be the result of compensative neural plasticity in spared neural circuit, and the Experience-dependent synaptic AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic-acid) receptor (AMPAR) delivery underlies behaviors that require neural plasticity such as learning. We found that a small compound, edonerpic-maleate (also known as T-817MA), facilitated experience-driven synaptic glutamate AMPA receptor delivery and resulted in the acceleration of motor function recovery after cortical or spinal cord cryoinjury. Edonerpic bound to collapsin-response-mediator-protein 2 (CRMP2), a downstream molecule of semaphorin, and is thought to be related to synaptic plasticity and learning. Furthermore, we detected CRMP2-dependent activation of ADF/cofilin by edonerpic maleate in the plasticity-inducing condition. Indicating edonerpic could facilitate synaptic AMPAR delivery through the regulation of actin dynamics. Thus, edonerpic-maleate, a neural plasticity enhancer, could be a clinically potent small compound to accelerate rehabilitation after damage of CNS.

1-S09-1 Symposium9

Toward the creation of a researcher database and succession of research techniques: From the first questionnaire survey

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Among the goals set by the Japanese Pharmacological Society, "Dissemination of knowledge about pharmacological science and applied research" is mentioned, and <new drug development> is positioned as one of the goals. However, remarkable progress has been made in cell and molecular biology research methods among pharmacological research methods, while isolated organ systems (extracted organs and tissue specimens) and in vivo animal models (pathological models, etc.) This has led to a situation in which research methods using scientists are shunned, leaving the evaluation system as the exit for preclinical research. This phenomenon is the same not only in Japan but also in the United States and Europe, and the International Union of Pharmacology (IUPHAR) has made a proposal for the development of researchers who use isolated organ systems and in vivo animal models. .

Based on this, the Pharmacological Society of Japan also created a database of researchers with research techniques using isolated organ systems and in vivo animal models, and collaborated with IUPHAR to pass on valuable research techniques and future Aimed at the advancement of pharmacology, the first questionnaire was conducted for academic councilors. This time, we will disclose the results and reflect the results in the second questionnaire for all members of the university.

1-S09-2 Symposium9

Pharmacological training with small animals in Kochi University

Motoaki Saito

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The use of animals for scientific purposes is still a subject of debate. The current regulatory position in most western countries allows regulated animal use to occur because of the perceived benefits in generating new knowledge.
The use of animals for scientific purposes engenders a wide range of ethical perspectives, with some people looking for the complete termination of animal use, and others strongly support their continued use. Although regulatory systems vary from country to country, in most jurisdictions, research and teaching institutions are required to ensure that staff and students using animals for scientific purposes are appropriately trained, that animals are well cared for, and that the ethical review process for projects is robust.
In the curriculum of the Kochi Medical School, it is mandatory for all medical students to perform laboratory exercises in the class of Pharmacology. For the purpose of this experimental class it is common to use small animals in these exercises. However, in recent years in many countries, alternative methods to replace the use of small animals have been introduced. Such methods are experiment simulations with the use of computers and they have been used in some medical schools. In this symposium, I will make an introduction on how we perform pharmacological laboratory exercises with the use of small animals in Kochi Medical School. Additionally, I would like to discuss with the audience, the necessity of the use of small animals in exercises as part of the training of medical students.

Efforts by The Japanese Association for Experimental Animal Technologists (JAEAT) : Scheduled technical training courses and their future development

Norio Yata[1,2]

[1]Advanced Science Research Center, Okayama Univ., [2]Director of The Japanese Association for Experimental Animal Technologists (JAEAT)

We, laboratory animal technicians, are always the closest to laboratory animals. We always have practiced breeding management, pre- and post-operative care, experimental treatment etc. by making the best use of our knowledge and techniques. That bring accurate experimental results. It is our significance.

"Skill" is individual and the only way to earn it is by training. On the other hand, "technique" is objective and can be transmitted by words and letters. Our goal is not to acquire skill called "SHOKUNINWAZA" in Japanese, but it is to make it universal as an objective technique that anyone can practice. The training course plays the important role to attain our goal.

The Japanese Association for Experimental Animal Technologists (JAEAT) was founded in 1966. We organize annual meeting, lecture and technical training course. We, JAEAT KANSAI branch, actively hold the training courses 4 times a year. In every training, one-on-one instruction is highly esteemed.

We created DVD video for basic experimental techniques. And graphical chart was published 2 years ago. Which were laminated, to bring in our bloody workspace. We can use them anytime, anywhere.

Technology is only meaningful if passed down to the next generation. Even failure is a valuable experience there. Whether it worked or not, everything is not a personal experience. From there we take out the universal contents and pass on them to the next generation based on animal welfare. It is our mission and we have no doubt.

In the presentation, I introduce our efforts to pass on the laboratory animal technique to next generation.

1-S10-1 Symposium10

Drug Discovery for Genetic Diseases Caused by Aberrant mRNA Splicing

Masatoshi Hagiwara

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Patients of congenital diseases have abnormalities in their chromosomes and/or genes. Therefore, it has been considered that drug treatments can serve to do little for these patients more than to patch over each symptom temporarily when it arises. Although we cannot normalize their chromosomes and genes with chemical drugs, we may be able to manipulate the amounts and patterns of mRNAs transcribed from patients DNAs with small chemicals. Based on this simple idea, we have looked for chemical compounds which can be applicable for human diseases targeting kinase families of CDKs, CLKs and DYRKs which are involved in the regulation of gene expression, and eventually succeeded to find FIT039 (1), TG003 (2), and ALGERNON (3) as potential therapeutic drugs to cure diseases such as viral infections, Duchenne muscular dystrophy, and Down syndrome, respectively. In addition, we established splicing reporter assay with dual color (SPREADD) using a segment of pathogenic genes, and found a splicing modulator, RECTAS (4), which can rectify the aberrant IKBKAP splicing in patient iPS cells of Familial dysautonomia. EDA-ID (anhidrotic ectodermal dysplasia with immunodeficiency), cardiac Fabry diseas**,** and type V cystic fibrosis are often induced by pseudo-exon recognition, and our chemical therapeutics can normalize the splicing patterns (5).

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Regulation of higher order structures and function of RNA by RNA-binding small molecules

Kazuhiko Nakatani

ISIR, Osaka Univ.

When we learned central dogma many years ago, RNA was just a blueprint of proteins. However, nowadays, RNAs are recognized as functional molecules to participate in several important biological reactions. ENCODE (the encyclopedia of DNA elements) project revealed that less than 3% of our human genome was eventually translated into proteins, whereas 76% was transcribed into RNA but not translated into proteins. These non-coding RNAs have been appointed to the crucial targets for future drug discovery. MicroRNAs (miRNAs) are typical examples of those functional non-coding RNAs and toxic RNA having long repeat sequences sequestered RNA-binding proteins in the nucleus, which led to the dysfunction of the proteins. In the presentation, our recent studies focused on the modulation of the maturation process of microRNA and the targeting toxic RNAs related to neurological disorders by our RNA-binding small ligand will be discussed. These studies showed that small molecules have much potential to modulate the structure and function of biologically relevant RNAs.

1-S10-3 Symposium10

Pyrrole-Imidazole Polyamides as artificial genetic switches

Hiroshi Sugiyama

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We have been undertaking original research on the molecular recognition of DNA by antitumor antibiotics, and the analysis of atom-specific chemical reaction on DNA. By reconstituting such knowledge, various functionalized sequence-specific DNA binding pyrrole-imidazole polyamides (PIPs) were synthesized as an artificial genetic switch, which can switch on and switch off the gene expression on demand. We recently developed alkylating PIP that could switches off cancer related KRAS gene and RUNX 1-3 controling genes. To switch on the gene expression we need to consider Epigenetics. We developed a SAHA-PIP containing sequence-specific pyrrole-imidazole polyamides (PIPs) and HDAC inhibiting SAHA. Evaluation of the effect of SAHA-PIPs on genome-wide gene expression in human dermal fibroblasts (HDFs) divulged that each SAHA-PIP could differentially activate the therapeutically important genes. Conjugation of DNA binding domain of 'I' with HAT activating CTB remarkably activated identical cluster of genes as SAHA-PIP 'I' to substantiate the role of PIP in sequence-specific gene regulation. Recently we introduced Bromodomain inhibitor to PIP to activate gene expression in sequence-specific manner. To extend recognition sequence, we introduce host-guest system to facilitate cooperative binding to target sequence. In this talk recent progress of regulation of the gene expression using designed PIPs will be discussed.

From the structure analysis of RNA to the identification of novel drug targets

Gota Kawai

Dept. Life Science, Chiba Inst. Tech.

It is well known that local structures in long RNAs, such as mRNAs, viral genomic RNAs and long non-coding RNAs, are required for their functions. We are analyzing interactions between small molecules and RNAs by the NMR spectroscopy to confirm that RNA local structures can be the target of small molecule drug. For example, a small molecule BzDANP having a three-ring benzo[c][1,8]naphthyridine system can bind to an RNA stem having C-bulge and inhibit the pre-miRNA-136 processing (Bioorg Med Chem. 27, 2140-2148, 2019). NMR is quite useful for the interaction analysis between small molecules and RNAs. Imino proton resonances can be observed selectively typically in 15-12 ppm where no other signals are observed. Some examples for the interaction analysis as well as the structure determination by NMR will be shown.

To identify the structured region in long RNAs, we improved the RNA secondary structure prediction method (http://www.rna.it-chiba.ac.jp/vsfold5/) and developed a system to visualize the possible structured regions in long RNAs (http://www.rna.it-chiba.ac.jp/～vswindow/). These systems can be used to find the drug target in long RNAs. Now, it is time to start the long RNA target small molecule drug discovery.

1-S12 Symposium12

Toward clinical application of torpor: active hypometabolism research in mice

Genshiro Sunagawa

BDR, RIKEN

Some mammals enter a hypometabolic state either daily torpor (minutes to hours in length) or hibernation (days to weeks), when reducing metabolism would benefit survival. The metabolic rate drops to 1～30% of normal rates, and animals sacrifice their vital biological functions such as consciousness and mobility to save metabolism, which makes them look offline. The mechanisms for such hypothermia-resistance and hypometabolism-resistance is not understood.

Hibernators demonstrate deep torpor by reducing both the sensitivity (H) and the theoretical set-point temperature (T_R) of the thermogenesis system, resulting in extreme hypothermia close to ambient temperature (T_A). We have developed a stable torpor induction method for mice and evaluated minimal body temperature (T_B) and oxygen consumption rate (VO_2) of fasting-induced torpor in C57BL/6J mice (B6J) under various T_As. As in hibernators, H decreased 91.5% during daily torpor while T_R only decreased 3.79 ° C in mice (Sunagawa GA and Takahashi M, *Sci Rep*, 2016). Furthermore, we have found that C57BL/6N (B6N) has a higher metabolic rate during torpor than B6J (GA Sunagawa, 2018, *BioRxiv* 374975).

Interestingly, in both B6J and B6N mice strains, H is decreased as hibernators, but T_R remains relatively unchanged during daily torpor. To investigate whether the stable T_R during torpor is a common feature in mice, we have evaluated various inbred strains and found that in some strains, T_R may be reduced than B6J or B6N mice. Because T_R is suspected to be controlled centrally in mammals, we are attempting to control T_R by stimulating the central nervous system.

1-S12-1 Symposium12

SIK family regulates sleep/wakefulness in mice

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Sleep is a behavior conserved from invertebrates to vertebrates, and tightly regulated in a homeostatic manner. The molecular and cellular mechanism determining the amount of sleep remains unknown. We established Sleepy mutant pedigrees through EEG/EMG-based screening of randomly mutagenized mice. The causative mutation for Sleepy mutant was a splicing mutation of Sik3 gene. The mutant SIK3 protein lacks a region containing a well-conserved protein kinase A-phosphorylated site, S551. We further investigated the molecular basis of sleep need using quantitative phosphoproteomic analysis of the sleep-deprived and Sik3 mutant mouse. The brain proteome of Sik3 mutant mice exhibits hyperphosphorylation, similar to that seen in that of sleep-deprived mice. The substitution of S551 into alanine residue resulted in a decreased time spent in wakefulness as original Sleepy mutant mice. I will present several on-going projects.

1-S12-2 Symposium12

CaMKII-dependent control of sleep duration in mammals

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The sleep-wake cycle is regulated through circadian clocks that drive the near-24 hrs rhythmicity of many physiological processes and sleep homeostasis that determines the sleep duration required per day. The discovery of core circadian clock components was initiated by the finding of *period* gene that controls the clock speed bidirectionally. On the other hand, it is still unknown whether there is a single gene that controls sleep duration bidirectionally. We focused on the role of Ca2+/calmodulin-dependent kinase II (CaMKII) in the regulation of sleep duration. We reported this enzyme as a sleep-promoting kinase by showing that the knock-out of *Camk2a* or *Camk2b* results in the significant reduction of sleep duration in mice (Tatsuki et al. Neuron 2016). CaMKII α/β dodecamer is activated by Ca2+/CaM and undergoes a large scale conformational change. The conformational change exposing the kinase domain affects the protein-protein interaction between CaMKII α/β and other binding partners. The exposure of the kinase domain also triggers the auto-phosphorylation that stimulates or suppresses the kinase activity of CaMKII α/β, depending on the phosphorylation sites. In this presentation, we will introduce our recent study aiming to understand what biochemical property of CaMKII α/β is most prominent to control the sleep duration and to create a gain-of-function mutant of CaMKII α/β that can, contrary to the phenotype of *Camk2a/b* knock-out, lengthen the sleep duration.

1-S12-3 Symposium12

Small molecule modulators of the circadian clock function

Tsuyoshi Hirota

ITbM, Nagoya Univ.

In mammals, circadian rhythms are generated through transcriptional regulatory networks of the clock genes. To search for novel clock modifiers, we applied chemical biology approaches. From hundreds of thousands of small molecules with diverse structure, we identified a number of compounds that potently change the period of the circadian clock in human cells. Among the period lengthening compounds, we previously discovered the first small molecule targeting the core clock protein CRY. The compound KL001 interacts with FAD-binding pocket of CRY and inhibits FBXL3-dependent degradation. By analyzing KL001 derivatives, we found 10 times more potent compound KL044. KL001 and KL044 share carbazole group and act on both CRY1 and CRY2. We further identified novel period lengthening compounds KL101 and TH301 that do not have carbazole group. Surprisingly, we discovered that KL101 is selective against CRY1 while TH301 shows much higher effect on CRY2. To understand molecular basis of the CRY1/CRY2 selectivity, we determined the X-ray crystal structures of CRY1-KL101, CRY1-TH301, CRY2-TH301, and CRY1-KL044 complexes. In this presentation, I will discuss these unique compounds that will enable atomic-level dissection of the functional difference between CRY1 and CRY2 proteins and their selective manipulation.

1-S13-1 Symposium13

Novel therapeutic approach mediated by microglia for rare brain diseases

Takeshi Ikeuchi

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Microglia are derived from primitive macrophages in the yolk sac and maintained by local precursors that colonize in brain independently from circulating mononuclear cells. Micrglia are critical effectors and regulators of changes in CNS homeostasis during development and in healthy and pathological conditions. New evidence has emerged that primary microglial dysfunction substantially contribute to the pathogenesis of the rare neurological diseases. This condition is now recognized as primary microgliopathy. Adult-onset leukoencephalopathy with spheroids and pigmented glia (ALSP) is an example of human primary microgliopathy. The causative gene for ASLP is *colony stimulating factor-1 receptor* (*CSF1R*), which is predominantly expressed in microglia. We previously showed that ALSP is caused by haploinsufficiency of CSF1R or loss of CSF1R-mediated signaling. The neuropathological examination revealed that density and number of microglia decreased in autopsied brain of ASLP. Moreover, individual microglia in ALSP brain demonstrated their characteristic morphology with thin processes and many knot-like structures. These findings have suggested that microglia dysfunction associated with loss of CSF1R signaling play an essential role in the pathogenesis of ALSP. Recently, bi-allelic mutations in *CSF1R* cause childhood-onset leukoencephalopathy that can be associated with a skeletal dysplasia. Almost complete loss of microglia was observed in brain of the patients for bi-allelic CSF1R mutation. Recently, hematopoietic stem cell transplantation (HSCT) was performed in three patients with ALSP, which provided a stabilization of disease progression. These findings raise the possibility that diseases caused by genetic defects in the microglial pathway as well as the other types of leukodencephalopathies could be benefically treated by HSCT. The microglia replacement in CNS may be facilitated by HSCT; however, this issue warrants further investigations.

Glial cells and pharmacological targets in Sandhoff disease

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Sandhoff disease (SD) is a genetic disorder caused by a mutation in the beta-hexosaminidase B (*HEXB*) gene in humans. This results in the massive accumulation of GM2 gangliosides in the nervous system, causing progressive neurodegeneration. The symptoms of SD include muscle weakness, seizures, and mental illness; along with loss of muscle coordination, vision, and hearing. In the most severe form, the onset begins during early infancy, and death usually occurs within 2-5 years of age. The established animal model, *Hexb*-deficient ($Hexb^{-/-}$) mouse, shows abnormalities that resemble the severe phenotype found in human infants. We have previously reported that activated microglia causes astrogliosis in $Hexb^{-/-}$ mouse at the early stage of development that can be ameliorated via immunosuppression. Moreover, within the cerebral cortices of $Hexb^{-/-}$ mouse, reactive astrocytes were found to express adenosine A_{2A} receptors in later inflammatory phases. Inhibiting this receptor with istradefylline decreases the number of activated microglial cells and inflammatory cytokines/chemokines. Thus, we underline the importance of the astrocytic A_{2A} receptor as a sensor, in regulating microglial activation in the late phase of inflammation.

1-S13-3 Symposium13

Pathological role of astrocytes in neurodegeneration of multiple sclerosis

Mika Takarada-Iemata, Thuong Manh Le, Nahoko Okitani, Osamu Hori

Dept. Neuroanat., Grad. Sch. Med. Sci., Kanazawa Univ.

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS). MS is characterized by extensive immune cell infiltration leading to inflammation, demyelination, and neurodegeneration. Recently, accumulating evidence has suggested that glial cells may contribute to the development of MS pathology. However, the molecular mechanism underlying the regulation of neuronal degeneration in MS remains largely unknown. N-myc downstream-regulated gene 2 (NDRG2) is a differentiation- and stress-associated molecule, and predominantly expressed in astrocytes in the CNS. In this study, we examined the relevance of NDRG2 in experimental autoimmune encephalomyelitis (EAE), a mouse model of MS. The expression of NDRG2 was enhanced in the acute and chronic phase after induction of EAE. Genetic deletion of NDRG2 ameliorated the clinical course and demyelination after EAE induction. Although the loss of NDRG2 slightly affected the inflammatory response, it significantly reduced neurodegeneration both in the acute and chronic phase. Further analysis revealed that deletion of NDRG2 restored the EAE-related decreases in the expression of astrocytic glutamate transporters. Thus, our findings suggest that NDRG2, expressed in astrocytes, may play a key role in the pathology of MS by modulating neuronal vulnerability to glutamate toxicity.

Research on the molecular pathogenesis of "primary astrocyte disorder" Alexander disease.

Kozo Saito, Eiji Shigetomi, Schuichi Koizumi

Dept. Neuropharmacol., Interdiscipl. Grad. Sch. Med., Univ. Yamanashi

Alexander disease (AxD) is a rare neurodegenerative disorder caused by the mutations in glial fibrillary acidic protein (GFAP) gene. AxD is classified clinically into cerebral type and bulbospinal type, based on neurological symptoms and brain MRI findings. Rosenthal fiber formations in astrocytes are the pathological hallmarks of AxD. Astrocyte dysfunction in the AxD brain is considered to be involved in the pathogenesis, which is poorly understood. Here, we show aberrant Ca^{2+} responses in astrocytes as playing a causative role in AxD. Transcriptome analysis of astrocytes from a model of AxD showed age-dependent upregulation of GFAP, several markers for neurotoxic reactive astrocytes, and downregulation of Ca^{2+} homeostasis molecules. *In situ* AxD model astrocytes produced aberrant extra-large Ca^{2+} signals (> 300 um^2), "AxCa signals", which increased with age, correlated with GFAP upregulation, and were dependent on stored Ca^{2+}. Inhibition of AxCa signals by deletion of inositol 1,4,5-trisphosphate type 2 receptors decreased the expression levels of GFAP and other reactive astrocyte molecules. Taken together, AxCa signals in the model astrocytes would be a cause of AxD pathogenesis.

Our serendipitous encounter with CICR

Makoto Endo

Dept. Pharmacol., Univ. of Tokyo

In 1967, Dr. Tanaka and I were working on the effect of caffeine on SR in skinned fibers. In the presence of 50 μM EGTA, 0.2 mM caffeine induced a large transient contraction in the skinned fiber after a long latent period. To our surprise, similar contractions spontaneously recurred at intervals of minutes. The falling phase of the transient contraction must have been caused by removal of Ca^{2+} from the fiber space due to diffusion and binding to EGTA. Ca^{2+} would have then been taken up again by the SR with its strong Ca^{2+} pump forming the basis of the repeated contraction. However, a question remained. The peak tension of the repeated contractions was close to the maximum tension of the same fiber, which indicated that Ca^{2+} was released along the entire length of the fiber. In the first contraction the whole fiber was exposed to caffeine simultaneously. However, after many minutes Ca^{2+} release would still occur again along the entire length of the fiber. This suggested propagation of the release of Ca^{2+} through the entire length of the fiber. We believed that some consequence of Ca^{2+} release must induce further Ca^{2+} release to form a positive feedback loop. All the results of contraction, mechanical stress or increases in the concentration of ADP or Pi could not induce Ca^{2+} release. As a result, we finally found out that Ca^{2+} itself can induce further release of Ca^{2+}.

Analysis of CICR control mechanism using molecular dynamics simulation and malignant hyperthermia model mouse

Toshiko Yamazawa

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Mutations in type 1 ryanodine receptor (*RyR1*) gene cause severe muscle diseases, such as malignant hyperthermia (MH), which is a disorder of Ca^{2+}-induced Ca^{2+} release via RyR1 in the skeletal muscle. We combined functional studies and molecular dynamics (MD) simulations of RyR1 bearing disease-associated mutations at the N-terminal region. When expressed in HEK293 cells, the mutant RyR1 caused abnormalities in Ca^{2+} homeostasis. MD simulation of the mutant RyR1 revealed that alterations of hydrogen bonds/salt bridges between N-terminal domains (NTD), consisting of A, B and C domains, strongly correlate with the channel function of RyR1. Next, we tested therapeutic effects of RyR1 inhibitor on MH model mice carrying mutation in the *RYR1* gene. RyR1 inhibitor suppressed caffeine-induced contraction in skeletal muscle from heterozygous MH mice. The heterozygous mice died with an increased body temperature when they were anesthetized by isoflurane. Pre-administration of RyR1 inhibitor completely prevented rise in the body temperature and death. In addition, RyR1 inhibitor rescued the mice after they developed MH episodes. These results suggest that RyR1 mutant mice will be promising model mice for MH pathogenesis and screening of new drugs.

Abnormal RyR2 in arrhythmogenic disorders and CICR

Nagomi Kurebayashi

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Ca^{2+} induced Ca^{2+} release (CICR) via ryanodine receptor 2 (RyR2) plays a central role in E-C coupling in cardiac cells, i.e., Ca^{2+} influx via L-type Ca^{2+} channels during action potential (AP) activates RyR2 to release Ca^{2+} from the ER and causes muscle contractions. Many arrhythmogenic mutations in RyR2 are reported to increase AP-independent spontaneous Ca^{2+} release from ER that often lead to arrhythmia. Two explanations have been proposed for the increased propensity of spontaneous Ca^{2+} release: (1) CPVT mutations increase the cytoplasmic Ca^{2+} sensitivity of RyR2 to enhance CICR, or (2) mutations decrease threshold for store-overload induced Ca^{2+} release (SOICR) by reducing luminal Ca^{2+} sensitivity of RyR2. To understand the underlying mechanism for the increased spontaneous Ca^{2+} release by the mutations, we performed quantitative evaluation of CICR activity and cytoplasmic and ER Ca^{2+} signals in HEK293 cells expressing mutant RyR2s. Furthermore, the effects of RyR2 inhibitors, which had been found by recently established high-throughput screening method, were examined on Ca^{2+} signals in RyR2-HEK cells and cardiomyocytes from adult mice. Our results indicate that CICR plays critical role in generation of spontaneous Ca^{2+} release and that regulation of CICR is important in suppression of arrhythmia.

Structural insight of Ca^{2+} induced Ca^{2+} release mechanism revealed by cryo-EM

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Ryanodine receptor (RyR) is a Ca^{2+} release channel in the sarcoplasmic reticulum of skeletal and cardiac muscles and plays a key role in excitation-contraction coupling. It is widely known that the cardiac specific isoform of the receptor (RyR2) mediates Ca^{2+} induced Ca^{2+} release (CICR) that opens the channel by binding of Ca^{2+} to the RyR2. Although number of structures of the RyR1 and RyR2 have already been determined by cryo-EM with near atomic resolution, there is no reported open structure just with bound Ca^{2+}, and most structures in the open state were created by adding extra molecules such as caffeine and ATP in addition to Ca^{2+}. Therefore, the mechanism of CICR is still largely unknown. Here, we have successfully obtained high-resolution cryo-EM structure of recombinant RyR2 in the open state just with bound Ca^{2+}. By comparing with structure in the closed state, we are finally able to discuss the mechanism of CICR clearly. The results of functional studies with numerous mutant RyR2 channels strongly support our mechanism.

1-S15-1 Symposium15

GPCRs for ketone bodies and energy homeostasis

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Ketone bodies such as β-hydroxybutyrate and acetoacetate are important alternative energy sources under nutrient deprivation. Ketone bodies are produced in liver during starvation, exercise, type I diabetes or feeding low-carbohydrate, medium-chain triglyceride diets, and have been shown to affect cellular signaling including the activity of histone deacetylases (HDACs) and G-protein coupled receptors (GPCRs). For example, β-hydroxybutyrate also acts as a signaling molecule via specific GPCRs such as GPR109A and GPR41 which are expressed in various tissues and involved in a variety of metabolic processes. However, the specific GPCRs for acetoacetate and its physiological functions remain unclear. Here, we identified acetoacetate as an endogenous agonist for a specific GPCR by ligand screening in heterologous expression system. Acetoacetate-GPCR signaling maintains energy homeostasis through lipid metabolism under ketogenic conditions. These observations provide insight into the role of ketone bodies in energy metabolism and highlight their therapeutic potential for ketogenic metabolic disorders.

1-S15-2 Symposium15

Physiological functions of GPCRs sensing Long-chain Free Fatty Acids

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A strategy to deorphanize G-protein-coupled receptors (GPCRs) identified a series of receptors, which are activated by Free Fatty Acids (FFAs). Hence, FFAs are now recognized as not only essential nutritional components but also signaling molecules in various physiological processes. A number of previous studies showed that GPCRs sensing FFAs play significant roles in nutritional regulation. In this free fatty acid receptor family, FFAR1 (GPR40) and FFAR4 (GPR120) are activated by long-chain FFAs. FFAR1 regulates insulin secretion in pancreatic beta-cells. FFAR4 promotes the secretion of glucagon-like peptide-1 (GLP-1) in the intestine, mediates anti-inflammatory effects of docosahexaenoic acid in macrophages and acts as a lipid sensor in adipose tissue to sense dietary fat and control systemic energy homeostasis. Furthermore, we recently found novel roles of these fatty acid receptors in the relationship between gut microbiota and host energy metabolism via the metabolites of dietary fatty acids. In this symposium, I will introduce recent advances in the physiological roles of FFAR1 and FFAR4, and I further present a summary of current understandings of their pharmacological characterization and their potential as drug targets.

1-S15-3 Symposium15

Regulation of physiological lipid homeostasis by prostaglandin receptor

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Adipose tissue is important not only for energy storage but also as an endocrine organ that regulates energy homeostasis and insulin sensitivity by secreting adipokines such as adiponectin and leptin. Excess lipid accumulation in adipose tissue results in an imbalance in the secretion of adipokines, leading to diabetes and other metabolic disorders. Hence, understanding of molecular mechanisms underlying physiological regulation of adipogenesis and lipid metabolism is an important issue both in biological and clinical aspects. Prostaglandins (PGs) are the arachidonate metabolites synthesized by the action of cyclooxygenase as a rate-limiting enzyme. It has been shown that several PGs regulate adipocyte defferentiation or lipolysis in cell culture system. Indeed, we previously identified that PGE_2-EP4 signaling suppresses adipocyte differentiation from 3T3-L1 preadipocytes and mouse embryonic fibroblasts. However it has not been examined the physiological roles of PG receptors in adipogenesis or adipocyte function in vivo. In this presentation, we would like to show the phenotypes regarding adipose tissue development and insulin response of PG receptor-KO mice and discuss on the physiological role of PG receptor signaling in the maintenance of lipid homeostasis.

V1b vasopressin receptor accelerates interaction between β-arrestin and μ-type opioid receptor and enhances opioid tolerance and adenylate cyclase sensitization

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Chronic and repeated exposures of morphine accelerate adenylate cyclase (AC) signaling and reduce analgesic efficacy, a condition known as opioid tolerance. Non-opioid neurotransmitters can modulate the development of opioid tolerance, but the mechanism has not been fully clarified. We found that analgesic tolerance to morphine developed with significant delay in mice lacking vasopressin V1b receptors (V1bRs) and in mice administered with a V1bR antagonist, but not in V1a-deficient mice. In rostral ventromedial medulla (RVM), V1bRs and μ-type opioid receptors (MORs) were co-expressed. In HEK cell model, the V1bR was constitutively phosphorylated and associated with β-arrestin 2. Complex formation between V1b-β-arrestin 2 and MOR was necessary for AC sensitization. Genome editing and deletion of the leucine-rich segment in V1bR carboxyl-terminus, which was necessary for β-arrestin 2 binding, increased morphine analgesia. These findings indicated that inhibition of V1bR provides a novel approach to enhance morphine analgesia without accelerating analgesic tolerance.

An encouragement of role-play ～ the utility of student-centered physician-patient role-play for practical pharmacotherapy education ～

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The active learning of practical pharmacotherapy has been performed at 18 medical schools as a part of pharmacology education from 2010 to 2019. This active learning program is a new pharmacology education technique which enables the simulated experience of explaining the features of medical treatment from medical doctors to patients through student-centered physician-patient role-play. This is named Case & Communication based approach (C&C approach), since it is studied through communication between doctors and patients based on several typical cases presented beforehand. The educational effectiveness was evaluated by a questionnaire survey. The questionnaire topics were (a) understanding of medical treatment, (b) understanding patient's feelings, (c) improvement of awareness and motivation as a doctor, and (d) positive influence upon study attitude. Furthermore, impressions of the role-play, written as free description. The highly effectiveness of role-play in pharmacology education was observed regarding all four topics. In addition, many students realized the importance of physician-patient communication, understanding of patient's feeling, and accurate and extensive knowledge of pharmacotherapy in their free description. This role-play program in pharmacology education may be effective for better understanding of pharmacotherapy, patient's feeling, and improvement of students' awareness and motivation as a doctor.
In this symposium, we would like to show the detail of this program and possibility of contribution to medical education.

Pharmacokinetic training of aspirin for medical undergraduate students to understand the concept of ion trap

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Medical undergraduate students who will become medical doctors should have enough knowledge and understandings of medication since they will administer medical drugs to cure or examine the patients in hospital. Our current pharmacological practice is intended for the students to learn pharmacokinetics of drugs by focusing on administration, metabolism and excretion of aspirin. In this practice, the volunteers are recruited from students who take aspirin with or without another agent that changes the urine to acidic or basic pH. They collect the urine during 3.5 hours after taking aspirin and analyze the metabolites of aspirin in the urine. Through this practice, the students understand how aspirin is metabolized and excreted to urine and also how the urine pH affects the excretion of the metabolites in urine. The students, who will need to administer medicine to patients as physicians, are expected to acquire basic aspects of pharmacokinetics and clinical trials through this practice.

Laboratory practice of pharmacology for medical students to learn pathophysiological science

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本学医学部の薬理学実習は、免疫学、微生物・感染症学、薬理学および病理学の4ユニットより構成される「病態の科学実習」の一部として2年次の9～10月に実施されている。病態の科学実習の主たる学修目標は、「科学的問題の発見・解決」のために必要な論理的思考と研究技法を学び、医学研究を実践するための基盤を身につけることとしている。臨床薬理学実習では、被験者になる事の意味を学生自身が被験者となり体験し、基礎薬理学実習では、すべてPCのシミュレーションソフトを用いて実施し学生個々人が独立して課題に取り組む。実習は午後半日（60分3コマ）を7回実施する。（1）実習の全体説明・実習前試験、（2）臨床薬理学実習：倫理委員会の指針に沿った二重盲検比較試験と臨床研究の実際（臨床試験の説明・同意・実施およびデータ解析）を学修、（3）薬物血中濃度モニタリングシミュレーション：科学的根拠に基づいた薬物投与量の調整法を修得、（4）心拍数と血圧に対する薬物の作用：薬物による患者の血圧調節の方法および作用機序を修得、（5）心室筋細胞の数理モデルを用いた活動電位シミュレーション：心臓電気薬理学を修得、（6）腸管平滑筋に対する薬物の作用：腸管運動異常に対する薬物治療戦略を修得、（7）総合討論・発表会・実習後試験である。評価項目は、知識、技能、態度である。知識は実習前と実習後試験で、技能は各実習レポートで、態度は実習への取り組みを加点方式で評価する。シンポジウムでは各実習の具体的な内容および準備・実施する上で実際に経験した種々の課題と解決策を紹介する。

Activity of the consortium of pharmacological training in Chugoku-Shikoku region

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Practice conducted in the Department of Pharmacology in medical schools plays an important role in learning the effect and risk of drugs through animal experiments as not only pharmacological but also ethical education. However, due to the budget reduction in the practice and cost cut for the instructors, performing practice with enough quality is so difficult in almost all medical schools. Thus, we have started a consortium of practice and education together with five pharmacology departments in the Chugoku and Shikoku regions to share practice tools and equipment, to develop human resource of instructors and to collaborate with each other. Here, we demonstrate our approach.

医学部薬理学実習は、医師として患者に実際に薬物投与する前に、動物実験を通して薬の作用と怖さを実感する、薬理学的観点だけでなく倫理面の教育としても重要な役割を担っている。また、創薬や薬理作用に関連したin vitroやin silicoの実習なども行われており、多岐にわたる。しかし、昨今実習リソースの新規申請・整備費用の予算削減に加え、実習を指導するスタッフの絶対的な減少という環境下に置かれており、薬理学実習の質の低下を防ぐ取り組みは喫緊の課題であるが各大学の個別の対応では限界がある。一方で実習期間は限定的であり、多くの大学で実習内容に類似性があるため、共通のリソースを用いてスタッフの人的交流を行うことができれば、大学間で機器を共有し薬理学実習の質の保証を担保する教育システムを構築することも可能であるとも考えられる。そこで中四国５大学が中四国薬理学教育コンソーシアムを結成し、実験器具の共有と人的交流を通した技術交流を図り、大学間で連携を行うことによる薬理学教育の質の向上を目指す試みを始めた。予算はまだまだ少なく、まずはLabChartの最新ソフトを購入し、各大学に持参して動物実験の際の解析に使用するなど、第一歩を踏み出したところであるが、今後はPowerLabなどの周辺機器の共有を進め、古くなった機器の整備や更新を進めて行きたい。このコンソーシアムの大きな目的のひとつは、大学間での薬理学実習を通じた人材交流である。上述したように、薬理学実習は多くの大学で類似した内容であることが全国アンケート調査で明らかとなったが、その質についての客観的評価は難しい。動物実験やin vitroの実験、in silicoの実習やシミュレーションなど、各大学の得意とするところを出し合い、指導し合うことにより、学生にとって、最もいい実習を体感できる実習システムを構築していきたいと考えている。

1-S17-1 Symposium17

Optogenetic examination of effects of stress on the orbitofrontal-amygdala synaptic transmission

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The orbitofrontal cortex (OFC) has important roles for processing of negative emotion and has recently been highlighted as a critical region in stress-related psychiatric disorders such as depression. However, mechanisms how stress affect OFC circuit and induce psychiatric symptoms were less understood. OFC sends dense projection to the amygdala, which is one of the key nodes for generation of negative emotion. Taken together, there is a possibility that stress affects the information processing in the OFC-amygdala pathway, and it underlies stress-induced emotional alteration. To address this possibility, we isolated the synaptic transmission form OFC to the basolateral nucleus of the amygdala (BLA) using optogenetic and whole-cell patch-clamp methods in mice, and examined the effect of repeated stress on the OFC-BLA synapse. Interestingly, repeated tail-shock stress induced postsynaptic plasticity as shown by increase of AMPA/NMDA currents ratio and inward rectification in OFC-BLA synapse. Furthermore, Optogenetic activation and chemogenetic inactivation of the OFC-BLA transmission during the tail-suspension test increased and decreased stress-related behavior in mice, respectively. Our findings suggested that synaptic changes in the OFC-BLA pathway were one of the neural bases in stress-induced behavioral alterations.

Mechanism of axonal degeneration: from molecular signaling to the development of therapeutic applications

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Research on the mechanism of injury-induced axonal degeneration (Wallerian degeneration) revealed that axonal degeneration is an active process involving transcriptional regulations and enzymatic reactions. Axonal degeneration is observed as part of the pathology in many neurological disorders, including neurodegenerative disorders, and causes key symptoms. Subcellular reactions regulating axonal degeneration are independent from those for apoptosis, in principle, and therefore, prevention of axonal degeneration may constitute an important therapeutic strategy against neurodegenerative disorders.

We have previously shown that ZNRF1, a ubiquitin ligase, regulates stability of microtubules constituting cytoskeletal structure in axons via regulating degradation of AKT and thereby controlling its downstream phosphorylation reaction cascade. We also found that the phosphorylation cascade also promotes autophagy in degenerating axons. In this symposium presentation, we will summarize our findings on the mechanism of ZNRF1-dependent regulation of axonal degeneration.

Novel defense strategy against progressive neurodegeneration by controlling intracellular Zn^{2+} dysregulation

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The causes of progressive neurodegeneration, i.e., Alzheimer's disease (AD) and Parkinson's disease (PD) are unknown. The basal (static) concentration of intracellular Zn^{2+} is estimated to be approximately 100 pM and is extremely lower than that of intracellular Ca^{2+} (～100 nM), suggesting that intracellular Zn^{2+} homeostasis is crucial for neural function. Moreover, the basal concentration of extracellular Zn^{2+} is estimated to be approximately 10 nM and is age-relatedly increased based on age-related increase in brain extracellular Zn. We postulated that progressive neurodegeneration is due to age-related intracellular Zn^{2+} dysregulation, which is induced by rapid influx of extracellular Zn^{2+}. Neuronal amyloid $\beta_{1\text{-}42}$ ($A\beta_{1\text{-}42}$) accumulation is considered an upstream event in the AD pathogenesis. Here we report that Zn-$A\beta_{1\text{-}42}$ oligomers formed in the extracellular compartment are synaptic activity-independently taken up into neurons, followed by rapid intracellular Zn^{2+} dysregulation. PD is characterized by a selective loss of dopaminergic neurons in the substantia nigra pars compacta of the brain. Here, we report a unique mechanism of nigral dopaminergic degeneration, in which rapid intracellular Zn^{2+} dysregulation via the production of reactive oxygen species, especially hydrogen peroxide causes PD in rats, which is induced by paraquart and 6-hydroxydopamine. I will talk about novel defense strategy against progressive neurodegeneration by controlling intracellular Zn^{2+} dysregulation.

1-S17-4 Symposium17

Therapeutic strategy for Parkinson's disease: Targeting zinc-binding protein in astrocytes

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Parkinson's disease (PD) is a progressive neurodegenerative disease with motor symptoms, such as tremor, akinesia/bradykinesia, rigidity and postural instability due to a loss of nigrostriatal dopaminergic neurons, and non-motor symptoms, such as orthostatic hypotension and constipation. Although nosotropic treatments to improve the motor disability in PD are being assessed at present, the main challenge remains to develop of neuroprotective or disease-modifying treatments. Therefore, it is desirable to find approaches that can inhibit the progression of dopaminergic neurodegeneration. Astrocytes are known to play an important role in the maintenance of the neuronal environment and exert neuroprotective effects by production of antioxidants, release of neurotrophic factors, and uptake of potentially neurotoxic molecules. In the previous study, we demonstrated that astrocytes produced antioxidative molecules metallothionein (MT)-1/2 in response to oxidative stress, and protected dopaminergic neurons against oxidative stress. MTs are cysteine-rich and metal-binding proteins such as zinc, copper, and cadmium to function in zinc homeostasis and metal detoxification. In this symposium, we will outline a new therapeutic strategy of neuroprotection against dopaminergic neurodegeneration by targeting MTs in astrocytes.

Neuroimmune system associated with brain development and neurodevelopmental disorders

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In the developing brain, generation of neurons and glial cells is rigorously regulated by various factors. In particular, recent studies demonstrated that immune system in the brain, called neuroimmune system, is a key player in brain development and neurodevelopmental disorders. However, it remains unclear whether peripheral lymphocytes contribute to brain development and the pathogenesis of neurodevelopmental disorders. In this study, we investigated the role of lymphocytes in the brain development and found that some lymphocytes are involved in oligodendrogenesis and the proliferation of neural progenitors in the neonatal brain. These findings prompt us to investigate whether neuroimmune system in the developing brain is also involved in the pathogenesis of neurodevelopmental disorders. To resolve this, we examined the proportion and role of immune cells in the pathological condition of neonatal brain. In this symposium, we will introduce the contribution of neuroimmune system to brain development and neurodevelopmental disorders.