Award Presentations

March 22 (Tue) — March 24 (Thu) , Hall AB

SAP1～SAP6	Hiroshi and Aya Irisawa Memorial Promotion Award for Young Physiologists
SAP7	Hiroshi and Aya Irisawa Memorial Award for Excellent Papers on Research in Circulation in the Journal of Physiological Sciences
SAP8	Aya Irisawa Memorial Promotion Award for Excellence by Women Physiologists
SAP9～SAP12	Promotion Award of the Physiological Society of Japan for Young Scientists
3S20A1	Symposium on the Hiroshi and Aya Irisawa Memorial Award for JPS Excellent Papers Award

SAP1

Molecular basis of the species-specific agonist activity by diamide insecticide

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The diamondback moth is the most widely distributed major pest of crucifers. Flubendiamide and chlorantraniliprole, diamide insecticides, show selective insecticidal activity against lepidopterous insects such as the diamondback moth. The diamide insecticides act to prolong ryanodine receptor (RyR) channel opening, resulting in uncoordinated muscle contractions in intoxicated pest insects. However, the molecular mechanisms underlying the species-specific action of diamide insecticides are unclear. Affinity labeling of the lepidopterous silkworm RyR (sRyR) revealed that flubendiamide is mainly incorporated into the divergent region 1 (DR1). DR1 shows a low sequence homology among species. Replacement of a 379 amino acid segment in DR1 of sRyR with that from rabbit RyR2 significantly impaired the response to flubendiamide, but only marginally reduced the sensitivity to caffeine, a general RyR activator. These findings indicate that DR1 plays an important role in the formation of an action site for flubendiamide. (COI:No)

SAP2

Analyses of subcellular localization of CALHM1 channel

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Calcium homeostasis modulator 1 (CALHM1) has been identified as a voltage-gated ion channel with a pore of ~14Å diameter, through which signaling molecules including ATP can travel across the plasma membrane. In taste buds of the tongue, CALHM1 serves as the release machinery of the primary neurotransmitter, ATP, which conveys taste information from taste cells to gustatory nerves. Mice lacking *Calhm1* are severely deficient in taste recognition due to impaired taste-evoked ATP release from taste buds.
In the meantime, CALHM1 activation in heterologous systems is toxic to most cells because it allows most cellular neutrients and inorganic ions, including amino acids and Ca^{2+} to leak out and in. Therefore, CALHM1 activity in vivo is likely confined to a specific region for its function to minimize its cytotoxicity. There have, however, been no insights into regulation of subcellular localization of CALHM1. We have been attempting to analyze the subcellular localization of CALHM1 in vivo by means of polyclonal antibodies produced in rabbits, viral gene transfection systems, and a transgenic mouse model. In addition, we have established MDCKII cells expressing CALHM1 which enable us to explore apical/basolateral sorting mechanisms of CALHM1 in polarized epithelial cells. Our recent results will be discussed. (COI:No)

SAP3

Impaired cardiac vagal control and treatment of dilated cardiomyopathy

Zhan Dongyun[1]、Du Chengkun[1]、Akiyama Tsuyoshi[1]、Morimoto Sachio[2]、Shimizu Shuji[1]、Kawada Toru[1]、Kangawa Kenji[1]、Shirai Mikiyasu[1]

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First, to evaluate cardiac vagal nerve activity in a knock-in (KI) mouse model of dilated cardiomyopathy (DCM) with δK210 mutation in the cardiac troponin T gene, we applied microdialysis technique to the left ventricular myocardium of anesthetized mice and myocardial interstitial acetylcholine (ACh) levels were measured by HPLC as an index of ACh release from cardiac vagal nerve endings. The effects of electrical stimulation of cervical vagal nerve (peripheral vagal control) and α_2-adrenergic stimulation by intravenous medetomidine (central vagal control) were examined in wild type and DCM KI mice. In DCM KI mouse, peripheral vagal control including ACh release from vagal nerve endings and postsynaptic function was preserved, but central vagal control through α_2-adrenergic receptor was impaired.
Second, to test whether cardiac vagal nerve activation ameliorates the mortality of DCM KI mouse, we examined the therapeutic efficacy of ghrelin, which centrally activates cardiac vagal nerve activity, in DCM KI mouse. Ghrelin ameliorated survival rate as well as cardiac function and remodeling in DCM KI mouse. Heart rate variability analysis indicated the decrease in cardiac sympathetic nerve activity as well as increase in cardiac parasympathetic nerve activity by ghrelin.
In DCM KI mouse, central cardiac vagal control is impaired and ghrelin, which centrally enhances cardiac vagal nerve activity, ameliorates mortality and cardiac function.
(COI:No)

SAP4

Mechnismas of cardiovascular regulation throught the autonomic nerves by nesfatin-1

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Nesfatin-1, one of neural peptides, causes anorexia and increase in energy expenditure. Recent our study found that intracerebroventricular (ICV) administration of nesfatin-1 elevated renal sympathetic nerve activity (RSNA) and blood pressure (BP) in anesthetized rats. Here, to clear hypothalamic mechanisms of nesfatin-1 action on RSNA and BP, we noticed intracellular signals in hypothalamic neurons and examined effects of ICV nesfatin-1 on hypothalamic signal cascades. Nesfatin-1 injection increased the extracellular signal-regulated kinase (ERK) activity in rats and p-ERK-positive neurons in the paraventricular hypothalamic nucleus (PVN) and the arcuate nucleus (ARC). In addition, nesfatin-1 microinjection into the PVN but not the ARC activated RSNA and BP. Next, we found that increased p-ERK1/2-positive neurons in the PVN by nesfatin-1 were co-expressed in neurons expressing corticotropin-releasing hormone (CRH) and that ICV injection of nesfatin-1 increased CRH levels in the PVN. Both CRH signaling blocker and ERK blocker suppressed renal sympathetic and hypertensive actions by nesfatin-1. These findings suggest that nesfatin-1 regulates the sympathetic nervous system through ERK signaling in the hypothalamic PVN-CRH neurons to maintain cardiovascular function. (COI:No)

SAP5

The role of Epac1 in the regulatory mechanisms of vascular smooth muscle cell migration and neointimal formation

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Vascular smooth muscle cell (VSMC) migration causes neointima after mechanical injury. We reported that an exchange protein activated by cAMP (Epac) 1 was upregulated in mouse arterial neointima and promoted VSMC migration. In this study, we examined the molecular mechanisms of Epac1-induced VSMC migration and the role of Epac1 in neointimal formation *in vivo*.
VSMCs were obtained from the $Epac1^{+/+}$ ($Epac1^{+/+}$-VSMCs) and $Epac1^{-/-}$ mice. Platelet-derived growth factor (PDGF)-BB-induced VSMC migration was attenuated in $Epac1^{-/-}$-VSMCs. PDGF-BB-mediated intracellular Ca^{2+} elevation of $Epac1^{-/-}$-VSMCs was also reduced. PDGF-BB or an Epac-selective cAMP analog-mediated lamellipodia formation accompanied by cofilin activation was rarely observed in $Epac1^{-/-}$-VSMCs. Next, $Epac1^{+/+}$ and $Epac1^{-/-}$ mice were subjected to femoral arteral injury to examine the effect of Epac1 on neointimal formation. In accordance with *in vitro* data, four weeks after injury, neointimal formation was attenuated in $Epac1^{-/-}$ mice in which cofilin activation was inhibited. Lastly, we generated the chimeric mice by bone marrow cell transplantation from $Epac1^{+/+}$ into $Epac1^{-/-}$ mice and vice versa to evaluate the contribution of bone marrow-derived cells. The genetic background of vascular tissues including VSMCs rather than of bone marrow-derived cells affected Epac1-mediated neointimal formation.
In conclusion, these data suggest that Epac1 plays a role in VSMC migration via facilitating Ca^{2+} influx and a cofilin-mediated lamellipodia formation, thereby promoting neointimal formation. (COI:No)

SAP6

In vivo nano-imaging and a thermal manipulation of myocardial contractions

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Changes in myocardial sarcomere lengths of merely ~100 nm dramatically change the heart's pump functions. Therefore, high-performance nano-imaging of cardiac sarcomeres *in vivo* is paramount to fully understand the contributions of SL dynamics to cardiac pump functions. In the present study, we developed a high-speed (100 fps), high-resolution (20 nm) nano-imaging system for myocardial sarcomeres in living mice. We expressed α-actinin-AcGFP to visualize the Z-disks in cardiomyocytes of the left ventricle *in vivo*. The length of a single sarcomere was determined by the peak-to-peak distance of the α-actinin-AcGFP fluorescence profile. Using this system, we conducted three-dimensional analyses of sarcomere dynamics during the cardiac cycle, simultaneously with electrocardiogram and left ventricular pressure measurements. These results provided the first direct evidence for the tight coupling of sarcomere dynamics and ventricular pump functions. Likewise, we developed a thermal stimulation system with infrared laser to modulate myocardial contractions. Accordingly, we found that microscopic heat pulses reproducibly induced contractions with no changes in intracellular calcium dynamics. This technique is likely to have a potential in systematically understanding the mechano-thermal coupling in cardiac as well as skeletal muscles. At the meeting, we will discuss the recent advances in cardiac nano-physiology as revealed by using cutting-edge optical technologies. (COI:No)

SAP7

Differential contribution of aortic and carotid sinus baroreflexes to control of heart rate and renal sympathetic nerve activity

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We have examined the roles of aortic and carotid sinus baroreceptors in control of heart rate (HR) and renal sympathetic nerve activity (RSNA) in 17 decerebrate rats. The baroreflex curves between the changes in mean arterial blood pressure (MAP) and HR or RSNA in response to intravenous injection of phenylephrine (10-20 μg/kg) or nitroprusside (10 μg/kg) were identified before and following sequential denervation of all four baroafferent nerves. The slope of the MAP-HR curve in the pressor range was decreased ($P < 0.05$) to 31 ± 7% of the control following denervation of bilateral aortic nerves, whereas it remained substantially (72 ± 10%) following denervation of bilateral carotid sinus nerves. The slope for HR became negligible following complete denervation of all four baroafferent nerves. In contrast, the slope of the MAP-RSNA curve decreased as the sequential baroafferent denervation progressed, irrespective of the denervation order, and it remained well as long as any single baroafferent was intact. The similar influences of sequential baroafferent denervation on the responses of HR and RSNA were observed in the depressor range. Thus it is likely that aortic and carotid sinus baroreceptors play differential roles in control of HR but they contribute similarly to control of RSNA (COI:No).

SAP8

Eye position-dependent responses to visual memory trace remapped by saccades in cortical area MST

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Despite our eye movements, we can perceive the visual world as stable and continuous. Two neural mechanisms to achieve this have been proposed. One is known as trans-saccadic remapping, in which neurons shift their receptive fields (RFs), providing anticipatory activity in accordance with each saccade (i.e., "predictive remapping.") The second is spatiotopic representation, which provides image location information by modulating neuronal responses in relation to the eye position (i.e., "eye position gain field.") Neural correlates of these two mechanisms have been reported in various areas, but the relationship and/or interaction between them have not yet been studied.
We recently reported that after a saccade, most neurons in the medial superior temporal area (MST) of rhesus monkeys respond to the visual stimulus that pre-existed inside their post-saccadic RFs and then turned off before the saccade (i.e., "memory remapping.") In the present study, to characterize the trans-saccadic memory in spatiotopic coordinates, we investigated the dependence of the remapped memory traces of the visual stimulus on eye positions in the MST. We found that the responses of most MST neurons after saccades were modulated by the gaze angle to both the real visual stimuli and the visual memory traces remapped by the saccades. Thus, the two mechanisms work together in individual MST neurons to represent a continuous and stable visual world. (COI:No)

SAP9

Deficiency of tRNA modification causes the development of X-linked mental retardation

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Genetic mutations in X chromosome-linked genes have been associated with mental retardation (XLMR). Recently, linkage analyses performed in Belgian, Chinese and Japanese families have identified Ftsj1 gene as a novel candidate gene. Ftsj1 shares homology with a bacterial 23S rRNA methyltransferase FTSJ. However, the molecular function of Ftsj1 and its pathological relevance in mental retardation have remained unknown. We generated Ftsj1 knockout (KO) mice and performed a comprehensive analysis to reveal the physiological functions. Ftsj1 is responsible for the 2-O-methylation of cytosolic transfer RNAs (tRNAs) at position 32 and 34. The deficiency of Ftsj1-mediated methylation caused a significant decrease of protein translation in KO mice. Accordingly, there was a marked decreased of synaptic proteins including glutamate receptors and signaling molecules. The KO mice exhibited abnormal spine morphology and decreased LTP/LTD levels, which ultimately contributed to the impaired spatial learning. There results suggest that the hypomodified tRNAs impairs neuronal protein synthesis, which contributes to the development of mental retardation in Ftsj1-deficient mice and human. (COI:No)

SAP10

Cortical Top-Down inputs during sleep consolidates perceptual memory in mice

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It is hypothesized that the internal (i.e. top-down) inputs have crucial roles for memory consolidation, however this has never been tested before. Here we studied when and how bottom-up and top-down cortical circuits contribute to tactile or visual recognition memory in freely moving mice. We applied optogenetic pathway-specific silencing technique to cortical bidirectional circuits between the primary somatosensory cortex (S1) and the secondary motor cortex (M2), a higher order frontal region. Silencing either the bottom-up (S1 to M2) or top-down (M2 to S1) pathway during the learning or retrieval phase impaired novelty discrimination in a tactile recognition test, while not affecting visual recognition memory. This data suggests that online tactile processing includes cortical top-down and bottom-up processing. Next, we applied closed-loop optogenetic stimulation to silence cortical circuits in a sleep state-specific fashion. During post-learning slow wave sleep (SWS), silencing the top-down but not the bottom-up pathway impaired the consolidation of tactile recognition memory. This top-down pathway silencing during post-learning SWS impaired the slow oscillation flow in top-down direction. Furthermore, the pathway silencing impaired memory reactivation in S1. These data reveal that slow oscillation flow in top-down direction is essential contributors to memory reactivation and consolidation. Take all data together, while the cortical bottom-up and top-down bidirectional pathways cooperate for online processing of sensory information, cortical top-down pathway unidirectionally contributes to memory consolidation during SWS. (COI:No)

SAP11

Peripheral administration of TRPA1 agonists increase food intake: involvement of the novel subpopulation of vagal afferents with orexigenic function

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Spices added in the meal are experientially used to stimulate appetite. However, their functional ingredients and action mechanisms are not revealed. Many spices have the agonistic activity for transient receptor potential ankyrin 1 (TRPA1). Vagal afferents innervate almost all peripheral organs, and sense the peripheral energy state and convey them to the brain to regulate feeding. Thus, we hypothesized that TRPA1 agonists exert their orexigenic action via directly interacting with vagal afferents. We examined whether a subpopulation of vagal afferent neurons is activated by TRPA1 agonists, allyl isothiocyanate (AITC) from wasabi and dially trisulfide (DATS) from garlic. AITC and DATS increased cytosolic Ca^{2+} concentration in 40% of single nodose ganglion (NG) neurons isolated from mouse. Cholecystokinin (CCK), a satiety hormone, activates approximately 40% of vagal afferents. We found that the majority (70%) of TRPA1 agonist-responsive NG neurons are unresponsive to CCK, suggesting that TRPA1 agonist- and CCK-responsive neurons are mostly separated subpopulations and TRPA1 agonist-responsive NG neurons may represent a distinct subpopulation with orexigenic function. Moreover, we found that intraperitoneal or oral administration of AITC or DATS increased food intake in mice, and these effects were blunted by desensitization of vagal afferents using capsaicin. In conclusion, we demonstrate that peripheral administration of TRPA1 agonist increases food intake by interacting with the novel subpopulation of vagal afferents with orexigenic role. (COI:No)

SAP12

VIP and AVP signaling regulate circadian cellular networks in the suprachiasmatic nucleus during postnatal development

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In mammals, the suprachiasmatic nucleus (SCN) in the hypothalamus plays a critical role in the expression of circadian rhythms. According to the current hypothesis, cellular circadian rhythms are generated by a transcription and translation feedback loop which involves several clock genes and their protein products. Recently we found that mice lacking *Cry1* and *Cry2* (*Cry1,2*$^{-/-}$) exhibited robust circadian rhythms in the cultured neonatal SCN slices. However, the circadian rhythms on the SCN tissue level disappeared during postnatal development, due to desynchronization among cellular rhythms. These results suggest the differential networks for circadian rhythm expression on the SCN tissue level between neonatal period and adulthood. The SCN contains two major neuropeptides arginine vasopressin (AVP) and vasoactive intestinal polypeptide (VIP) in the shell and core regions, respectively. They have been regarded as important factors for cellular signaling in the SCN, but it remains unclear how these molecules work in the network during postnatal development. Here we show that in *Cry1,2*$^{-/-}$ SCN, VIP signaling is critical for the tissue-level circadian rhythms in the neonates, and AVP signaling is substantially attenuated throughout the life. We also found multiple cellular clusters with different circadian periods in the SCN. They were differently integrated by VIP and AVP signaling depending on postnatal development. (COI:No)

Symposium on the Hiroshi and Aya Irisawa Memorial Award for JPS Excellent Papers Award

March 24 (Thu), 9:00-10:30, Room A

3S20A1-1

Role of P2Y1-mediated Ca^{2+} signals of astrocytes in neuronal excitability.

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Astrocytes express a plethora of Gq-protein coupled receptors (GqPCR), activation of which cause massive Ca^{2+} elevation. Activation of GqPCRs in astrocytes can lead to gliotransmitter release, in turn, to regulate synapses. Thus, GqPCRs in astrocytes could greatly affect brain functions and brain disorders. P2Y1 receptor (P2Y1) has a central role in astrocyte Ca^{2+} signals both *in vitro* and *in vivo*. Gene and functional expression of P2Y1 are upregulated in pathophysiology, such as stroke, Alzheimer disease and epilepsy. However, it is not clear if upregulation of P2Y1 is the cause or result of such diseases, since expression of many genes also are altered. We recently generated a novel transgenic mice in which astrocytes specifically overexpress P2Y1 using Tet-off system. In situ hybridization data show upregulation of P2ry1 mRNAs which were co-localized with GFAP, an astrocyte marker. Astrocytes overexpressing P2Y1 in the dentate gyrus displayed three-fold larger Ca^{2+} signals evoked by P2Y1 agonist than its control. Astrocytes with P2Y1 overexpression displayed more spontaneous Ca^{2+} signals (sponta Ca^{2+}) which were largely synchronized between neighboring astrocytes. Pharmacological experiments suggest that sponta Ca^{2+} seemed to be mediated by endogenous ligands (e.g. ATP) released through hemichannels. The frequency of spontaneous EPSCs recorded from granule cells was increased without affecting the amplitude in astrocyte P2Y1 overexpression mice. Overall, our data suggest that astrocyte overexpressing P2Y1 communicate each other more tightly, which may increase excitability of neurons. (COI:No)

3S20A1-2

Findings and remaining concerns of “Early-life stress increases the motility of microglia in adulthood” published in Journal of Physiological Science.

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As it is well known, early-life stress causes several neuropsychological disorders in adulthood. Such disorders may be partly induced as a result of instability of neuronal circuits and/or synaptic formation. Glial cells, such as astrocyte and microglia may also contribute the change. However, the mechanisms underlying the change have not yet been clearly understood. We previously reported in Journal of Physiological Science that the mushroom spine in the somatosensory cortex (SSC) is unstable in early-life stressed mice not only in the juvenile stage but also in adulthood. We recently also reported that, the number and motility of filopodia-like protrusions of microglial processes tended to increase in the SSC of early-life stressed mice. Interestingly, the motility of protrusions significantly correlated with the nociceptive threshold level measured by the von Frey test. These results indicated that the activity of microglia affected the neuronal function in early-life stressed mice. In this symposium, I summaries findings of our previous study which mainly reported in JPS and also want to discuss about the limitation and remaining concern of study. (COI:No)

3S20A1-3

In vivo two-photon imaging of cortex to hippocampal dentate gyrus using a newly developed a high–peak power 1064-nm light source

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Conventional *in vivo* two-photon microscopy has revealed vital information about neural activity in relation to brain function, despite its limitations in imaging events at depths greater than several hundred micrometers from the surface of the brain. In this study, we developed a novel two-photon microscope consisting of a 1064-nm gain-switched laser diode–based light source with average power above 4 W, pulse width of 7.5 picoseconds, repetition rate of 10 MHz, and a high-sensitivity photomultiplier tube for efficient detection of fluorescence. By applying this newly developed two-photon microscope to *in vivo* imaging, we were able to successfully visualize hippocampal neurons in dentate gyrus and panoramic views of CA1 pyramidal neurons and cerebral cortex, in both young adult and adult mice. Fine structures of dendrites in CA1 neurons could be visualized with a high peak-signal-to background ratio that could not be achieved by titanium sapphire laser excitation. Furthermore, our system achieved multicolor imaging with neurons and blood vessels in the hippocampal region *in vivo*. We hope that our two-photon microscopy system will be applicable to investigations of various neural functions, including the morphological changes undergone by neurons during physiological phenomena. (COI:No)