Award Posters of the PSJ

（March 21, 12:45～14:00）

AP-1～AP-2	Promotion Award of the Physiological Society of Japan for Young Scientists
AP-3～AP-7	Hiroshi and Aya Irisawa Memorial Promotion Award for Young Physiologists
AP-8	Hiroshi and Aya Irisawa Memorial Promotion Award for Cardiovascular Physiologists
AP-9	Aya Irisawa Memorial Promotion Award for Excellence by Women Physiologists

AP-1 (P1-206)

TRPM2 protects mice against polymicrobial sepsis by enhancing bacterial clearance

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TRPM2 is an oxidative stress-activated nonselective Ca^{2+} permeable channel abundantly expressed in macrophages to regulate production of inflammatory mediators. However, the role and mechanism of TRPM2 in polymicrobial sepsis remains unclear. Using CLP-induced polymicrobial sepsis model, Trpm2-KO mice had increased mortality compared with wild-type (WT) mice. The increased mortality was associated with increased bacterial burden, organ injury, and systemic inflammation. TRPM2-mediated Ca^{2+} influx plays an important role in LPS or CLP-induced HO-1 expression in macrophage. HO-1 up-regulation decreased bacterial burden both in WT BMDMs and in CLP-induced septic WT mice. Disruption of TRPM2 decreased HO-1 expression and increased bacterial burden in BMDMs. Interestingly, pretreatment of Trpm2-KO BMDMs with HO-1 inducer markedly increased HO-1 expression and decreased bacterial burden. Moreover, pretreatment of Trpm2-KO mice with HO-1 inducer reversed the susceptibility of Trpm2-KO mice to sepsis by enhancing bacterial clearance. In addition, septic patients with lower monocytic TRPM2 and HO-1 mRNA levels had a worse outcome compared with septic patients with normal monocytic TRPM2 and HO-1 mRNA levels. TRPM2 levels correlated with HO-1 levels in septic patients. Our data demonstrate a protective role of TRPM2 in controlling bacterial clearance during polymicrobial sepsis possibly by regulating HO-1 expression.

(COI: No)

AP-2 (P1-067)

Identification of retrograde signals required for synapse elimination in the developing brain

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Precise formation of neural circuits during development is a prerequisite for proper brain functions. Neurons form exuberant synapses with target cells early in development. Then, necessary synapses are selectively strengthened whereas unnercessary connections are weakened and eventually eliminated during the course of postnatal development. This process is known as synapse elimination. Synapse elimination is an important step to shape initial redundant neural circuits into functionally mature circuits, and the disruption is likely linked to mental disorder and brain dysfunction. While the underlying mechanism is still unclear in any systems, several lines of evidence suggest that retrograde signaling from postsynaptic cells regulates synapse elimination. However, these retrograde signals remain to be identified. We have screened retrograde molecules required for synapse elimination of climbing fiber to Purkinje cell connection in the developing cerebellum. We identified some key retrograde molecules which strengthen necessary synapses and eliminate unnecessary synapses. Here I am going to talk about the role of these retrograde molecules in synapse elimination.

(COI: No)

AP-3 (P1-012)

Voltage-dependent movement of the catalytic domain of voltage-sensing phosphatase, VSP, probed by the site-specific incorporation of a fluorescent unnatural amino acid

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Voltage-sensing phosphatase, VSP, consists of a voltage sensor and a cytoplasmic catalytic domain (CD). The enzymatic activity has been shown to be coupled to the voltage sensor movement. It has been proposed that the voltage-sensor activation induces the conformation change of CD. However, the direct evidence has been lacking. To monitor the voltage-dependent conformation change of CD, we genetically incorporated a fluorescent unnatural amino acid, Anap, into CD. First, Anap was incorporated into "gating loop" which has been claimed to make large conformational change for switching enzymatic activity based on the crystallographic study of CD of VSP. Anap fluorescence was changed in a voltage-dependent manner, indicating that CD changes its conformation upon the voltage-sensor activation. Besides the conformation change, it is also possible that the voltage sensor regulates the distance between CD and the plasma membrane. Since the substrate of the enzyme is phosphoinositides which are membrane components, membrane binding of CD may be crucial for the enzymatic activity. To detect the change of the distance between CD and the plasma membrane, CD and the plasma membrane were labeled by Anap and dipicrylamine(DPA), respectively. We verified that DPA works as a FRET acceptor of Anap on VSP and are currently testing if the distance between CD and the plasma membrane is changed during the voltage-dependent phosphatase activity.

(COI: No)

AP-4 (P1-059)

Cancer cell-specific crosstalk between Na^+, K^+-ATPase and volume-sensitive anion channel in membrane microdomains exerts anti-proliferative activity

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Na^+, K^+-ATPase is a potential target for anti-cancer therapy, because cardiac glycosides, inhibitors of Na^+, K^+-ATPase, potently block cancer cell growth. However, the mechanism underlying the anti-cancer effects of cardiac glycosides is not fully understood. In the present study, we found that ouabain, a cardiac glycoside, inhibited cancer cell proliferation via activation of volume-sensitive outwardly rectifying (VSOR) anion channel. The effects were suppressed by DCPIB, a selective inhibitor of VSOR channel, and the knockdown of Na^+, K^+-ATPase α1-isoform (α1NaK) or VSOR channel component LRRC8A (SWELL1). The disruption of membrane microdomains by methyl-β-cyclodextrin and the attenuation of the production of reactive oxygen species (ROS) by the inhibitors of NADPH oxidase (NOX) significantly suppressed the ouabain-induced VSOR activation and inhibition of cell proliferation. On the other hand, the ouabain-induced effects were not observed in non-cancer cells. These results suggest that α1NaK, NOX and VSOR channels form a signalosome in the membrane microdomains of cancer cells, and that the cardiac glycoside exerts anti-cancer activity through the cancer-specific signalosome.

(COI: No)

AP-5 (P2-220)

In vivo assessment of cardiac autonomic nerve activities and identification of cardioprotective agents for heart failure treatment using atrial microdialysis technique

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Introduction: Sympathoexcitation and vagal withdrawal are causes of heart failure progression. Therefore, sympatho-suppression using beta-blockers has been a gold standard treatment for heart failure. We developed the atrial microdialysis technique to simultaneously assess cardiac sympathetic and vagal activities. Using this technique, we examined the effects of various pharmacological agents on cardiac autonomic nerve activities to identify cardioprotective agents.
Methods: In anesthetized rabbits, a dialysis probe was implanted into the right atrial myocardium near the sinoatrial node and was perfused by the Ringer's solution. Dialysate norepinephrine (NE) and acetylcholine (ACh) concentrations were analyzed as indices of cardiac autonomic nerve activities using high-performance liquid chromatography.
Results: 1) Electrical stimulation of sympathetic nerve or vagal nerve significantly increased dialysate NE or ACh concentration in a frequency-dependent manner. 2) Intravenous injection of medetomidine or guanfacine significantly increased dialysate ACh concentration. Furthermore, medetomidine significantly suppressed sympathetic NE release. 3) Intracerebroventricular injection of ghrelin significantly enhanced vagal ACh release to the heart.
Conclusions: Atrial microdialysis technique enabled us to simultaneously monitor cardiac sympathetic and vagal nerve activities. This technique may be useful for the identification of cardioprotective agents.

(COI: No)

AP-6 (P1-034)

Molecular mechanism and regulation of partial agonism of the M2 muscarinic receptor-activated K^+ currents

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

(COI: No)

AP-7 (P2-217)

Rapid cholinergic and delayed β-adrenergic vasodilatation in non-contracting muscles during one-armed cranking

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We have reported that the rapid cholinergic and delayed β-adrenergic vasodilatation increases blood flow of non-contracting vastus lateralis (VL) muscle during one-legged cycling (Ishii et al. 2013, 2014). It was unclear whether such mechanisms contribute to vasodilatation in non-contracting muscles during one-armed exercise. We examined the influences of atropine and/or propranolol on the blood flow responses of the contralateral biceps and triceps brachii and forearm extensor muscles and VL muscle during moderate one-armed cranking for 1 min (n=7). As an index of muscle tissue blood flow, relative concentration in oxygenated-hemoglobin (Oxy-Hb) was measured using near-infrared spectroscopy. The Oxy-Hb of the muscles increased during one-armed cranking. The increase in Oxy-Hb at the early period of exercise was blunted by atropine, whereas propranolol attenuated the later increase in Oxy-Hb during the exercise. Following combined atropine and propranolol, the Oxy-Hb decreased during the exercise. The influences of the autonomic blockades on the Oxy-Hb response were not different among the muscles. It was concluded that the rapid cholinergic and delayed β-adrenergic vasodilatation increased the blood flows of non-contracting arm and leg muscles during one-armed exercise.

(COI: No)

AP-8

Discharges of aortic and carotid sinus baroreceptors during spontaneous motor activity- and pharmacologically-evoked pressor interventions

Matsukawa, Kanji; Ishii, Kei; Kadowaki, Akito; Ishida, Tomoko; Idesako, Mitsuhiro; Liang, Nan (*Dept Integrative Physiol, Grad Sch Biomed Health Sci, Hiroshima Univ, Hiroshima, Japan*)

We have shown that the cardiomotor component of aortic baroreflex is inhibited at onset of spontaneous motor activity in decerebrate cats, without altering carotid sinus baroreflex. The dissociation may be attributed to a difference in the responses between aortic nerve activity (AoNA) and carotid sinus nerve activity (CsNA). The stimulus-response curves of mean AoNA and CsNA per beat against mean arterial blood pressure (MAP) were compared between the pressor interventions evoked by spontaneous motor activity and by iv injection of phenylephrine or norepinephrine, in which the responses in heart rate (HR) were opposite (i.e., tachycardia vs. baroreflex bradycardia) despite the identical increase in MAP. The stimulus-response curves of the AoNA and CsNA matched between spontaneous motor activity- and pharmacologically-evoked pressor intervention and the slopes of the relative percent AoNA and CsNA were equal between the two interventions. Diastolic AoNA and CsNA (defined as the minimal value within a beat) during spontaneous motor activity showed a greater increase in association with tachycardia, which was abolished by fixing HR at the intrinsic cardiac frequency. Thus mean mass activities of aortic and carotid sinus baroreceptors can faithfully encode the beat-by-beat changes in MAP not only at rest but also during spontaneous motor activity and cannot explain the spontaneous motor activity-related inhibition of the cardiomotor component of aortic baroreflex.

(COI: No)

AP-9

Mechanism of thalamic network remodeling after the peripheral nerve injury

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Peripheral sensory nerve injury causes large-scale somatotopic reorganization in the brain. However, neural circuit mechanisms by which the reorganization occurs remain largely unknown. A relay neuron in the mouse whisker sensory thalamus (V2 VPm) receives generally a single afferent fiber originating from the whisker-representing trigeminal nucleus (PrV2). We here found that this one-to-one synaptic relationship was disrupted within one week after transection of the whisker sensory nerve: newly afferent fibers were recruited onto a relay neuron after the nerve transection. Using the Krox20-Ai14 transgenic mouse, in which PrV2-origin afferent fibers are specifically labeled with fluorescent protein, we found that non-PrV2-origin afferent terminals significantly increased in the V2 VPm after the injury, whereas PrV2-origin afferent terminals decreased and weakened around the same time as the synaptic remodeling. Origins of non-PrV2-origin afferent fibers after the transection included the mandibular (V3) subregions of trigeminal nuclei and the dorsal column nuclei, which normally represent body parts other than whiskers. These results indicate that the transection of whisker sensory nerve induces considerable retraction of PrV2-origin afferent fibers and invasion of non-PrV2-origin ones in the V2 VPm, thereby induces large-scale somatotopic reorganization.
We also found tonic $GABA_A$ receptor current was potentiated much earlier than the remodeling of lemniscal fibers onto VPM neurons. Moreover, we found that chronic infusion of tonic GABA agonist into the VPM of normal mice could recruit additional lemniscal fibers onto VPM neurons, whereas lack of tonic $GABA_A$ receptor currents prevented the remodeling of lemniscal fibers after the IONC. These results indicate that enhancement of tonic $GABA_A$ receptor current was crucial for the remodeling of lemniscal fibers after the injury.

(COI: No)