Award Presentations (Poster)

Award Presentations(Poster)
Promotion Award of the Physiological Society of Japan for Young Scientists

SPK-1 (3PK-213)

BDNF secretion regulated by secretory vesicle-associated protein CAPS2

Shinoda, Yo[1,2]; Sadakata, Tetsushi[3]; Furuichi, Teiichi[1,2,4] ([1]*Dept. of Appl. Biol. Sci., Fac. of Sci. and Technol., Tokyo Univ. of Sci., Chiba, Japan;* [2]*JST/CREST, Saitama, Japan;* [3]*Adv. Sci. Res. Leaders Develop. Unit, Gumma Univ. Gumma, Japan;* [4]*RIKEN BSI, Saitama, Japan*)

Calcium-dependent protein for secretion 2 (CAPS2) is known to be associated with the secretion of dense-core vesicles (DCVs) that contain peptides, hormones and proteins. Brain derived neurotrophic factor (BDNF) is one of the most critical protein which is involved in the neural generation, proliferation, differentiation, network construction and plasticity, which is thought to be contained in DCVs. Through the use of CAPS2 knockout (KO) mice, the present study analyzed the role of CAPS2 in BDNF secretion. CAPS2 KO mice had reduced hippocampal BDNF levels, and overexpression of exogenous CAPS2 significantly increased frequency, amplitude, and kinetics of depolarization-induced BDNF vesicle exocytosis in CAPS2 KO hippocampal neurons. The CAPS2 KO hippocampus displayed impaired GABAergic interneuron systems, including decreased GABAergic neuronal numbers in the juvenile stage, decreased number of synaptic vesicles in inhibitory synapses, and reduced frequency and amplitude of miniature inhibitory postsynaptic currents. Moreover, the CAPS2 KO mice exhibited reduced late-phase long-term potentiation (L-LTP) in CA3-CA1 synapses, decreased hippocampal theta oscillation frequencies, and increased anxiety-like behavior. These results suggest that CAPS2 promotes activity-dependent BDNF secretion, which is critical for the formation of a hippocampal GABAergic interneuronal network and their related behavior.

Award Presentations(Poster)
Hiroshi and Aya Irisawa Memorial Promotion Award for Cardiovascular Physiologists

SPK-2

Evaluation of left ventricular mechanical work and energetics of normal hearts in SERCA2a transgenic rats

Zhang, Guo-Xing[1,2]; Obata, Koji[1]; Takeshita, Daisuke[1]; Mitsuyama, Shinichi[1]; Nakashima, Tamiji[3]; Kikuta, Akio[3]; Hirabayashi, Masumi[4]; Tomita, Koichi[4]; Vetter, Roland[5]; Dillmann, Wolfgang H.[6]; Takaki, Miyako[1] ([1]*Dept. Physiol II. Nara Medical Univ. Kashihara, Japan;* [2]*Dept. Physiol. Soochow Univ. Suzhou, China;* [3]*Dept. Anat. Sch. Med. Univ. Occup. & Environ. Health, Kitakyushu, Japan;* [4]*NIPS, Okazaki, Japan;* [5]*Charité-Univ. Berlin, Berlin, Germany.;* [6]*Dept. Med. Univ. California, CA. USA*)

We established two lines of SERCA2a-overexpressed transgenic rats (TGI and II) to analyze cardiac mechanical works and energetics in normal hearts at 300-bpm pacing. Left ventricular (LV) end-systolic pressure (ESP) and systolic pressure-volume area (PVA ; a total mechanical energy per beat) at midrange LVV (mLVV) were significantly larger in TGI rats and were unchanged in TGII rats, compared to those in non-TG (WT) littermates. Myocardial oxygen consumption per minute for E-C coupling was significantly increased, and the mean slope of myocardial oxygen consumption per beat (VO_2)-PVA linear relation was smaller, but the overall O_2 cost of LV contractility for Ca^{2+} is unchanged in all TG rats. Ca^{2+} concentration exerting maximal ESP_{mLVV} in TGII rats was significantly higher than that in WT rats. Ca^{2+} overloading protocol did not elicit mitochondrial swelling in TGII rats. In conclusion, long-term SERCA2a overexpression enhanced or maintained LV mechanics, improved contractile efficiency under higher energy expenditure for Ca^{2+} handling and improved Ca^{2+} tolerance, but did not change overall O_2 cost of LV contractility for Ca^{2+} in normal hearts of TG rats.

Key words : pressure-volume area, SERCA2a, transgenic rat

Award Presentations(Poster)
Hiroshi and Aya Irisawa Memorial Promotion Award for Young Physiologists

SPK-3 (1PK-049)

The deltaC splice-variant of TRPM2 is the hypertonicity-induced cation channel(HICC)in HeLa cells, and the ecto-enzyme CD38 mediates its activation

Numata, Tomohiro[1,2,3]; Sato, Kaori[3]; Christmann, Jens[4]; Marx, Romy[4]; Mori, Yasuo[1,2]; Okada, Yasunobu[3]; Wehner, Frank[4] ([1]*Grad. Sch of Env. Stud, Kyoto Univ. Kyoto, Japan;* [2]*Dep. of Synth. Chem. and Biol. Chem., Kyoto Univ. Kyoto, Japan;* [3]*Dep. of Cell Physiol., Nat. Ins. of Physiol. Sci., Okazaki, Japan;* [4]*Dep. of System. Cell Biol., Max-Planck-Inst. of Mol. Physiol., Dortmund, Germany*)

Hypertonicity-induced cation channels (HICCs) are key-players in proliferation and apoptosis. However, the actual molecular entity of HICCs has not yet been identified. We report that in HeLa cells, intracellular adenosine diphosphate ribose (ADPr) and cyclic ADPr, as activators of TRPM2, elicited cation currents the characteristics of which are identical to those of HICC currents activated by hyperosmolarity. Silencing of TRPM2 and CD38 (as the supposed source of ADPr and cADPr) inhibited hypertonicity- and nucleotide-induced currents and the regulatory volume increase. Systematic analysis of intracellular cADPr and extracellular application of nucleotides revealed that the outwardly directed gradient, rather than the intracellular activity, of ADPr and cADPr triggers activation of TRPM2. Cloning of TRPM2 verified the deltaC-splice variant as the molecular correlate of the HICC, which was supported by quantification of Ca^{2+} selectivity. Pull-down and FRET/FLIM experiments revealed a close proximity of TRPM2 and CD38, and we thus propose a transport related to nucleotide export via CD38 as a novel mechanism of TRPM2 activation.

SPK-4 (1PK-053)

The sensor for the inner membrane lipid modulating the activation gating of the KcsA potassium channel

Iwamoto, Masayuki; Oiki, Shigetoshi (*Dept. Mol. Physiol. Biophys., Univ. Fukui Facult. Med. Sci., Japan*)

The membrane lipids act as cofactor for the function of the ion channel proteins and specific lipid molecules are indispensable for maintaining channel activities. For the KcsA potassium channel, the presence of anionic phospholipids such as phosphatidylglycerol (PG) is prerequisite for the channel activity. Previously we demonstrated by means of single-channel current recordings in the asymmetric lipid bilayer that the PG molecule on the inner leaflet, rather than the outer leaflet, renders the KcsA channel highly active. In this study the lipid effect for the KcsA channel activity was further analyzed. The fluorescent method revealed that the helix-bundle gate is kept open in the PG liposome but not much in the liposomes made of neutral or cationic phospholipids. To elucidate the underlying mechanism of the interaction between anionic lipids on the inner leaflet and the activation gate, charge-neutralizing mutations to positively charged residues were introduced. Several amino acid residues lying at the inner boundary of the membrane were found to be sensitive to the PG effect on the gating. Mechanism underlying lipid-mediated regulation of the activation gating of the KcsA channel will be discussed.

SPK-5 (1PK-072)

Mitochondrial NCX controls directional migration of B lymphocyte

Kim, Bongju[1]; Takeuchi, Ayako[2]; Matsuoka, Satoshi[1] ([1]*Center for Innovation in Immunoregulative Technology and Therapeutics, Gradu ate School of Medicine, Kyoto University, Kyoto, Japan;* [2]*Department of Physiology and Biophysics, Graduate School of Medicine, Kyoto University, Japan*)

To clarify the roles of mitochondria Ca^{2+} handling on chemotaxis of B lymphocyte, we studied CXCL12-induced migration of A20 B lymphocytes. CXCL12 (100 ng/ml) increased transwell migration from 4.6± 0.5% to 12.6±0.6%. This increase was dose-dependently inhibited by CGP-37157 (an inhibitor of mitochondrial Na-Ca exchange (NCX_m, NCLX)). In cells which NCLX was knocked down by siRNA, the transwell migration was inhibited (control siRNA : 7.3±0.2% vs. NCLX siRNA : 1.8±0.2%). These data suggest that NCXm is related to chemotaxis of A20 B lymphocytes. In 8 hrs observation of cell migration without CXCL12, mean displacement of NCLX siRNA transfected cells was larger (21.4±1.5 μm) than control cells (13.5±2.2 μm). Applying CXCL12 gradient did not increase mean displacement and directional migration to chemokine in NCLX siRNA cells. Above data indicate that NCLX knock down accelerates random migration and inhibits directional migration. Intracellular Ca^{2+} was higher in NCLX siRNA cells (fura-2 ratio 0.49±0.01) than the control cells (0.45±0.01, $P<0.05$) in the absence of CXCL12. After 2 hrs CXCL12 stimulation, the intracellular Ca^{2+} increased in control (0.51±0.01) but not in NCLX siRNA cells. Mitochondria redistributed at the rear side (uropod) during migration in control but not in NCLX siRNA cells. Mitochondrial and cytosolic Ca^{2+}, and localization of mitochondria maybe related to NCLX mediated control of migration.

SPK-6 (2PK-013)

Blockade of GABAergic inputs into the RVLM neurons enhances respiratory modulation of the cardiovascular sympathetic nerve in the *in situ* arterially-perfused preparation of rats

Koganezawa, Tadachika; Kitazaki, Hideaki (*Depart. Physiol., Div. Biomed. Sci., Fac. Med., Univ. Tsukuba, Tsukuba, Japan*)

It has been known that neurons in the rostral ventrolateral medulla (RVLM neurons) generate the activity of the cardiovascular sympathetic nerve (SNA), and receive the respiratory modulation from the respiratory center. Recently, we have reported that respiratory-related inhibitory inputs into the RVLM neurons in hypertensive rats are attenuated than that in normotensive rats. However, it is still unclear what kinds of inhibitory inputs are related with the respiratory-related inhibitory inputs. In this study, we evaluated effects of blockade of GABAergic inputs into the RVLM neurons on respiratory modulation of the SNA in the *in situ* arterially perfused preparation of rats. We injected a GABAA receptor antagonist, bicuculline (5 mM, 50 nL), into the RVLM bilaterally, and analyzed the effect on respiratory modulation of the SNA by the phrenic nerve activity-triggered average of the SNA. As a result, blockade of GABAergic inputs into the RVLM neurons elevated the basal SNA and enhanced the respiratory related SNA. The respiratory-phase relation of SNA in the presence of bicuculline in normotensive rats was similar with that in the absence of bicuculline in hypertensive rats. These data may indicate that enhancement of respiratory related modulation of cardiovascular sympathetic nerve in hypertensive rats is caused by attenuation of GABAergic inputs into the RVLM neurons.

Award Presentations(Poster)
Aya Irisawa Memorial Promotion Award for Excellence by Women Physiologists

SPK-7 (2PK-086)

Structural and functional analysis of membrane microdomain as a platform for cell signaling pathway of Ca^{2+}-sensitization of vascular smooth muscle contraction

Kajiya, Katsuko; Nojimoto, Kazutaka; Kishi, Hiroko; Takada, Yuichi; Zhang, Ying; Kobayashi, Sei (*Dept. Mol. Physiol. & Med. Bioreg., Yamaguchi Univ. Grad. Sch. Med., Ube, Japan*)

Abnormal vascular smooth muscle (VSM) contractions such as vasospasm are caused by a Rho-kinase (ROK)-mediated Ca^{2+}-sensitization of VSM contraction. As an upstream mediator of the Ca^{2+}-sensitization, we previously identified sphingosylphosphorylcholine (SPC). The degrees of SPC-induced Ca^{2+}-sensitization correlated well with serum total and LDL-cholesterol (Chol) levels, and inversely with HDL-Chol levels. Furthermore, depletion of VSM Chol destroyed Chol-enriched membrane microdomains such as caveolae and lipid rafts, and abolished the SPC-induced Ca^{2+}-sensitization. However, mechanisms by which SPC transduces the Ca^{2+}-sensitizing signals exclusively through membrane microdomains are unknown. In this study, we tested if SPC preferably interacts with membrane microdomains and affects their structural homeostasis. Firstly, we examined the interaction of human VSM cells with SPC using the surface plasmon resonance measurement. We obtained the first direct evidence that VSM cells have very high affinity for *d*-SPC, but not *l*-SPC, indicating highly structural specificity of SPC. Secondly, we examined the effects of SPC on the surface structure of the VSM cells using scanning and transmission electron microscope. SPC altered dramatically surface structural characteristics of the membrane microdomain. These results support the important role of membrane microdomains such as caveolae and lipid rafts in SPC-induced Ca^{2+}-sensitization of VSM contraction.

SPK-8

Identification of novel voltage-sensing proteins and its biological and biophysical significance

Sasaki, Mari (*Grad. Sch. Med., Ehime Univ., Ehime, Japan*)

We have found two novel voltage sensing domain (VSD) containing molecules without pore domain ; VSP (voltage sensing phosphatase) and VSOP (voltage-sensor only protein). VSP displays channel-like "gating" currents and directly translates changes in membrane potential into the turnover of phosphoinositides. VSP dephosphorirate PIP_2 and PIP_3. This finding indicates that VSD can function beyond channel proteins and thus more ubiquitously than previously appreciated. Recently, voltage-sensor of VSP was used to develop voltage sensitive fluorescent probes. VSOP is another molecule which has a four transmembrane domain similar to the voltage sensor domain of voltage-gated ion channels. We show that VSOP functions as a voltage-gated proton channel even if it does not have pore domain. (VSOP is also called Hv1 or HVCN1.) We also demonstrate that knockout mice of HVCN1 gene show splenomagaly, autoantibodies and nephritis, which are reminiscent of phenotypes of autoimmune disorders. These studies indicate that membrane potential is important not only in neuron or myocyte but also in many cell types including sperm and immune cells.