Neurobiology: From Synapse to Memory

**J-1**

TRPM8 mediated calcium influx in mast cells can induce menthol and cold allergy
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Allergy has long been recognized as an immune disorder in human and deteriorated quality of life. Numerous irritants, named allergen were causing agents of allergy. One of them, menthol and cold temperature can cause allergy and cold uticaria but the molecular mechanism of their action was not known. Among the transient receptor potential channel, subtype melastatin 8 (TRPM8) can respond to menthol and cold. In rat basophilic leukemia mast cell line, RBL-2H3, cold temperature and menthol induced calcium influx. TRPM8 expression was also identified. TRPM8 activation by menthol mediated calcium influx and increase of histamine release about 50% of total histamine content. EC50 of menthol 25-35 treatment, the calcium influx was 2.62 mM and that of histamine release was 1.29 mM. When TRPM8 expression level was reduced by small interfering RNA, menthol and cold temperature induced calcium influx was reduced as well as histamine release. These results implicate menthol and cold temperature induced allergy can be mediated by TRPM8.

**J-2**

TRP channel ligands modulate human platelet aggregation through PLC inhibition
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Activated platelet serves haemostatic functions in a variety of biological situations for example, arterial thrombosis and inflammation. Its aggregation is the key process to achieve the haemostatic functions and fine modulated of the platelet aggregation can be beneficial under some pathological conditions having activated platelets. From a number of natural sensory compounds, we found some suppressor or enhancer compounds for platelet aggregation. We tested the effect of each compound on ADP-induced aggregation of human platelets in vitro and those in vitro suppression effects were compared with the effect of reference drugs (acetylsalicylic acid and cinnamaldehyde). Among tested compounds, camphor was most effective in suppression of ADP-induced platelet aggregation in a concentration-dependent manner. In contrast, menthol showed opposite effects to camphor at millimolar concentration. Those two compounds showed significant change of Ca2+ influx of human platelets upon application and our western blot results shows a couple of sensory transient receptor potential ion channels are expressed in rat and human platelets. These findings suggest camphor and menthol possibly have their effect on platelet aggregation via changes in activity of some ion channels.

**J-3**

The striatum enriched tyrosine phosphatase regulates the dopaminergic neuronal development
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The striatum enriched tyrosine phosphatase (STEP) is highly expressed within dopaminergic neurons in the striatum. The localization of STEP within dopaminergic neurons raised the possibility that STEP may interact with the dopamine receptors. In this study, we investigated the role of STEP on dopamine D2 receptors (D2R)-mediated extracellular signal-related kinase (ERK) signaling pathway. STEP appeared to be dephosphorylated and activated upon the stimulation of D2R. The developmental and regional expression of STEP in mouse brain announced that STEP is probably involved in the control of dopaminergic neuronal development. The treatment of oligomeric STEP siRNA (sSTEP) significantly blunted the quinpirole-induced increase in the number of dopaminergic neurons from WT mice, while this was not detected in primary mesencephalic cultures from D2R-/- mice. Furthermore, the silencing of STEP expression perturbed D2R-mediated ERK signaling in dopaminergic neuronal cells from WT mice, but not in D2R-/- mice. These results suggest that the regulation of ERK signaling by STEP plays a critical role in D2R-mediated dopaminergic neuronal development. [This work was supported by basic research grant from KOSSEP (Grant No. R01-2004-000-10671-0) and by a grant (Grant No. M1039V001014-06K2201-0140) from Brain Research Center of the 21st Century Frontier Research Program funded by the Korean Ministry of Science and Technology.]

**J-4**

The interaction between the ankyrin-rich membrane spanning protein and the septin Sept5/CDCCrel-1.
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Neurotrophins are very important for regulating neuronal plasticity, survival and differentiation in the nervous system. They bind to two structurally different receptors, the Trk receptor tyrosine kinase and the p75 neurotrophin receptor, which are closely associated. Trk and p75 receptors utilize specific adaptor proteins to transmit signals into the cell. An ankyrin-rich membrane spanning protein (ARM60) or Kisd2/c220, originally identified as a p75 interacting protein and as a PKD substrate, serves as a novel downstream target of Trk receptor tyrosine kinase. In order to identify proteins interacting with ARMS, we performed a yeast two hybrid screening. We found out that C-terminus of ARM60 specifically interacts with Sept5/CDCCrel-1, a mammalian septin. Sept5 binds to and inhibits the SNARE-protein syntaxin, thereby negatively modulating neurotransmitter release. Co-immunoprecipitation using lysates from transiently transfected HEK-293 cells showed the specific interaction between ARMS and Sept5. ARMS and Sept5 also interact with each other endogenously in primary hippocampal neurons. In addition, we mapped the Sept5 domains involved in binding to ARMS. The direct interaction between ARMS and Sept5/CDCCrel-1 suggests the possible role of septin in neurotrophin-mediated intracellular signaling events, such as neurotransmitter release. This research was supported by a grant (M-SC) from the Korea Research Foundation, Republic of Korea.

**J-5**

The change of synaptic proteins at pre- and post-synapse by AG25-35 treatment leads to loss of synaptic function in rat organotypic hippocampal slice cultures
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Accumulation of amyloid β(Aβ) in Alzheimer’s disease(AD) is the primary driver of neurodegeneration and cognitive decline leading to dementia. Dementia is attributed not only to the neuronal cell death, but also to a synapse failure including pre- and post-synaptic functional loss. In presynapse, synaptophysin, a specific marker for presynaptic terminals is an integral membrane protein localized to synaptic vesicles. SNAP25 is a presynaptic plasma membrane protein, which acts as one component of core complex in synaptic vesicle docking and fusion. A major component of postsynaptic density at the postsynaptic is a CaMK II, which interacts with the NR2B subunits of NMDA receptors as well. Other substrate of CaMK II is GluR1 of AMPA receptors implicated in neuronal plasticity and memory formation. To study the effect of Aβ25-35 on synaptic loss in OHSaL, western blot or immunofluorescence staining and electron microscopy were used. By Aβ25-35 treatment, the immunofluorescence levels of SNAP25, synaptophysin, NR2B and PS25, decreases in the stratum lucidum of CA3 and membrane layer of dentate gyrus(DG). The fluorescence of GluR1 and CaMK II are concentrated in the cell bodies of pyramidal layer, but the total protein levels are not significantly changed in a western blotting analysis. In the electron microscopic observation, dying cells, degenerating myelin and damaged synapses are observed. These results indicate that Aβ25-35 induces change of synaptic proteins at presynapse and postsynapse suggesting synaptic dysfunction in AD. [Supported by grants from Korean Research Foundation R04-2004-000-10019-0]

**J-6**

Structural constraint of the loop connecting two RCK domains in BKCa channel revealed by mutational analysis
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Large-conductance calcium-activated potassium (BKCa) channels play a key role in modulating many important physiological processes. These channels are dually activated by intracellular Ca2+ and membrane voltage. Calcium-dependent gating of mammalian BKCa channels is mediated by intracellular carboxyl terminus containing two domains of the regulator of K+ conductance (RCK). Although the two RCK domains (RCK1 and RCK2) are separated by a protein segment of 101 residues conserved poorly and predicted to have no regular secondary structure, the direct interaction between the two RCK domains is known to be essential for Ca2+-dependent activation of the channel. In order to understand the functional importance of this segment, we constructed a series of deletion mutations and expressed the mutant channels in a heterologous system. When we investigated the effects of the mutations using electrophysiological means, we found that the length rather than the specific sequence of the segment is critical for the functionality of the channel. These results set a minimum length connecting the two RCK domains and provide structural constraints for the ‘gating ring’ composed of eight RCK domains rendered by the tetrameric channel. We are currently working on the functional reconstitution of BKCa channel from two polypeptides disconnected at the segment by expressing simultaneously.

Poster Session