S 3-2

ACTIVATION MECHANISMS OF TRPV1

Uhtaek Oh

Sensory Res Center, CRI, College of Pharmacy, Seoul National University, Seoul, Korea

TRPV1, a cloned capsaicin receptor, is a molecular sensor for detecting adverse stimuli and a key element for inflammatory nociception and represents biophysical properties of native channel. Although the physiological role or biophysical properties of VR1 are known, its activation mechanisms by ligands are poorly understood. Here, we show that TRPV1 requires phosphorylation by Ca^{2+}-calmodulin-dependent kinase II (CaMKII) for its activation by capsaicin. In contrast, dephosphorylation by calcineurin, leads to desensitization of the receptor. Point mutation of TRPV1 at two putative consensus sites for CaMKII fails to elicit capsaicin-sensitive currents with concomitant reduction in phosphorylation of TRPV1 *in vivo*. The mutant also lost the high-affinity binding of ^{3}H-resiniferatoxin, a potent capsaicin-receptor agonist. We conclude that the dynamic balance between phosphorylation and dephosphorylation of the channel by CaMKII and calcineurin controls the activation/desensitization state by regulating the binding property. Furthermore, since sensitization by protein kinase A and C converges on these sites, phosphorylation stress in the cell appears to control a wide range of excitability in response to various adverse stimuli. In addition, we introduce an association protein that controls activity of TRPV1. Even though TRPV1 retains similar channel properties as that of native capsaicin receptor in sensory neurons, there appears to be a marked difference between TRPV1 and native capsaicin receptors in the pharmacological response profiles to vanilloids or acid. One plausible explanation for this overt discrepancy is the presence of regulatory proteins associated with TRPV1. Here, we identify Fas-associated factor 1 (FAF1) as a regulatory factor, which is co-expressed with and binds to TRPV1 in sensory neurons. When expressed heterologously, FAF1 reduces the responses of TRPV1 to capsaicin, acid and heat, to the pharmacological level of native capsaicin receptor in sensory neurons. Furthermore, silencing FAF1 by RNA interference augments capsaicin-sensitive current in native sensory neurons. We therefore conclude that FAF1 forms an integral component of the vanilloid receptor complex, and that it constitutively modulates the sensitivity of TRPV1 to various noxious stimuli in sensory neurons.

Key Words: TRPV1, FAF1, CAMKII, Pain

S 3-3

ROLES OF PREFRONTAL AND CINGULATE SYNAPTIC PLASTICITY IN BEHAVIORAL FEAR MEMORY AND PERSISTENT PAIN

Min Zhuo

Department of Physiology, Faculty of Medicine, University of Toronto Centre for the Study of Pain, University of Toronto, Toronto, Canada

Neuronal synapses in the central nervous systems are plastic, and can undergo long-term changes throughout life. Studies of molecular and cellular mechanisms of such changes not only provide important insight into how we learn and store new knowledge in our brains, but also reveal the mechanisms of pathological changes occurring following an noxious stimulus such as pain and fear. Using integrative approaches including genetic, pharmacological, electrophysiological and behavioral studies, we explore the synaptic mechanisms for LTP and LTD in the cingulate and prefrontal cortex of adult mice. We found that activation of postsynaptic NMDA receptor is required for the induction of synaptic LTP. The expression of cingulate LTP is likely mediated by postsynaptic AMPA receptors, while presynaptic form of paired-pulse facilitation remained unchanged during synaptic potentiation. Activation of calcium-calmodulin stimulated adenylyl cyclase AC1 is required for the induction of LTP. Similar to the hippocampus, NMDA NR2A subtype receptor is required for the induction of LTP. NMDA NR2B receptors, however, also contribute to synaptic potentiation. Genetic reduction of NR2B expression or pharmacological inhibition of NR2B receptor by selective antagonists reduced behavioral contexual fear memory. The possible contribution of the ACC to the formation of fear memory is its role in pain perception. Supporting this hypothesis, inhibition of NMDA NR2NB receptors in the ACC inhibited behavioral sensitization to non-noxious stimuli. Our results provide strong evidence that synaptic potentiation within the cingulate/prefrontal cortex play important roles physiological and pathological responses to noxious sensory stimuli and injury, including emotional fear and persistent pain.