[S1-4] *[4/21/2000 (Fri) 11:50~12:30/Byulkwan Auditorium, Bldg 26]*

Intracellular ATP Increases the Activity of Capsaicin-activated Channels in a Phosphorylation-independent Way

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Capsaicin (CAP) is the pungent ingredient of red hot peppers and excites small sensory neurons, causing pain or neurogenic inflammation. The excitation of sensory neurons by CAP is associated with influxes of cations. A ligand-gated, non-selective cation channel specifically activated by CAP was identified in cultured DRG neurons (Oh et al., 1996). The outward-rectifier with slope conductance of 45 pS at 60 mV accounts largely for the current response of sensory neurons to CAP. Unlike other ligand-gated channels, however, for fast synaptic transmission, CAP binds to the intracellular sites of the channel (Jung et al., 1999). A cDNA (VR1) encoding for a channel sensitive to CAP was cloned (Caterina et al., 1997). When expressed heterologously, VR1 resembles the native channels in sensory neurons in many respects such as channel properties. One of the striking properties of VR1 is its activation by temperature in noxious heat range (Tominaga et al., 1998). Recently, lipids such as anandamide or various metabolic products of lipoxygenases such as 12-hydroperoxyeicosatetraenoic acid or leukotriene B_4 activate the channel (Hwang et al., 2000). Three-dimensional structures of some metabolic products of lipoxygenases fit well to that of capsaicin, providing evidence that these lipids would be endogenous capsaicin-like substances. Therefore, the channel is now thought to be a chemical sensor as well in mediating nociception.

Activity of ion channels is often modulated by intracellular signals such as kinase actions. Thus, modulation of channel activity by phosphorylation is one way of controlling cell excitability by intracellular signals in excitable cells. In the present study, we investigated whether ATP modulates the activity of CAP-channel via phosphorylation. Intracellular application of ATP, one of major energy metabolites, augments greatly the activity of CAP-

activated channels (*icap)* in isolated membrane patches. The augmenting effect of ATP is Mg^{2+}-independent and is not blocked by various kinase inhibitors. Non-hydrolyzable analogs of ATP such as AMPPNP and ATPγS also augmented *icap*. Other nucleotides including GTP failed to increase *icap*. EC_{50} of ATP in augmenting *icap* was 1.6 mM and thus much higher than required as a substrate for protein kinase. Unlike our initial expectation, these results suggest that, intracellular ATP augments the *icap* in a phosphorylation-independent way, possibly by acting as an allosteric regulator.

If ATP increases *icap* by allosteric binding to the channel protein, we would expect that the possible presence of nucleotide-binding site in the CAP channel. The cloned CAP channel, VR1, contained two homology sequences of nucleotide binding sites, the Walker A and B motifs (Walker et al., 1982), in each cytosolic terminal. We, therefore, mutated VR1 on each nucleotide-binding motif to clarify the implication of ATP binding. We firstly tested whether the augmenting effect of ATP was present in VR1. CAP evoked single-channel currents in an inside-out excised membrane patch isolated from *Xenopus* oocyte expressing wild type VR1. In this patch, the addition of 2 mM ATP greatly augmented *icap* by 232% ($p < 0.005$, $n = 7$), suggesting that the augmenting effect of ATP is also present in the cloned channel. We now found that VR1 contained the two putative nucleotide-binding consensus sequences, the Walker A- and B-type motifs. The Walker A motif forms glycine-rich, phosphate-binding loop and its terminal lysine is invariant. We, therefore, substituted the lysine to arginine (VR1-K735R) to investigate the role of Walker A motif in the augmenting effect of ATP in VR1. In oocytes expressing VR1-K735R, CAP activated *icap* as normally observed in wild type VR1. But, the mutant (VR1-K735R) completely blocked the augmenting effect of ATP. The Walker B motif is identified generally by a stretch of four to six hydrophobic amino acids followed by a highly conserved aspartic acid (Walker et al., 1982). In addition, mutation at this invariant aspartate in the putative Walker B motif to asparagine (D178N) also blocked the augmenting effect of ATP. Oocytes expressing a VR1 mutant having mutations at both Walker-type A and B motifs (D178N/ K735R) blocked the ATP augmenting effect ($n = 12$). These results clearly indicate that the augmenting effect of ATP requires binding of ATP to the channel at these loci.

The allosteric modulation of *icap* by intracellular ATP would contribute to controlling the sensitivity of the CAP channel evoked by noxious stimuli. Although we observed the allosteric modulation of the channel by ATP, we can not exclude the possibility that intracellular ATP helps the CAP receptors undergo changes in activity via phosphorylation or dephosphorylation

(Oh et al., 1998). Therefore, it is highly likely that intracellular ATP would help to set the sensitivity of the channel via allosteric modulation as well as conformational changes by phosphorylation of the channels. The precise physiological significance, however, of the ATP modulation of the channel in sensory neurons is yet to be studied further.

References

- Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997). *Nature* 389: 816-824.
- Hwang SW, Cho H, Kwak J, Lee S-Y, Kang C-J, Jung J, Cho S, Min KH, Suh Y-G, Kim D, Oh U (2000) *Proc Natl Acad Soc USA (in press)*
- Jung J, Hwang SW, Kwak J, Lee S-Y, Kang C-J, Kim WB, Kim D, Oh U (1999) *J Neurosci* 19: 529-538.
- Oh U, Hwang SW, Kim D (1996) *J Neurosci* 16: 1659-1667.
- Oh U, Shin JS, Hwang SW, Cho HW, Jung JY, Kwak J, Kim M, Lee S-Y, Kang CJ (1998) *Soc Neurosci Abstr* 24: 727.7
- Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D (1998) *Neuron* 21: 531-543.
- Walker J, Saraste M, Runswick MJ, Gay NJ (1982) *EMBO J* 1: 945-951.