[12:00～12:40]

Inflammatory Signals to TRPV1, the Capsaicin Channel

Uhtaek Oh
Sensory Research Center, *CRI,*
Seoul National University, College of Pharmacy, Seoul, Korea

Capsaicin (CAP) is a pungent ingredient in hot peppers. CAP has a unique action on the pain sensory system. CAP causes a pain when applied to the skin. The hyperalgesic action of CAP is mediated by the excitation of sensory neurons. CAP is known to activate ion channels that allow cation influxes, thus, depolarizing sensory neurons. Recently, we identified a CAP-activated ion channel and described properties of the channels (Oh et al., 1996). The channel is a ligand-gated channel and permeable to cations. In addition, we found that CAP, the ligand, binds to the intracellular side of the channel. Recently, the channel is cloned and dubbed as VR1 (vanilloid receptor 1) (Caterina et al., 1997). Primary structure of VR1 shows that VR1 belongs to transient receptor potential (TRP) channel family, having 6 transmembrane domains with two long cytosolic amino acid sequence in each N- or C- terminus. The presence of the CAP channels in sensory neurons evidently raises a question what is the endogenous ligand of the receptors.

Single-Channel Properties of CAP Channel

Single-channel currents activated by CAP are sought to identify the CAP-activated channels in cultured sensory neurons isolated from 1-2 day old neonatal rats (Oh et al., 1996). In solution containing 140 mM Na^+ as a charge carrier, extracellular application of CAP to outside-out patches causes a great activation of an ion channel in a concentration-dependent manner (EC_{50}, 1.1 μM), that is blocked by the CAP antagonist, capsazepine. The channel is also activated by 2-10 nM resiniferatoxin, a potent analog of CAP. In symmetrical 140 mM Na^+, the single-channel slope conductances are 45 and 80 pS at -60 and +60 mV, respectively, showing outward rectification. The reversal potential does not shift significantly when Na^+ is replaced by

K^+, Cs^+, Rb^+ or Li^+, indicating that the channel discriminates poorly among cations. The channel is also permeable to Ca^{2+}. The channel is classified as a ligand-gated ion channel because openings of the channel continues after forming isolated membrane patches where soluble cytosolic components are lacking. This means the channel is directly activated by ligands, not through the actions of second messengers or other proteins.

Thus, these experiments clearly demonstrate the presence of the CAP-activated ion channel in DRG neuron and describe some of the important properties of the CAP channel. Openings of the channel give rise to the whole-cell current activated by CAP and thus are responsible for the excitation of sensory neurons.

Agonist-Recognition Sites in the Cytosolic Tails of VR 1

Although the function and the biophysical properties of the channel are now substantially known, the regions of VR1 that recognize ligands are largely unknown. Because capsaicin acts on VR1 at the intracellular side, certain regions in the cytosolic tails are implicated as a potential binding site. By the stepwise deletion of VR1, we localized two charged amino acids in the N- and C-cytosolic tails that determined ligand binding (Jung et al., 2002). Point mutations of the two residues resulted in a loss of sensitivity to capsaicin, and a concomitant loss of specific binding to [^{3}H]-resiniferatoxin, a potent vanilloid. Furthermore, changes in the charges of the two amino acids stopped capsaicin-sensitive currents and ligand binding without affecting current responses to heat. Thus, these two regions in the cytoplasmic tails of VR1 provide structural elements for hydrophilic interaction with vanilloids, and might constitute a long-suspected binding pocket together with the third transmembrane domain, which is implicated in the hydrophobic interaction between VR1 and vanilloids.

Products of Lipoxygenases as Endogenous Activators

Although the presence of CAP receptor and its functional role in the pain sensory system are now known, an endogenous activator of the receptor has not yet been found. Furthermore, the hyperalgesic neural response such as c-fos expression in the dorsal horn of the spinal cord induced by inflammation is blocked by capsazepine

(Kwak et al., 1998), a CAP receptor blocker (Bevan et al., 1992), suggesting that an endogenous capsaicin-like substance is produced and causes hyperalgesia by opening capsaicin-activated channels. Because the channel is activated when CAP and other ligands are applied to the intracellular side of patches, thus, the endogenous ligands are likely present in the cell. We tested many intracellular messengers on the CAP channel to determine whether they activate the channel. We now identify that products of lipoxygenases (LO) are capable of activating the channel.

Interestingly enough, products of LOs are implicated in mediating inflammatory nociception because various LO products are produced during inflammation (Samuelsson, 1983) and cause hyperalgesia when injected intradermally (Levine et al., 1984; Levine et al., 1986). In addition, products of LOs often function as intracellular messengers in neurons. Among their actions, products of LOs act directly on K^+ channels in *Aplysia* sensory neurons (Piomelli et al., 1987) and mammalian cardiac muscle cells (Kim et al., 1989).

Here we present evidence that products of LOs directly activate the CAP receptor in isolated membrane patches of sensory neurons (Hwang et al., 2000). When applied to the bath of inside-out patches, 12-hydroperoxytetraenoic acid (12-HPETE) activates single-channel currents that were sensitive to capsazepine in isolated membrane patches. The IV curve of single-channel currents activated by 12-HPETE is outwardly-rectifying and identical to that obtained by CAP. The amplitude of single-channel currents activated by both 12-HPETE and CAP are not different. Theses results indicate that the channel currents activated by 12-HPETE are identical to those activated by CAP. The channels activated by 12-HPETE are permeable to various cations. LO products also activate VR1, the cloned CAP receptor, expressed HEK293 cells. Products of LOs other than 12-HPETE also activated the CAP channels. Among them, 12- and 15-HPETE, 5- and 15-(S)-hydroxyeicosatetraenoic acids, and leukotriene B_4 possess the highest potency. Dose-response relationships reveal that the potencies of 12-HPETE, 15-HPETE, leukotrien B4, and 5-HETE are 8.0, 8.7, 9.2, and 11.7 μM, respectively, showing much lower potency than CAP. Anandamide, the endogenous ligand for cannabinoid receptors also activates the channel with half-maximal dose of 11.7 μM. Because prostaglandins (PGs) are known to be related to pain, various PGs are

applied to the CAP receptors. PGs, however, fail to activate the channel. Other saturated or unsaturated fatty acids are also tested for its activation of CAP channels. They all fail to activate the channels.

Results of our study indicate that CAP and various eicosanoids act on the capsaicin receptor, suggesting a structural similarity between CAP and eicosanoids. Thus, structures of eicosanoids and CAP in the energy-minimized state are superimposed to compare three-dimensional structures. Three-dimensional structures of 12-(S)-HPETE, 15-(S)-HPETE, 5-(S)-HETE, and LTB_4 are compared with that of CAP. To do this, we extract structures in the energy-minimized state first using SYBYL molecular mechanics and then aligned the structures using a genetic similarity-algorithm program (GASP). Interestingly, CAP in the energy-minimized state fits well to the S-shaped 12-HPETE. In particular, the phenolic hydroxide and amide moieties in CAP overlap precisely with the carboxylic acid and hydroperoxide moieties in 12-HPETE, respectively. The two key regions in CAP or 12-(S)-HPETE are known to have dipolar property that allows hydrogen bond interactions with the CAP receptor (30). In addition, the aliphatic chain region of the 12-(S)-HPETE fits well with the alkyl chain of CAP. In contrast, 15-HPETE, 5-HETE and LTB_4, shared less structural similarity with CAP (Hwang et al., 2000).

Bradykinin signaling pathway: Upstream signal to the 12-LO/VR1 pathway

Because products of LO activate the channel, it seems obvious to ask what stimulates the LO/VR1 pathway in order to cause pain. Although bradykinin (BK) is a powerful pain causing inflammatory mediator, but its activation mechanism of sensory neurons is not known. Because BK releases arachidonic acid, a key substrate for LO in sensory neurons, we hypothesized that BK activates VR1 via the PLA_2/LO pathway. In order to prove the hypothesis, we performed electrophysiological experiments, Ca^{2+}-imaging, and chemical analysis of LO products. As a result, we observed that BK-evoked whole-cell currents recorded from sensory neurons were significantly reduced by capsazepine (CZP), a capsaicin receptor antagonist. In the skin nerve preparation, CZP and quinacrine, a PLA_2 inhibitor, and NDGA, a LO inhibitor reduced BK-induced excitation of sensory nerves. In addition, quinacrine, NDGA and CZP blocked BK-induced Ca^{2+}-influx. To examine if sensory neurons can, in fact, release the lipid

products of LO by BK, we used HPLC-coupled with radioisotope to detect the lipid products. As results, we confirmed that 12-HETE, an immediate downstream metabolite of 12-HPETE was indeed released from sensory neurons after the BK application (Shin et al., 2002).

This study demonstrates that bradykinin excites sensory nerve endings by activating VR1 via production of 12-LO metabolites of arachidonic acid by activated PLA_2. This finding identifies a mechanism that might be targeted in the development of new therapeutic strategies for the treatment of inflammatory pain.

Histamine signaling pathway: In addition, we present unequivocal evidence that histamine, another inflammatory mediator, also uses the PLA2/LO/VR1 pathway for excitation of sensory neurons. Because histamine is a major pruritogenic (itch causing) substance, identification of the histamine signaling pathway is much helpful to developing anti-pruritogenic substance to cure itch sensation in atopic dermatitis patients.

References

Bevan S, Hothi S, Hughes G, James IF, Rang HP, Shah K, Walpole CSJ, Yeats JC Capsazepine: a competitive antagonist of the sensory neurone excitant capsaicin. *Br J Pharmacol* 107, 544-552 (1992).

Docherty, R.J., Yeats, J.C., Bevan, S., and Boddeke, H.W. Inhibition of calcineurin inhibits the desensitization of capsaicin-evoked currents in cultured dorsal root ganglion neurones from adult rats. *Pflugers Arch* 431, 828-837 (1996).

Hwang, S.W., Cho, H., Kwak, J., Lee, S.-Y., Kang, C.-J., Jung, J., Cho, S., Min, K.H., Suh, Y.-G., Kim, D. and Oh, U. Direct activation of capsaicin receptors by products of lipoxygenases: Endogenous capsaicin-like substances. *Proc. Natl. Acad. Sci.* (USA) 97(11):6155-6160 (2000).

Jung J, Lee S-Y, Hwang SW, Cho H, Shin J, Kang Y-S, Kim S, and **Oh U.** Agonist Recognition Sites in the Cytosolic Tails of Vanilloid Receptor 1. *J Biol Chem* 277: 44448-44454 (2002).

Kim, D., Lewis, D.L., Graziadei, L., Neer, E.J., Bar-Sagi, D. and Clapham, D.E. G-protein βγ-subunits activate the cardiac muscarinic K+-channel via phospholipase A_2. *Nature* 337, 557-560 (1989).

Kwak, J.Y., Jung, J.Y., Hwang, S.W., Lee, W.T. and Oh, U. A capsaicin-receptor antagonist, capsazepine, reduces inflammation-induced hyperalgesic responses: evidence for an endogenous capsaicin-like substance. *Neuroscience* 86, 619-616 (1998).

Levine, J.D., Lau, W. and Kwiat, G. Leukotriene B4 produces hyperalgesia that is dependent on polymorphonuclear leukocytes. *Science* 225, 743-745 (1984).

Levine, J.D., Lam, D., Taiwo, Y.O., Donatoni, P. and Goetzl, E.J. Hyperalgesic properties of 15-lipoxygenase products of arachidonic acid. *Proc Natl Acad Sci USA* 83, 5331-5334 (1986).

Oh, U., Hwang, S.W. and Kim, D. Capsaicin Activates a Non-selective Cation Channel in Cultured Neonatal Rat Dorsal-root Ganglion Neurons. *J. Neurosci.* 16(5), 1659-1667 (1996).

Oh, U., Shin, J.S., Hwang, S.W., Cho, H.W., Jung, J.Y., Kwak, J.Y., Kim, M.R., Lee, S.Y. and Kang, C.J. The activity of a capsaicin-activated channel is reduced by dephosphorylation. Soc. Neurosci., 24(2), 721.7 (1998).

Piomelli, D., Volterra, A., Dale, N., Siegelbaum, S.A., Kandel, E.R., Schwartz, J.H. and Belardetti, F. Lipoxygenase metabolites of arachidonic acid as second messengers for presynaptic inhibition of *Aplysia* sensory cells. *Nature* 328, 38-43 (1987).

Samuelsson, B. Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science* 220, 568-575 (1983).

Shin J, Cho H, Hwang SW, Jung J, Shin CY, Lee S-Y, Kim, SH, Lee M-G, Choi YH, Kim J, Haber NA, Reichling DB, Khasar S, Levine JD, and Oh U. Bradykinin-12-lipoxygenase-VR1 signaling pathway for inflammatory hyperalgesia. *Proc Natl Acad Sci USA* 99(15): 10150-10155 (2002).

S13 14:00~14:10 November 12, 2004 (Fri)

Recent Topics of Genetic Polymorphism in Drug Therapy

Tetsuo Satoh (Chiba University, Japan)

S14 14:10~14:50 November 12, 2004 (Fri)

Current Status and Perspective on the Development of Therapeutic Drugs for Alzheimer's Disease

Yoshiharu Yamanishi (Eisai Company, Japan)

S15 14:50~15:30 November 12, 2004 (Fri)

S-Ally-L-Cysteine, a Garlic Compound, Selectively Protects Cultured Hippocampal Neurons from ER Stress-Induced Neuronal Death

Yoshihisa Ito (Nihon University, Japan)