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Molecular Mechanisms of Trigeminal Nociception and Sensation of Pungency

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Cloning and characterization of capsaicin receptor TRPV1

One characteristic shared by many nociceptive neurons is sensitivity to capsaicin, the main pungent ingredient of 'hot' chili peppers (Szallasi and Blumberg, 1999). When a capsaicin receptor was isolated using a Ca^{2+}-imaging-based expression cloning method, it was designated vanilloid receptor subtype 1 (VR1) because a vanilloid moiety constitutes an essential chemical component of capsaicin (Caterina *et al.*, 1997). Capsaicin receptor TRPV1 is a member of the large TRP (transient receptor potential) superfamily of ion channels (Clapham, 2003), whose prototypical member, TRP, was found to be deficient in a *Drosophila* mutant exhibiting abnormal responsiveness to continuous light (Montell and Rubin, 1989). Patch-clamp recordings from HEK293 cells expressing TRPV1 revealed that capsaicin-activated TRPV1 currents exhibit non-selective cation permeability with outwardly rectifying current-voltage relationship. TRPV1 can be activated by at least three different pain-producing stimuli—capsaicin, heat (>43°C) or protons (acidification)—all of which are known to cause pain *in vivo* (Caterina *et al.*, 1997; Tominaga *et al.*, 1998). In addition to capsaicin, piperine and gingerone, the main pungent ingredient of black pepper and ginger respectively, were found to activate TRPV1. TRPV1 transcript and protein were found to be most highly expressed in sensory neurons including trigeminal neurons. Moreover, *in situ* hybridization and immunostaining revealed that, within dorsal root and trigeminal sensory ganglia (DRG and TG, respectively), TRPV1 expression predominated in small diameter cell bodies, most of which give rise to unmyelinated C-fibers. Furthermore, analyses of mice lacking TRPV1 have shown that TRPV1 is essential for selective modalities of pain sensation and for tissue injury-induced thermal hyperalgesia (Caterina *et al.*, 2000). Thus, TRPV1 is a central molecule for detecting nociceptive stimuli.

Sensitization of TRPV1

Inflammatory pain is initiated by tissue damage/inflammation and is characterized by hypersensitivity both at the site of damage and in adjacent tissue. One mechanism underlying these phenomena is the modulation (sensitization) of ion channels such as TRPV1. Sensitization is triggered by extracellular inflammatory mediators that are released *in vivo* from surrounding damaged or inflamed tissues and from nociceptive neurons themselves (i.e. neurogenic inflammation) (Julius and Basbaum, 2001). Mediators known to cause sensitization include prostaglandins, adenosine, serotonin, bradykinin, ATP and some proteinases. Among the inflammatory mediators, extracellular ATP, bradykinin and pryptase or trypsin have been reported to potentiate TRPV1 responses through metabotropic $P2Y_2$, B2 and proteinase-activated receptor 2 (PAR2) receptors, respectively, in a PKC-dependent manner in both a heterologous expression system and native sensory neurons (Tominaga *et al.*, 2001; Sugiura *et al.*, 2002; Moriyama *et al.*, 2003; Dai *et al.*, 2004). In addition to potentiating capsaicin- or proton-evoked currents, ATP, bradykinin or PAR2 agonists also lower the temperature threshold for heat activation of TRPV1 to as low as 30°C, such that normally non-painful thermal stimuli (i.e. normal body temperature) are capable of activating TRPV1. This represents a novel mechanism through which the large amounts of ATP, bradykinin, trypsin or tryptase released from different cells in inflammation might trigger a sensation of pain. Two serine residues in the cytoplasmic domain of TRPV1 were identified as substrates for PKC-dependent phosphorylation (Numazaki *et al.*, 2002). A PKA-dependent pathway also seems to be involved in TRPV1 sensitization, and PKA-dependent phosphorylation of serine and threonine residues on TRPV1 has been reported (Bhave *et al.*, 2002; Rathee *et al.*, 2002). In addition, a reduction of direct PIP_2-mediated TRPV1 inhibition and generation of lipoxygenase derived products through PLA_2 activation are also reported to be involved in the potentiation of TRPV1 activity following Gq-coupled receptor activation (Chuang *et al.*, 2001; Shin *et al.*, 2002).

Tissue acidification is induced in pathological conditions, and such acidification exacerbates or causes pain. In addition to the direct activation of TRPV1, acidification also shifts temperature-response curve of TRPV1 to the left so that the channel can be activated at lower temperatures (lower than body temperature) and responses to heat are bigger at a given suprathreshold temperature (Tominaga *et al.*, 1998). This phenomenon might also contribute to inflammatory pain.

TRPV1 activation and induction of nociceptive response by a non-pungent capsaicin-like compound, capsiate

Capsiate has been extracted from a non-pungent cultivar of red pepper, CH-19 sweet, and shown to be a capsaicin analogue called capsinoid that has an ester bond instead of the amide bond between vanillyl moiety and fatty acid chain (Kobata *et al.*, 1999). The burning sensation we feel when eating hot chili peppers is attributed to the activation of capsaicin receptors on sensory neurons innervating the oral cavity. It is likely that capsiate is non-pungent either because it does not activate TRPV1 efficiently or because it cannot gain access to TRPV1-expressing sensory neurons from the mouth cavity. To elucidate the mechanisms underlying the non-pungency of capsiate, we examined whether capsiate activates TRPV1 and excites sensory neurons. First, capsaicin and capsiate were applied to the oral cavity of mice (Iida *et al.*, 2003). The number of pungency-related behaviors, chin rubbing, chip digging or gaping after capsaicin application was significantly greater than that observed upon capsiate application, indicating that capsiate does not cause pungency-related behaviors in mice. To confirm the inability of capsiate to excite sensory neurons whose cell bodies are located in TG, we performed an eye irritancy assay. The number of wiping reactions was significantly smaller in mice treated with capsiate compared with capsaicin, indicating that capsiate does not cause eye irritancy. However, patch-clamp experiments revealed that capsiate activates TRPV1 expressed in HEK293 cells as well as causes inward currents in rat DRG neurons (Iida *et al.*, 2003). Capsiate injection into a hindpaw induced nociceptive behavior but failed to induce classical signs of irritation when applied to the mouth or skin. Capsiate seemed not to reach the trigeminal nerve endings when

applied to mouth. Detailed analyses of capsiate revealed that it exhibits high lipophilicity and instability in the aqueous condition, suggesting that high lipophilicity and instability might be critical determinants for the pungency.

Thermosensitive TRP channels

We feel a wide range of temperatures spanning from cold to heat. Within this range, temperatures >~43°C and <~15°C evoke not only a thermal sensation, but also a feeling of pain. In mammals, six thermosensitive ion channels have been reported, all of which belong to the TRP super family. These include TRPV1 (VR1), TRPV2 (VRL-1), TRPV3, TRPV4, TRPM8 (CMR1) and TRPA1 (ANKTM1) (Patapoutian *et al.*, 2003). These channels exhibit distinct thermal activation thresholds (>43°C for TRPV1, >52°C for TRPV2, >~34–38°C for TRPV3, >~27–35°C for TRPV4, <~25–28°C for TRPM8 and <17°C for TRPA1) and are expressed in primary sensory neurons, including trigeminal ones as well as other tissues. Some of the thermosensitive TRP channels have been found to be activated by pungent chemical substances in our mouth; capsaicin for TRPV1, menthol for TRPM8 and allyl isothiocyanate (main ingredient of wasabi and mustard oil) for TRPA1 (Caterina *et al.*, 1999; McKemy *et al.*, 2002; Peier *et al.*, 2002; Bandell *et al.*, 2004; Jordt *et al.*, 2004). Other thremosensitive TRP channels expressed in trigeminal neurons could have such chemical stimuli.

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