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Molecular and Cellular Mechanisms of Trigeminal Chemosensation

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Abstract

Three sensory systems, olfaction, taste, and somatosensation, are dedicated to the detection of chemicals in the environment. Trigeminal somatosensory neurons enable us to detect a wide range of environmental stimuli, including pressure, temperature, and chemical irritants, within the oral and nasal mucosa. Natural plant-derived irritants have served as powerful pharmacological tools for identifying receptors underlying somatosensation. This is illustrated by the use of capsaicin, menthol, and wasabi to identify the heat-sensitive ion channel TRPV1, the cold-sensitive ion channel TRPM8, and the irritant receptor TRPA1, respectively. In addition to TRP channels, members of the two-pore potassium channel family have also been implicated in trigeminal chemosensation. KCNK18 was recently identified as a target for hydroxy- α -sanshool, the tingling and numbing compound produced in Schezuan peppers and other members of the *Xanthoxylum* genus. The role of these channels in trigeminal thermosensation and pain will be discussed.

Keywords

chemosensation; trigeminal neuron; somatosensation; chemical irritant; TRP channel

Introduction

Our chemical senses include somatosensation, gustation, and olfaction. Trigeminal somatosensory neurons mediate the detection of thermal, mechanical, and chemical stimuli in the head and neck region. Areas of particularly dense innervation include the nasal and oral cavities, where olfaction and taste are traditionally thought of as the main chemical senses. However, a number of studies have demonstrated that the trigeminal system plays a major role in chemosensation in the oral and nasal mucosa. For example, anosmic subjects or animals lacking functioning olfactory systems retain the ability to detect a variety of chemical irritants via the trigeminal system.^{1–4} These studies and others have established the trigeminal system as a detector of noxious chemicals and a trigger for initiating protective respiratory reflexes.

There is much interest in identifying the molecular and cellular mechanisms underlying chemical irritant detection by the trigeminal system. One of the most powerful strategies in the study of somatosensation has been the use of natural plant products to discover molecular mechanisms underlying thermal and chemical detection. Several chemical irritants, such as

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Conflicts of Interest

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menthol and capsaicin, have been used to identify ion channels that play key roles in somatosensory function. In this review, we will discuss the use of capsaicin, menthol, mustard oil, and hydroxy- α -sanshool to identify their cognate receptors in somatosensory neurons (Fig. 1).

Capsaicin

The heat-like effects of fruit from the *Capsicum* genus have been investigated since the late 1800s. Purified capsaicin produces a variety of effects including acute pain, vasodilation, hyperventilation, and change in blood pressure.⁵ Likewise, similar physiological effects are induced by resiniferotoxin, a potent capsaicin analog isolated from the cactus-like plant, *Euphorbia resinifera*.⁶ Early studies suggested that capsaicin exerts its effects by activating a receptor on a subset of somatosensory neurons that detect noxious heat.⁷ Caterina *et al.* used an expression cloning strategy to functionally search for capsaicin receptors. Using this approach, they identified a member of the transient receptor potential (TRP) ion channel family, TRPV1, as a capsaicin receptor.⁸

TRPV1 is a ligand-gated, inwardly rectifying, nonspecific cation channel, typical of other members of the TRP channel family.⁹ Capsaicin activates TRPV1 by binding to an intracellular region of the channel, adjacent to the second and third transmembrane domains.¹⁰ TRPV1 is highly expressed by a subset of primary somatosensory neurons in both the trigeminal and dorsal root sensory ganglia. In addition to being activated by capsaicin, heterologously expressed TRPV1 also responds to heat, with a threshold of activation of approximately 43°C and a coefficient of temperature dependence (Q_{10}) of more than 20. These properties match those of heat-sensitive somatosensory neurons, suggesting that TRPV1 functions as an endogenous heat transducer.⁸ In addition, TRPV1 activity can be modulated by a variety of inflammatory mediators that are released following nerve injury or inflammation, including protons, lipid-derived second messengers, such as anandamide, and bioactive peptides, such as bradykinin.^{11,12}

Analyses of TRPV1-deficient animals have revealed a key role for TRPV1 in both acute heat detection and thermal hypersensitivity following injury and inflammation. Genetic ablation of TRPV1 attenuates responses to noxious heat and capsaicin at both the cellular and behavioral level.¹³ Studies have also demonstrated that TRPV1 is required for behavioral thermal hypersensitivity following nerve injury or injection of inflammatory mediators, such as nerve growth factor, protons, or carageenan.^{13,14} TRPV1-deficient animals display little aversion to capsaicin in water aversion assays, unlike their wild-type littermates, indicating that TRPV1 expression in trigeminal neurons is sufficient to drive oral behavior.¹³ Thus, TRPV1 acts as a polymodal integrator of both heat and chemical stimuli.

Menthol

Menthol is the cooling agent produced by plants of the Lamiaceae, or mint, family. Hensel and Zotterman were the first to show that menthol acts directly on cold-sensitive somatosensory neurons. They demonstrated that menthol shifts the sensitivity of cold-activated neurons to warmer temperatures, and hypothesized that menthol alters the activity of a protein involved in cold transduction.¹⁵

A variety of transduction mechanisms have been proposed to mediate cold sensation. These include the opening of nonselective cation channels, inhibition of background potassium channels, and blockade of ionic transporters. Like the use of capsaicin to identify TRPV1, McKemy *et al.* used menthol to search for molecules that mediate the detection of cold. Using expression cloning, they identified another member of the TRP ion channel family, TRPM8, as a molecular target of menthol action.¹⁶ Mutagenesis studies have revealed two regions of

the TRPM8 channel that are crucial for menthol binding, the second transmembrane domain and a region of the C-terminal domain, known as the TRP box.¹⁷

When expressed heterologously, TRPM8 displays a number of physiological properties that are shared by native cold receptors, including a thermal threshold of ~25°C, activation by menthol, and desensitization to both menthol and cold. In addition, TRPM8 is activated by the super-cooling agent icilin (AG-3-5), which is ~300 times more potent than menthol-evoked activation of the channel.^{16,18} These results, combined with immunohistochemistry showing TRPM8 expression in a unique subset of cold-sensitive, small-diameter nociceptors, made TRPM8 a likely transducer of *in vivo* cold detection.

An essential role for TRPM8 in cold sensation has now been demonstrated by analysis of mice lacking functional TRPM8 channels. Sensory neurons isolated from knockout mice have attenuated responses to menthol, icilin, and cold. Behavioral studies reveal severe deficits in cold-evoked responses in knockout mice, as measured by acetone evaporative cooling, cold plate, and two-choice temperature paradigm assays.¹⁹⁻²¹ Furthermore, TRPM8 is also required for injury- or inflammation-evoked hypersensitivity to cold and cold-mediated analgesia.^{20, 21} Two lines of evidence suggest that TRPM8 is not the sole transducer of cold stimuli. First, there is a population of cold-sensitive, menthol-insensitive neurons that have a low threshold of activation of approximately 12°C and persist in the absence of functional TRPM8 channels. Second, TRPM8-deficient mice show normal cold-evoked behavior in response to noxious cold (<10°C).¹⁹⁻²¹ Future studies using TRPM8-deficient mice are necessary for identifying the molecular mechanism(s) underlying noxious cold detection.

Mustard Oil

Isothiocyanate compounds constitute the pungent ingredients in wasabi, horseradish, and other mustard extracts. Topical application of mustard oil (allyl isothiocyanate) has long been known to activate somatosensory neurons, resulting in acute pain and neurogenic inflammation through peripheral release of neuropeptides from the primary afferent nerve terminal. This, in turn, produces robust hypersensitivity to thermal and mechanical stimuli. Although the physiological consequences of mustard oil exposure were well known, the cellular and molecular sites of isothiocyanate action remained a mystery for decades.

In search of a mustard oil receptor, Jordt *et al.* discovered that yet another member of the TRP channel family, TRPA1, is activated by a wide range of isothiocyanate compounds.²² TRPA1 is highly expressed in a subset of capsaicin-sensitive, TRPV1-expressing, peptidergic somatosensory neurons and was originally proposed to function as a noxious cold transducer.²³ TRPA1 is also activated by pungent thiosulfinates produced in garlic and other members of the *Allium* genus, cinnamaldehyde, a pungent compound from cinnamon, and THC, a plant derived cannabinoid.^{22,24,25}

Analysis of TRPA1-deficient mice has shown that TRPA1 is required both for acute behavioral responses to mustard oil, and for the prolonged mechanical and thermal hypersensitivity following mustard oil exposure. TRPA1 is also required for inflammatory responses to formalin, thiosulfinates, cinnamaldehyde, and the α,β -unsaturated aldehyde acrolein, an airway irritant present in tear gas, vehicle exhaust, and smoke.^{26,27} These diverse agonists activate TRPA1 through covalent modification of cysteines on the intracellular C-terminal domain of the channel.^{28,29}

In general, TRPA1 appears to act as a sensor of inflammatory state. It is activated directly by prostaglandins, such as 15dPGJ₂, PGA₂, and Δ^{12} -PGJ₂^{30,31} and can be modulated by PLC-coupled receptors that mediate inflammation, such as the bradykinin receptor.^{24,26,27,32} TRPA1 also appears to be activated directly by reactive oxygen species including hydrogen

peroxide and the lipid peroxidation products 4-HNE, 4-ONE, and 4-HHE.^{30,33,34} Taken together, these studies show that TRPA1 acts as a general mediator of inflammation that can be activated by a plethora of endogenous and exogenous irritants.

Sanshool

Szechuan peppers and other members of the *Zanthoxylum* genus have been used in traditional folk medicine to treat toothache and other types of trigeminal pain. In contrast to the intense burning pain associated with hot peppers of the *Capsicum* genus, Szechuan peppers elicit a robust, benign buzzing and tingling paresthesia, suggestive of an interaction with neurons involved in tactile sensation. Psychophysical studies in humans have shown that the alkylamide, hydroxy- α -sanshool, is the active ingredient in Szechuan peppers.³⁵ Extracellular recordings from rat trigeminal fibers suggested that purified hydroxy- α -sanshool activates trigeminal neurons involved in the detection of innocuous light touch or mild cooling.³⁶

A variety of molecular mechanisms were proposed to account for the sensations elicited by hydroxy- α -sanshool, including TRPV1 and TRPA1 activation.^{35,37} However, two findings suggest that hydroxy- α -sanshool has a distinct molecular target. First, hydroxy- α -sanshool activates somatosensory neurons that are insensitive to capsaicin and mustard oil. Second, mice lacking functional TRPA1 and TRPV1 channels display the same aversion to hydroxy- α -sanshool-containing water as their wild-type littermates. Bautista *et al.*³⁸ found that three members of the KCNK two-pore potassium channel family serve as hydroxy- α -sanshool receptors. In contrast to the opening of TRP channels by irritants, hydroxy- α -sanshool inhibits KCNK3, 9, and 18. This inhibition of background potassium conductance results in somatosensory neuron activation. KCNK18 appears to be the primary target of sanshool action in somatosensory neurons.³⁸ However, analysis of KCNK-deficient animals is required to definitively know whether KCNK channels represent the sole molecular target by which sanshool exerts its pungency.

Conclusions

Natural products offer a unique opportunity to identify the molecular mechanisms of trigeminal somatosensation. While some molecules that mediate somatosensation have been identified, many mechanisms, particularly in regard to mechanosensation, remain elusive. Identifying molecular transducers is just the first step in understanding the coding of chemical stimuli. A clear understanding of chemosensation requires elucidation of the individual contributions of the olfactory, gustatory, and somatosensory system, as well as their interactions.

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Figure 1.

Schematic of natural plant products and their cognate receptors. Extracts from the fruits of the *Capsicum* genus contain capsaicin, an agonist of TRPV1. Leaves from the *Mentha* genus contain high concentrations of menthol, an agonist of TRPM8. Allicin is the primary pungent compound found in garlic and is one of many TRPA1 agonists. Hydroxy- α -sanshool, the numbing compound in prickly ash, inhibits KCNK3, 9, and 18 potassium channels. (In color in *Annals* online.)