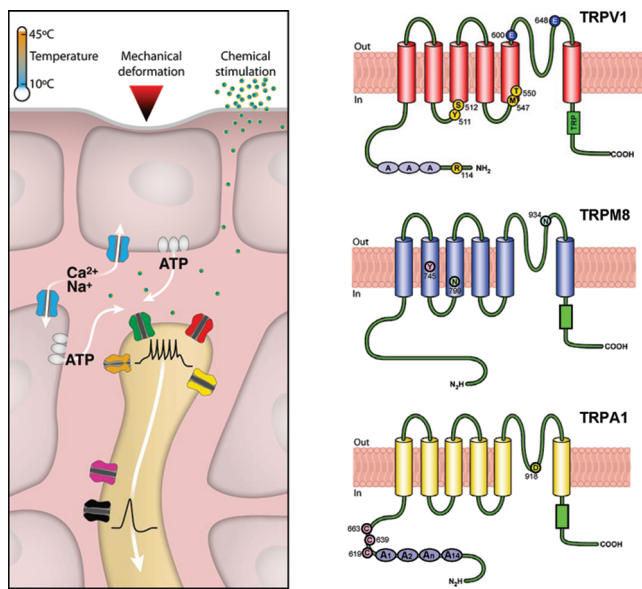


Chemosensory Properties of the Trigeminal System

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Abstract



The capacity of cutaneous, including trigeminal endings, to detect chemicals is known as chemesthesis or cutaneous chemosensation. This sensory function involves the activation of nociceptor and thermoreceptor endings and has a protective or defensive function, as many of these substances are irritants or poisonous. However, humans have also developed a liking for the distinct sharpness or pungency of many foods, beverages, and spices following activation of the same sensory afferents. Our understanding of the cellular and molecular mechanisms of chemosensation in the trigeminal system has experienced enormous progress in the past decade, following the cloning and functional characterization of several ion channels activated by physical and chemical stimuli. This brief review attempts to summarize our current knowledge in this field, including a functional description of various sensory channels, especially TRP channels, involved in trigeminal chemosensitivity. Finally, some of these new findings are discussed in the context of the pathophysiology of trigeminal chemosensation, including pain, pruritus, migraine, cough, airway inflammation, and ophthalmic diseases.

Keywords: Chemesthesis, pungency, spices, capsaicin, menthol, TRP channel, TRPV1, TRPV2, TRPV3, TRPA1, TRPM8, KCNK, pain, cough, asthma, dry eye

A large variety of plant-derived natural products and other chemical agents evoke sensory responses with an infinite shade of perceptual qualities. The perception of chemical stimuli by sensory means is referred to as chemosensation or chemoreception. In humans, the olfactory and the gustatory systems are the principal chemosensory systems and the substrates for the senses of smell and taste, respectively (1). The perception of chemical agents, such as a perfume or the smell of a fruit, is strongly linked to memory and can evoke pleasant feelings. They are also important in nutrition and food selection. However, detection of many chemicals can also have a protective or defensive function, as many are irritants or are poisonous. Their presence in plants is precisely to act as deterrents against animals searching for food.

Albeit less well recognized, the trigeminal somatosensory system also plays a fundamental role in chemosensation and the overall “flavor” of foods. Sensory endings of the trigeminal (V cranial) nerve innervate the skin covering the face, the mucous membranes of the nasal and oral cavities, and the cornea and conjunctiva of the eye (Figure 1). These endings can be activated by physical stimuli (mechanical forces and temperature) and by a huge array of chemical agents (2), and evoke sensations of touch, temperature, and pain. The capacity of cutaneous, including trigeminal endings, to detect chemicals is known as chemesthesis or cutaneous chemosensation (3). Chemosensitive glossopharyngeal (IX cranial) and vagal (X cranial) nerve fibers innervating the oropharynx and respiratory tract fall in the same category. Oral chemesthesis explains the pungent or sharp feel of many different foods and spices such as chili peppers, horseradish, wasabi roots, and Szechuan pepper, the coolness of peppermint, the tingle of carbonated drinks, and the irritation produced by substances such as nicotine or raw garlic extracts (Table 1). Protective responses evoked by trigeminal stimulation include salivation, tearing, coughing, respiratory depression, and sneezing. Moreover, pungent irritation or burning pain is a very common side effect of drugs applied topically to the skin.

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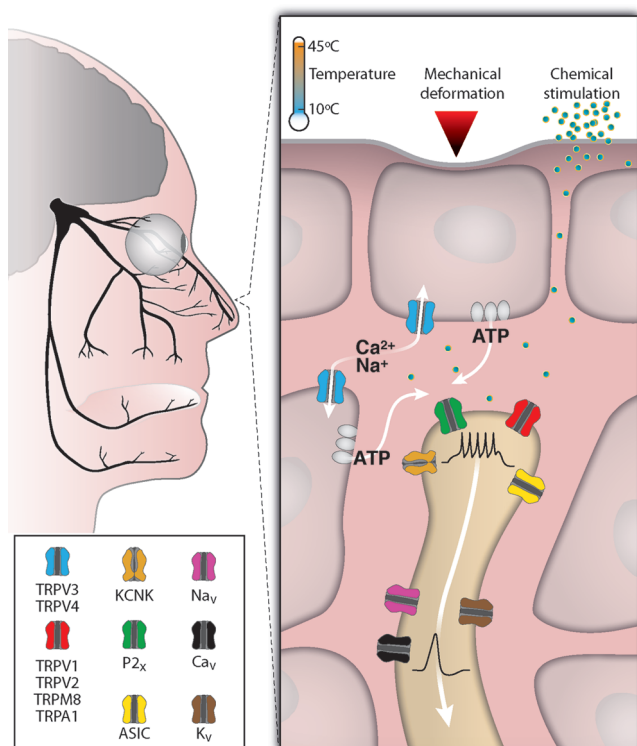


Figure 1. Molecular determinants of chemosensation in trigeminal nerve terminals. Branches of the human trigeminal nerve innervating the face, the eye, and the nasal and oral cavities. Temperature and many chemical agents can stimulate chemosensitive channels directly. These channels are expressed in sensory nerve terminals and mucosal epithelial cells or skin keratinocytes. The opening of cationic channels (TRPs, ASICs) or the closing of potassium-selective channels (KCNK) generate a graded transduction current, depolarization, and the firing of action potentials. Voltage gated sodium (Na_V), calcium (Ca_V), and potassium (K_V) channels participate in action potential electrogenesis and propagation of the nerve impulse to the brainstem. Mechanical deformation of the skin can trigger the release of ATP from keratinocytes and the activation of purinergic (P_{2X}) receptors from sensory nerve terminals.

The sensitivity of somatosensory terminals to chemical irritants was first recognized by Parker in 1912 while studying aversive responses in fish, coining the term “common chemical sense” to describe this property (2, 4, 5). Some early researchers, including Parker, described this sense as distinct from taste, odor, touch and pain, while others defended the view that pain and the common chemical sensitivity were mediated by the same nerve endings (6). Most modern authors agree that cutaneous chemosensation is determined primarily by the chemical activation of nociceptors and thermoreceptors (7, 8).

Chemical signaling of taste and smell involves a metabotropic mechanism; binding of the chemical agent to the receptor activates a heterotrimeric G protein that initiates a transduction cascade (1). In contrast, activation of somatosensory afferents by chemicals involves the direct gating of an ion channel by the chemical stimulus and is called ionotropic transduction. In either

case, the chemical signal leads to a change in ionic permeability and the depolarization of the sensory receptor. The sensations evoked by chemical agents applied to the skin or mucous membranes vary in their quality (e.g., burning, stinging, itch, tingling, cold), temporal profile, and intensity. Even sensations evoked by individual chemicals are complex. An explanation for this complexity is provided by the overlapping sensitivity of individual ion channels to chemical agonists (Table 1), which results in the combinatorial activation of different classes of endings by a single chemical (9, 10).

In recent years, a tremendous progress has been achieved in our understanding of how different chemicals are detected by trigeminal endings (8, 11, 12). A landmark finding was the use of capsaicin to clone the vanilloid receptor and the identification of transient receptor potential (TRP) channels as transducers of physical (e.g., temperature) and chemical signals in mammalian sensory fibers (13) (Figure 2). In addition to TRP channels, other ion channels expressed in trigeminal neurons appear to play important roles in chemosensation (14, 15). Other natural products (e.g., menthol) were also fundamental in the identification and functional characterization of other sensory channels (16). This brief review attempts to summarize our current knowledge of the cellular and molecular mechanisms of chemosensation in the trigeminal system. Some of these new findings are discussed in the context of the pathophysiology of trigeminal chemosensation. Other authors have addressed some aspects of this subject in recent years (17–19).

Pungency

Pungency refers to the sharp or biting quality of sensation evoked by some foods, spices, or volatile compounds in contact with trigeminal endings of the nasal or oral epithelium. The response threshold of trigeminal fibers to chemical stimuli is generally 1–2 log units higher than olfactory thresholds for the same compounds (2, 20). This is not true for the corneal endings in the eye that can be extremely sensitive to chemical irritants. To reach the nerve endings and evoke a sensation, the agonists first need to pass through the lipid phase of the epithelial barrier or through tight junctions. It is somewhat puzzling why some potent agonists of nociceptive transduction channels lack pungency when applied to the skin or mucosal surfaces. A classical study by Szolcsanyi and Jancso-Gabor showed that some capsaicin analogues produced rapid desensitization of the sensory terminals but lacked pungency characteristics (21). Olvanil (*N*-9-*Z*-octadecenoyl-vanillamide) is another agonist of TRPV1 channels lacking the pungency of capsaicin. Also, SDZ249-665, a potent agonist of TRPV1 channels, shows little irritancy when instilled in the nose or eye of guinea pigs (22). This is in contrast with the

Table 1. Plant-Derived Agonists of Chemosensitive Ion Channels^a

chemical agonist	natural source	receptor	ref
capsaicin	chili peppers (<i>Capsicum</i> sp.)	V1	13, 17
resiniferatoxin	dried latex of the cactus (<i>Euphorbia resinifera</i>)	V1	13, 72
piperine	black pepper (<i>Piper nigrum</i>)	V1 > A1	24, 161, 162
Δ 9-tetrahydrocannabinol	marijuana plant (<i>Cannabis sativa</i>)	A1, V2	83, 84, 109
cannabidiol	marijuana plant (<i>Cannabis sativa</i>)	V1, V2	83, 84
camphor	camphor laurel tree (<i>Cinnamomum camphora</i>)	V3, V1	59, 85, 87
incensole acetate	incense (<i>Boswellia</i> sp.)	V3	89
thymol	thyme (<i>Thymus vulgaris</i>)	V3, A1	88, 100, 115,
carvacrol	oregano (<i>Origanum vulgare</i>)	V3, A1	88, 100
eugenol	clove oil (<i>Eugenia charophyllata</i> , <i>Syzygium aromaticum</i>)	V3, V1, A1	96, 100
allyl isothiocyanate	mustard oil (<i>Brassica nigra</i>), horseradish (<i>Armoracia rusticana</i>) wasabi (<i>Wasabia japonica</i>)	A1 > V1	96, 97, 107, 109
cinnamaldehyde	cinnamon oil (<i>Cinnamomum cassia</i>)	A1	96, 103, 110,
allicin	garlic (<i>Allium sativum</i>)	A1 > V1	98, 99, 163
diallyl disulfide	garlic (<i>Allium sativum</i>)	A1 > V1	98, 99, 164
1'-acetoxychavicol acetate	galangal (<i>Alpinia galanga</i> , <i>Kaempferia galanga</i>)	A1	165
methyl salicylate	wintergreen oil (<i>Betula lenta</i> , <i>Spiraea</i> sp., <i>Gaultheria</i> sp.)	A1, V1	96, 107
gingerols	ginger (<i>Zingiber officinale</i>)	A1, V1	96, 166
menthol	peppermint oil (<i>Mentha piperita</i>)	M8, A1 > V3	16, 59, 115, 117, 123
eucalyptol	eucalyptus oil (<i>Eucalyptus polybractea</i>)	M8	16, 123
linalool	coriander (<i>Coriandrum sativum</i>), laurel (<i>Laurus nobilis</i>), Szechuan pepper (<i>Zanthoxylum piperitum</i>)	M8, A1	123, 129
citral	lemongrass (<i>Cymbopogon citratus</i>)	M8 > V1 > A1 > V3	86 ^b
nicotine	tobacco plant (<i>Nicotiana tabacum</i>)	A1	114
hydroxy- α -sanshool	Szechuan pepper (<i>Zanthoxylum piperitum</i>)	KCNK, A1, V1	14, 129

^a The references cited focus on data obtained from recombinant channels expressed in heterologous systems. In many of the same references, responses to the same agonists were also observed in trigeminal or DRG sensory neurons. The symbol > denotes that the receptor on the left shows a higher affinity, or responds stronger, to the agonist. Note that in most cases the natural source of a given agonist includes other species belonging to the same or other genera. ^b Reference 123 could not demonstrate effects of citral on mouse TRPM8.

pungent irritation produced by capsaicin, which is followed by a prolonged desensitization, a feature exploited clinically to achieve antinociception. Dermal application of anandamide, an endogenous cannabinoid, produces skin microvessel dilation via a TRPV1-dependent mechanism (23). Interestingly, anandamide application on the skin does not result in pain.

The mechanisms explaining this lack of pungency of some agonists of TRPV1 are not clear. In the case of anandamide, a nonselective agonist of TRPV1, it is possible that well-known additional effects, such as inhibition of sodium and calcium channels, and activation of potassium channels could prevent the electrical propagation of the afferent message. Another possibility is that binding to the receptor by nonpungent agonists is slow, producing a progressive depolarization and inactivation of the receptor potential at the nerve terminals.

Recently, Ursu et al. evaluated the pungency of several TRPV1 agonists covering a large range of potencies and found a strong correlation between the kinetics of calcium flux, pungency (estimated by the increment in eye-wipes produced by the compound), and lipophilicity (24). The authors evaluated the effect of agonists on membrane potential and action potential firing, with current-clamp recordings of DRG neurons, and found that the onset of the depolarization induced by olvanil (i.e., nonpungent agonist) was much slower, and irreversible, compared to the effects induced by capsaicin. This slow depolarization, albeit of similar magnitude to that produced by capsaicin, translated into a low probability of evoking action potentials. A molecular understanding of the mechanisms involved in long lasting TRPV1 desensitization by capsaicin agonists is critical for its potential application in therapies targeting the

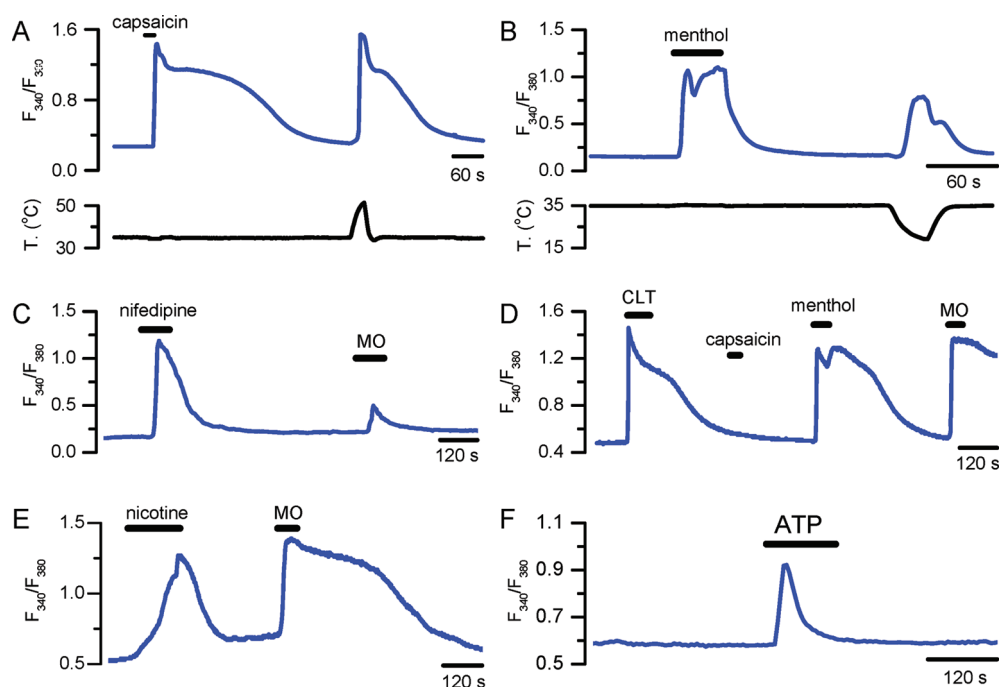


Figure 2. Chemosensitive responses of cultured mice trigeminal sensory neurons. Fura-2 calcium imaging of agonist-evoked responses of individual sensory neurons to capsaicin (100 nM) and warm temperature (A), menthol (100 μ M) and cold temperature (B), nifedipine (10 μ M) and mustard oil (100 μ M) (C), clotrimazole (10 μ M), capsaicin (100 μ M), menthol (100 μ M), and mustard oil (100 μ M) (D), nicotine (1 mM) and mustard oil (100 μ M) (E), ATP (1 mM) (F). Responses were obtained with different imaging systems, and absolute amplitude cannot be compared. (A) by R. Madrid (unpublished), (B) from Madrid et al. (159), (C) from Fajardo et al. (113), (D) from Meseguer et al. (160), (E) by V. Meseguer (unpublished), and (F) by E. de la Pena (unpublished). The record in (D) was obtained from a *Trpv1*^{-/-} mouse.

TRPV1 receptor, including treatment of chronic pain and overactive bladder syndrome.

Perceptual Interaction between Temperature and Chemical Sensitivity

In the trigeminal system, the sensations evoked by many chemical agents show clear cross-interactions with temperature (25). Many chemical agents (e.g., capsaicin) sensitize the perception of temperature. In addition, temperature and touch introduce additional perceptual qualities to the sensory experience evoked by chemicals in contact with the trigeminal system. Menthol potentiates cold sensations (26). In agreement with these psychophysical findings, menthol sensitizes responses of trigeminal endings to cold temperature (27).

These interactions are easily explained by the allosteric gating of TRP channels by chemical and thermal stimuli (see below) (13, 28, 29). TRP channels with a marked sensitivity to temperature are referred to as thermoTRPs (30).

Chemosensory Signaling between Skin Cells and Nerve Fibers

A special type of chemosensory communication is provided by the signaling between cells in the skin (e.g., keratinocytes, mast cells) and neighboring nerve

endings (31, 32). Chemosensing by non-neuronal cells is also important in tissues normally exposed to food- or blood-borne substances, such as the gastrointestinal epithelium.

It has become apparent that certain thermoTRP channels are functionally expressed in numerous non-neuronal cell types of the skin. It has been postulated that traumatic or thermal activation of skin cells can release diffusible molecules that activate neighboring nerve endings (Figure 1). In a co-culture system, mechanical activation of keratinocytes results in a propagated Ca^{2+} wave and the activation of adjacent DRG neurons by a mechanism involving released ATP (33). A recent elegant study provided strong evidence for the thermal activation of TRPV3 channels on keratinocytes and the subsequent release of ATP to the extracellular space (34). ATP is an accepted transmitter or co-transmitter in different sensory nerves (35, 36). Indeed, sensory neurons placed in close proximity to activated keratinocytes responded with a depolarizing inward current (34).

The Sensation of Itch

Itch, formally known as pruritus, is defined as an unpleasant sensation that triggers an overwhelming urge to scratch. Severe pruritus is a frequent medical problem with poor treatment. A number of chemical

agents behave as mediators of pruritus (i.e., pruritogens), including endogenous substances such as histamine, neuropeptides, prostaglandin E₂, proteases, and cytokines. Drugs such as chloroquine, clonidine, and morphine can also induce pruritus.

Itch and pain are closely related sensations, with important similarities (e.g., sensitization), but nevertheless are distinct in their behavioral manifestations. There is a strong overlap between mediators of itch and pain, as well as neuronal pathways involved in their expression (37). However, recent studies provide strong support for the concept of a specific sensory labeled line for itch separate from the one reporting pain. Thus, selective ablation of a specific class of interneurons in the dorsal horn of the spinal cord can abolish itch while pain sensations remain completely normal (38). In contrast, the loss of a different population of inhibitory interneurons in the superficial dorsal horn potentiates itch responses with no change in nociception (39). Also in this line, selective removal of the glutamate transporter VGLut2 from sensory afferents expressing TRPV1 results in a strong potentiation of spontaneous scratching behaviors in mice (40). Finally, recent work implicates altered function of TRPV3 channels in causing allergic and pruritic dermatitis in rodents (41).

Chemical Sensitivity to Protons

Extracellular acidification is typical of tissues under conditions of ischemia or inflammation. Acidic solutions applied to the skin or mucosae induce pain (reviewed in ref 42). These solutions activate and sensitize trigeminal nerve terminals of polymodal nociceptors (43, 44) and induce inward currents in small diameter trigeminal sensory neurons (45) (46). Currents in individual neurons have different kinetic properties, pharmacology, and pH sensitivity, suggesting that they are mediated by more than one receptor type. Several ion channels expressed in trigeminal nociceptors are sensitive to low pH, including TRPV1, ASICs, and TASK channels.

The acid-sensing ion channels (ASICs), members of the DEG/ENaC superfamily, are activated by extracellular protons and are overexpressed during inflammation. Several ASICs are expressed in TG neurons (e.g., ASIC- α , ASIC- β , DRASIC). Amiloride reduces or eliminates proton-gated currents in many TG neurons, suggesting an important contribution of ASICs (46). Moreover, psychophysical and pharmacological data in humans suggest that ASICs have an important role in the sensation of cutaneous acid-induced pain.

Extracellular protons also activate TRPV1 channels and strongly sensitize the response to vanilloid agonists (13). Many studies have identified a pH-sensitive current blocked by specific TRPV1 antagonists (e.g., capsazepine) in TG neurons (13, 46, 47). Unlike

ASIC-mediated currents, these currents are generally noninactivating.

Among the 2P-domain K⁺ channels, TASK subunits (TASK-1, TASK-2, TASK-3) represent background outward rectifiers that are constitutively active at resting membrane potential and are inhibited by extracellular acidic pH values (48). Closure of these channels will depolarize the neuron. TASK-1 and TASK-3 channels have been found in some sensory neurons with nociceptive properties (47). Moreover, the pH-sensitive current in these neurons is only partially sensitive to capsazepine.

Chemical Sensitivity to CO₂

Carbonated drinks, containing dissolved CO₂, elicit a very distinct sensation that combines a stinging and pungent quality. This complex sensation is certainly responsible for the popularity of carbonated beverages among consumers. Until recently, the neural basis of this sensation remained somewhat obscure; some authors favored a mechanical hypothesis (i.e., the bursting of tiny CO₂ bubbles would excite mechanoreceptors) over a chemosensitive mechanism. It is well established that CO₂ activates trigeminal nociceptors in the cornea and nasal epithelium (49, 50). Although many CO₂-sensitive fibers are capsaicin-sensitive, the mechanism of action appears to be independent of TRPV1 because the activation is not blocked by capsazepine and is only partially desensitized by capsaicin (51). In agreement with the neurophysiological data, the sensations evoked by CO₂ solutions to the tongue are only partially desensitized by capsaicin (52). Some studies postulated the conversion of CO₂ into carbonic acid, a reaction catalyzed by carbonic anhydrase, and the stimulation of polymodal nociceptors by protons. However, a large fraction of lingual nerve fibers sensitive to carbonated water are not activated by acidic solution, indicating that changes in mucosal pH are not the direct mediators of the response to CO₂ (53). The mechanism was clarified recently in an elegant study (see below) and appears to be mediated by intracellular acidification of trigeminal nerve terminals and activation of TRPA1 channels (54). It is interesting that cooling enhances the pungent and tingling sensation of carbonated water (55). Whether this is due to physicochemical factors (i.e., the effect of temperature on CO₂ solubility) or to potentiation of CO₂-mediated responses in TRPA1 nociceptors by low temperature is unknown (56).

TRP Channels as Polymodal Transducers

The transient receptor potential (TRP) ion channel superfamily, which was first described in the fruit fly *Drosophila melanogaster*, forms a large group of cation-permeable ion channels conserved in yeast, worms, insect, fish, and mammals (57). These receptors share

structural similarities with voltage-gated K^+ channels and are classified according to their primary amino acid sequence (58). They are probably tetrameric, and each subunit is predicted to have six transmembrane (TM) spanning domains with a short pore-forming hydrophobic loop between TM domains 5 and 6.

A distinct feature of many TRP channels is their polymodal activation by physical (e.g., temperature, mechanical forces) and chemical stimuli (9, 59). This characteristic is ideal to serve as a detector of environmental stimuli, acting as a molecular interface between the external world and the nervous system. Many TRP channels are expressed in sensory neurons (60) and skin cells, and can be activated by a variety of physical and chemical stimuli to function as signal integrators (61). Importantly, the different stimuli show allosteric interactions in the gating of the channels: exposure to one stimulus sensitizes the response to another (29). The prototype for this characteristic is the TRPV1 receptor that integrates noxious heat, tissue acidosis, and chemical stimuli which are all known to cause pain (13, 62).

Sensory Ion Channels and Chemosensation

Next, I will briefly address the chemical sensitivity of individual ion channels expressed in trigeminal sensory neurons (Figure 1). Emphasis is placed on the functional relevance of the different channels to the detection of external stimuli. The reader is referred to a number of recent reviews which provide an extensive account of the pharmacological properties and chemical sensitivity of some of these transduction channels (19, 63–66).

TRPV1

TRPV1, also known as the “capsaicin or vanilloid receptor”, is a Ca^{2+} -permeable, nonselective cationic channel that belongs to the TRP family of ion channels. The identification and functional characterization of this protein as a polymodal transducing molecule for physical and chemical stimuli in the mammalian somatosensory system represents a milestone in molecular studies of pain and trigeminal chemosensation (13). TRPV1 is activated by capsaicin (13, 67), the pungent component of “hot” chilli peppers long since known as a selective activator of polymodal nociceptor endings (reviewed in refs 68 and 69). Topical application of capsaicin to the skin or mucosae elicits a sensation of burning pain (70). Fittingly, the channel is also activated by noxious heat temperatures (threshold ~ 43 °C) and by extracellular protons (reviewed in ref 71). The early response to capsaicin application includes an augmented sensitivity to noxious and innocuous stimuli.

Other vanilloids also activate the TRPV1 receptor (17, 66). Among them, resiniferotoxin, an alkaloid extracted from some species of *Euphorbia* cactuslike

plants, is one of the most potent chemical agonists of TRPV1 known (72). Various fatty acid derivatives, referred to as endovanilloids, are activators of TRPV1 channels (66). Recently, some animal toxins were identified as TRPV1 agonists (73).

TRPV1 is preferentially expressed in small size, unmyelinated primary sensory neurons of the trigeminal and DRG ganglia, the classical polymodal nociceptors. Therefore, TRPV1 was proposed as the molecular substrate for polymodality in these neurons, providing them with the ability to respond to heat and to potentially injurious chemical stimuli causing pain (13, 62) (Figure 2A). More recently, TRPV1 has been detected in other tissues, including keratinocytes, urothelium, endothelial cells, and brain nuclei, expanding its biological role.

Bird orthologues of TRPV1 are insensitive to capsaicin. This characteristic was exploited by Jordt and Julius, making chimeric proteins between mammalian and avian TRPV1 receptors, to identify structural elements in TRPV1 important for capsaicin sensitivity (67). The authors found that Tyr 511 and Ser 512, located in the region between the second intracellular loop and the third TM domain, are critical for the action of vanilloid ligands. Further studies have established the importance of additional residues in capsaicin sensitivity (reviewed in ref 71). Other studies have established the molecular determinants of heat and proton sensitivity in TRPV1. The acidic residue Glu 600, located within the extracellular loop close to the channel pore, is involved in proton potentiation of TRPV1 activity, whereas Glu 648 is critical for proton-evoked activation of TRPV1 (74).

An important functional characteristic of TRPV1 channels is their high permeability to Ca^{2+} ions (13). In addition, prolonged exposure of TRPV1 to vanilloid agonists results in a modification of the ionic pore properties (i.e., “pore dilation”) (75) with a threefold increase in the relative permeability to Ca^{2+} (as compared to sodium). This results in a Ca^{2+} overload of capsaicin-sensitive nerve terminals, the retraction of epidermal nerve fibers, and the long-lasting functional desensitization of nociceptive sensory endings (76). These structural and functional changes are the basis for the therapeutic use of skin patches with high capsaicin content for the treatment of peripheral neuropathic conditions, including postherpetic neuralgia, diabetic neuropathy, and sensory neuropathy associated to HIV infection (77, 78). This functional pore dilation of TRPV1 by agonists is also the basis of a novel delivery method of the membrane-impermeant local anesthetic QX-314 to TRPV1-expressing sensory nerve terminals, leading to their selective block (79, 80).

TRPV2

TRPV2 is an ion channel with an amino acid sequence $\sim 50\%$ identical to TRPV1 (81). It is expressed in

sensory neurons of medium and large diameter. The channel is insensitive to capsaicin and low external pH. TRPV2 is activated by noxious heat, with an activation threshold (~ 52 °C) higher than that of TRPV1 (81) and to changes in osmolarity (82). Very few natural chemical agonists of TRPV2 are known, and none are considered selective. $\Delta 9$ -Tetrahydrocannabinol and cannabidiol of the marijuana plant activate the channel (83, 84).

TRPV3

TRPV3 is an ion channel structurally analogous to TRPV1 that is strongly expressed in skin keratinocytes. Expression of TRPV3 in peripheral sensory neurons is a controversial subject (85, 86). When transfected into mammalian cells, this channel responds to temperature with a threshold around 33 °C but not to capsaicin or pH changes (reviewed in ref 30). TRPV3 null mice have strong deficits in responses to innocuous and noxious heat, indicating that it participates in thermosensation (85).

TRPV3 is activated by a number of natural chemical compounds, including camphor (87) and carvacrol, a monoterpenoid phenol present at high concentrations in the essential oil of oregano. Other monoterpenes such as 6-*tert*-butyl-*m*-cresol, eugenol (derived from cloves), dihydrocarveol, thymol (from thyme), carveol, and (+)-borneol also activate TRPV3 (87, 88). Incensole acetate, a compound in the resin obtained from *Boswellia* trees, also activates TRPV3 (89). Farnesyl pyrophosphate (FPP), an intermediate metabolite in the mevalonate pathway, is a specific activator of TRPV3. The cutaneous sensations produced by heating and cooling can be enhanced by camphor coapplication (90).

TRPA1

TRPA1 is a nonselective cationic TRP channel (1119 amino acids in humans) phylogenetically distant to other mammalian TRP proteins. Like other TRPs, TRPA1 channels have six putative transmembrane-spanning domains, with cytoplasmic N- and C-terminal ends (91, 92). Activation of this channel is responsible for the pungent feel of many foods and condiments, including isothiocyanates occurring naturally in fruits and plants such as mustard greens and capers (Table 1). Most notably, the chemical sensitivity of this receptor has been preserved during evolution, being similar in humans and flies (93).

Chemical activators of TRPA1 have been reviewed recently in several publications (59, 91, 92, 94, 95). The list of TRPA1 agonists keeps growing daily and includes many natural and synthetic irritants such as allyl isothiocyanate (in mustard oil) (96, 97) (Figure 2C), cinnamaldehyde (in cinnamon oil) (96), allicin and diallyl disulfide (in garlic extract) (98, 99), methyl salicylate (in wintergreen oil) (96), ginger (96), carvacrol (in oregano) (100), formalin (101), natural fungal deterrents

like isovelleral (102), unsaturated aldehydes like acrolein (103), isocyanates (104), and oxidizing agents like hypochlorite (OCl^-) and hydrogen peroxide (H_2O_2) (64, 105). Several odorants (α -terpineol, amyl acetate, benzaldehyde, toluene) known to activate trigeminal fibers also activate TRPA1 channels (106). The unique chemical sensitivity of this ion channel for some of these irritants was clarified in TRPA1 knockout mice (103). These animals were completely insensitive to mustard oil and allicin (but see ref 107). In addition, these animals had pronounced deficits in bradykinin-evoked nociceptor excitation, highlighting a fundamental role for TRPA1 in neurogenic inflammatory pain (96, 97, 103, 108).

Mechanisms of TRPA1 Activation

An old puzzle in the pain literature concerned the diverse physicochemical properties of chemical sensory irritants. Recent work has unveiled the unique molecular mechanism of activation of TRPA1 channels, explaining its capacity to sense many structurally unrelated chemicals. Unlike traditional chemoreceptors (e.g., olfactory receptors) that can discriminate between closely related chemical structures, TRPA1 is activated by structurally different molecules with high chemical reactivity. These highly electrophilic compounds interact with thiol groups in side chains of intracellular cysteines in the intracellular N-terminal region of the TRPA1 molecule, forming covalent bonds (109, 110). These covalent modifications produce long lasting channel openings that can be reversed by reducing agents such as dithiothreitol (110). Mutational studies have identified several cysteine and lysine residues involved in channel activation (109, 110). In human TRPA1, crucial residues for channel activation by allyl isothiocyanate include a cluster of cysteines (Cys619, Cys639, and Cys663) and Lys708 (109). Actually, activation of TRPA1 by electrophilic compounds is more complex than outlined here (for further details, consult refs 91, 95, and 111). For example, certain Cys-reacting compounds show species-specific responses in TRPA1 channels (112).

Other agonists of TRPA1 appear to act by conventional reversible ligand–receptor interactions. They include, among others, icilin, nifedipine (113), nicotine (114) (Figure 2E), menthol (115) (Figure 2D), eugenol, carvacrol, and $\Delta 9$ -tetrahydrocannabinol. Mutating the critical cysteines to nonreactive serines does not prevent the agonist action of some of these compounds (109, 110).

Dissolved CO_2 activates TRPA1-expressing trigeminal neurons. This activation is abrogated in neurons obtained from TRPA1^{-/-} animals. Furthermore, intracellular protons activate TRPA1 channels in excised patches in a dose dependent manner, suggesting that the mechanism of activation in intact neurons involves

diffusion of CO₂ across the plasma membrane and intracellular acidification by the re-equilibration of CO₂ and H₂O in bicarbonate (HCO₃⁻) and a free proton (H⁺) (54).

TRPM8

The transient receptor potential melastatin 8 channel (TRPM8) is a nonselective cation channel activated by mild cold temperatures (threshold around 25 °C in heterologous systems) and cooling compounds such as menthol. The TRPM8 protein is 1104 amino acids long (16, 116, 117) and is found in a subpopulation (10–15%) of small-diameter, cold-sensitive peripheral sensory neurons (16, 117). The same neurons are activated by cooling compounds (Figure 2B). Thermosensitive nerve endings of these sensory neurons innervate the skin and mucosae (cornea, oral cavity) (118–120) where TRPM8 plays a clear physiological role in the detection of low temperature ambient signals (reviewed in refs 121 and 122)).

In addition to cold, TRPM8 can be activated by natural and synthetic cooling mimetic agents such as icilin (AG-3-5), eucalyptol, menthol, and an abundance of menthol analogues (123–125), with WS-12 being the most potent one described to date in the scientific literature. Other natural weak agonists of TRPM8 include hydroxy-citronellal, geraniol, and linalool (123). Chemicals modulators of TRPM8 and their mechanisms of channel gating have been reviewed recently (63).

KCNK Channels

Alkylamides are a unique class of compounds that elicit a distinct tingling sensation when applied to the tongue (126). Natural plant-derived alkylamides, such as α -hydroxy-sanshool, are found in Szechuan pepper (*Zanthoxylum piperitum*) and are heavily used in oriental cooking. At the cellular level, α -hydroxy-sanshool activates mechanoreceptors and thermosensitive trigeminal neurons (127, 128). The sensation evoked is clearly distinct from the pungent sensation evoked by capsaicin or isothiocyanates, specific activators of TRPV1 and TRPA1, respectively. This suggests a distinct molecular mechanism of action. Recently, Bautista et al. found that natural alkylamides inhibit a particular class of background or leak potassium channels with two pore domains (KCNK3, KCNK9, and KCNK18) (14). Closure of these potassium-selective channels leads to depolarization and firing. In vitro recordings from sensory fibers established that sanshool has a potent excitatory effect on D-hair cells, an ultrasensitive light-touch receptor, and A β mechanoreceptors. Sanshool also activated a subpopulation of very slowly conducting C fibers (128). The firing evoked by sanshool was not suppressed by TRPA1 and TRPV1 antagonists. However, other studies have established excitatory effects of sanshool on TRPV1 and TRPA1 channels (129).

Purinergic Receptors

Application of ATP to skin blisters is painful. Normally, ATP can be coreleased with noradrenaline from sympathetic nerve terminals or from skin cell in response to mechanical deformation (e.g., shear stress) (36). Application of ATP or α,β -methylene ATP excites capsaicin-sensitive trigeminal lingual afferents (130) (Figure 2F). Primary sensory neurons and nerve terminals express all subtypes of ionotropic purinergic receptors (P2X). The P2X₃ subunit is selectively expressed in IB4(+) nociceptors, forming heteromeric assemblies with P2X₂ subunits (15). In addition, a high percentage of sensory neurons express metabotropic P2Y receptors.

Patophysiology of Trigeminal Chemosensitivity

Trigeminal irritant stimuli applied repeatedly at short interstimulus intervals produce a sensitization of the response (3). Also, during inflammation, trigeminal nociceptor terminals can become sensitized, increasing their excitability and responding inadequately to innocuous stimuli (allodynia) and augmenting their response to noxious stimuli (hyperalgesia) (131).

The molecular mechanisms of nociceptor sensitization following injury are complex (131, 132). One mechanism involves the sustained activation, posttranslational modifications, and membrane translocation of TRPV1 channels by a variety of endogenous agents released after injury or inflammation, including bradykinin and endogenous lipids, leading to the sensitizing/excitatory action of polymodal nociceptor neurons during these pathological states (133–138). TRPV1 channels are critical in inflammatory thermal hyperalgesia (139).

The expression of TRPV1 and TRPA1 channels in vagal chemosensory unmyelinated afferents innervating the lung and airways (140, 141) suggests that activation of these receptors could be involved in the symptomatology and pathogenesis of different airway diseases. Indeed, TRP channels are emerging as important mediators of pathologies such as asthma, chemical hypersensitivity, chronic cough, and chronic obstructive pulmonary disease (reviewed in refs 95 and 142–144)). Many TRPA1 agonists are volatile organic compounds, present in cigarette smoke and smog, which trigger eye irritation, sneezing, cough, bronchoconstriction, respiratory depression, mucous secretion, and neurogenic inflammation of the airways, reflexes aimed at limiting exposure of the affected tissues to potentially harmful chemicals.

Capsaicin is a powerful tussive agent, and TRPV1 is upregulated during airway inflammation (144). TRPA1 agonists, including acrolein and crotonaldehyde, two α,β -unsaturated aldehydes present in cigarette smoke (145), can also trigger cough reflexes, a response inhibited by HC-030031, a selective TRPA1 antagonist (146, 147).

Endogenous unsaturated aldehydes, such as 4-hydroxynonenal and 4-oxo-nonenal, also activate TRPA1 - (105, 148) and vagal bronchopulmonary fibers (149). These α,β -unsaturated aldehydes are produced during oxidation of membrane phospholipids in response to tissue injury, inflammation, and oxidative stress. Inhibition of TRPA1 with HC-030031 prevents neuropeptide-mediated constriction of bronchial rings produced by exposure to cigarette smoke or α,β -unsaturated aldehydes (145). Other endogenous products of oxidative stress also activate TRPA1 including hydrogen peroxide (H_2O_2) and the cyclopentenone prostaglandin, 15-deoxy- $\Delta(12,14)$ -prostaglandin J2, an electrophilic compound - (105, 150, 151). Hydrogen sulfide (H_2S) is a gaseous molecule formed endogenously during inflammation. It activates mouse and human TRPA1 in recombinant systems and induces bladder detrusor overactivity, suggesting a role for this inflammatory mediator in symptoms secondary to lower urinary tract infection - (152). Nitrooleic acid, a byproduct of nitrate stress also activates TRPA1 via covalent modification (153). Hypochlorite, the oxidizing mediator of chlorine, activates recombinant TRPA1 channels and TRPA1-expressing neurons (64).

A study in an animal model suggests that TRPA1 channels are involved in the etiology of the airway inflammation and hyperreactivity characteristic of asthma (154). Following an ovalbumin challenge in sensitized mice, the inflammatory and allergic response, characterized by an increase in leukocytes (primarily eosinophils) and T_H2 interleukins and TNF- α , was abrogated by genetic ablation or pharmacological inhibition of TRPA1 (154). Moreover, airway hyperreactivity to acetylcholine was absent in the TRPA1 ko mice or after specific TRPA1 block.

Exposures to many volatile chemical irritants, such as formaldehyde, toluene, or acrolein, can trigger headache. A recent study by Kunkler et al. showed that activation of TRPA1 channels and release of CGRP are critical for the meningeal vasodilatation induced by environmental irritants (155). These findings suggest that TRPA1 participates in the activation of the trigeminovascular system following exposure to volatile irritants, unveiling a novel potential therapeutic target for the treatment of migraine headache.

Basal tearing is essential for maintaining ocular surface wetness. In a recent study, Parra and colleagues found a reduced basal tearing rate in TRPM8 ko mice - (120). These animals showed a complete absence of spontaneous and cold-evoked activity in trigeminal corneal thermoreceptor endings, and the authors hypothesize that a reduced reflex input of cold- and menthol-activated corneal sensory afferents to lacrimal glands reduces basal tearing. Consistent with this hypothesis, increasing the surface temperature of the cornea in

human volunteers, a maneuver that is expected to silence corneal cold thermoreceptors, also decreased basal tearing. These findings have important implications for the pathophysiology and potential treatment of dry eye disease and other pathologies of mucosal surfaces such as burning mouth syndrome and vaginal dryness.

Mice lacking the *Trpv3* gene have skin alterations and curly whiskers (156). In keratinocytes, the channel forms a signaling complex with TGF- α /EGFR. Activation of EGFR leads to increased TRPV3 channel activity and Ca^{2+} influx, which in turn stimulates TGF- α release, an important effector in hair morphogenesis and epidermal development (156). A recent study by Yoshioka et al. suggests an important role of TRPV3 in the development of allergic and pruritic dermatitis (41). Mice with a gain-of-function mutation (G573S) of TRPV3 (157) expressed higher TRPV3 levels in epidermal keratinocytes, were hairless, and developed typical signs of dermatitis, including erythema, edema, dry skin, skin erosions, and excoriations (41). These mice also exhibited significantly increased scratching behavior compared with wildtype, suggesting the spontaneous development of pruritus. These findings make TRPV3 channels promising candidates for the development of new therapies for the treatment of itch (158).

Concluding Remarks

Trigeminal chemosensitivity is an important aspect of human sensory physiology, relevant to food enjoyment, nutrition, peripheral mechanisms of itch and pain, thermoregulation, skin physiology, environmental toxicology, respiratory diseases, and eye pathologies. Work over the past decade has expanded tremendously our understanding of the cellular and molecular aspects of trigeminal chemosensation. The identification of specific ion channels, especially TRP channels, involved in different pathophysiological mechanisms including pain, pruritus, headache, chronic cough, and asthma, opens new opportunities for their treatment. Unfortunately, the number of specific antagonists targeting TRP channels is still very limited and their characterization using *in vivo* models of disease is very fragmentary. However, the investment in drug discovery programs by major pharmaceutical companies in this area is high. There is significant hope that the pace of progress in this field will speed up, and significant developments are expected in the upcoming years, hopefully resulting in novel therapeutics for some of these diseases.

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