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TRPs in Taste and Chemesthesis

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Abstract

TRP channels are expressed in taste buds, nerve fibers, and keratinocytes in the oronasal cavity. These channels play integral roles in transducing chemical stimuli, giving rise to sensations of taste, irritation, warmth, coolness, and pungency. Specifically, TRPM5 acts downstream of taste receptors in the taste transduction pathway. TRPM5 channels convert taste-evoked intracellular Ca^{2+} release into membrane depolarization to trigger taste transmitter secretion. PKD2L1 is expressed in acid-sensitive (sour) taste bud cells but is unlikely to be the transducer for sour taste. TRPV1 is a receptor for pungent chemical stimuli such as capsaicin and for several irritants (chemesthesis). It is controversial whether TRPV1 is present in the taste buds and plays a direct role in taste. Instead, TRPV1 is expressed in non-gustatory sensory afferent fibers and in keratinocytes of the oronasal cavity. In many sensory fibers and epithelial cells lining the oronasal cavity, TRPA1 is also co-expressed with TRPV1. As with TRPV1, TRPA1 transduces a wide variety of irritants and, in combination with TRPV1, assures that there is a broad response to noxious chemical stimuli. Other TRP channels, including TRPM8, TRPV3, and TRPV4, play less prominent roles in chemesthesis and no known role in taste, *per se*. The pungency of foods and beverages is likely highly influenced by the temperature at which they are consumed, their acidity, and, for beverages, their carbonation. All these factors modulate the activity of TRP channels in taste buds and in the oronasal mucosa.

Keywords

TRPM5; TRPV1; TRPA1; Gustation; Chemesthesis; Sour; Salty; Irritants; Spices; Carbonated sodas; Oronasal cavity; Trigeminal

1 Introduction

Consistent with their role in sensory mechanisms elsewhere in the body, TRP channels are key players in the chemical senses. This chapter reviews findings over the past decades up to the present about TRP channels in two chemical senses—taste and chemesthesis. The other major chemical sense, olfaction, although closely linked with chemesthesis (Cain and Murphy 1980; Frasnelli et al. 2007), is discussed elsewhere, in the chapter “TRPs in Olfaction.” Taste comprises sensations of sweet, sour, salty, bitter, umami, and possibly fat, as generated in the peripheral end organs, the taste buds. Chemesthesis refers to the more

general chemical sensitivity of the skin and mucous membranes, perceived as irritation, pungency, cooling, warmth, or heat. The following pages catalog and discuss the different TRP channels that may be involved in gustation and chemesthesis. Of the entire superfamily of TRP channels, only a limited number have known functions in taste and chemesthesis. A review of the literature reveals that certain of these TRP channels figure more prominently in taste and others in chemesthesis. Simply enumerating references to a given TRP channel in abstracts of these publications shows that the channel that is mentioned most often in taste literature is TRPM5, and in chemesthesis is TRPV1 (Fig. 1). This might indicate that these particular channels are the main players in those senses, or at least the ones most thoroughly investigated to date.

After briefly introducing the cellular organization of taste buds and the anatomy of the chemesthetic sense (next), I will present and describe the expression pattern and functional properties of TRPM5, TRPV1, and other TRP channels involved in taste and chemesthesis in the order of how often they are cited in the literature, as shown in Fig. 1.

2 What Types of Cells Are Found in Taste Buds?

Taste buds represent a community of cells that express different chemosensory transduction proteins and carry out distinct functions. Taste cells are classified into three main categories, originally based on their histology and ultrastructure, but more recently distinguished by their expression patterns of key proteins involved in taste transduction (Chaudhari and Roper 2010). These categories are type I, type II, and type III taste bud cells. TRP channels are differentially expressed among these taste cell types (discussed below).

Type I cells are the most numerous cells in taste buds, comprising $\frac{1}{2}$ to $\frac{2}{3}$ of the total population of 70–85 taste cells in rodents (Ma et al. 2007). These cells envelop adjacent taste cells with lamellar processes. These lamellar processes are endowed with a powerful ecto-ATPase (Bartel et al. 2006). This surface-bound enzyme rapidly breaks down and limits the diffusion and lifetime of ATP, a transmitter released by type II cells during taste excitation (see next). Type I cells also are believed to participate in the spatial buffering of K^+ ions released into the interstitial spaces deep within a taste bud when taste cells are stimulated and they generate bursts of action potentials (Dvoryanchikov et al. 2009). This combination of functions suggests that type I cells represent a type of glial or supporting cell within taste buds (Chaudhari and Roper 2010). Type I cells have also been implicated in salt taste (Vandenbeuch et al. 2008). This function remains to be confirmed with additional studies.

Type II taste cells make up about 20–30 % of the taste bud¹. These taste cells possess G protein-coupled receptors (GPCRs) for sweet, bitter, and umami taste compounds. Taste GPCRs for sweet and umami compounds consist of heterodimers of T1R1, T1R2, and T1R3. Bitter taste receptors (T2Rs) form a family of about two dozen (depending on species) GPCRs that differ markedly from the T1Rs. Type II cells have also been called Receptor taste cells because they express these taste GPCRs for sweet, umami, or bitter.

¹The proportions of types I, II, and III cells stated here are based on taste tissues from mice and rats, immunostained for cell markers that characterize these types (Ma et al. 2007). These proportions vary markedly depending on the location of the taste buds within the oral cavity.

Some bitter-sensitive Receptor cells also respond to aversively high salt (NaCl, KCl) concentrations (Oka et al. 2013). Receptor (type II) taste cells express TRPM5 channels which are prominently involved in taste transduction, as will be shown below. Upon taste stimulation, these cells secrete ATP via membrane channels (Huang et al. 2007; Romanov et al. 2007; Taruno et al. 2013). ATP is a neurotransmitter that excites gustatory sensory afferents (Bo et al. 1999; Finger et al. 2005). ATP, acting as a paracrine transmitter, also excites adjacent type III taste cells (Huang et al. 2007).

Type III cells represent a small population of cells within taste buds, ~15 %.¹ These cells respond directly to acid (sour) taste stimuli (Huang et al. 2006, 2008b; Chang et al. 2010). Type III cells may also respond to high concentrations of NaCl and KCl (Oka et al. 2013). In addition, type III cells also respond indirectly to sweet, bitter, and umami tastes via the aforementioned purinergic paracrine pathway from Receptor (type II) cells (Tomchik et al. 2007). Type III cells are the only taste cells that have ultrastructural specializations associated with synapses. Correspondingly, these cells selectively express proteins associated with synapses, such as SNAP25, and they store and release the neurotransmitters serotonin and GABA. Because of these characteristics, type III cells have been named Presynaptic cells. Stimulation of Presynaptic (type III) cells releases serotonin and GABA into the interstitial space of the taste bud. Serotonin, and perhaps also GABA, mediates an inhibitory feedback onto Receptor (type II) taste bud cells during taste excitation (Chaudhari and Roper 2010). Presynaptic (type III) cells also express the TRP channel PKD2L1, whose function remains debatable, as will be discussed below.

These cells and their connections are reviewed in Chaudhari and Roper (2010) and Roper (2013), and diagrammed in Fig. 2.

3 What Is the Anatomical Basis for Chemesthesis?

The sense of irritation, pungency, warmth, or cooling elicited by a wide variety of chemical compounds acting on the skin and mucus membranes, coined the “common chemical sense” (Parker 1912), is now termed chemesthesis (Green et al. 1990; Green 2012). This multimodal sense is related to but distinct from taste, olfaction, pain, temperature, and itch (Laska et al. 1997; Green 2000). Examples of chemesthesis include the irritating effects of concentrated saline solutions (e.g., seawater) on the nasal mucosa, or painful sensations evoked by a high concentration of CO₂. Other examples are the irritation of wasabi or hot chili peppers (capsaicin), the astringency of tannins in tea, and the unpleasant sensation from methyl salicylate. Chemesthesis also refers to nonirritating sensations such as the cooling of menthol, used in mouthwash, toothpaste, decongestants, and other products. Chemesthesis also includes pleasant sensations, for instance, those produced by aromatic spices such as oregano and mint. Given this broad range of sensations, it is not surprising that the receptor mechanisms subserving chemesthesis are equally diverse and are present in a wide array of sensory structures, including nociceptors, other free nerve endings, and keratinocytes.

Although formally, chemesthesis represents the general chemical sensitivity of the skin and mucus membranes throughout the body, this term commonly refers to sensations in the oronasal cavities (Fig. 3) and eyes (specifically, the cornea). Oronasal chemesthetic signals

are believed to be conducted by somatosensory fibers in the trigeminal (V), glossopharyngeal (IX), and vagus (X) nerves. Chemesthetic stimuli are transduced by unmyelinated terminals of fibers traveling in these nerves, by isolated chemosensory cells innervated by afferent axons travelling in these nerves, and possibly by epithelial keratinocytes, as discussed below.

4 TRP Channels in Taste Buds

4.1 TRPM5 Channels Are Downstream Effectors for G Protein-Coupled Taste Receptors

The first TRP channel identified unambiguously in taste tissue was TRPM5. Consequently, there is an abundant literature on the expression and function of this channel in taste cells. Antibodies to TRPM5 and transgenic mice expressing GFP on the TRPM5 promoter have proven to be key markers for certain taste bud cells and have been invaluable for investigating taste bud structure and function.

4.1.1 TRPM5 Expression in Taste Buds—Margolskee and his colleagues (Perez et al. 2002) used single-cell PCR to search for RNAs differentially distributed among taste cells expressing or lacking gustducin, a taste-specific G_α protein associated with sweet and bitter transduction. Their search identified TRPM5 in a subset of taste bud cells. Perez et al. (2002) and, shortly after, Zhang et al. (2003) confirmed that this TRP channel is selectively expressed in cells that also possess transduction machinery for sweet and bitter tastes. Transgenic mice expressing GFP on the TRPM5 promoter and/or anti-TRPM5 immunostaining verified the association of TRPM5 with Receptor (type II) taste cells (Clapp et al. 2006; Bezencon et al. 2007; Kaske et al. 2007). Consequently, TRPM5 immunostaining or GFP expression driven by TRPM5 has become a reliable and robust marker for Receptor (type II) taste cells.

4.1.2 TRPM5 Function in Taste Buds—Based on its cellular location, Perez et al. (2002) presumed that TRPM5 participated in transduction of sweet and bitter tastes. Heterologous expression of their transcripts in CHO cells and *Xenopus* oocytes led the researchers to conclude that this TRP channel mediated Ca^{2+} influx during taste transduction. They surmised that the immediate events following gustatory activation of taste GPCRs was an IP₃-mediated depletion of intracellular Ca^{2+} stores, and that this depletion triggered TRPM5 to open. Shortly following that publication, Montell and his laboratory (Hofmann et al. 2003), Liu and Liman (2003), and Prawitt et al. (2003) clarified that TRPM5 was a monovalent cation channel that was impermeable to Ca^{2+} . These researchers and Zhang et al. (2007) also reported that this channel was triggered open by a rise in, not a depletion of, intracellular Ca^{2+} consequent to taste stimulation. This is now accepted as how TRPM5 participates in taste transduction (Liman 2007). Interestingly, TRPM5 is one of only two TRP channels (the other being TRPM4) that do not permeate Ca^{2+} . They are selectively permeable to monovalent cations. Because Na^+ and K^+ ions permeate TRPM5 channels, this channel is believed to generate depolarizing receptor potentials in Receptor (type II) cells. The consensus chemotransduction pathway for taste GPCRs is outlined in Fig. 4.

Huang and Roper (2010) demonstrated the importance of TRPM5 for taste transmitter secretion, the final step in the above transduction pathway. They showed that during taste-evoked responses, the depolarization generated by TRPM5 acts in concert with Ca^{2+} released from intracellular stores to elicit non-vesicular ATP secretion, presumably through pannexin 1 and/or CAHLM1 channels (Huang et al. 2007; Romanov et al. 2007; Huang and Roper 2010; Taruno et al. 2013).

4.1.3 Genetic Ablation of *Trpm5*: Knockout Studies in Taste—Initial reports of genetically modified mice lacking functional TRPM5 protein showed the mice lacked normal taste responses to sweet, bitter, or umami compounds (Zhang et al. 2003). This finding cemented a role for TRPM5 in taste transduction. Later studies that used a different *Trpm5* knockout mouse strain reported that taste responses were significantly reduced, but not entirely absent (Damak et al. 2006; Oliveira-Maia et al. 2009). Those studies underlined the importance of TRPM5 in taste but also revealed taste transduction mechanisms for sweet, bitter, and umami that are independent of TRPM5. Genetically engineered mice lacking TRPM5 also have a substantially reduced response to aversively high concentrations of sodium and potassium salts (Oka et al. 2013). Specifically how TRPM5 channels participate in aversive salt taste transduction is not presently known. Lastly, Liu et al. (2011) showed that knockout mice lacking TRPM5 had reduced taste responses to linoleic acid, indicating that this TRP channel is involved in the chemotransduction pathway for fatty taste in rodents. The receptors for fatty taste are currently being hotly pursued. Whether fatty is a basic taste is currently actively debated.²

4.1.4 Pharmacological Block of TRPM5 Channels in Taste Buds—In addition to genetic knockout experiments, researchers have used pharmacological agents to block TRPM5 channel activity and assay how this affects taste. Talavera et al. (2008) showed that quinine, a pharmacological antagonist of TRPM5, reduced sweet-evoked gustatory nerve responses in mice, consistent with the role in taste transduction outlined above. To confirm that TRPM5 was the proximate target for quinine, these researchers showed that quinine had no effect in *Trpm5* knockout mice.³ These findings may be related to the ability of the bitterness of quinine to reduce sweet, a taste quality transduced by TRPM5 (Lawless 1979; Keast and Breslin 2003; Frank et al. 2005). Sweet/bitter mixture suppression due to central cognitive effects is equally plausible (Kroeze and Bartoshuk 1985). Curiously, Talavera et al. (2008) found that quinine did not significantly inhibit taste nerve responses evoked by umami stimuli, even though umami, like sweet and bitter taste transduction, is believed to utilize TRPM5 channels. A likely explanation is that, like sweet, umami taste is transduced via multiple parallel, TRPM5-dependent and TRPM5-independent pathways (Talavera et al. 2008; Tokita and Boughter 2012; Kusuhara et al. 2013).

Other studies have tested alternative TRPM5 blockers. Palmer et al. (2010) demonstrated that triphenylphosphine oxide (TPPO), a selective antagonist for TRPM5, blocked currents

²It remains arguable whether fatty acids or fats in general stimulate a primary taste quality in humans, as opposed to the sensations of olfaction and texture that fats elicit (Tucker and Mattes 2012).

³Talavera et al. (2008) used the same strain of *Trpm5* knockout mice that had been shown to exhibit reduced, but reliable, TRPM5-independent sweet responses (Damak et al. 2006). Quinine did not alter these TRPM5-independent responses.

in excised patches of mouse taste cells. Taste-evoked responses were not investigated in that study. Topical application of high concentrations of TPPO (~100× IC₅₀) onto the rat tongue during taste stimulation altered salt responses and reduced gustatory nerve responses to the bitter tastant quinine (Ren et al. 2013). Findings showing responses to aversively strong salt stimulation are reduced in *Trpm5* knockout mice (Oka et al. 2013) are consistent with these effects of TPPO. However, using quinine as a bitter taste stimulus is complicated given that Talavera et al. (2008) had shown that quinine itself is a TRPM5 antagonist. It is fair to say that the detailed actions of TPPO on taste responses have not been well characterized.

Finally, another approach to blocking TRPM5 has been to eliminate inward current through this channel using ion substitutions. As previously cited, Huang and Roper (2010) eliminated TRPM5 function in isolated mouse taste Receptor (type II) cells by replacing Na⁺ in the bathing medium with NMDG⁺, a cation that does not permeate TRPM5 channels. Exchanging Na⁺ with NMDG⁺ blocked taste-evoked transmitter (ATP) release from the cells, consistent with the proposed role for TRPM5 in taste transduction.

4.1.5 Factors That Modulate TRPM5 Channel Activity in Taste Buds—Acidic solutions potently block TRPM5 when this channel is expressed in HEK cells. The effect of acid has an IC₅₀ at pH 6.2 with nearly complete block at pH 5.4 (Liu et al. 2005). Intracellular acidification does not affect TRPM5 channels. The pronounced effect of low pH on TRPM5 is curious insofar as one might predict acidic solutions might reduce tastes that are transduced by TRPM5 channels, namely sweet, bitter, and umami. However, combining acid (sour) taste with sweet or bitter results in variable and complex outcomes, including no interaction, suppression, or enhancement, depending on concentrations of the two stimuli (Schiffman et al. 2000; Green et al. 2010; see Keast and Breslin 2003). A possible explanation for these disparate effects of acid stimulation in taste mixtures is that TRPM5 channels in situ may have different properties than when expressed in HEK cells, such as forming channel multimers that are pH insensitive or that TRPM5 channels in situ are situated on the basolateral surface of taste cells and protected from fluctuations of pH in the oral cavity, as suggested by Liu et al. (2005). Alternatively, there may be overriding central neural mechanisms (Savant and McDaniel 2004). Furthermore, acidic compounds will stimulate Presynaptic (type III) taste cells, thereby activating inhibitory paracrine pathways (serotonergic, GABAergic) in taste buds (Fig. 2), complicating any direct actions of low pH on TRPM5 channels in Receptor (type II) cells.

Temperature also modulates TRPM5 activity. Warming HEK cells expressing TRPM5 from 15° to 35 °C activates this channel (Talavera et al. 2005). This means that temperatures to which taste buds are routinely exposed when one consumes cold or hot food and beverages can affect TRPM5. Talavera et al. (2005) showed that this heat sensitivity of TRPM5 indeed confers a temperature modulation of certain taste responses. Specifically, warming taste stimuli to 35 °C enhanced responses to sweet compounds in chorda tympani nerve recordings from mice. Sweet taste responses in *Trpm5* knockout mice were unaltered by temperature changes. This temperature sensitivity of TRPM5 is believed to explain how warming or cooling the tongue gives rise to gustatory nerve responses and taste sensations even in the absence of chemical stimuli (Sato et al. 1975; Cruz and Green 2000). Warming the tongue would be expected to activate TRPM5 directly, bypassing taste receptors and

IP3-mediated Ca^{2+} release and eliciting transmitter release. This simple mechanism cannot completely explain thermal taste, however, because cooling the tongue, which would have the opposite effect and suppress TRPM5 channels, elicits sour or bitter tastes (Cruz and Green 2000). Furthermore, warming solutions of taste stimuli selectively enhanced responses to sweet compounds, yet TRPM5 also transduces bitter and umami compounds. Why sweet taste is specifically enhanced was not explained. Lastly, presenting a taste stimulus at different temperatures onto the tongue elicits different responses in gustatory neurons in the brain stem (Wilson and Lemon 2013). This temperature modulation of taste-evoked responses was attributed in part to the thermal sensitivity of TRPM5.

Other regulators of TRPM5 include phosphatidylinositol-4,5-bisphosphate (PIP2) (Liu and Liman 2003) and arachidonic acid (Oike et al. 2006). How or whether these intracellular factors figure into taste modulation via alterations in TRPM5 channel activity during taste transduction remains undetermined.

4.1.6 TRPM4 in Taste Buds—There is only very indirect evidence that another member of the melastatin-related branch of TRP channels, TRPM4, is present in taste buds. TRPM4 channels, like TRPM5, do not pass Ca^{2+} , which sets these channels apart from other TRP channels. Neither the transcripts for TRPM4 nor the protein itself has been detected in taste buds to date. Yet, using patch clamp recordings on taste bud cells from mice lacking TRPM5 channels, researchers have observed Ca^{2+} -activated currents with properties suggestive of TRPM4 (Zhang et al. 2007). A role for this current in taste cells, or even whether it is generated by TRPM4, is not known.

4.1.7 Conclusions Regarding TRPM4 and TRPM5 Channels in Taste Buds⁴—There is strong, unambiguous, and compelling evidence that exists for TRPM5 channels in a subset of taste bud cells, namely in Receptor (type II) taste cells. This Ca^{2+} -activated monovalent cation channel functions as a downstream effector to link taste GPCR activation and release of intracellular Ca^{2+} with membrane depolarization during taste stimulation.

⁴TRPM5 channels are also found in chemical-sensing cells that express “taste” GPCRs but that are located outside the taste buds. For instance, solitary chemosensory cells in the upper air tract express taste receptors and TRPM5 channels (Kaske et al. 2007; Lin et al. 2008). These cells are discussed in greater detail later in this chapter. Also, isolated receptor cells in the lower respiratory tract that are innervated by vagal sensory fibers (“brush cells”) express bitter taste receptors and other components of the taste receptor transduction pathway, including TRPM5 (Kaske et al. 2007; Tizzano et al. 2010; Krasteva et al. 2011). In the intestinal tract, nutrient-sensing cells express taste GPCRs as well as TRPM5 channels (Wu et al. 2002; Fonfria et al. 2006; Bezencon et al. 2007; Jang et al. 2007; Kidd et al. 2008; Kokrashvili et al. 2009; Young et al. 2009; Janssen et al. 2011). These gut chemoreceptor cells may employ TRPM5 in a similar transduction pathway as do taste cells. Also, TRPM5 and taste receptors are expressed in the pancreas (Taniguchi 2004; Fonfria et al. 2006; Reimann et al. 2008; Nakagawa et al. 2009; Colsoul et al. 2010). TRPM5 channels are required for normal glucose-stimulated insulin secretion from the pancreas (Uchida and Tominaga 2011); *Trpm5* knockout mice have impaired glucose tolerance, and pancreatic islets from these mice show defective glucose-induced insulin release (Colsoul et al. 2010). Nakagawa et al. (2009) showed that the insulin-secreting pancreatic β cell line, MIN6, that had previously been shown to express TRPM5 channels (Prawitt et al. 2003) expresses sweet taste receptors. They reported that artificial sweeteners and glucose promoted insulin secretion from these cells. These data reinforce the notion that there may be a transduction pathway in pancreatic β cells resembling that in taste Receptor (Type II) cells. [Curiously, without referring to the earlier study showing TRPM5 expression in MIN6 cells (Prawitt et al. 2003), Nakagawa et al. (2009) reported that TRPM5 was *not* present in their MIN6 cells.] Finally, taste receptors and TRPM5 are co-expressed in spermatids (Iwatsuki et al. 2010; Li and Zhou 2012; Meyer et al. 2012; Mosinger et al. 2013). The function of this chemoreceptor transduction pathway remains to be elucidated, though it appears to be involved in spermatid differentiation and maturation (Mosinger et al. 2013).

As a generality, one might argue that many chemosensory cells throughout the body that express “taste” GPCRs also express TRPM5 and likely mobilize intracellular Ca^{2+} in a manner similar to the canonical taste transduction pathway (Fig. 4). Perhaps the nomenclature for “taste” receptor genes, “TASRs,” should be reconsidered and renamed to apply more broadly to chemical sensors situated far distant from the end organs of taste in the oral cavity.

TRPM5 is found in many chemoreceptor cells located outside of taste buds that also have “taste” GPCRs, suggesting a common linkage between TRPM5 and taste receptors. Evidence for expression of the related channel, TRPM4, in taste buds is considerably weaker.

4.2 Do Taste Bud Cells Express TRPV1 Channels?

The next most intensely studied TRP channel after TRPM5 in taste is TRPV1, the capsaicin receptor. Two sensations related to gustation are believed to involve TRPV1—pungency and salty taste. Pungent sensations elicited by capsaicin, the active ingredient of hot chili peppers, were investigated long before its cognate receptor TRPV1 (formerly, VR1) was cloned (Szolcsanyi 1977; Rozin et al. 1981; Lawless et al. 1985). The consensus is that this spicy ingredient mainly affects somatosensory afferents (nociceptors, see chemesthesis, below). However, there may be some ill-defined interactions between capsaicin and taste bud cells. More recently, salt taste has been postulated to involve TRPV1 or a TRPV1-like channel (Lyll et al. 2004). Despite these associations between TRPV1 and taste, the notion that this channel is present and functional in taste buds is arguable, as reviewed below.

4.2.1 TRPV1 Expression in Taste Buds—It is debatable whether TRPV1 is expressed in taste bud cells. The findings are equivocal. TRPV1 transcripts have been identified using RT-PCR on RNA extracted from rat taste buds (Liu and Simon 2001; Lyall et al. 2004; Moon et al. 2010). It is impossible in these experiments, however, to rule out contamination from somatosensory nerve terminals (e.g., trigeminal) that might contribute TRPV1 RNA (Whitehead et al. 1999; Tohda et al. 2001) or contamination from epithelial cells that also express TRPV1 (Marincsak et al. 2009). Highlighting this problem, Ishida et al. (2002) and Kido et al. (2003) independently observed immunostaining for TRPV1 protein (then named VR1) in nerve fibers surrounding and penetrating into rat taste buds. Both laboratories specifically noted the absence of TRPV1-immunopositive taste bud cells. Those findings were contradicted by another group who claimed to find TRPV1 immunostaining in rat taste bud cells that were also immunopositive for taste receptors (Gu et al. 2009; Moon et al. 2010). These latter authors offered no explanation for the discrepancy between their findings and those of previous researchers. It must be noted that because their tissue source was rat lingual samples, Gu et al. (2009) and Moon et al. (2010) were unable to conduct the gold standard control—immunostaining tissue from *Trpv1*-knockout animals. Failing this control and given the nonselectivity of some anti-TRPV1 antibodies (Everaerts et al. 2009), the claim of TRPV1-immunopositive taste cells should be viewed with some reservation. The contradictory findings remain unresolved.

If gustatory sensory afferents, *per se*, express TRPV1, as claimed in some of the above publications, one might expect to find evidence for TRPV1 expression in the parent neurons—geniculate and petrosal ganglion cells. Given that geniculate ganglion neurons predominantly (but not exclusively) innervate taste buds, that ganglion is well suited to investigate TRPV1 expression in gustatory afferents. A comprehensive study of cranial ganglia from rats failed to reveal expression of TRPV1 in geniculate ganglion neurons, though expression was abundant in the neighboring trigeminal ganglion (Matsumoto et al. 2001). In contrast, Katsura et al. (2006) reported TRPV1 immunostaining and in situ

hybridization for TRPV1 in roughly 20 % of rat geniculate ganglion cells, a finding that might be consistent with the aforementioned TRPV1 expression in fibers innervating taste buds. Yet, a portion of geniculate ganglion neurons innervates the skin of the outer ear via the posterior auricular nerve. This population might include TRPV1-expressing somatosensory nociceptors.⁵ Katsura et al. (2006) did not refer to or explain their discrepancy with the earlier findings of Matsumoto et al. (2001). As explained above, rat tissue does not lend itself to immunostaining controls using knockout tissue. Furthermore, it was not clear in Katsura et al. (2006) that control probes (sense vs. antisense) were included in their in situ hybridization. In short, the absence of critical controls for in situ hybridization and immunostaining substantially weakens findings claiming expression of TRPV1 in the geniculate ganglion.

In an interesting alternative approach to exploring expression of TRPV1 in sensory fibers that innervate taste buds and specifically in chorda tympani nerve fibers, Hiura et al. (1990) injected a limited number of mice ($n = 3$) with capsaicin shortly after birth. This procedure destroys neurons that express TRPV1. Using electron microscopy, they counted the number of axons remaining in the chorda tympani nerves. Injecting capsaicin caused no loss of chorda tympani axons relative to uninjected controls, leading Hiura et al. to conclude that geniculate ganglion neurons and their axons in the chorda tympani do not have capsaicin receptors (later identified as TRPV1).⁶ In earlier studies using this technique on neonatal rats, Nagy et al. (1982) had shown that peptidergic (Substance P) fibers surrounding the taste buds were destroyed by capsaicin treatment, entirely consistent with the destruction of somatosensory, capsaicin-sensitive fibers (see below). That is, neurons that express TRPV1, such as those in the trigeminal ganglion, are destroyed by neonatal capsaicin injection. Neurons lacking TRPV1 are refractory to this procedure. The above findings stress the absence of TRPV1 in sensory neurons and their axons that innervate taste buds.

4.2.2 TRPV1 Function in Taste Buds—If there is no definitive evidence for the expression of TRPV1 RNA or protein in taste buds, is there instead functional evidence for this channel in the end organs of taste? Some researchers report that in isolated rat taste receptor cells, 50–100 μM capsaicin stimulates Ca^{2+} influx and inhibits K^{+} conductance, though not necessarily via TRPV1 (Park et al. 2003; Costa et al. 2005).⁷ Additionally, Liu and Simon (2001) found no functional evidence for TRPV1 in isolated rat taste bud cells. Liu and Simon (2001) based their conclusion on the inability of an established TRPV1 antagonist, capsazepine, to block proton-activated taste cell responses. Those findings were supported by their later study where they speculated that capsaicin elicits effects independent of TRPV1, perhaps by partitioning into the plasma membrane and influencing ion channels and GRPCs nonselectively (Costa et al. 2005).

⁵However, using patch-clamp recordings, Nakamura and Bradley (2011) reported that geniculate ganglion neurons with axons in the posterior auricular nerve specifically were insensitive to capsaicin, a TRPV1 agonist. This finding argues against a population of geniculate ganglion nociceptive neurons dedicated to the posterior auricular nerve.

⁶Nakamura and Bradley (2011) also noted that geniculate ganglion neurons with axons in the chorda tympani nerve were insensitive to capsaicin, complementing the findings of Hiura et al. (1990). The only capsaicin-responsive geniculate ganglion neurons were those innervating the soft palate and having axons in the greater petrosal nerve (Nakamura and Bradley 2011).

⁷These are reasonable concentrations of capsaicin. Five to 100 μM capsaicin, when applied to the tongue, elicits a mild to moderate burning sensation (Simons et al. 2002).

Capsaicin (10 μ M) applied to the tongue elicits small, almost negligible responses in gustatory nerves innervating taste buds (Dahl et al. 1997), a finding that does not strongly argue for the existence of capsaicin receptors in taste bud cells or taste fibers. Capsaicin solutions, however, are able to *modulate* taste nerve responses to other compounds, particularly NaCl (Wang et al. 1995; Osada et al. 1997). This modulation was interpreted as being produced indirectly, via stimulation of non-gustatory, capsaicin-sensitive somatosensory nerve terminals in the lingual epithelium (e.g., trigeminal) that released peptides such as Substance P or CGRP onto taste cells, that is, by axon reflex (Finger 1986; Holzer 1988; Wang et al. 1995).

Other researchers extended the above findings and reached a different conclusion. Namely, Lyall et al. (2004) applied the TRPV1 agonists capsaicin and resiniferatoxin topically onto the tongue and also found these agents enhanced gustatory nerve (chorda tympani) responses to NaCl stimulation. This enhancement was specifically in amiloride-insensitive NaCl responses, that component of salt taste that appears to transduce aversive concentrations of Na⁺ and K⁺ salts (Oka et al. 2013). Capsaicin and resiniferatoxin were ineffective in enhancing responses in *Trpv1* knockout mice. Based on these findings and their RT-PCR data (see above), Lyall et al. (2004) concluded that TRPV1 or a TRPV1-like channel is expressed in taste bud cells and transduces amiloride-insensitive salt taste. However, these conclusions have been challenged by behavioral studies of salt taste in *Trpv1* knockout mice (see below).

Lastly, investigators have conducted physiological tests to search for evidence of TRPV1 in the sensory ganglion cells that innervate taste buds—geniculate ganglion neurons. Nakamura and Bradley (2011) reported that a subset of rat geniculate ganglion neurons, namely, 27 % of the small population of neurons that innervate the soft palate responded to capsaicin (10 μ M) with an inward current under patch clamp recordings. Geniculate ganglion neurons that innervated taste buds on the anterior tongue did not respond to capsaicin. Geniculate ganglion cells that responded to capsaicin had features associated with nociceptive neurons, such as large tetrodotoxin-resistance Na⁺ currents and immunopositivity for isolectin B₄ (IB4)-FITC. It was not possible to identify whether geniculate ganglion cells that responded to capsaicin innervated taste buds *per se*. In short, the findings of Katsura et al. (2006) and Nakamura and Bradley (2011), taken together, could be interpreted as suggesting that about ¼ of the geniculate ganglion neurons have characteristics of nociceptors and that these neurons innervate the soft palate, not the tongue. Because both those studies were conducted on rats, it was not possible to validate the observations in animals lacking TRPV1 channels (i.e., knockout, next).

4.2.3 Genetic Ablation of *Trpv1*, Knockout Studies in Taste—Mice normally avoid drinking solutions containing high concentrations of capsaicin (100 μ M), presumably due to the irritating effect generated by stimulating somato-sensory TRPV1 channels (chemesthesis, see below). Not surprisingly, *Trpv1* knockout mice consume solutions of 100 μ M capsaicin as readily as they do water (Costa et al. 2005). Yet, despite the absence of TRPV1 channels and the absence of capsaicin-evoked avoidance behavior, capsaicin was nonetheless able to alter taste responses in knockout mice. Specifically, 100 μ M capsaicin reduced the strong taste preference for sucrose solutions in *Trpv1* knockout mice (Costa et

al. 2005). The significance of these findings is that capsaicin acts on targets other than TRPV1. Reports of how capsaicin acts on taste buds and gustatory fibers should be interpreted with caution.

Trpv1 knockout mice have been studied intensively vis-à-vis the hypothesis that this vanilloid receptor transduces salt (NaCl) taste (above). Two independent studies showed that there are no changes, or only ambiguous ones, in salt taste behavior in *Trpv1* knockout mice (Ruiz et al. 2006; Treesukosol et al. 2007). Further investigations substantiated these findings and indicated that wild-type and *Trpv1* knockout mice did not differ in their perceived saltiness of moderate concentrations of NaCl solutions (Smith et al. 2012). Only when NaCl concentrations became sufficiently high to elicit irritation, possibly through nociceptors or osmoreceptors, were differences between wild-type and *Trpv1* knockout mice evident. Put simply, there is no evidence for alterations in salt taste in *Trpv1* knockout mice. Once again, the findings do not vouch for the presence of TRPV1 in taste buds or sensory fibers innervating taste buds.

4.2.4 Conclusions Regarding TRPV1 Channels in Taste⁸—In sum, neither the molecular expression of nor physiological evidence for TRPV1 in sensory gustatory afferent neurons or taste cells is particularly compelling. RT-PCR evidence for TRPV1 expression in taste buds may stem from contaminating fragments of somatosensory nerve fibers. Immunohistochemical findings lack critical controls using *Trpv1* knockout tissue. However, the lack of evidence for TRPV1 expression in taste buds or gustatory afferent neurons does not, of course, prove that this TRP channel is not present. Until and unless more complete experiments are conducted on knockout mice lacking TRPV1, or single-cell RT-PCR on RNA taken from isolated taste bud cells (DeFazio et al. 2006), a role of this channel in taste transduction remains uncertain, at best.

4.3 Are PKD2L1 Channels the Long-Sought Sour Transducer?

4.3.1 PKD2L1 Expression in Taste Buds—LopezJimenez et al. (2006) were the first to identify PKD2L1 (“polycystic kidney disease 2-Like 1,” aka TRPP2) channels co-expressed with the transmembrane protein PKD1L3 (“polycystic kidney disease 1-Like 3”) in a subpopulation of mouse circumvallate taste bud cells. These cells were distinct from those that transduce sweet, bitter, and umami compounds. Consequently, LopezJimenez et al. (2006) surmised that PKD2L1 together with PKD1L3 was involved in acid (sour) or salty taste. In situ hybridization and immunostaining showed that PKD2L1 is found in taste buds throughout the tongue and palate. By contrast, PKD1L3 expression is limited to taste buds in the posterior tongue (circumvallate and foliate taste buds) (Huang et al. 2006; Ishimaru et al. 2006). Kataoka et al. (2008) showed that PKD2L1 immunostaining specifically marked Presynaptic (type III) taste cells.

⁸Parenthetically, other gustatory-related sensations attributed to TRPV1 channels include metallic taste (Riera et al. 2009) and aversive off-tastes of artificial sweeteners (Riera et al. 2008). These conclusions were reached by investigations using heterologous expression systems and taste behavioral assays. The conclusions from the behavioral studies were verified using *Trpv1* knockout mice. How or whether those findings specifically implicate TRPV1 in taste bud cells or gustatory afferents remains to be examined in greater detail. TRPV1 also appears to play some role in the consumption of ethanol, though whether this involves taste per se is unlikely. Specifically, *Trpv1* knockout mice have a higher preference for and consumption of ethanol solutions. Moreover, *Trpv1* knockout mice have greater tolerance for the inebriating action of ethanol (Blednov and Harris 2009).

4.3.2 PKD2L1/PKD1L3 Function in Taste Buds—Shortly after its discovery in taste cells, the combination of PKD2L1 and PKD1L3 was expressed in HEK293T cells and shown to form a cation channel activated by acid (sour-tasting) solutions (Ishimaru et al. 2006). Moreover, mice in which Pre-synaptic (type III) taste cells (and specifically, cells expressing PKD2L1) were selectively ablated lacked responses to acid taste stimuli (Huang et al. 2006). A study on a very limited sample of human subjects ($n = 2$) who exhibited the rare taste disturbance sour ageusia reported that these individuals lacked expression of PKD2L1 and PKD1L3, among other genes (Huque et al. 2009). Interestingly, expressing PKD2L1 in drosophila chemoreceptor cells that normally sense sugars and elicit feeding showed a slight but statistically significant increase in the feeding preference to citric acid (Adachi et al. 2012). These findings all strongly implicated the combination of PKD2L1 and PKD1L3 in acid taste and have led many to conclude that acid taste transduction has finally been explained at the molecular level.

Yet, in the enthusiasm for embracing the view that PKD2L1/PKD1L3 explains acid (sour) taste, important inconsistencies had been overlooked. Namely, heterologously expressed PKD2L1/PKD1L3 has a high threshold for acid activation (e.g., $EC_{50} = \text{pH } 2.9$ with acetic acid) compared with that for sour-sensing taste bud cells, and the expressed channels respond mainly to the *removal* of acid stimulation (offset), not its onset (Ishimaru et al. 2006; Inada et al. 2008; Ishii et al. 2009; Kawaguchi et al. 2010). Further, taste buds in the anterior tongue (fungiform taste buds) and palate, which respond quite well to sour stimuli, lack the PKD1L3 transmembrane protein (Huang et al. 2006; Ishimaru et al. 2006). Finally, many researchers had not taken into account the longstanding awareness that sour taste is not simply activated by acid solutions (i.e., by extracellular protons) (Harvey 1920). Indeed, a proximate stimulus for sourness is *intracellular* acidification (Lyall et al. 2001; Richter et al. 2003; Huang et al. 2008b) produced when fully protonated (i.e., uncharged) acid molecules cross the plasma membrane and dissociate in the cytosol (see (Roper 2007)).⁹ In sum, certain details of PKD2L1/PKD1L3 activation do not align well with sour taste activation in taste buds.

4.3.3 Genetic Ablation of Pkd2l1 and Pkd1l3, Knockout Studies in Taste—A final argument challenging the notion that PKD2L1/PKD1L3 channels transduce acid (sour) taste is the finding that genetic knockout of PKD2L1 and/or PKD1L3 in mice reduces acid taste responses only somewhat (Horio et al. 2011) or not at all (Nelson et al. 2010). There is no doubt that these PKD channels are expressed in acid-sensitive taste cells and that ablating taste cells that express PKD2L1 eliminates acid taste (Huang et al. 2006). However, PKD2L1/PKD1L3 channels themselves may play other roles in cellular function apart from transducing acid taste.

⁹Organic (“weak”) acids such as acetic acid exist as a mixture of protonated and dissociated acid molecules at levels of pH that are sharply sour tasting. Mineral (“strong”) acids such as HCl that are fully dissociated in aqueous solution do not readily cross the plasma membrane (i.e., the plasma membrane is tolerably impermeable to protons). Mineral acids are not as sour tasting as organic acids at equivalent pH values. Protons can only gain access to the cytosol via H^+ -permeable ion channels and transporters, neither of which are features of the PKD2L1/PKD1L3 dimer. Parenthetically, one such H^+ -permeable channel has been identified in Presynaptic (type III) taste bud cells. This channel may contribute to the sour taste of mineral acids (Chang et al. 2010).

4.3.4 Conclusions Regarding PKD2L1 in Sour Taste—In sum, TRP channels may participate in some as-yet unexplained manner to acid taste or to the physiology of acid-sensing taste bud cells, but PKD2L1/PKD1L3 channels *per se* do not appear either to be necessary or sufficient for transducing this taste quality. Mechanisms other than those involving PKD2L1 channels must explain acid taste transduction (Roper 2007; Chang et al. 2010).

4.4 Other, Lesser-Studied TRP Channels in Taste Buds

4.4.1 TRPV4 in Taste Buds—TRPV4 immunostaining was recently identified in zebrafish taste buds (Amato et al. 2012). In other tissues, this TRP channel is associated with sensing cell volume, warm temperatures, and mechanical perturbations (Watanabe et al. 2002; Suzuki et al. 2003; Vriens et al. 2004; Becker et al. 2005; McKemy 2007). The function of TRPV4 channels in zebrafish taste buds was not investigated, but these authors speculated that TRPV4 functioned as a chemoreceptor (Amato et al. 2012). In a separate study, Bradley (2000) argued that certain mammalian taste buds, namely, those in the pharynx, are tonicity detectors. This could provide a possible role for TRPV4 in taste if this TRP channel should be found to be expressed in mammalian pharyngeal taste bud cells. Further, there has been a longstanding comparison between mechano- and osmosensing cutaneous Merkel cells and Merkel-like cells present in taste buds, at least in teleosts and amphibia (Toyoshima 1989; Whitear 1989). In rodents, cutaneous Merkel cells and their innervation in rodents express TRPV4 (Suzuki et al. 2003; Boulais et al. 2009) providing further speculation for a possible mechano- or osmosensing role for TRPV4 in taste buds. Yet, to date, there are no reports of TRPV4 expression in mammalian taste buds. All the aforementioned implied functions for this channel in taste buds are completely speculative.

4.4.2 TRPM8 in Taste Buds—TRPM8 channels are discussed below with reference to chemesthesis. There is one report describing TRPM8 expression in taste buds in the soft palate and pharynx of rats (Sato et al. 2013). Because TRPM8 channels are associated with sensing cool temperatures, these researchers surmised that palatal and pharyngeal taste buds are stimulated during swallowing cold food and drinks.

5 TRP Channels in Chemesthesis

Chemesthesis is often associated with afferent terminals of sensory ganglion neurons innervating the oronasal cavities. A comprehensive quantitative analysis of TRP channels expressed in the neurons from one of these sensory ganglia—mouse trigeminal ganglion neurons—revealed 17 of the possible 28 TRP genes (Vandewauw et al. 2013). A subset of these TRP channels—namely TRPM2,4,7,8; TRPA1; TRPML1; PKD2L1; and TRPV1,2,4—were present at levels significantly higher than the others, perhaps suggesting these channels have especially important sensory functions. Certain of these highly expressed TRP channels transduce thermosensations and, when activated by plant-derived ligands, stimulate a sense of warmth or cooling (Fig. 5). A selection of these TRP channels is discussed below (also see reviews by Gerhold and Bautista 2009; Nilius and Appendino 2011; Viana 2011; Nilius and Appendino 2013).

5.1 TRPV1 Channels Play a Major Role in Chemesthesis

By far, TRPV1 is the predominant TRP channel mentioned in the scientific literature of chemesthesis (Fig. 1) and thus will be presented first. A common experience when cooking with hot chili spices is reflex coughing when kitchen vapors are inhaled. The primary irritant in chili peppers is capsaicin, the prototypic TRPV1 agonist. Capsaicin is a well-established chemesthetic irritant of the nasal cavities (Collier and Fuller 1984) as well as a pungent spice for the palate (Nilius and Appendino 2013). TRPV1, however, is involved in responses to a much broader palette of chemical irritants than just capsaicin, as discussed next.

5.1.1 TRPV1 Expression in the Oronasal Cavities—The capsaicin receptor TRPV1 is robustly expressed in somatosensory neurons and their peripheral nerve fibers (e.g., trigeminal) of the oral and nasal cavities (Caterina et al. 1997; Ishida et al. 2002; Kido et al. 2003; O'Hanlon et al. 2007; Yilmaz et al. 2007; Vandewauw et al. 2013). This widespread expression reflects a major role for this TRP channel in chemesthesis, nociception, thermoreception, and the pungency of hot spices (see reviews by Gerhold and Bautista 2009 and Nilius and Appendino 2013). A significant number (between 20 % and 56 %) of trigeminal ganglion neurons are immunopositive for TRPV1 channels (Ichikawa and Sugimoto 2001; Dussor et al. 2003; Bae et al. 2004; Tanimoto et al. 2005). Yet, as previously cited (Everaerts et al. 2009), caution must be taken interpreting positive results with TRPV1 antibodies, especially in tissues where *Trpv1* knockout animals are not available.

TRPV1 expression has also been documented by immunostaining and RT-PCR in keratinocytes and dermal mast cells, including those in the oronasal mucosa (Kido et al. 2003; Stander et al. 2004; Seki et al. 2006; Kawashima et al. 2012; Kun et al. 2012). This suggests a much broader presence of this TRP channel than just in sensory neurons. As mentioned, however, great caution should be taken interpreting immunostaining with anti-TRPV1 antibodies and RT-PCR may detect TRPV1 expression from axon fragments included in epithelial samples.

5.1.2 TRPV1 Function in Chemesthesis—The seminal work of Julius, Caterina, and others has firmly established the polymodal role of TRPV1 in sensing elevated temperature, generating the pungency of chili peppers (capsaicin) and black pepper (piperine), and eliciting respiratory reflexes (e.g., coughing) (Caterina et al. 1997, 1999; Tominaga et al. 1998; Jordt et al. 2000; Trevisani et al. 2004; McNamara et al. 2005; Adcock 2009).¹⁰ TRPV1 is activated by many chemical compounds in addition to capsaicin and piperine, including acids, ammonia, allicin, mustard oil, cannabinoids such as anandamide, and numerous other compounds (Tominaga et al. 1998; Silver et al. 2006; Veronesi and Oortgiesen 2006; Ohta et al. 2007; Dhaka et al. 2009).

Not all acidic irritants stimulate TRPV1 effectively. Volatilized citric acid but not acetic acid elicits TRPV1-mediated respiratory reflexes (Symanowicz et al. 2004; Trevisani et al.

¹⁰An interesting aside is that TRPV1 in birds lacks the molecular binding domain for capsaicin. Consequently, birds are indifferent to the irritation of capsaicin. This allows birds to consume and disperse seeds of plants such as chili peppers that otherwise repel animals (Jordt and Julius 2002).

2004). However, acetic acid excites TRPV1 channels in the somatosensory axons that innervate the oral cavity (Arai et al. 2010). Differences between acetic and citric acids may be explained by the relative inability of citric acid, compared with acetic acid, to cross cell membranes and effect intracellular acidosis (Ishii et al. 2012). *Extracellular* protons (e.g., from citric acid) activate TRPV1 (Jordt et al. 2000), but intracellular acidification (e.g., from acetic acid) has the reverse action—it inhibits this channel (Chung et al. 2011).¹¹ *Intracellular* protons instead activate another TRP channel present in trigeminal axons, TRPA1 (Wang et al. 2010, 2011) (see below). Thus, whether TRPV1 channels are stimulated in a given situation may depend on the balance of extracellular tissue pH versus intracellular, cytosolic acidification.

Sensory afferent axons expressing TRPV1 channels not only transmit chemesthetic information orthodromically to the CNS, but they also stimulate surrounding (peripheral) tissues via antidromic axon reflex and release of glutamate and peptides from their terminals (Holzer 1988; Jęftinija et al. 1991; Omote et al. 1998). Peptides secreted by axon reflexes include Substance P and calcitonin gene-related peptide (CGRP). This local effector role of sensory nerve endings underlies inflammatory responses in the skin, such as vasodilation when nociceptors are stimulated. Indeed, Finger (1986) and Wang et al. (1995) speculated that stimulating the tongue with capsaicin releases peptides from lingual nerve fibers that terminate near or within taste buds and modulate gustatory responses. Solitary chemosensory receptor cells in the airway mucosa, discussed below, are innervated by peptidergic trigeminal fibers expressing TRPV1 (Tizzano et al. 2010). Chemesthetic stimulation of these TRPV1-rich trigeminal fibers might similarly be expected to elicit Substance P, CGRP, and glutamate release onto the chemosensory cells via axon reflex, possibly affecting the sensitivity of the solitary chemosensory cells.

A function for TRPV1 channels in keratinocytes and other nonneural tissues in the oronasal cavities is not well studied. As briefly discussed below, there are indications that TRPV1 expressed in nonneural tissues may be involved in inflammatory responses (Fernandes et al. 2012), similar to what has been found for TRPV3 in thermal hyperalgesia (Huang et al. 2008a).

5.1.3 Genetic Ablation of *Trpv1*, Knockout Studies in Chemesthesis—The original studies on *Trpv1* knockout mice demonstrated the central role of this TRP channel in pain and thermoreception (Caterina et al. 2000; Davis et al. 2000). Decades before *Trpv1* was cloned, capsaicin-sensitive neurons were ablated in rodents by neonatal injections of the capsinoid. Among other sensory phenomena, this pharmacological ablation showed that capsaicin-sensitive fibers were responsible for certain chemesthetic responses. For instance, rats lacking capsaicin-sensitive afferents did not respond to cigarette smoke as do intact rats

¹¹This is a simplification. By definition, at equi-pH, citric and acetic acid solutions contribute the same $[H^+]_0$. However, at a given pH, fully protonated acetic acid molecules will more readily cross the cell membrane and contribute to intracellular acidification more effectively than will partially protonated citric acid. For example, given their dissociation constants for the fully protonated, neutral moieties (presumably the molecule that is most membrane-permeant), a 10 mM solution of citric acid ($pK_{a1} = 3.13$) at pH = 3.13 will contribute 0.75 mM H^+ and 4.9 mM triprotonated (neutral, membrane-permeant) acid molecules ($H_3Citrate$). The remaining 5.1 mM consists of $H_2Citrate^-$, $H_1Citrate^{2-}$, and $Citrate^{3-}$. By contrast, the same concentration of acetic acid (10 mM, $pK_a = 4.75$) at this same pH will also contribute 0.75 mM H^+ but a twofold higher concentration (9.8 mM) of the uncharged, membrane-permeant molecule, Acetate.

(Lundberg and Saria 1983). Modern genetic ablation shows that *Trpv1* knockout mice lack responses to certain irritants but maintain normal avoidance to other chemesthetic compounds. For instance, in an isolated mouse tracheal preparation, acid-stimulated secretion of CGRP, a bronchostimulator and index of tracheal irritation, was significantly reduced in *Trpv1* knockout compared with wild-type mice (Kichko and Reeh 2009). Further, Saunders et al. (2013) demonstrated that wild-type C57Bl mice avoid cotton swabs saturated with capsaicin or cyclohexanone, but *Trpv1* knockout mice have a somewhat neutral response to the swabs. Yet, in that same study, *Trpv1* knockout mice had mixed responses or no deficiencies in reacting aversively to benzaldehyde, eugenol, amyl acetate, nicotine, toluene, and acetic acid. Similarly, mice lacking TRPV1 channels maintained normal protective respiratory reflexes against irritation by acetic acid and other volatilized irritants (Symanowicz et al. 2004). In other words, somatosensory afferents express chemesthetic receptors other than TRPV1, as will be discussed below. As Saunders et al. (2013) state, polymodal afferent fibers express multiple TRP channels besides TRPV1 to provide redundant mechanisms for detecting irritants. Eliminating TRPV1 only reduces, but does not eliminate, the capacity to respond to irritant stimuli.

How knocking out TRPV1 expression in *nonneural* tissues impacts chemesthesia has not been investigated substantially. Okada et al. (2011) reported that TRPV1 channels in basal epithelial cells in the cornea participate in inflammation and scarring after chemical irritation (alkali treatment). Corneal inflammation was reduced and healing enhanced in *Trpv1* knockout mice. A solid connection between TRPV1 and neurogenic inflammation, of course, is well established (Cortright and Szallasi 2009).

5.1.4 Modulation of TRPV1 Channels—There is a rich literature on influences that modulate TRPV1 activity, including such varied factors as bradykinin, membrane lipids, lysophosphatidic acid, heat, and acids (Sugiura et al. 2002; Tominaga and Tominaga 2005; Vay et al. 2010; Morales-Lazaro et al. 2013). Of interest for the present chapter, the chemesthetic irritant nicotine sensitizes TRPV1 channels to capsaicin, but curiously not to heat (Liu et al. 2004). This sensitization of TRPV1 channels by nicotine does not appear to affect cough reflexes. That is, the presence of nicotine did not alter capsaicin-evoked coughing (Hansson et al. 1994). Parenthetically, nicotine itself is not believed to stimulate TRPV1 channels (Silver et al. 2006).

Equally important for its role in chemesthesia, protons stimulate TRPV1 and enhance capsaicin activation of this channel (Jordt et al. 2000). This may have implications for how certain beverages and foods interact, discussed below. Briefly, acidic soft drinks such as colas might be expected to potentiate TRPV1-mediated pungency of hot spicy foods. This has not been systematically investigated, although there are numerous anecdotal references to this phenomenon.

Finally, as mentioned above, heat directly activates TRPV1. However, when tested on TRPV1-expressing HEK cells, increasing the temperature (22° to 50 °C) actually decreased the potency for capsaicin (Sprague et al. 2001). It is surprising, then, that human subjects perceive heated solutions of capsaicin (34° to 60 °C) as more pungent than cooled solutions (2° to 18 °C) (Sizer and Harris 1985; Green 1986). Yet, another report recorded little effect

of temperature upon capsaicin-evoked oral irritation (Prescott et al. 1993), perhaps explained by methodological differences among the different studies. An altered pungency with increased temperature would have obvious implications for serving food spiced with chili peppers. Interactions between capsaicin and temperature on TRPV1, however, are complicated by their mutual desensitization of the channel.

5.1.5 Conclusions Regarding TRPV1 in Chemesthesis—There is no question that TRPV1 channels in somatosensory afferent fibers underlie responses to many oronasal chemesthetic stimuli, especially volatilized acids, capsaicin, and a variety of other irritants. Yet, TRPV1 immunolocalization to specific cells and tissues is clouded by uncertainties regarding anti-TRPV1 antibodies. Knockout studies show that receptors other than TRPV1 in somatosensory fibers are also activated by many chemical irritants. Redundant mechanisms for sensing oronasal chemesthetic stimuli provide a safety net to insure potentially harmful chemicals are detected. TRPV1 expression in nonneural cells such as keratinocytes may contribute to chemesthetic responses in some as-yet poorly explained manner, possibly involving inflammatory cellular responses. The ability of acidic foods and beverages to enhance TRPV1-mediated responses in the oronasal cavity is a distinct, but as-yet largely unexplored possibility.

5.2 TRPA1 Channels are Co-Expressed with TRPV1

The next most-studied TRP channel in chemesthesis is TRPA1. TRPA1 channels have been implicated in thermo- (cold), mechano-, and chemosensory reception, as well as in other physiological processes (Nilius et al. 2012). TRPA1 is co-expressed with TRPV1 in many somatosensory cells and elsewhere in the oronasal cavities. The two TRP channels TRPA1 and TRPV1 cooperate to assure that an individual responds to chemesthetic irritants.

5.2.1 TRPA1 Expression in the Oronasal Cavities—As with TRPV1 channels, TRPA1 is prominently expressed in trigeminal neurons and fibers innervating nasal and oral epithelium (Kobayashi et al. 2005; Peyrot des Gachons et al. 2011; Vandewauw et al. 2013). TRPA1 channels are also found in nonneural tissues, including keratinocytes (Anand et al. 2008; Atoyan et al. 2009; Nassini et al. 2012). TRPA1 is often co-expressed with TRPV1 (Story et al. 2003; Kobayashi et al. 2005; Akopian 2011; Vandewauw et al. 2013). For instance, in one study on rat trigeminal ganglion cells, 44 % of the neurons expressed TRPV1 and ~83 % of these cells also expressed TRPA1 (Kobayashi et al. 2005). All cells expressing TRPA1 co-expressed TRPV1. These relationships are illustrated in Fig. 6. Although, to my knowledge, the ratio of TRPV1 to TRPA1 expression from cell to cell in sensory ganglia has not been quantified, it seems unlikely that this is uniformly constant across all neurons. Instead, it seems more plausible that the relative expression of TRPV1 to TRPA1 may vary from neuron to neuron and may even be dynamic (Diogenes et al. 2007). This variation could impart different sensitivities to chemesthetic irritants in the oronasal cavities (Rentmeister-Bryant and Green 1997). Sensory fibers with proportionately different TRPA1/TRPV1 expression might have distinctive distributions throughout the mucosa.

5.2.2 TRPA1 Function in Chemesthesis—TRPA1 channels are activated by compounds as diverse as allyl isothiocyanate (“mustard oil,” found in horseradish, wasabi),

allicin (garlic), methyl salicylate (“wintergreen oil,” found in mouthwashes), cinnamaldehyde (main constituent of cinnamon oil, present in foods, chewing gums, and toothpastes) (Bandell et al. 2004; Jordt et al. 2004; Bautista et al. 2005; Macpherson et al. 2005; Gerhold and Bautista 2009; Nilius and Appendino 2013), and even cannabinoids (Akopian et al. 2008) and ozone (Taylor-Clark and Undem 2010). The inflammatory pain evoked by lipopolysaccharide (LPS) is attributed to activation of TRPA1 channels (Andersson et al. 2012). Interestingly, LPS appears to stimulate TRPA1 not by binding to a particular molecular domain within the channel protein, but instead by inserting into the plasma membrane surrounding the channels and changing the membrane rigidity (Talavera et al. 2013). That is, LPS activates TRPA1 channels via mechanical perturbation rather than chemostimulation. Menthol, long recognized as an agonist of TRPM8 (cool sensing) channels, exerts concentration-dependent actions on TRPA1. Low concentrations of menthol activate TRPA1; high concentrations inhibit this channel (Karashima et al. 2007). Menthol may be influencing TRPA1 activity not only by ligand binding, but, as with LPS, by altering plasma membrane fluidity (Kashiwayanagi et al. 1990).¹²

Interestingly, TRPA1 channels in somatosensory fibers innervating the throat are stimulated by oleocanthal, a pungent compound present in high-quality extra-virgin olive oil. Excitation of these fibers underlies a reflexive cough elicited by consuming high-quality olive oil (Peyrot des Gachons et al. 2011).

High concentrations of CO₂ in the oral cavity and nasal passages elicit a stinging or pungent sensation. This is now understood to be transduced by diffusion of CO₂ across cell membranes of nociceptors, causing cytosolic acidification and activation of TRPA1 channels (Wang et al. 2010). Similarly, heterologously expressed TRPA1 is strongly activated by organic (weak) acids. This is explained by the same mechanism as for CO₂, namely, diffusion of the uncharged, fully protonated acid across the cell membrane, followed by intracellular acidification (Wang et al. 2010).

Stimulating TRPA1 channels in peripheral sensory fibers, as does TRPV1 activation, triggers release of Substance P and CGRP via axon reflex (Trevisani et al. 2007). These peptides initiate inflammatory responses in the surrounding tissues. One might speculate that activating TRPA1 channels in keratinocytes might trigger ATP release and stimulation of nearby somatosensory nerve endings (Dixon et al. 1999; Rong et al. 2000; Koizumi et al. 2004; Lee and Caterina 2005; Lumpkin and Caterina 2007), similar to how stimulating TRPV1 in uroepithelial keratinocytes causes them to secrete ATP and excite nociceptors (Birder et al. 2002).

TRPA1 is also associated with sensing noxious cold temperatures (Story et al. 2003), but this is more related to its role in nociception and is not further discussed here.

Finally, as mentioned above, TRPA1 and TRPV1 are co-expressed in many cells. The two TRP channels appear to form a complex at the plasma membrane and influence each other's function (Staruschenko et al. 2010; Akopian 2011). For instance, responses of neurons to

¹²Parentetically, altering the physical properties of the plasma membrane is how menthol might be modulating TRPM8 channel activity (Morenilla-Palao et al. 2009).

mustard oil differ depending on the presence of TRPV1 (Salas et al. 2009; Staruschenko et al. 2010), in part, because mustard oil is an agonist for both these TRP channels. Similar modulation of TRPA1 responses to other chemesthetic ligands is likely. Indeed, regional differences in the sensitivity of the oral and nasal cavities to common culinary spices such as wasabi, piperine, and capsaicin may reflect the different distribution pattern of cranial nerves innervating these regions (Rentmeister-Bryant and Green 1997) as well as the presence of nerve fibers co-expressing variable proportions of TRPV1 and TRPA1.

5.2.3 Genetic Ablation of *Trpa1*, Knockout Studies in Chemesthesis—Mutant mice lacking TRPA1 channels have been used to validate many of the functional studies cited above. For instance, an early study using *Trpa1* knockout mice showed that TRPA1^{-/-} mice consume water containing the irritant mustard oil (allyl isothiocyanate), whereas wild-type mice strongly avoid this solution (Kwan et al. 2006). In more recent studies, researchers showed that trigeminal neurons isolated from *Trpa1* knockout mice do not respond to cinnamaldehyde and only weakly to propionic acid (a weak acid that acidifies the cytosol), whereas neurons isolated from wild-type mice exhibit robust responses (Wang et al. 2011). These data support the argument that intracellular acidification stimulates TRPA1 channels. Buffer solutions saturated with ozone (10 μM) stimulate neurons isolated from vagal ganglion of wild-type mice, but do not do so for vagal ganglion neurons from *Trpa1* knockout mice. This confirms that TRPA1 is a target for ozone stimulation of vagal airway fibers (Taylor-Clark and Udem 2010). Styrene and naphthalene elicit respiratory reflexes (reduce breathing) mediated by nasal trigeminal nerves in wild-type mice but not in *Trpa1* knockout mice (Lanosa et al. 2010).

5.2.4 Conclusions Regarding TRPA1 in Chemesthesis—In summary, TRPA1 channels in somatosensory nerve fibers (and possibly in mucosal epithelial cells, too) are major targets for diverse chemesthetic stimuli. The co-expression of TRPV1 with TRPA1 in many of these same fibers and cells may modify responses to TRPA1 ligands and contribute to polymodal chemesthetic responses.

5.3 TRPM8 Channels Mediate a Cooling Sensation

After TRPV1 and TRPA1, TRPM8 is the next most commonly referenced TRP channel in publications regarding chemesthesis. TRPM8 was initially discovered in prostate epithelial cells and seems to play a poorly understood role in regulating cell growth in that tissue (Tsavalier et al. 2001; Malkia et al. 2011). Shortly after its discovery in the prostate, this channel was identified in cool-sensing thermo-receptive sensory neurons. Pertinent to the present review, TRPM8 was also found to respond to chemesthetic stimuli, specifically the cooling sensation of menthol (e.g., peppermint oil) and the synthetic compound icilin (McKemy et al. 2002; Peier et al. 2002a).

5.3.1 TRPM8 Expression in the Oronasal Cavities—TRPM8 is prominently expressed in trigeminal ganglion cells (Ta et al. 2010; Vandewauw et al. 2013). When explored with single-cell RT-PCR or immunostaining, only a subset of trigeminal neurons express TRPM8 (Nealen et al. 2003; Kobayashi et al. 2005; Kim et al. 2011) (see Fig. 6). TRPM8 immunostaining has been reported in nerve fibers in the nasal cavity (specifically,

the olfactory epithelium, (Nakashimo et al. 2010), cornea (Bautista et al. 2007), and oral epithelium (Abe et al. 2005; Sato et al. 2013). Takashima et al. (2007) and Dhaka et al. (2008) genetically engineered mice to express EGFP in axons having TRPM8 channels and demonstrated that fibers with this channel innervate a variety of tissues, including dental pulp and lingual epithelium. Both Takashima et al. (2007) and Dhaka et al. (2008) state that in the tongue, TRPM8-containing fibers surround but do not penetrate into taste buds. This finding is consistent with the immunohistochemical findings of Abe et al. (2005). Contrasting this, Sato et al. (2013) reported TRPM8-immunopositive cells in structures that appear to be taste buds in the soft palate and pharynx from rats. Sato et al. (2013) concluded that taste buds in these regions differ from lingual taste buds.

5.3.2 TRPM8 Function and Genetic Ablation of *Trpm8* in Chemesthesis—

Naturally-occurring chemesthetic ligands that activate TRPM8, in addition to menthol from mint plants, include extracts from essential oils and aromatic spices used in cooking, mouthwashes, and fragrances, such as geraniol (from rose oil or citronella oil) and eucalyptol (from eucalyptus) (Vetter and Lewis 2011). Knocking out TRPM8 expression in mice results in a marked decrease in menthol-evoked responses recorded from sensory ganglion cells, including trigeminal neurons, isolated from these mice. Moreover, the mice themselves are significantly less sensitive to innocuous cool temperatures or topical application of the cool irritant acetone (Bautista et al. 2007; Colburn et al. 2007; Dhaka et al. 2007). Lastly, mice in which neurons that express TRPM8 were ablated did not respond to innocuous or noxious cold temperatures (Knowlton et al. 2013). These results indicate that although TRPM8 channels *per se* may not transduce temperatures <10 °C, they are co-expressed in sensory neurons with other cold receptors that do respond to these low temperatures.

5.3.3 Conclusions Regarding TRPM8 in Chemesthesis—TRPM8 is expressed in polymodal sensory neurons that respond to cool temperatures and noxious cold, though this channel itself is not responsible for transducing temperatures below ~10 °C. Chemesthetic stimuli that elicit cooling sensations, notably menthol, activate this channel in somatosensory neurons.

5.4 TRPM5 Channels also Play a Role in Chemesthesis

TRPM5 is only moderately expressed in trigeminal neurons and not nearly to the same extent as TRPV1, TRPA1, or TRPM8 and other TRP channels (Vandewauw et al. 2013). A prominent role for TRPM5 in chemesthesis is more likely due to the presence of these TRP channels in cells other than somatosensory neurons. Specifically, respiratory and laryngeal epithelium has a population of isolated, chemical-sensing receptor cells that respond to irritants. These cells exist as individual sensors, not aggregated into clusters such as taste buds. This cell population has been termed the diffuse chemosensory system (Sbarbati and Osculati 2005). Receptor cells in the diffuse chemosensory system, termed *solitary chemosensory cells*, express G protein-coupled taste receptors (specifically, T2R bitter receptors) and downstream effectors, including TRPM5 (Kaske et al. 2007; Lin et al. 2008). Solitary chemosensory cells respond to noxious chemicals and bacterial secretions, release transmitters that excite trigeminal nerve fibers, and elicit protective respiratory reflexes

(Finger et al. 2003; Kaske et al. 2007; Tizzano et al. 2010; Barham et al. 2013), presumably by employing TRPM5 channels in a similar manner as with chemotransduction in taste cells. In rodents, solitary chemosensory cells also control access to the pheromone-sensing vomeronasal organ in the nasal cavity.¹³ TRPM5 is required for this function (Ogura et al. 2010). Stimulation of solitary chemosensory cells can release transmitters such as acetylcholine onto sensory afferents (Krasteva et al. 2011) and elicit respiratory responses (e.g., sneezing, reduction of respiration) and epithelial inflammation. Conversely, as mentioned above, axon reflex release of glutamate and peptides (substance P, CGRP) from these same afferent terminals may modulate responses of solitary chemosensory cells.

5.4.1 Conclusions Regarding TRPM5 in Chemesthesis—The best-documented role for TRPM5 in chemesthesis is as a downstream transducer channel for “taste” GPCRs expressed in solitary chemoreceptor cells of the airway mucosa and involved in sensing chemicals released by bacteria. Other TRPM5 functions include control of access to pheromone-sensing cells, a function also mediated by solitary chemoreceptor cells.

5.5 Less-studied TRP Channels in Chemesthesis: TRPV3, TRPV4, and TRPV2

There is a scant literature on the presence and function of TRPV2, TRPV3, and TRPV4 channels in the oronasal cavities. These TRP channels have been associated with sensing temperatures $>25^{\circ}$ (Watanabe et al. 2002; Chung et al. 2003; Vriens et al. 2004; McKemy 2007). However, more recent experiments show that *Trpv3/Trpv4* double-knockout mice display no marked difference from wild-type mice regarding a preference for thermal gradients or response to heat-evoked nociception (Huang et al. 2011). Additionally, TRPV4 appears to play a role in osmosensing, mechanoreception, and stimulating pain associated with ultraviolet radiation (sunburn) (Suzuki et al. 2003; Vriens et al. 2004; Becker et al. 2005; Moore et al. 2013).

5.5.1 TRPV3,V4 Expression in the Oronasal Cavities—TRPV3 is expressed only at very low levels in trigeminal neurons from mice (Vandewauw et al. 2013), though this channel is present at significantly higher levels in trigeminal neurons from monkey (Xu et al. 2002). TRPV4 is significantly expressed in mouse trigeminal ganglion cells (Vandewauw et al. 2013). TRPV3 and TRPV4 presumably are also present in the nerve terminals of somatosensory fibers that innervate the oral and nasal epithelium. However, Nakashimo et al. (2010) failed to detect TRPV4 immunostaining in nerve fibers of the olfactory epithelium in mice.

Keratinocytes, including in the oral and nasal epithelium, express TRPV3 and TRPV4 channels (Peier et al. 2002b; Xu et al. 2002, 2006; Chung et al. 2003; Bandell et al. 2007; Nakatsuka and Iwai 2009; Kida et al. 2012). Most functional studies (see next) indicate a function for TRPV3 and TRPV4 in keratinocytes.

5.5.2 TRPV3,V4 function in Chemesthesis—TRPV3 in keratinocytes is believed to contribute to the sensations of warmth when consuming foods spiced with ingredients such

¹³Humans do not possess a comparable anatomical structure and the existence of human pheromones is debatable.

as oregano, savory, clove, or thyme, representing chemostimulation by carvacrol, eugenol, and thymol (Xu et al. 2006). TRPV3 may also mediate irritation or thermosensations in the nose and throat elicited by inhaling camphor (e.g., Vicks VapoRub); camphor is a TRPV3 agonist (Moqrich et al. 2005) and icilin is an antagonist (Sherkheli et al. 2012). TRPV3 is even believed to transduce the mystical exhilaration induced by certain incenses, though this effect was attributed to TRPV3 channels in the CNS, not in oronasal keratinocytes (Moussaieff et al. 2008). How stimulation of TRPV3 channels in keratinocytes is transmitted to sensory afferent fibers is not well understood, but may involve the release of neuroactive compounds such as nitric oxide and ATP, as with TRPV4 (next) (Lumpkin and Caterina 2007; Mihara et al. 2011).

Few ligands from natural products are known for TRPV4. One is bisandrographolide A extracted from the Asian herb, “King of Bitter” (*Andrographis paniculata*) (Smith et al. 2006). This plant is used in traditional medicine in India and China. It is unlikely that its bitter taste is related to TRPV4 activity insofar as this channel is not known to be expressed in taste bud cells. As with TRPV3, in keratinocytes, TRPV4 channels may participate in thermal sensations (Watanabe et al. 2002; Chung et al. 2003; McKemy 2007). However, TRPV4 also plays a significant role in regulating cell–cell junctions and maintaining the transepidermal barrier. Genetically engineered mice lacking TRPV4 have defective skin barrier function (Sokabe et al. 2010), and activating TRPV4 (e.g., with warm temperatures, 33°) promotes this barrier and its recovery after damage (Kida et al. 2012). The barrier function of TRPV4 in keratinocytes may be related to its ability to regulate cell volume (Strotmann et al. 2000; Becker et al. 2005). By upholding the keratinocyte barrier, TRPV4 may regulate access of topically applied chemical stimuli to underlying somatosensory free nerve terminals. Yet, assigning any one particular role for TRPV4 in chemesthesis is complicated by the polymodal nature of the stimuli that activate this channel and the heterogeneity of cellular processes in which it is involved (Vriens et al. 2004).

5.5.3 Conclusions Regarding TRPV3,V4 in Chemesthesis—TRPV3 and TRPV4 are abundantly expressed in trigeminal neurons, at least in monkeys. However, there is more documentation for a function for these TRP channels in keratinocytes, where they may participate in thermal sensations and, for TRPV4, possibly controlling transepidermal barrier of the oronasal mucosa to chemical compounds. There is little direct information concerning chemesthetic responses transduced by TRPV3 or TRPV4.

5.5.4 TRPV2 and Chemesthesis: Expression, Function, and Genetic Ablation—TRPV2 is a channel that is associated with sensing high temperatures (McKemy 2007). This TRP channel is abundantly expressed in the mouse trigeminal ganglion (Vandewauw et al. 2013), and TRPV2-immunopositive fibers are present in oral and nasal epithelium (Nakashimo et al. 2010; Sasaki et al. 2013). Dendritic and Langerhans cells within the epithelium also are reported to be TRPV2 immunopositive (Shimohira et al. 2009; Sasaki et al. 2013). Yet even given this pattern of expression in fibers and oronasal epithelium, a role for TRPV2 in chemesthesis is not readily apparent. TRPV2 is insensitive to capsaicin and related hot spicy compounds (Caterina et al. 1999). Few natural compounds are known that activate TRPV2. Interestingly, TRPV2 agonists include the non-psychotropic compounds

found in *Cannabis sativa*—cannabidiol, 9-tetrahydrocannabinol, and cannabitol (Qin et al. 2008).

Instead, TRPV2 channels have been associated with noxious stimulation, especially hot burning pain and mechanical nociception (McKemy 2007). Heterologous expression of rodent TRPV2 showed channel activation at temperatures $>52^{\circ}$ (Neeper et al. 2007). Yet, *Trpv2* knockout mice have normal responses to heat or mechanical stimulation (Park et al. 2011) and heterologous expression of human TRPV2 did not confer heat sensitivity to HEK cells (Neeper et al. 2007). These findings challenge the notion that this channel senses heat and mechanical nociception.

Concluding Remarks: TRP Channels and Implications for Consuming Food and Beverages

The quantitative study by Vandewauw et al. (2013) indicates that a number of TRP channels not mentioned above are expressed in relatively high levels in the mouse trigeminal ganglion, specifically TRPM2, TRPM4, TRPM7, TRPML1, and PKD2L1. Although there is little information about the function of these channels in chemesthesis, their presence in this ganglion suggests that they may play such a role. Additionally, as a generality, many TRP channels are co-expressed in cells and interact to modify each other's properties (Akopian 2011; Fernandes et al. 2012; see the TRIP Database: <http://trpchannel.org/>, Shin et al. 2011). Thus, the presence of multiple TRP channels in somatosensory ganglion cells with varying proportions of co-expression may confer a rich variety of responses to a wide spectrum of chemical stimuli in the oronasal cavities.

Additionally, many of the TRP channels discussed in this review on taste and chemesthesis are regulated by pH. Classic examples include TRPV1 channels that are directly activated by extracellular protons (Tominaga et al. 1998) and the binary complex of PKD2L1/PKD1L3 which is stimulated by the withdrawal of protons (Ishimaru et al. 2006; Inada et al. 2008; Ishii et al. 2009; Kawaguchi et al. 2010). By contrast, extracellular acidification rapidly blocks TRPM5 and slowly enhances inactivation (Liu et al. 2005). Protons in the extracellular solution activate human TRPA1 but block rodent TRPA1 (de la Roche et al. 2013). These are but a few examples of how protons act on the extracellular domains of TRP channels to regulate their opening.

Conversely, intracellular protons can also profoundly modulate TRP channels. For instance, cytosolic acidification opens rodent TRPA1 channels (Wang et al. 2010) but inhibits TRPV1 channels (Chung et al. 2011).

Interestingly, soft drinks such as sodas are often quite acidic and they are carbonated. Carbonated drinks release CO_2 that readily passes across cell membranes and acidifies the cytosol. This lowers the intracellular pH of the superficial oral epithelium and possibly somatosensory fibers coursing through this epithelium as well (Carstens et al. 2002). Intracellular acidification activates TRPA1 channels, at least in the rodent (Wang et al. 2010), and would be expected to enhance TRPA1 activity in response to agonists. Furthermore, consuming foods acidified with organic ("weak") acids such as acetic acid

(vinegar) would similarly be expected to acidify both the extra- and intracellular milieu of cells (Richter et al. 2003; Roper 2007) and might be expected to modulate TRP channel responses (Fig. 7).

The acidity of many popular soft drinks, especially colas, is achieved by a mineral (“strong”) acid, phosphoric acid ($pK_1 = 2.148$). Phosphoric acid is largely dissociated at pH levels found in colas and thus will not readily pass across cell membranes. Instead, protons largely remain in the extracellular spaces. For example, Coca Cola and Pepsi Cola are acidified with phosphoric acid to pH 2.5 (Jain et al. 2007). Thus, assuming no buffering, ~70 % of the phosphoric acid is dissociated and $[H^+]_o$ may reach ~3 mM in the oral cavity.¹⁴ These drinks will deposit extracellular protons in the epithelium and around somatosensory fibers in the oral cavity, depending on how well the acid penetrates into the oral epithelium and how much buffering takes place in the mucosa. It is likely that the interstitial spaces may become quite acidic, at least in the superficial epithelium. Such extracellular acidification of the tissue spaces would be expected to modulate the activity of TRP channels on cells and fibers there. As an example, one would anticipate that the low extracellular pH would enhance TRPV1 activity, as it does in experiments on isolated sensory neurons (Petersen and LaMotte 1993; Martenson et al. 1994; Liu and Simon 2000) (Fig. 7).

Consequently, by combining carbonation with acidic pH, soda drinks would be expected to have a strong influence on foods containing TRP agonists. Indeed, the oral tingling and irritation of carbonated drinks has been documented (Carstens et al. 2002; Hewson et al. 2009). Further, interactions between TRPV1 and the artificial sweeteners used in many sodas has also been investigated (Riera et al. 2008). Yet, any specific interaction such as enhancement of TRPV1- and TRPA1-mediated pungency by drinking colas has not been systematically studied despite the abundance of anecdotes to this effect on the Internet. This includes a Facebook page dedicated to the burn of sodas and capsaicin (<https://www.facebook.com/pages/Eating-spicy-food-then-accidentally-reaching-for-some-soda-to-cool-down/115325535176212>). Interactions between carbonation, acidity, and pungency might be generalized beyond those elicited by soft drinks. It is likely that the rich pallet of flavors and spices during a meal is constantly in flux and amplified by interactions between food chemicals, food temperature, and the TRP channels expressed in taste buds, somatosensory fibers, and oral keratinocytes.

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¹⁴As an aside, this low pH contributes little sourness to colas because protons do not readily cross the plasma membrane and stimulate sour taste. To put this pH into perspective, vinegar is ~700 mM acetic acid, pH 2.3 to 2.6 (see discussion in Roper 2007).

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Fig. 1. “Word clouds” showing the prominence of literature references to TRP channels in taste (top) and chemesthesis (bottom)

Abstracts of publications describing TRP channels in studies on taste and chemesthesis were searched. The size of the font is proportional to the number of times a given TRP channel is mentioned in these abstracts (see <http://www.wordle.net/>). This representation gives an approximation of how well-studied are these TRP channels in taste and chemesthesis. For taste, TRPM5 > TRPV1 >> PKD2L1 ~ TRPA1 > PKD1L3 > TRPM8 > TRPV4 >> TRPV3. For chemesthesis, TRPV1 >> TRPA1 > TRPM8 >> TRPM5 > TRPV4 > TRPV2 > TRPV3

Fig. 2. The three major classes of taste cells
This classification incorporates ultrastructural features, patterns of gene expression, and the functions of each of types I, II (Receptor), and III (Presynaptic) taste cells. Type I cells (grey) degrade or absorb neurotransmitters. They also may clear extracellular K⁺ that accumulates after action potentials (shown as starburst symbols) in Receptor (yellow) and Presynaptic (green) cells. K⁺ may be extruded through an apical K channel such as ROMK. Salty taste may be transduced by some type I cells, but this remains uncertain. Sweet, bitter, and umami taste compounds activate Receptor cells, inducing them to release ATP through pannexin1 (Panx1) (Huang et al. 2007; Romanov et al. 2007) or CALHM1 (Taruno et al. 2013) channels. The extracellular ATP excites ATP receptors (P2X, P2Y) on sensory nerve

fibers and on taste cells. Presynaptic cells, in turn, release serotonin (5-HT) and GABA, which inhibit Receptor cells. Sour stimuli (and carbonation, not depicted) directly activate presynaptic cells. Only presynaptic cells form ultrastructurally identifiable synapses with nerves. Starburst symbols denote action potentials. Tables below the cells list some of the proteins that are expressed in a cell type—selective manner. Modified from Chaudhari and Roper (2010)

Fig. 3. The oronasal cavity and its innervation, showing TRP channels involved in taste and chemesthesis

TRP channels are expressed in sensory ganglion neurons, their axon terminals, and in epithelial keratinocytes throughout the oronasal cavity. The TRP channels illustrated here have known or implied functions in gustation and chemesthesis. Other TRP channels are expressed in these sites, but their functions in taste and chemesthesis are not well understood. Drawing courtesy of Patrick Lynch, Yale University

Fig. 4. Canonical transduction pathway for sweet, bitter, and umami taste stimuli

These taste compounds activate G protein-coupled taste receptors that are expressed in taste Receptor (type II) cells. Taste receptor activation leads to IP3-mediated intracellular Ca²⁺ release, TRPM5 opening, and ultimately, transmitter secretion from Receptor cells.

Reviewed in Chaudhari and Roper (2010)

Fig. 5. Certain TRP channels involved in chemesthesis are also thermoreceptors

Mammalian TRP ion channels detect a broad range of temperatures. Their in vitro properties predict the thermal zones each channel potentially mediates. A role for TRPV3 and TRPV4 in thermal detection, however, is debatable (Huang et al. 2011). Many of these channels are also activated by plant derivatives, some of which provide distinct sensations of temperature. Examples of these plants are shown at top of figure. Modified from McKemy (2007)

Fig. 6. Distribution of rat trigeminal ganglion neurons that express TRPM8, TRPA1, and TRPV1

There is extensive co-expression of TRPA1 and TRPV1 in many of these sensory ganglion cells. A separate population of neurons expresses TRPM8. This Venn diagram was created from data published in Kobayashi et al. (2005)

Fig. 7. Acidic food and drink are likely to influence TRP channel activity in the oronasal epithelium

Mineral (strong) acids, for example, phosphoric acid in cola drinks, might be expected to penetrate into and acidify the interstitial fluid spaces in the mucosal epithelium (increase $[\text{H}^+]_o$). This would enhance TRPV1 channel activity in cells and nerve fibers there.

Alternatively, weak acids such as acetic acid or CO_2 from carbonated drinks will diffuse across cell membranes, dissociate inside the cells, and acidify the cytosol (increase $[\text{H}^+]_i$). This would be expected to occur inside nerve terminals, keratinocytes, and taste bud cells, as shown here. Intracellular acidification enhances TRPA1 channel activity and suppresses TRPV1 channels. Drawing of taste bud from Parker (1912)