Chapter 6
TRP Channels as Targets for Modulation of Taste Transduction

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Abstract The sense of taste endows organisms with the ability to distinguish nutritious from potentially harmful food components and to orchestrate adaptive attractive or aversive behaviors. Several members of the Transient Receptor Potential (TRP) family of cation channels have been implicated in the perception of canonical and non-canonical taste modalities, TRPM5 for sweet, bitter, umami and fat, TRPP3/PKD1L3 for sour and TRPV1 for salt and metallic tastes. However, TRPM5 is the only one for which there is consensus on its contribution to taste transduction. Here I review recent findings on the role of this channel in taste perception at the peripheral level. Emphasis is made on reported mechanisms of TRPM5 channel modulation that may have an impact on gustatory transduction. Understanding these mechanisms allows learning about basic taste signaling processes and their modulation (e.g. by temperature and taste-taste interactions), and is essential for the design of optimal therapeutic strategies targeting the gustatory system.

Keywords Taste transduction · Sweet · Bitter · Umami · Salt · Sour · TRPM5 · TRPV1 · TRPP3/PKD1L3

6.1 Introduction

Animal adaptation requires constant monitoring of the chemical environment. In mammals, chemosensation is based on three main mechanisms, olfaction, taste and chemesthesis. The first two allow the detection and identification of chemical compounds that are airborne or dissolved in the saliva, respectively, whereas the third

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is based on detection at the level of the skin, mucosa and viscera. The initial step in these processes is the interaction of compounds with specialized receptors expressed in sensory cells. This event is then transduced into electric signals, which are carried by nerve fibers to high centers of the nervous system. In the case of taste, transduction takes place in taste buds located in the tongue and palate. These structures contain four types of taste cells (I–IV) and the endings of gustatory nerves (chorda tympani and glossopharyngeal). Type I cells seem to provide mechanical support to the rest of the structure (Pumplin et al. 1997), play buffering functions that maintain low levels of extracellular ATP (Bartel et al. 2006; Vandenbeuch et al. 2013) and K^+ (Dvoryanchikov et al. 2009) and are thought to contribute to salt taste transduction (Vandenbeuch et al. 2008). Type II cells express G protein-coupled receptors (GPCRs) for molecules that we perceive as sweet, bitter or umami (glutamate). Expression of these receptors is more or less exclusive, in the sense that GPCRs for bitter compounds (T2Rs) are not co-expressed with receptors for sweet and umami (heterodimers of T1R1, T1R2 and T1R3) (Chaudhari and Roper 2010). However, it is important to note that the taste quality of a particular taste receptor cell is not determined by the receptors they express, but rather the nerve fiber which they are connected to (Mueller et al. 2005). Type III cells are stimulated by sour and by salts. Type II and type III cells differ in the way they connect to the taste sensory nerves. Upon stimulation, type II cells release ATP and there is evidence indicating that this may occur through the passage of ATP through pannexin 1 (Huang et al. 2007; Romanov et al. 2007; Huang and Roper 2010) and through CALHM1 channels (Taruno et al. 2013a; Taruno et al. 2013b), but it remains unclear whether these mechanisms are mutually exclusive. Extracellular ATP activates P2X receptors in taste nerves and P2Y receptors in Type III cells (Kinnamon and Finger 2013). The latter are endowed with classical synaptic proteins, as well as with voltage-gated Ca^{2+} channels. Type III cells, also known as 'presynaptic', are thought to communicate with the gustatory nerves via vesicular release of GABA and serotonin (Chaudhari and Roper 2010). Finally, type IV cells, also called basal cells, are considered to be precursors of taste receptor cells. These structural and functional aspects of taste were covered by recent reviews (Feng et al. 2012; Liman et al. 2014; Roper 2014).

Several TRP channels have been implicated in distinct aspects of taste transduction. For instance, TRPV1 has been proposed in multiple studies as mediator of the amiloride-insensitive component of salt taste transduction (Lyall et al. 2004, 2005, 2009; Treesukosol et al. 2007; Katsumata et al. 2008). In addition, TRPP3/PKD2L3 were suggested as sour receptors (Ishimaru et al. 2006; LopezJimenez et al. 2006; Ishimaru and Matsunami 2009). However, negative or inconclusive evidence from knockout mice, and lack of definitive proof for the functional expression of these channels in taste receptor cells, have been raised as doubts for their classification as *bona fide* elements of taste transduction pathways (Roper 2014). In contrast, although many issues remain unclear, TRPM5 has drawn consensus about its role in the transduction of several taste qualities. For these reasons, and given the availability of very recent excellent reviews on this subject (Kinnamon 2012; Nilius and Appendino 2013; Liman 2014; Liman et al. 2014; Roper 2014), this chapter focuses on

the most salient basic properties of TRPM5 and how the mechanisms of modulation of this channel influence gustatory transduction at the level of taste receptor cells.

6.2 TRPM5 and its Role in Gustatory Transduction and Modulation

6.2.1 Basic Properties in Heterologous and Native Expression Systems

Of all TRP channels thought to be involved in taste transduction TRPM5 is the best characterized. TRPM5 was first identified in a differential screening of cDNAs from taste receptor cells and was shown to co-express with molecules known to participate in taste signaling, including α-gusducin and phospholipase C-β_2 (Perez et al. 2002, 2003). TRPM5 was originally described as a Ca^{2+}-permeable store-operated channel (Perez et al. 2002, 2003), but soon afterwards it was established that it is in fact a non-selective Ca^{2+}-impermeable cation channel activated by increase in intracellular Ca^{2+} concentration (Hofmann et al. 2003; Liu and Liman 2003; Prawitt et al. 2003).

The effective intracellular Ca^{2+} concentration (EC_{50}) for TRPM5 activation has been reported for recombinant channels between 0.7 and 32 μM (Hofmann et al. 2003; Liu and Liman 2003; Ullrich et al. 2005) for mouse and 0.84 μM for human (Prawitt et al. 2003). The reasons for these divergences seem to be related to the degree of desensitization of the channel in different recording conditions. Indeed, Ullrich et al. showed that in the whole-cell configuration the EC_{50} was 0.7 μM, whereas in inside-out patches it was 32 μM (Ullrich et al. 2005). This desensitization was found to be Ca^{2+}-dependent and partially reverted by phosphatidylinositol-4,5-bisphosphate (PIP_2) (Liu and Liman 2003). The mechanisms underlying the sensitivity of TRPM5 to intracellular Ca^{2+} remain poorly understood, although it has been discarded that it is mediated by calmodulin (Hofmann et al. 2003). Intracellular Ca^{2+} uncaging experiments revealed that TRPM5 is rapidly activated ($\tau < 2$ ms) upon a strong increase of intracellular Ca^{2+} concentration (Ullrich et al. 2005).

The characterization of the biophysical properties of TRPM5 has been limited. The single-channel conductance has been estimated between 16 and 25 pS (Hofmann et al. 2003; Liu and Liman 2003; Prawitt et al. 2003; Zhang et al. 2007), but detailed properties and modulation of single-channel kinetics have not been reported. It is known though that TRPM5 is a voltage-gated channel, activated by membrane depolarization. Like for other voltage-gated TRP channels, the voltage-dependence of TRPM5 activation is quite shallow, with a slope factor between 53 and 58 mV (Talavera et al. 2008c; Gees et al. 2014). This is suggestive of a low apparent gating valence of ~0.6 per channel (Talavera et al. 2005), similar to what has been directly estimated for TRPM8 (Voets et al. 2007). In regard to the kinet-

ics of voltage-dependent gating, it has been shown that current relaxation can be described with a single exponential time course. The resulting time constant has a bell-shaped voltage dependence, which is a classical indicative of faster channel closing and opening at very negative and very positive potentials, respectively (Talavera et al. 2008c; Gees et al. 2014). The relationship between the voltage and Ca^{2+} dependencies of gating is not yet known, and no gating models have been put forward. As detailed below, TRPM5 was shown to be also strongly stimulated by heat (Talavera et al. 2005).

Most of the properties of recombinant TRPM5 channels have been confirmed in the native environment of taste receptor cells. These include lack of selectivity between Na^+ and Cs^+ as charge carriers, impermeability to $NMDG^+$ and Ca^{2+}, stimulation by increase in intracellular Ca^{2+} concentration and membrane depolarization and high sensitivity to heat (Romanov et al. 2007; Zhang et al. 2007).

6.2.2 TRPM5 in the Perception of Sweet, Bitter and Umami: Two Knockout Mice Tell Slightly Different Stories

The role of TRPM5 in taste transduction has been directly tested by evaluating the responses of *Trpm5* knockout mice. The first report, by Zhang et al., utilized mice with a partial deletion of the *Trpm5* gene and retaining the promoter region, which suggest that a large part of the amino terminal of TRPM5 could be still transcribed (Zhang et al. 2003). Such mice were reported not to be different from wild type mice regarding the responses to salt and acid stimuli, but completely insensitive to sweet, bitter and umami stimuli at the level of behavior and gustatory nerve responses (Zhang et al. 2003). Thus, it was concluded that TRPM5 is absolutely required for the transduction of these taste modalities. However, other studies that used knockout mice fully lacking the TRPM5 protein consistently yielded different results. In several separate experimental series it was found that gustatory nerve responses to sweet, bitter and umami stimuli were significantly reduced, but not completely abolished in knockout mice (Talavera et al. 2005, 2008c; Damak et al. 2006). Damak et al. (2006) found that *Trpm5* knockout mice were sensitive to sweet, bitter and umami tastes in a long access two-bottle preference test but not in a brief-access licking test. It was argued that this difference could result from a lower sensitivity of the brief-access test and/or from the influence of post-ingestive taste-independent effects (Damak et al. 2006). Interestingly, it was later found that the sweet-blind *Trpm5* knockout mice from Zhang et al. displayed preference for sucrose in a long-term test due to post-ingestive reward (de Araujo et al. 2008). This preference was associated with calorie intake because the non-energetic sweetener sucralose failed to trigger preference over water in the same test.

The reasons underlying the differences in the results from studies with the two *Trpm5* knockout strains remain unknown. It has been suggested that the lack of response of knockout mice in the study of Zhang et al. could be due to a putative dominant-negative action of the TRPM5 N-terminal protein fragment that is

presumably produced in this mice on alternative transduction pathways (Damak et al. 2006). Additionally, differences in genetic background of these mice were also put forward (Damak et al. 2006). However, none of these hypotheses has been tested. Nevertheless, the observations of Damak et al. (2006) and several other studies (Devantier et al. 2008; Eddy et al. 2012 and see below) tilt the balance in favor of a model of transduction of sweet, bitter and umami featuring TRPM5-dependent as well as TRPM5-independent mechanisms. Not surprisingly, the relative importance of these mechanisms depends on the tastant, experimental paradigm and gustatory field being evaluated (Damak et al. 2006).

These and other studies served to establish a qualitative model for the role of TRPM5 in transduction of sweet, bitter and umami stimuli (Fig. 6.1). Activation of G-protein-coupled taste receptors by these tastants stimulates PLCβ2 with the consequent production of IP_3 and Ca^{2+} release from the endoplasmic reticulum through type 3 IP_3 receptors (IP_3R3) (Hisatsune et al. 2007). The increase in intracellular Ca^{2+} concentration activates TRPM5, leading to cation influx and depolarization of the taste receptor cell (Hofmann et al. 2003; Liu and Liman 2003; Prawitt et al. 2003; Zhang et al. 2003). This depolarization activates voltage-gated Na^+ channels leading to action potential firing (Gao et al. 2009; Vandenbeuch and Kinnamon 2009). It seems that the combination of increase in intracellular Ca^{2+} concentration and depolarization triggers the release of ATP through pannexin 1 (Huang et al. 2007; Huang and Roper 2010) and/or CALHM1 channels (Taruno et al. 2013a, b).

6.2.3 Role of TRPM5 in the Perception of Salts and Fats

Intriguingly, the *Trpm5* knockout mice from Damak et al. (2006) displayed significantly lower responses to NaCl than wild type mice. This again contrasts with the results of Zhang et al. who reported that their *Trpm5* knockout and wild type mice have the same sensitivity to NaCl, but are in agreement with reduced responses to NaCl in *α-gustducin* (Glendinning et al. 2005) and of *Plcβ2* (Dotson et al. 2005) knockout mice. Notably, if we consider TRPM5 to be exclusively expressed in cells transducing sweet, bitter or umami stimuli we should conclude that the TRMP5-dependent responses to NaCl do not elicit salty taste. Instead, they should elicit sweet, bitter and/or umami qualities. Alternatively, TRPM5 may participate in the transduction of saltiness of NaCl in a small proportion of taste receptor cells reported to express this channel but not α-gustducin (Damak et al. 2006) or T1R or T2R (Zhang et al. 2003). This latter possibility is supported by the findings that *Trpm5* knockout mice, but not *Plcβ2* knockout mice, display smaller chorda tympani responses to low concentrations (100 mM) of NaCl in the presence of benzamil, a potent blocker of the epithelial Na^+ channel ENaC (Ren et al. 2013). On the other hand, the responses of the chorda tympani to salts at high concentrations (500 mM KCl or 500 mM NaCl plus amiloride) were shown to be about 50% weaker in *Trpm5* knockout mice and *Plcβ2* knockout mice than in wild type animals (Oka et al. 2013). Interestingly, wild type mice clearly preferred solutions containing

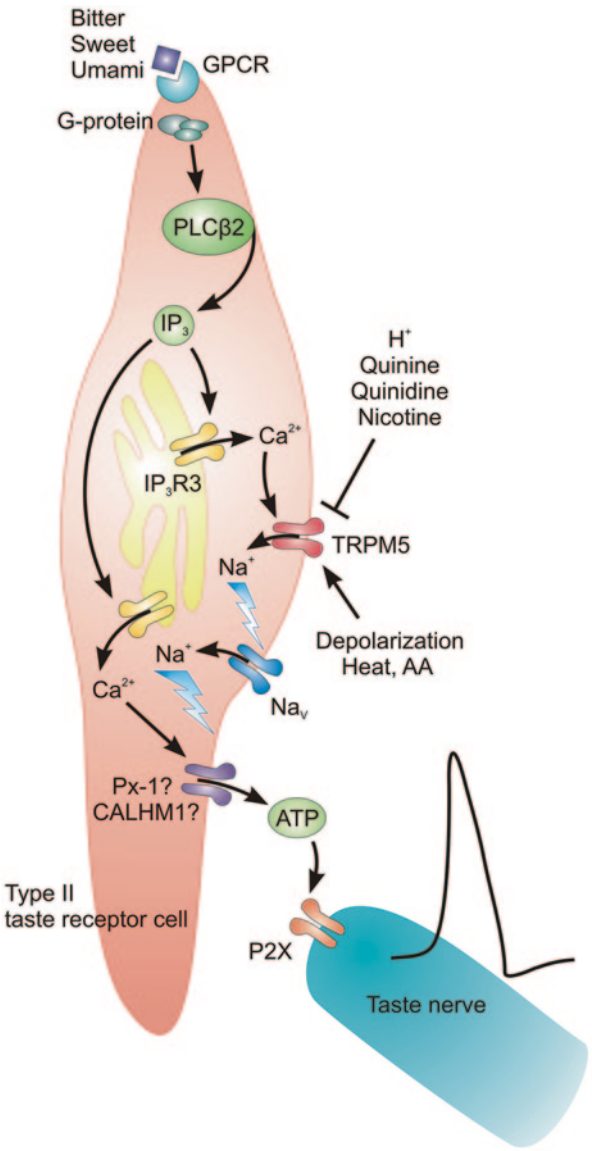

Fig. 6.1 Cartoon of the TRPM5-dependent signaling pathway for the detection of sweet, bitter and umami stimuli. Type II taste receptor cells express TRPM5 in the basolateral membrane. TRPM5 channels are activated by increase in intracellular Ca^{2+} that results from the activation of G-protein-coupled taste receptors sensitive to bitter, sweet and umami compounds. TRPM5 is inhibited by extracellular acidosis, quinine, quinidine and nicotine and stimulated by membrane depolarization, heat and arachidonic acid (*AA*)

low concentrations of divalent salts such as $FeSO_4$ and $ZnSO_4$ over water, but this was not observed in *Trpm5* knockout mice nor in *T1r3* knockout mice (Riera et al. 2009). Furthermore, recent evidence indicates that T1R3 is involved in the detection of calcium and magnesium (Tordoff et al. 2008, 2012).

Finally, there is one study showing that linoleic acid elicits non-selective monovalent cation currents in taste receptor cells that are reduced by application of the TRPM5 inhibitor triphenylphosphine oxide (TPPO) and by genetic ablation of

Trpm5 (Liu et al. 2011). However, it remains unclear why these maneuvers also reduced the increase in intracellular Ca^{2+} concentration triggered by linoleic acid. This is not expected because TRPM5 activation is supposed to be downstream the Ca^{2+} signal. Of note, *Trpm5* knockout mice were found to be deficient in the detection of linoleic acid in tests for long-term preference and brief-access licking recordings after conditioned taste aversion. This body of evidence indicates that TRPM5 is involved in the detection of salts and fats, but the precise underlying pathways remain to be fully characterized.

6.2.4 Modulators of TRPM5 and their Influence on Taste Transduction

The key role of TRPM5 in transduction mechanisms in the taste receptor cells implies that factors that modulate this channel are expected to affect taste. It is notable, however, that the influences of the Ca^{2+}-dependent desensitization and the modulation by PIP_2 and voltage on taste transduction remain completely unknown.

As other voltage-gated TRP channels (Nilius et al. 2005; Talavera et al. 2008a; b), TRPM5 displays a strong thermal sensitivity, being stimulated by heating. The underlying mechanism entails a shift of the voltage-dependence of channel activation to negative potentials (Talavera et al. 2005), very much like that described for activation of TRPV1 (Voets et al. 2004). Of note, channel stimulation by either depolarization or heating can not be observed at low intracellular Ca^{2+} concentration (Talavera et al. 2005). Notably, it was shown that heating strongly enhances chorda tympani responses to sweet compounds in wild type but not in *Trpm5* knockout mice. The strong sensitivity of TRPM5 to heat was therefore proposed to underlie the enhanced sweetness perception at high temperatures. It is also tempting to speculate that heat-induced stimulation of TRPM5 mediates "thermal taste", which consists in the trigger of taste sensations in the absence of tastants by warming the tongue from 20 to 30 °C (Cruz and Green 2000). This effect would require the stimulation of TRPM5 currents by heating at basal (low) intracellular Ca^{2+} concentrations. Alternatively, heating could induce on its own slight increase in the intracellular Ca^{2+} concentration and act as initial trigger for Ca^{2+}-induced activation of TRPM5. However, none of these possibilities have been addressed experimentally. It has been argued that, besides TRPM5, other elements of the taste transduction pathways could be sensitive to heat and contribute to enhanced taste response (Liman 2006, 2014). However, for any element to be significantly important, it would have to feature a thermal sensitivity similar to that of TRPM5 ($Q_{10} \sim 10$ between 25 and 35 °C). For any TRP channel lover who recognizes that the "TRP-centric" view of sensory biology has done more harm than good (Meseguer et al. 2011), it would be extremely interesting to identify such heat-sensitive element outside the TRP protein family. At the moment, TRPM5 remains the most plausible candidate (Talavera et al. 2007).

It has been also pointed out that, considering the role of TRPM5 in the transduction of bitter and umami, heat-induced activation of TRPM5 should result in enhanced responses to these stimuli (Liman et al. 2014; Roper 2014). However, this was not observed experimentally (Talavera et al. 2005). To explain this apparent contradiction, it is important to notice that only the responses that strongly rely on TRPM5 activation are expected to be significantly enhanced by heating. In the original report, which used the *Trpm5* knockout strain generated by Damak et al. (2006), only the chorda tympani responses to sweeteners were strongly reduced in the mice lacking TRPM5 (Talavera et al. 2005). As explained below, a similar discussion rose around the inhibitory effect of quinine on TRPM5.

Another potent modulator of TRPM5 is extracellular acidosis. Liu et al. showed that TRPM5 currents were sharply inhibited by drops in extracellular pH between 7 and 5.5 (Liu et al. 2005). This effect was voltage-independent, which is in accordance with the location of residues important for the inhibitory effect at the extracellular face of the channel. Notably, acidosis enhanced the desensitization of TRPM5 currents. Given the restricted localization of TRPM5 at the basolateral membrane of taste receptor cells, the relevance of its inhibition by extracellular protons has been taken with caution (Roper 2014). Also with reserves one should take the reports on stimulation of TRPM5 by arachidonic acid (Oike et al. 2006) and on the inhibition of this channel by extracellular Zn^{2+} (Uchida and Tominaga 2013). It is currently not clear whether and how these effects are relevant for taste transduction.

The rather extensive literature on interactions between bitter and sweet tastants motivated the study of the possible effects of membrane-permeable bitter compounds on TRPM5 (Talavera et al. 2008c; Gees et al. 2014). It was found that quinine, the bitter additive in tonic waters, and its stereoisomer quinidine inhibit TRPM5 at micromolar concentrations. Furthermore, both compounds inhibited the responses of the chorda tympani to sweet tastants in wild type mice, but did not alter the residual responses of sweet-sensitive gustatory fibers of *Trpm5* knockout mice (Talavera et al. 2008c) (Fig. 6.2). These findings can readily explain the long-known inhibitory effect of quinine on sweet taste perception (Öhrwall 1891) and its partial localization at the gustatory periphery (Formaker and Frank 1996). This phenomenon was considered as a bitter-sweet taste interaction, but it is now clear that the quinine-induced inhibition of the sweet pathway is at least in part dissociated from the detection and processing of the bitter signal because the bitter tastant inhibits directly the sweet transduction pathway.

TRPM5 currents were later found to be inhibited also by nicotine, a compound that has been usually linked to gustatory transduction (Grunberg 1982, 1985; Simons et al. 2006; Lyall et al. 2007; Tomassini et al. 2007). The exact way in which nicotine acts on the perception of these tastes is still not well understood, but very likely multiple different mechanisms are involved. These include nicotine-induced reduction of the size of fungiform papillae and the number of taste cells (Tomassini et al. 2007), inhibition of chorda tympani responses to KCl and NaCl (Lyall et al. 2007), suppression of gustatory responses of neurons in the nucleus of the solitary tract (Simons et al. 2006), inhibition of voltage-dependent sodium channels, sensitization of TRPV1 (Liu et al. 2004) and activation of TRPA1 (Talavera et al. 2009).

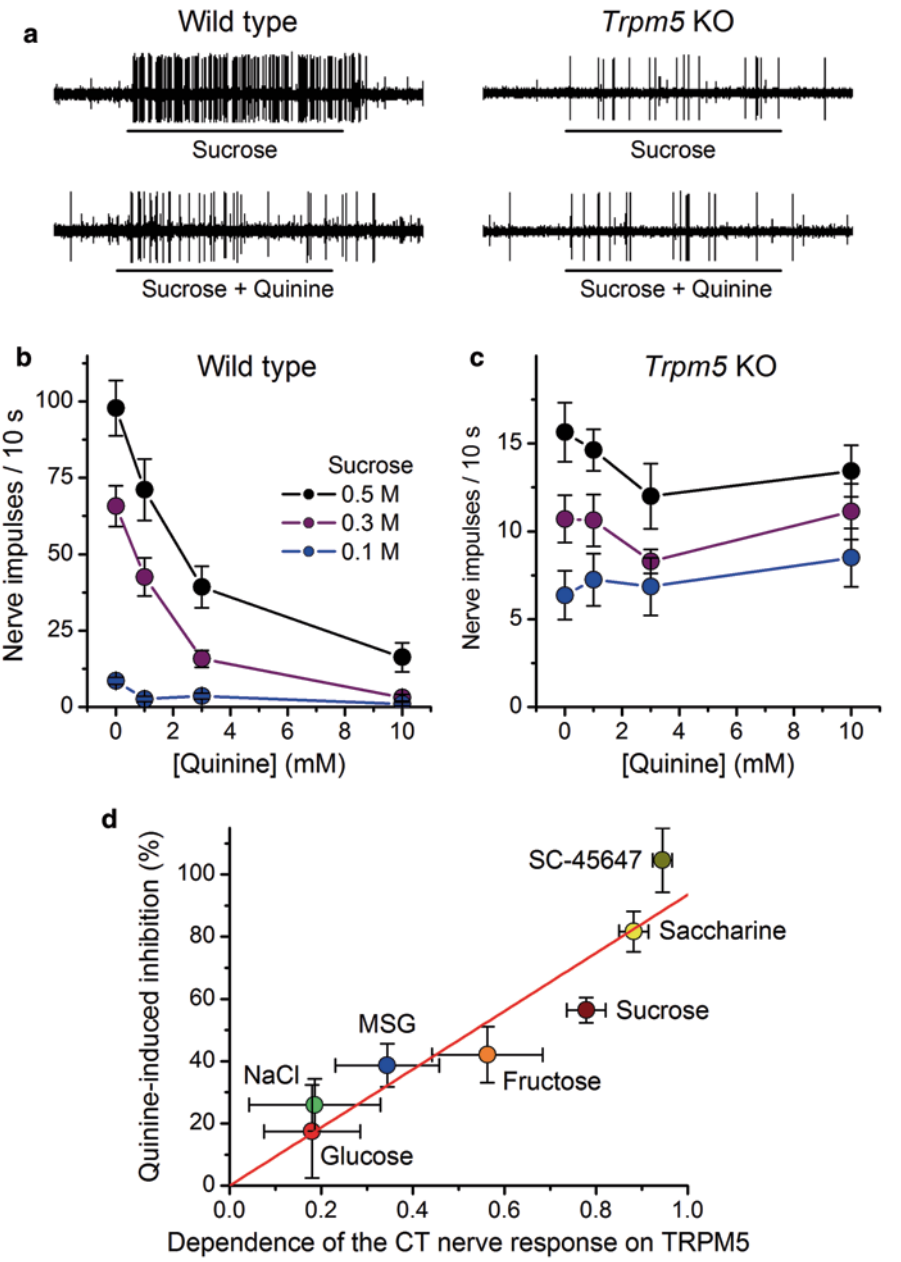

Fig. 6.2 Quinine inhibits the responses of the chorda tympani to sucrose in wild type but not in *Trpm5* knockout mice. **a** Effects of quinine (10 mM) on the responses of single sweet-sensitive fibers from wild type and *Trpm5* knockout mice to sucrose (0.5 M). **b, c** Concentration-dependent effects of quinine on the number of nerve impulses elicited by sucrose in single-sweet sensitive fibers from wild type and *Trpm5* knockout mice. **d** Correlation between the inhibitory effects of quinine of the responses of mouse chorda tympani to different tastants with the degree to which these responses depend on TRPM5. (Figure adapted from (Talavera et al. 2008c), with permission)

The inhibition of TRPM5 by nicotine represents yet another peripheral mechanism of inhibition of gustatory perception. Given the relatively high nicotine concentrations required for TRPM5 inhibition (IC_{50} of 1.3 mM at −50 mV), it is virtually impossible that this effect could be relevant during normal smoking. On the other hand, it could be relevant during tobacco chewing and the use of snuss, in nicotine-replacement therapies, and certainly for the interpretation of experimental studies that used nicotine concentrations of up to 600 mM (Carstens et al. 1998; Boucher et al. 2003; Simons et al. 2006).

Besides shedding light on the mechanisms underlying bitter-sweet taste interactions, the effects of quinine and nicotine on TRPM5 have important implications for our understanding of the mechanisms of bitter taste transduction. The inhibitory effects of these compounds on TRPM5 currents indicate that the perception of their bitter taste cannot be mediated solely by a process requiring TRPM5 activation. Alternative pathways should be recruited to elicit the aversive behavior towards quinine and nicotine at high concentrations. Accordingly, it has been recently shown that nicotine also activates a nAChR-dependent and TRPM5-independent gustatory pathway (Oliveira-Maia et al. 2009). Notably, it is expected that quinine, quinidine and nicotine may also interfere with the perception of umami. However, as for the case of the stimulatory effect of heat on TRPM5 (see above), the effect of TRMP5 inhibitors on the transduction of a certain compound must be related to the extent to which this process depends on TRPM5 activation. This contention was clearly demonstrated for quinine (Talavera et al. 2008c) (Fig. 6.2).

In contrast to quinine, quinidine and nicotine, other bitter compounds known to have an influence in sweet taste perception, including saccharine, caffeine and the related xanthines theophylline and theobromine had no effect on TRPM5 currents, indicating multiple mechanisms of bitter-sweet taste interactions (Gees et al. 2014).

6.3 Concluding Remarks

In recent years we have witnessed evident advances in the understanding of the signaling processes underlying taste transduction, but many issues remain unsolved. For instance, it is particularly challenging that despite the large amount of evidence implicating TRPV1 and TRPP channels, no definitive conclusions have been reached. Regarding TRPM5 we are in a more luxurious position, as some tools, including knockout mice, channel modulators and the inhibitor TPPO, are available to study the roles of this channel. We are particularly expectant about the publication of a full report on the discovery and characterization of a group of selective agonists that may help developing new taste modulators and treatments for type II diabetes (Philippaert 2013). However, it is clear that we still know very little about the details of how this channel is gated in basal conditions and how it is modulated. Furthermore, most findings have been obtained in heterologous expression systems and need to be confirmed in taste cells. It is also clear that studying the pharmaco-

logical properties of individual compounds in detail is of outmost importance for the understanding of their specific chemosensory properties.

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References

Bartel DL, Sullivan SL, Lavoie EG, Sevigny J, Finger TE (2006) Nucleoside triphosphate diphosphohydrolase-2 is the ecto-ATPase of type I cells in taste buds. J Comp Neurol 497:1–12

Boucher Y, Simons CT, Cuellar JM, Jung SW, Carstens MI, Carstens E (2003) Activation of brain stem neurons by irritant chemical stimulation of the throat assessed by c-fos immunohistochemistry. Exp Brain Res 148:211–218

Carstens E, Kuenzler N, Handwerker HO (1998) Activation of neurons in rat trigeminal subnucleus caudalis by different irritant chemicals applied to oral or ocular mucosa. J Neurophysiol 80:465–492

Chaudhari N, Roper SD (2010) The cell biology of taste. J Cell Biol 190:285–296

Cruz A, Green BG (2000) Thermal stimulation of taste. Nature 403:889–892

Damak S, Rong M, Yasumatsu K, Kokrashvili Z, Perez CA, Shigemura N, Yoshida R, Mosinger B Jr, Glendinning JI, Ninomiya Y, Margolskee RF (2006) *Trpm5* null mice respond to bitter, sweet, and umami compounds. Chem Senses 31:253–264

de Araujo IE, Oliveira-Maia AJ, Sotnikova TD, Gainetdinov RR, Caron MG, Nicolelis MA, Simon SA (2008) Food reward in the absence of taste receptor signaling. Neuron 57:930–941

Devantier HR, Long DJ, Brennan FX, Carlucci SA, Hendrix C, Bryant RW, Salemme FR, Palmer RK (2008) Quantitative assessment of TRPM5-dependent oral aversiveness of pharmaceuticals using a mouse brief-access taste aversion assay. Behav Pharmacol 19:673–682

Dotson CD, Roper SD, Spector AC (2005) PLCb2-independent behavioral avoidance of prototypical bitter-tasting ligands. Chem Senses 30:593–600

Dvoryanchikov G, Sinclair MS, Perea-Martinez I, Wang T, Chaudhari N (2009) Inward rectifier channel, ROMK, is localized to the apical tips of glial-like cells in mouse taste buds. J Comp Neurol 517:1–14

Eddy MC, Eschle BK, Peterson D, Lauras N, Margolskee RF, Delay ER (2012) A conditioned aversion study of sucrose and SC45647 taste in TRPM5 knockout mice. Chem Senses 37:391–401

Feng P, Huang L, Wang H (2012) Taste bud homeostasis in health, disease, and aging. Chem Senses 39:3–16

Formaker BK, Frank ME (1996) Responses of the hamster chorda tympani nerve to binary component taste stimuli: evidence for peripheral gustatory mixture interactions. Brain Res 727:79–90

Gao N, Lu M, Echeverri F, Laita B, Kalabat D, Williams ME, Hevezi P, Zlotnik A, Moyer BD (2009) Voltage-gated sodium channels in taste bud cells. BMC Neurosci 10:20

Gees M, Alpizar YA, Luyten T, Parys JB, Nilius B, Bultynck G, Voets T, Talavera K (2014) Differential effects of bitter compounds on the taste transduction channels TRPM5 and IP_3 receptor type 3. Chem Senses 39:295–311

Glendinning JI, Bloom LD, Onishi M, Zheng KH, Damak S, Margolskee RF, Spector AC (2005) Contribution of alpha-gustducin to taste-guided licking responses of mice. Chem Senses 30:299–316

Grunberg NE (1982) The effects of nicotine and cigarette smoking on food consumption and taste preferences. Addict Behav 7:317–331

Grunberg NE (1985) Nicotine, cigarette smoking, and body weight. Br J Addict 80:369–377

Hisatsune C, Yasumatsu K, Takahashi-Iwanaga H, Ogawa N, Kuroda Y, Yoshida R, Ninomiya Y, Mikoshiba K (2007) Abnormal taste perception in mice lacking the type 3 inositol 1,4,5-trisphosphate receptor. J Biol Chem 282:37225–37231

Hofmann T, Chubanov V, Gudermann T, Montell C (2003) TRPM5 is a voltage-modulated and Ca^{2+}-activated monovalent selective cation channel. Curr Biol 13:1153–1158

Huang YA, Roper SD (2010) Intracellular Ca^{2+} and TRPM5-mediated membrane depolarization produce ATP secretion from taste receptor cells. J Physiol 588:2343–2350

Huang YJ, Maruyama Y, Dvoryanchikov G, Pereira E, Chaudhari N, Roper SD (2007) The role of pannexin 1 hemichannels in ATP release and cell-cell communication in mouse taste buds. Proc Natl Acad Sci U S A 104:6436–6441

Ishimaru Y, Matsunami H (2009) Transient receptor potential (TRP) channels and taste sensation. J Dent Res 88:212–218

Ishimaru Y, Inada H, Kubota M, Zhuang H, Tominaga M, Matsunami H (2006) Transient receptor potential family members PKD1L3 and PKD2L1 form a candidate sour taste receptor. Proc Natl Acad Sci U S A 103:12569–12574

Katsumata T, Nakakuki H, Tokunaga C, Fujii N, Egi M, Phan TH, Mummalaneni S, Desimone JA, Lyall V (2008) Effect of Maillard reacted peptides on human salt taste and the amiloride-insensitive salt taste receptor (TRPV1t). Chem Senses 33:665–680

Kinnamon SC (2012) Taste receptor signalling—from tongues to lungs. Acta Physiol (Oxf) 204:158–168

Kinnamon SC, Finger TE (2013) A taste for ATP: neurotransmission in taste buds. Front Cell Neurosci 7:264

Liman ER (2006) Thermal gating of TRP ion channels: food for thought? Sci STKE 2006, pe12

Liman ER (2014) TRPM5. Handb Exp Pharmacol 222:489–502

Liman ER, Zhang YV, Montell C (2014) Peripheral coding of taste. Neuron 81:984–1000

Liu D, Liman ER (2003) Intracellular Ca^{2+} and the phospholipid PIP_2 regulate the taste transduction ion channel TRPM5. Proc Natl Acad Sci U S A 100:15160–15165

Liu L, Zhu W, Zhang ZS, Yang T, Grant A, Oxford G, Simon SA (2004) Nicotine inhibits voltage-dependent sodium channels and sensitizes vanilloid receptors. J Neurophysiol 91:1482–1491

Liu D, Zhang Z, Liman ER (2005) Extracellular acid block and acid-enhanced inactivation of the Ca^{2+}-activated cation channel TRPM5 involve residues in the S3-S4 and S5-S6 extracellular domains. J Biol Chem 280:20691–20699

Liu P, Shah BP, Croasdell S, Gilbertson TA (2011) Transient receptor potential channel type M5 is essential for fat taste. J Neurosci 31:8634–8642

Lopez Jimenez ND, Cavenagh MM, Sainz E, Cruz-Ithier MA, Battey JF, Sullivan SL (2006) Two members of the TRPP family of ion channels, PKD1L3 and PKD2L1, are co-expressed in a subset of taste receptor cells. J Neurochem 98:68–77

Lyall V, Heck GL, Vinnikova AK, Ghosh S, Phan TH, Alam RI, Russell OF, Malik SA, Bigbee JW, Desimone JA (2004) The mammalian amiloride-insensitive non-specific salt taste receptor is a vanilloid receptor-1 variant. J Physiol 558:147–159

Lyall V, Heck GL, Vinnikova AK, Ghosh S, Phan TH, Desimone JA (2005) A novel vanilloid receptor-1 (VR-1) variant mammalian salt taste receptor. Chem Senses 30(Suppl 1):i42–i43

Lyall V, Phan TH, Mummalaneni S, Mansouri M, Heck GL, Kobal G, Desimone JA (2007) Effect of nicotine on chorda tympani responses to salty and sour stimuli. J Neurophysiol 98:1662–1674

Lyall V, Phan TH, Mummalaneni S, Melone P, Mahavadi S, Murthy KS, Desimone JA (2009) Regulation of the benzamil-insensitive salt taste receptor by intracellular Ca^{2+}, protein kinase C, and calcineurin. J Neurophysiol 102:1591–1605

Meseguer VM, Denlinger BL, Talavera K (2011) Methodological considerations to understand the sensory function of TRP channels. Curr Pharm Biotechnol 12:3–11

Mueller KL, Hoon MA, Erlenbach I, Chandrashekar J, Zuker CS, Ryba NJ (2005) The receptors and coding logic for bitter taste. Nature 434:225–229

Nilius B, Appendino G (2013) Spices: the savory and beneficial science of pungency. Rev Physiol Biochem Pharmacol 164:1–76

Nilius B, Talavera K, Owsianik G, Prenen J, Droogmans G, Voets T (2005) Gating of TRP channels: a voltage connection? J Physiol 567:35–44

Öhrwall H (1891) Untersuchungen über den Geschmackssinn. Skand Arch Physiol 2:1–69

Oike H, Wakamori M, Mori Y, Nakanishi H, Taguchi R, Misaka T, Matsumoto I, Abe K (2006) Arachidonic acid can function as a signaling modulator by activating the TRPM5 cation channel in taste receptor cells. Biochim Biophys Acta 1761:1078–1084

Oka Y, Butnaru M, von Buchholtz L, Ryba NJ, Zuker CS (2013) High salt recruits aversive taste pathways. Nature 494:472–475

Oliveira-Maia AJ, Stapleton-Kotloski JR, Lyall V, Phan TH, Mummalaneni S, Melone P, Desimone JA, Nicolelis MA, Simon SA (2009) Nicotine activates TRPM5-dependent and independent taste pathways. Proc Natl Acad Sci U S A 106:1596–1601

Perez CA, Huang L, Rong M, Kozak JA, Preuss AK, Zhang H, Max M, Margolskee RF (2002) A transient receptor potential channel expressed in taste receptor cells. Nat Neurosci 5:1169–1176

Perez CA, Margolskee RF, Kinnamon SC, Ogura T (2003) Making sense with TRP channels: store-operated calcium entry and the ion channel TRPM5 in taste receptor cells. Cell Calcium 33:541–549

Philippaert KP (2013) Screening for and characterization of pharmacological tools to target the Ca^{2+} activated non-selective cation channels TRPM4 and TRPM5. In 22nd Congress of the European-Chemoreception-Research-Organization (ECRO), vol 39. ed. Press, O. U., pp 106–107, Leuven

Prawitt D, Monteilh-Zoller MK, Brixel L, Spangenberg C, Zabel B, Fleig A, Penner R (2003) TRPM5 is a transient Ca^{2+}-activated cation channel responding to rapid changes in $[Ca^{2+}]i$. Proc Natl Acad Sci U S A 100:15166–15171

Pumplin DW, Yu C, Smith DV (1997) Light and dark cells of rat vallate taste buds are morphologically distinct cell types. J Comp Neurol 378:389–410

Ren Z, Rhyu MR, Phan TH, Mummalaneni S, Murthy KS, Grider JR, Desimone JA, Lyall V (2013) TRPM5-dependent amiloride- and benzamil-insensitive NaCl chorda tympani taste nerve response. Am J Physiol Gastrointest Liver Physiol 305:G106–G117

Riera CE, Vogel H, Simon SA, Damak S, Le Coutre J (2009) Sensory attributes of complex tasting divalent salts are mediated by TRPM5 and TRPV1 channels. J Neurosci 29:2654–2662

Romanov RA, Rogachevskaja OA, Bystrova MF, Jiang P, Margolskee RF, Kolesnikov SS (2007) Afferent neurotransmission mediated by hemichannels in mammalian taste cells. EMBO J 26:657–667

Roper SD (2014) TRPs in taste and chemesthesis. Handb Exp Pharmacol 223:827–871

Simons CT, Boucher Y, Carstens MI, Carstens E (2006) Nicotine suppression of gustatory responses of neurons in the nucleus of the solitary tract. J Neurophysiol 96:1877–1886

Talavera K, Yasumatsu K, Voets T, Droogmans G, Shigemura N, Ninomiya Y, Margolskee RF, Nilius B (2005) Heat activation of TRPM5 underlies thermal sensitivity of sweet taste. Nature 438:1022–1025

Talavera K, Ninomiya Y, Winkel C, Voets T, Nilius B (2007) Influence of temperature on taste perception. Cell Mol Life Sci 64:377–381

Talavera K, Nilius B, Voets T (2008a) Neuronal TRP channels: thermometers, pathfinders and lifesavers. Trends Neurosci 31:287–295

Talavera K, Voets T, Nilius B (2008b) Mechanisms of thermosensation in TRP channels. In Martinac B (ed) Sensing with ion channels. Springer, pp 101–120

Talavera K, Yasumatsu K, Yoshida R, Margolskee RF, Voets T, Ninomiya Y, Nilius B (2008c) The taste transduction channel TRPM5 is a locus for bitter-sweet taste interactions. FASEB J 22:1343–1355

Talavera K, Gees M, Karashima Y, Meseguer VM, Vanoirbeek JA, Damann N, Everaerts W, Benoit M, Janssens A, Vennekens R, Viana F, Nemery B, Nilius B, Voets T (2009) Nicotine activates the chemosensory cation channel TRPA1. Nat Neurosci 12:1293–1299

Taruno A, Matsumoto I, Ma Z, Marambaud P, Foskett JK (2013a) How do taste cells lacking synapses mediate neurotransmission? CALHM1, a voltage-gated ATP channel. Bioessays 35:1111–1118

Taruno A, Vingtdeux V, Ohmoto M, Ma Z, Dvoryanchikov G, Li A, Adrien L, Zhao H, Leung S, Abernethy M, Koppel J, Davies P, Civan MM, Chaudhari N, Matsumoto I, Hellekant G, Tordoff MG, Marambaud P, Foskett JK (2013b) CALHM1 ion channel mediates purinergic neurotransmission of sweet, bitter and umami tastes. Nature 495:223–226

Tomassini S, Cuoghi V, Catalani E, Casini G, Bigiani A (2007) Long-term effects of nicotine on rat fungiform taste buds. Neuroscience 147:803–810

Tordoff MG, Shao H, Alarcon LK, Margolskee RF, Mosinger B, Bachmanov AA, Reed DR, Mccaughey S (2008) Involvement of T1R3 in calcium-magnesium taste. Physiol Genomics 34:338–348

Tordoff MG, Alarcon LK, Valmeki S, Jiang P (2012) T1R3: a human calcium taste receptor. Sci Rep 2:496

Treesukosol Y, Lyall V, Heck GL, Desimone JA, Spector AC (2007) A psychophysical and electrophysiological analysis of salt taste in *Trpv1* null mice. Am J Physiol Regul Integr Comp Physiol 292:R1799–R1809

Uchida K, Tominaga M (2013) Extracellular zinc ion regulates transient receptor potential melastatin 5 (TRPM5) channel activation through its interaction with a pore loop domain. J Biol Chem 288:25950–25955

Ullrich ND, Voets T, Prenen J, Vennekens R, Talavera K, Droogmans G, Nilius B (2005) Comparison of functional properties of the Ca^{2+}-activated cation channels TRPM4 and TRPM5 from mice. Cell Calcium 37:267–278

Vandenbeuch A, Kinnamon SC (2009) Why do taste cells generate action potentials? J Biol 8:42

Vandenbeuch A, Clapp TR, Kinnamon SC (2008) Amiloride-sensitive channels in type I fungiform taste cells in mouse. BMC Neurosci 9:1

Vandenbeuch A, Anderson CB, Parnes J, Enjyoji K, Robson SC, Finger TE, Kinnamon SC (2013) Role of the ectonucleotidase NTPDase2 in taste bud function. Proc Natl Acad Sci U S A 110:14789–14794

Voets T, Droogmans G, Wissenbach U, Janssens A, Flockerzi V, Nilius B (2004) The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. Nature 430:748–754

Voets T, Owsianik G, Janssens A, Talavera K, Nilius B (2007) TRPM8 voltage sensor mutants reveal a mechanism for integrating thermal and chemical stimuli. Nat Chem Biol 3:174–182

Zhang Y, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu D, Zuker CS, Ryba NJ (2003) Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell 112:293–301

Zhang Z, Zhao Z, Margolskee R, Liman E (2007) The transduction channel TRPM5 is gated by intracellular calcium in taste cells. J Neurosci 27:5777–5786