The taste transduction channel TRPM5 is a locus for bitter-sweet taste interactions

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ABSTRACT

Ordinary gustatory experiences, which are usually evoked by taste mixtures, are determined by multiple interactions between different taste stimuli. The most studied model for these gustatory interactions is the suppression of the responses to sweeteners by the prototype bitter compound quinine. Here we report that TRPM5, a cation channel involved in sweet taste transduction, is inhibited by quinine (EC50=50 μM at −50 mV) owing to a decrease in the maximal whole-cell TRPM5 conductance and an acceleration of channel closure. Notably, quinine inhibits the gustatory responses of sweet-sensitive gustatory nerves in wild-type (EC90=−1.6 mM) but not in Trpm5 knockout mice. Quinine induces a dose- and time-dependent inhibition of TRPM5-dependent responses of single sweet-sensitive fibers to sucrose, according to the restricted diffusion of the drug into the taste tissue. Quinidine, the stereoisomer of quinine, has similar effects on TRPM5 currents and on sweet-induced gustatory responses. In contrast, the chemically unrelated bitter compound denatonium benzoate has an ~100-fold weaker effect on TRPM5 currents and, accordingly, at 10 mM it does not alter gustatory responses to sucrose. The inhibition of TRPM5 by bitter compounds constitutes the molecular basis of a novel mechanism of taste interactions, whereby the bitter tastant inhibits directly the sweet transduction pathway.—Talavera, K., Yasumatsu, K., Yoshida, R., Margolskee, R. F., Voets, T., Ninomiya, Y., Nilius, B. The taste transduction channel TRPM5 is a locus for bitter-sweet taste interactions. FASEB J. 22, 000–000 (2008)

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More than 100 years ago, Öhrwall (1) demonstrated that quinine, a prototype bitter compound, inhibits human perception of sweet taste. This phenomenon, known as bitter-sweet taste interaction, is still one of the most intriguing and relevant models of gustatory sensory integration and has a strong physiological relevance. Bitter-tasting signals for poisonous compounds such as plant alkaloids and triggers aversive behavior, whereas sweet-tasting allows the identification of energy-enriched food sources. For bitter-sweet mixtures, the decrease in the resulting rewarding value due to the suppression of sweet perception by bitter tastants may represent a complementary, although important, protective mechanism (2, 3). In addition, bitter-sweet mixture interactions may determine the preference toward the consumption of vegetables (4) and sugar-sweetened beverages (5) and therefore have a significant effect on health.

Bitter-sweet mixture interactions have been studied almost exclusively with quinine, which elicits bitter taste perception in humans at micromolar concentrations (see, for example, refs. 6, 7). Although with quantitative differences, these interactions have been observed in several preparations exploring the various levels of taste perception and transduction (Table 1). Physiological experiments have shown that increasing concentrations of quinine above the micromolar range augment the sucrose concentration threshold for the detection of sweetness (8). On the other hand, concentrations at hundreds of micromolar levels are needed to inhibit the magnitude of the response elicited by suprathreshold stimulation with sweeteners (9, 10, 12–14). Electrophysiological experiments in hamsters have demonstrated that the inhibition of sweet-induced gustatory responses by quinine is also present at the levels of the brainstem nucleus and the gustatory periphery [chorda tympani (CT) nerve] (Table 1).

It has been proposed that gustatory mixture interactions may occur through multiple mechanisms: chemical reaction between tastants, competition of the tastants for the receptors, cross-talk between transduction cascades in the taste receptor cells, lateral cell-cell interactions within the taste buds, and interaction between gustatory signals at the levels of the papillae, afferent nerve fibers, central nervous system, and conscious experience (2, 3, 11, 19–21). However, electro-

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For instance, recent data strongly indicate that heat fibers (33–35). Thus, factors that modulate TRPM5 function are expected to have an influence on taste perception. For instance, recent data strongly indicate that heat activation of TRPM5 underlies the thermal modulation of sweet taste perception (27).

Interestingly, it has been shown that quinine inhibits TRPM5-like, nonselective cation channels in native cells (36–38). Thus, we hypothesized that the quinine-induced suppression of peripheral gustatory responses to sweet stimuli could be due to an inhibition of TRPM5 currents. To test this hypothesis, we studied the effects of quinine, quinidine, the stereoisomer of quinine, and the strongly bitter compound denatonium benzoate on TRPM5 currents and on TRPM5-dependent gustatory nerve responses in situ. In this work, we mainly analyzed the effects of quinine on the gustatory responses to sweeteners, as the effects on the response to other taste qualities are not as clear at the peripheral level of taste processing.

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Physiological studies have narrowed the main locus for the suppression of the responses to sweeteners by quinine to the gustatory periphery and, in particular, to the level of taste receptor cells (2, 3, 19).

TRPM5 is a $\text{Ca}^{2+}$- and voltage-activated nonselective cation channel and member of the transient receptor potential (TRP) superfamily (22–27) with a critical role in the transduction of sweet, bitter, and umami tastes (27–29) and other chemosensory stimuli (30, 31). This channel is proposed to be activated downstream of the activation of G-protein-coupled taste receptors (24, 28, 32) and to generate the depolarizing receptor potential needed for taste receptor cell communication with afferent taste fibers (33–35). Thus, factors that modulate TRPM5 function are expected to have an influence on taste perception. For instance, recent data strongly indicate that heat
Patch-clamp experiments

Murine TRPM5 and human TRPM4 were cloned in the pAGGS-IRESGFP vector and transiently transfected in HEK-293 cells using TransIT-293 reagents (Mirus, Madison, WI, USA). Cell culture and patch-clamp recordings were performed as described previously (27). TRPM5 currents were recorded in the whole-cell patch-clamp configuration, using an extracellular solution containing 150 mM NaCl, 5 mM CaCl₂, 1 mM MgCl₂, and 10 mM HEPES and was titrated to 7.4 with NaOH. The intracellular (pipette) solution contained 50 mM NaCl, 100 mM N-methyl-D-glucamine, and 10 mM HEPES and was titrated to pH 7.2 with NaOH. Intracellular Ca²⁺ was buffered to 500 nM free Ca²⁺ by adding 2 mM EGTA and 1.55 mM CaCl₂ as determined with the software Cabuf (G. Droogmans; ftp://ftp.cc.kuleuven.ac.be/pub/droogmans/cabuf.zip). Given that TRPM4 currents undergo a fast and irreversible desensitization in the whole-cell patch-clamp configuration (26, 39, 40), TRPM4 currents were recorded in the inside-out patch-clamp configuration. The extra- and intracellular solutions were the same as for TRPM5 recorded in the inside-out patch-clamp configuration (26, 39, 40). TRPM4 currents were recorded in the whole-nerve patch-clamp configuration (23–27). 

Inhibition of TRPM5 by quinine was investigated. Quinine depletes and accumulates in the intracellular milieu. All currents were fit by a function of the form

\[ \text{Inhibition} = \frac{A_0 - (A_b - A_1)}{1 + (EC_{50}/[D])^H} \]

where \( A_0 \) is the amplitude of the response in the absence of the drug, \( A_1 \) is the amplitude of the response at saturating drug concentrations, \( EC_{50} \) is the effective concentration, \([D]\) is the drug concentration, and \( H \) is the Hill coefficient.

RESULTS

Quinine inhibits TRPM5 and TRPM4 currents

We used the whole-cell patch-clamp technique to investigate the effects of quinine on TRPM5 channels heterologously expressed in HEK-293 cells. As previously reported (27), no sizeable background currents were detected in nontransfected HEK-293 cells nor when cells transfected with TRPM5 were dialyzed intracellularly with a Ca²⁺-free (10 mM 1,2-bis(2-aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid) solution (data not shown). To record TRPM5 currents, TRPM5-transfected cells were dialyzed intracellularly with 500 nM Ca²⁺ (23–27). Figure 1 shows that extracellular application of quinine inhibited TRPM5 currents in a dose-dependent and reversible manner. The inhibition was more pronounced at negative potentials, with \( EC_{50} \) values of 50 ± 5 and 148 ± 14 µM at −50 and +100 mV, respectively. \( H \) was 0.84 ± 0.07 at −50 mV and 0.84 ± 0.06 mV at +100 mV.

We further analyzed the effects of quinine on TRPM5 channels by determining the voltage dependence of the steady-state and kinetic properties of TRPM5 currents in the control and in the presence of 100 µM extracellular quinine. Currents were recorded during the application of voltage steps lasting 300 ms to +100 mV followed by a step to −50 mV (holding potential of +100 mV) (Fig. 2A, left). Current-voltage relationships were determined from the amplitude of steady-state currents. Figure 2B shows that quinine inhibits TRPM5 currents at all membrane potentials. However, consistent with the results shown above, the inhibition was stronger at negative potentials (Fig. 2C). Next, we determined the voltage dependence of instantaneous current amplitudes in the control and in the presence of quinine (n=4) (Fig. 2D–E). For both cases the data were well fit by linear functions, indicating that quinine does not affect the open pore rectification pattern. Current-voltage relationships were fit by a function of the form

\[ I(V) = \frac{G_{\text{max}}(V - V'_r)}{1 + \exp(-(V - V'_{act})/s_{act})} \]

where \( G_{\text{max}} \) is the maximal TRPM5 whole-cell conductance, \( V'_r \) is the reversal potential, \( V'_{act} \) is the potential of half-maximal activation, and \( s_{act} \) is the slope factor \( n=4 \) (Fig. 2B). Application of 100 µM quinine decreased \( G_{\text{max}} \) to 63 ± 5% of the control value. Quinine
shifted the activation curve to more positive potentials and increased the voltage sensitivity of channel activation ($V_{act} = 23 \pm 3$ mV and $s_{act} = 53 \pm 3$ mV in the control vs. $V_{act} = 33 \pm 9$ mV and $s_{act} = 42 \pm 3$ mV in the presence of quinine; $n=4$, $P<0.05$) (Fig. 2F). The reversal potential was not significantly different between the control (28.0 ± 0.2 mV) and the value in the presence of quinine (28.2 ± 0.6 mV). In addition, quinine reduced the time constant of current relaxation at negative potentials (Fig. 2G), which indicates that the voltage dependence of current inhibition (Fig. 2C) is due to an increase in the rate of channel closing.

TRPM4 is the closest homolog of TRPM5 in the whole TRP channel superfamily. These channels share some key functional characteristics such as voltage-dependent gating, activation by intracellular Ca$^{2+}$, and impermeability to Ca$^{2+}$ (26) and show more than 50% similarity in the amino acid sequence. Because both channels are coexpressed in some cell types (25, 26), it could be speculated that they provide for some level of redundancy in certain cellular processes. Moreover, it has been shown that taste cells of Trpm5 knockout (KO) mice express a channel with characteristics similar to those of TRPM4 (35), raising the possibility that this channel has a role in taste transduction. Thus, we tested whether quinine affects the function of human TRPM4 channels overexpressed in HEK-293 cells. Figure 3 shows that quinine inhibits TRPM4 currents in a reversible manner. Notably, in contrast with what was observed for TRPM5, the inhibition was more potent at positive potentials, with EC$_{50}$ values of 450 ± 50 and 113 ± 9 μM at −50 mV and +100 mV, respectively. H was 1.1 ± 0.1 at −50 mV and 0.97 ± 0.07 at +100 mV.

Quinine inhibits TRPM5-dependent gustatory responses to sweet stimuli

Next, we determined whether quinine influences TRPM5-dependent taste perception in situ, by recording the electrical responses of whole bundles and single fibers of the chorda tympani nerve from wild-type and Trpm5 knockout mice (27, 29). The CT nerve innervates the anterior taste field of the tongue, which is preferentially responsive to sweet over other taste stimuli. With use of a Trpm5 knockout mouse model different from our own it was shown that taste responses to sweet, bitter, and umami stimuli are completely impaired (28). However, it is important to note that our Trpm5 knockout mouse model is quantitatively different, as it shows decreased, but not totally abolished, behavioral and gustatory nerve responses to these stimuli (27, 29). This characteristic allows us to determine whether quinine affects the TRPM5-dependent pathway exclusively for perception of sweet taste stimuli.

First, we tested the effects of quinine on responses of the whole CT nerve to sucrose (0.3 M). For this experiment, CT nerves were stimulated with sucrose alone, with sucrose-quinine mixtures, and with quinine alone (Fig. 4A). The neural component corresponding to sucrose in the presence of quinine was determined by subtracting CT responses to quinine at different concentrations from the responses to the corresponding sucrose-quinine mixtures (2). This method is supported by the facts that sucrose and quinine responses are generated by different subsets of CT fibers and that sucrose does not affect CT responses to quinine (3, 19). As shown in Fig. 4B, quinine induced a dose-dependent inhibition of the responses to sucrose in wild-type mice with an EC$_{50}$ of 1.6 ± 0.2 mM. In contrast, residual responses to sucrose in Trpm5-null mice were not affected by the drug. Notably, with increasing quinine concentrations, the amplitude of the sucrose component in wild-type mice falls to the level of the response of the knockout mice. Likewise, quinine inhibited the
responses to three other sweeteners (fructose, saccharin, and SC45647) in wild-type but not in \textit{Trpm5}-null mice (Fig. 4C). On the other hand, quinine application had no statistically significant effect on the responses to umami [monosodium glutamate (MSG) in the presence of 10 \mu M amiloride to inhibit the response to Na\textsuperscript{+}] or salty (NaCl) stimuli in wild-type mice.

To obtain unequivocal evidence of the effects of quinine on the TRPM5-dependent responses to sweeteners we tested single sweet-sensitive fibers dissected from the CT nerve. These fibers were identified by their significantly stronger response to sucrose compared with other stimuli (Fig. 5A, B). Consistent with the results obtained in whole CT nerves, single fibers of \textit{Trpm5}-null mice were responsive to sucrose in a dose-dependent fashion, although to a much lower extent than fibers of wild-type mice (Fig. 5C, D). To quantify the effects of quinine on sweet-elicited activity we compared the number of nerve impulses evoked during a 10-s period of stimulation with sucrose with the number of nerve impulses elicited by mixtures of sucrose and quinine during the same period. In accord with the results obtained from the above-mentioned whole CT nerve experiments, quinine induced a dose-dependent inhibition of single sweet-labeled fiber responses to sucrose in wild-type but not in \textit{Trpm5} knock-out mice (Fig. 5B–D).

Notably, quinine was more effective in reducing TRPM5 currents in whole-cell patch-clamp experiments than in inhibiting the responses of CT and single sweet-sensitive fibers. From Fig. 5C it is possible to estimate that the quinine concentration needed to reduce the responses of single fibers to sucrose to 50\% was 20- to 40-fold larger than the EC\textsubscript{50} for inhibition of inward currents (50 \mu M at \sim 50 mV). This result is expected, as in whole-cell experiments the drug was applied directly on the cell membrane expressing the TRPM5 channels, whereas in the \textit{in situ} experiments quinine was applied to the apical side of the taste receptor cells and needed to diffuse to the basolateral membrane where TRPM5 channels are expressed.

If indeed limited diffusion was the cause of the relatively low effectiveness of quinine \textit{in situ}, one should expect that the inhibition of nerve responses to
sucrose increases with the time of exposure to quinine. To test this possibility, we determined the dose dependence of the effect of quinine on the firing of single sweet-sensitive fibers in the first and second halves of the 10-s period of stimulation with 0.3 M sucrose. As shown in Fig. 6A, quinine was more potent in decreasing the number of nerve impulses in the second half than in the first half of the stimulation period. In the 0- to 5-s interval, the estimated EC50 was 2.05 ± 0.08 mM and the Hill coefficient was 1.5 ± 0.1, whereas in the 5- to 10-s interval these magnitudes were 1.17 ± 0.01 mM and 2.5 ± 0.1, respectively. Consistent with this result, the inhibition of the responses to sucrose by quinine showed a marked increase with the time of quinine application, with a relaxation time constant of 2.0 ± 0.3 s (Fig. 6B).

Effects of quinidine and denatonium benzoate on TRPM5 currents and sweet-induced gustatory responses

To test the specificity of quinine inhibition of TRPM5 and sweet responses we tested the effects of two other bitter compounds: quinidine, a quinine stereoisomer, and denatonium benzoate, which is chemically unrelated. Figure 7A, B shows that quinidine induces a reversible and dose-dependent inhibition of TRPM5 currents. Notably, quinidine dose-response curves at −50 and +100 mV were very similar to those of quinine, with an EC50 of 42 ± 3 μM and H of 0.93 ± 0.06 at −50 mV and an EC50 of 120 ± 6 μM and H of 0.82 ± 0.03 at +100 mV (Fig. 7C). Next, we tested the effects of quinidine on responses of the whole CT nerve to sucrose (0.3 M). As for quinine, CT nerves were stimulated with sucrose alone, with sucrose-quinidine mixtures, and with quinidine alone (Fig. 7D). In wild-type mice, the sucrose component in the CT responses to sucrose-quinidine mixtures was reduced by increasing concentrations of quinidine with an EC50 of 1.22 ± 0.14 mM (Fig. 7E). This inhibition was, however, not observed in Trpm5 knockout mice. Again, these results are very similar to those obtained with quinine (Fig. 4B).

Denatonium benzoate had no significant effect on TRPM5 currents at concentrations up to 300 μM (Fig. 8A, B) but induced a significant decrease of the current amplitude at 1 and 3 mM (Fig. 8C, D). Application of higher concentrations of denatonium benzoate compromised the stability of the recordings, and no useful data were acquired in those conditions. To compare the effects of denatonium benzoate with those of quinine (Fig. 1C) and quinidine (Fig. 7C), we fitted the available data with a Hill function assuming a maximal inhibitory effect of 100% and a Hill coefficient of 1. This allowed us to estimate the EC50 values for denatonium benzoate to be 4.1 ± 0.5 and 9.4 ± 1.1 mM at −50 and +100 mV, respectively (Fig. 8E). In line with these observations, whole CT nerve recordings revealed that denatonium benzoate (10 mM) had no statistically significant effect on the amplitude of the sucrose component of the wild-type and Trpm5 KO responses to sucrose-denatonium benzoate mixtures (Fig. 8F).

DISCUSSION

We have found that quinine and its stereoisomer quinidine induce a dose-dependent inhibition of TRPM5 currents in HEK-293 cells and that both drugs inhibit TRPM5-dependent peripheral gustatory responses. The inhibition of TRPM5 currents by quinine is due to a
decrease in the whole-cell conductance and a voltage-dependent inhibition that can be explained by an increase in the rate of channel closing at negative potentials. Notably, the potency of the inhibitory effect is similar to that observed in TRPM4/TRPM5-like non-selective cation channels in a rat insulinoma cell line (36), a cell line derived from neonatal mouse mandibular glands (37), and human T-lymphocytes (38). Likewise, we have found that quinine effects on the integrated whole-nerve responses from chorda tympani nerve of wild-type and Trpm5 knockout mice to different taste stimuli: sweet (0.5 M sucrose, 0.5 M fructose, 20 mM saccharin, and 0.3 mM SC-45647), umami (0.1 M MSG plus 10 μM amiloride), and salty (0.1 M NaCl) (n=6 in all cases). The amplitude of the response component corresponding to each tastant in the tastant-quinine mixtures was determined by subtracting the response to quinine alone (10 mM, horizontally dashed bar) from the response to the corresponding tastant-quinine mixture. Wild-type responses to all sweeteners were significantly smaller in the presence of quinine than the responses to the sweeteners presented alone. *P < 0.05; **P < 0.01.

TRPM5 as a locus for modulating taste perception

We have previously shown that the modulation of TRPM5 gating by temperature underlies the thermal sensitivity of sweet taste perception (27). In addition, it has been proposed that chemical modulators of TRPM5, such as phosphatidylinositol-4,5-bisphosphate (24), extracellular protons (42), and arachidonic acid (43), may alter TRPM5-mediated gustatory responses, but these hypotheses have not been tested. Our present data demonstrate that not only physical but also pharmacological interventions on TRPM5 channels can modulate sweet taste perception in situ.

We have found that quinine inhibits inward TRPM5 currents in isolated cells with an EC₅₀ of 50 μM. However, although it is well known that quinine is able to rapidly diffuse into cells of isolated taste buds (44, 45) and reach the basolateral membrane where TRPM5 is expressed (22), effective inhibitory concentrations in vivo may be significantly higher than those in isolated cells because of restricted diffusion in the interstitial space of the taste buds. Our experiments on single sweet-sensitive fibers indeed show that the inhibitory effect of quinine is weaker than that for TRPM5 currents in isolated cells, but it increases with the time of application.
application (Fig. 6; see also refs. 2, 15). This finding is in accordance with restricted diffusion of the drug in the living tissue and explains the difference in potency between the whole-cell patch-clamp and the in situ experiments.

According to one previous report, quinine concentrations just greater than $10^{-11}$ M are high enough to increase the threshold for the human detection of the sweetness of sucrose (8). Our results indicate that this effect might not be mediated by the inhibition of TRPM5. Alternatively, it could be due to peripheral interactions between TRPM5-dependent bitter and sweet gustatory pathways and/or bitter-sweet interactions at high levels of gustatory integration (see below). In humans, quinine-induced inhibition of TRPM5 may be relevant to more common situations, in which high concentrations of sweeteners are used. Indeed, it has been shown that the suppression of suprathreshold sweet sensations requires hundreds of micromolar concentrations of quinine (Table 1). Of note, quinine is well-known for its commercial use in the composition of tonic water in concentrations up to 220 $\mu$M (Code of Federal Regulations, U.S. Food and Drug Administration; http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=172.575). Thus, it is expected that significant masking of the sweet taste occurs in these beverages owing to quinine suppression of sweet taste perception, which implies that these drinks may contain higher sugar concentrations than humans can actually detect.

It has been shown that TRPM5 is involved in the transduction of umami taste perception (28, 29). However, quinine application had no statistically significant effect on the CT response to MSG. To explain this result, it must be taken into account that, in contrast to the results reported for another Trpm5 knockout mouse model (28), we observed that in our Trpm5 knockout mice the CT nerve responses to MSG are reduced only by 33% with respect to the response in wild-type mice (Fig. 4C; see also refs. 27, 29). Therefore, the inhibitory effect of quinine on TRPM5 is expected to have a limited effect on the total wild-type CT response to MSG. These results are in agreement with previous data showing that the murine CT nerve re-
response to MSG (unlike the responses to sweeteners) has a weak temperature dependence (27).

Interestingly, using single facial fibers of the channel catfish, Ogawa et al. (46) did show clear inhibitory effects of quinine (1–10 mM) on the gustatory responses to several amino acids. Moreover, single amino acid-sensitive fibers in this preparation could be classified in two groups according to the sensitivity to quinine. Future experiments may address the possibility that single umami-sensitive CT fibers of the mouse can be divided into quinine-sensitive (TRPM5-dependent) and quinine-insensitive (TRPM5-independent) groups. Such a result could explain why we did not observe a statistically significant effect of quinine on the whole CT nerve responses to MSG. Further experiments may also test whether quinine inhibits TRPM5-dependent responses to other bitter compounds, as it was reported that this drug inhibits the responses of single fibers of the channel catfish to caffeine and denatonium benzoate, whereas the latter compounds did not affect the responses to amino acids (46).

The inhibition of TRPM5 by quinine predicts that the more a gustatory response depends on TRPM5, the more susceptible it will be to inhibition by quinine. Our CT data (Fig. 4C) indicate that, indeed, the magnitude of the quinine-induced inhibition of wild-type responses to different tastants strongly correlates (r=0.93) with the extent to which wild-type responses to these tastants depend on TRPM5 (Fig. 9). Note that, according to what is argued above, the point corresponding to MSG fits very well within this correlation.

TRPM4 is the closest homolog to TRPM5 within the TRP channel superfamily, showing multiple similarities in some key functional properties, such as voltage- and Ca^{2+}-dependent gating and impermeability to Ca^{2+}. Thus, the detection of native single channels in taste cells of Trpm5 KO mice with characteristics similar to those of TRPM4 (35) raises the possibility that this channel is involved in the transduction of taste stimuli. In this case, TRPM4 could be a good candidate to sustain the taste responses to sweet, bitter, and umami stimulus observed in Trpm5 KO mice (refs. 27, 29 and present results). We found that quinine inhibits TRPM4 channels overexpressed in HEK-293 cells. However, the EC_{50} for quinine at the physiologically relevant potential of −50 mV was ~9-fold larger than that for TRPM5 channels (Fig. 3C). Notably, quinine did not alter the amplitude of the gustatory responses of Trpm5 KO mice, which can be explained by either the lack of a role of TRPM4 in taste perception or, in the light of our results, its weak sensitivity to quinine at physiological potentials.

The inhibition of TRPM4 currents was stronger at +100 mV than at −50 mV, reflecting a voltage sensitivity that is opposite to that observed in TRPM5. At this point, it is difficult to speculate about the mechanism(s) underlying these differences, but it is interesting to note that the voltage dependence of the inhibition of HERG channels by quinine and quinidine can be reversed by mutations in the S6 segment (47). Future structure-function studies in TRPM4/TRPM5 channels may elucidate which structures underlie the opposite voltage dependence of the quinine-induced inhibition.

The results obtained with quinine indicate that other bitter compounds may affect the perception of sweet taste by altering TRPM5 function. Therefore, we tested the effects of quinidine, the stereoisomer of quinine, and denatonium benzoate, a chemically unrelated compound with marked bitter character. Interestingly, quinidine was nearly as effective as quinine in inhibiting whole-cell TRPM5 currents in HEK-293 cells and TRPM5-dependent responses to sucrose. However, denatonium benzoate showed a 100-fold weaker inhibitory potency on TRPM5 currents, with significant effects being observed only at concentrations of 1 mM and above. In accordance with this observation and the expected limited diffusion of denatonium benzoate in intact gustatory tissue, application of a 10 mM concen-
A molecular mechanism for bitter-sweet mixture suppression

Our results strongly indicate that TRPM5, the activation of which is essential for sweet taste perception (27–29), is a locus for taste modulation and, in particular, allow postulation that bitter compounds that have strong inhibitory effects on TRPM5 are expected to induce bitter-sweet mixture suppression in the gustatory periphery. Notably, this result is in full agreement with previous studies indicating that part of the interaction between quinine and the sweet transduction pathway occurs at the gustatory periphery, and, in particular, at the level of taste receptor cells (2, 3, 19). The inhibition of TRPM5 by quinine constitutes a novel mechanism of the so-called bitter-sweet taste interactions for two reasons. First, it provides for a molecular basis of the phenomenon and, second, it implies that the bitter tastant inhibits the sweet transduction pathway directly. This mechanism challenges the fundamental concept of bitter-sweet taste interaction, as it dissociates the quinine-induced inhibition of the sweet pathway from the detection and processing of the bitter signal. Furthermore, this mechanism reconciles the effects of quinine on sweet responses and the principle of segregated peripheral labeled lines for the perception and processing of bitter and sweet stimuli (28, 48–50).

It must be noted that our in situ experiments do not disprove the existence of bitter-sweet interactions at high levels of gustatory integration (18) nor the interactions between TRPM5-dependent sweet and bitter transduction pathways. However, they do demonstrate that the TRPM5-independent sweet and bitter taste pathways do not interact at the neural periphery (10, 12, 20, 21) nor via lateral inhibition...
between sweet- and bitter-labeled taste receptor cells (2, 19). Indeed, we observed that in contrast with wild-type mice, whereas whole CT responses of Trpm5 knockout mice to quinine were preserved in 60% with respect to those of wild type (Fig. 4C), the nerve responses of knockout mice to sweeteners were totally insensitive to quinine.

Sweet and bitter compounds induce a reciprocal suppression of their gustatory responses (12, 16–18, 51, 52). However, at least for quinine-sucrose mixtures, there is a functional asymmetry in the underlying peripheral mechanisms of inhibition, as quinine suppresses peripheral sucrose responses but not vice versa (2, 19). Our results provide a straightforward explanation for these observations, i.e., a direct inhibitory effect of quinine on the TRPM5-dependent sweet transduction pathway.

From the biological point of view, the elucidation of the mechanisms for suppression of sweet responses by bitter compounds remains a central question. As mentioned above, this phenomenon may constitute a mechanism of protection against the consumption of noxious components in calorie-enriched foods. In this context, a defensive mechanism based on the inhibition of a specific element of the transduction cascade (e.g., quinine on TRPM5) seems to be a very effective complement to neural-based mechanisms of negative feedback located either at the gustatory periphery or at higher integration levels. In any case, our results teach us that quinine, although useful to study peripheral

Figure 8. Effects of denatonium benzoate on TRPM5 currents and CT nerve responses to sucrose. A) Examples of TRPM5 currents recorded during the application of depolarizing pulses to +100 mV followed by a step to −50 mV (holding potential of +28 mV) in the control condition, in the presence of 10, 300, 100, or 30 μM denatonium benzoate (DB), and during the washout of this compound. B) Time course of steady-state TRPM5 current amplitudes at +100 and −50 mV. Extracellular application of denatonium benzoate is denoted by the horizontal bars. C) Examples of TRPM5 currents in the control, in the presence of 1 or 3 mM denatonium benzoate, and during the washout of this compound. D) Time course of steady-state TRPM5 current amplitudes at +100 and −50 mV. Extracellular application of denatonium benzoate is denoted by the horizontal bars. E) Average concentration-dependent inhibition of TRPM5 currents by denatonium benzoate at +100 and −50 mV (n=9–16). The dotted and dashed lines represent the corresponding dose-dependent effects of quinine at −50 and +100 mV, respectively (Fig. 1C). F) Comparison of the amplitude of whole chorda tympani nerve responses to sucrose in the absence and in the presence of denatonium benzoate for wild-type (WT) and Trpm5 knockout mice (n=6 each). The amplitude of the sucrose component in the response to the sucrose-denatonium benzoate mixtures was determined by subtracting the amplitude of the response to denatonium benzoate alone from the response to the corresponding mixture. Nerve responses were normalized to the response to 0.1 M NH₄Cl.

Figure 9. The effect of quinine on CT nerve responses correlates with the degree to which these responses depend on TRPM5. The inhibitory effect of quinine on CT nerve responses to different taste stimuli was determined with the formula: inhibition = 100 · (1−(Rₘ−Rₚ)/Rₚ), where Rₘ, Rₚ, and Rₜ are the magnitude of response to the mixture of the tastant and quinine (10 μM), to quinine alone (10 μM), and to the tastant alone, respectively (Fig. 5C). For each tastant, the degree of dependence of the wild-type CT nerve response on TRPM5 was estimated as 1 − Rₜ/KO/RₚWT, where Rₜ/KO and RₚWT are the average response of Trpm5 knockout and wild-type mice, respectively.
processes, is not the best choice to learn about the mechanisms of interaction between the sensory pathways for sweet and bitter tastes at high levels of gustatory signal integration.

Relevance to the mechanisms of bitter taste transduction

The effects of quinine on TRPM5 have strong implications for our understanding of the mechanisms of bitter taste transduction, as the role of TRPM5 in this process is still a matter of debate. On one hand, it has been proposed that the transduction of bitter taste (including that of quinine) is entirely mediated by the activation of TRPM5 (28). On the other hand, previous data suggested multiple pathways (45, 53, 54) and, more recently, Trpm5-null mice have been found to be responsive to quinine (refs. 27, 29; see also Fig. 3).

Our data demonstrate that quinine inhibits TRPM5 currents and therefore indicate that the perception of the bitter taste of this compound cannot be mediated solely by the activation of TRPM5 channels. At least one alternative pathway must come into play to trigger the aversive behavior toward quinine at high concentrations. The dose dependence of the suppression of TRPM5-dependent sweet responses by quinine may give an estimate of the concentrations at which the bitter taste of quinine may not be mediated by TRPM5 (i.e., the concentrations at which quinine inhibits TRPM5 in situ). We have found that 1 mM quinine inhibits >60% of the neural responses to 0.1 M sucrose (Fig. 5D). Assuming similar accessibility of quinine to the basolateral membrane of best sweet- and best bitter-labeled taste receptor cells, we estimate that TRPM5-dependent gustatory responses to quinine may be significantly reduced at concentrations >1 mM. Accordingly, Trpm5-null mice are endowed with a TRPM5-independent gustatory pathway, which supports neural and behavioral taste responses to quinine at concentrations higher than 1 mM (29).

In conclusion, we have identified the TRPM5 cation channel as a quinine-sensitive element in the sweet taste transduction pathway and provide the molecular basis of a novel mechanism of peripheral gustatory processing underlying bitter-sweet taste interactions. In addition, our data constitute additional independent evidence for the existence of at least one TRPM5-independent pathway for the transduction of bitter taste and demonstrate that TRPM5 can be used as a pharmacological target for the modulation of taste perception.

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