Taste Receptor Cell Responses to the Bitter Stimulus Denatonium Involve Ca\(^{2+}\) Influx Via Store-Operated Channels

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A variety of chemical compounds induce bitter taste responses via different mechanisms (see reviews in Gilbertson et al. 2000; Glendinning et al. 2000). One pathway involves activation of the T2R/TRB G protein-coupled membrane receptors (Adler et al. 2000; Chandrashekar et al. 2000; Matsunami et al. 2000; Ming et al. 1998). T2Rs activate gustducin, a chemo-sensory-specific heterotrimeric G protein composed of α-gustducin (McLaughlin et al. 1992), and its partners, β\(3\)γ13 (Huang et al. 1999). α-Gustducin activates phosphodiesterase (PDE), causing decreases in intracellular cAMP, while its partners activate phospholipase C (PLC\(\beta\)), to produce inositol-1,4,5-trisphosphate (IP\(_3\)) and diacylglycerol (DAG) (Huang et al. 1999; Rossler et al. 1998; Yan et al. 2001). While the physiological consequences of the reduced cAMP are not clear, IP\(_3\) binds to Type III IP\(_3\) receptors (Clapp et al. 2001) and elicits a release of Ca\(^{2+}\) from intracellular stores (Akabas et al. 1988; Ogura et al. 1997). These studies were conducted with brief stimulus exposures in Ca\(^{2+}\)-free solutions to demonstrate the involvement of intracellular stores in the [Ca\(^{2+}\)] response.

In this study we used a prolonged application of the bitter stimulus denatonium in the presence of extracellular Ca\(^{2+}\) to determine if Ca\(^{2+}\) influx also contributes to bitter transduction.

We used isolated taste cells of mudpuppy, Necturus maculosus, as well as taste cells of transgenic mice expressing green fluorescent protein (GFP) under the control of the α-gustducin promoter (Wong et al. 1999). The rationale for using mudpuppy taste cells is that more than 80% of the taste cells respond to denatonium with an IP\(_3\)-mediated release of Ca\(^{2+}\) from intracellular stores (Ogura et al. 1997), while <5% of mammalian taste cells respond to denatonium (Caicedo and Roper 2001). We report here that prolonged exposure to denatonium results in Ca\(^{2+}\) influx that is likely mediated by store-operated channels.

METHODS

Taste cells from Necturus lingual epithelium (Ogura et al. 1997) and mouse circumvallate papillae (Gilbertson et al. 1993) were isolated as described previously and plated onto Cell Tak-coated coverslips for Ca\(^{2+}\) imaging. [Ca\(^{2+}\)] response to denatonium in the presence of extracellular Ca\(^{2+}\) to determine if Ca\(^{2+}\) influx also contributes to bitter transduction.

Stimuli that have been obtained for bitter stimuli (Cubero-Castillo and Noble 2001). We report here that prolonged exposure to denatonium results in Ca\(^{2+}\) influx that is likely mediated by store-operated channels.
RESULTS

Denatonium induces Ca\(^{2+}\) influx in taste cells

In mudpuppy taste cells, sustained application of 2.5 mM denatonium resulted in a transient Ca\(^{2+}\) response followed by a sustained phase that lasted more than several minutes (Fig. 1A). When external Ca\(^{2+}\) was removed, the sustained phase disappeared, suggesting that Ca\(^{2+}\) influx was involved. When extracellular Ca\(^{2+}\) was returned to the medium, the Ca\(^{2+}\) influx returned (Fig. 1A). Similar responses were obtained in all denatonium-responsive taste cells tested (n = 12). In a subset of denatonium-responsive taste cells (5 of 12), prolonged stimulation resulted in oscillations of [Ca\(^{2+}\)] (Fig. 1B). The intensity and frequency of oscillations were variable among cells, with some cells showing dramatic responses and other cells showing little if any oscillation. The oscillations were superimposed on the elevated baseline, with a frequency of 1–5/min. The oscillatory response also disappeared in Ca\(^{2+}\)-free solution (Fig. 1B), suggesting that oscillations require the presence of extracellular Ca\(^{2+}\).

To determine if Ca\(^{2+}\) influx is also involved in response to denatonium in mammals, we recorded Ca\(^{2+}\) responses from isolated taste cells of transgenic mice expressing GFP under the control of the α-gustducin promoter. Since denatonium activates α-gustducin, we recorded selectively from GFP-expressing taste cells of circumvallate papillae. The results were similar to those of mudpuppy. Denatonium (1 mM) induced a transient increase in [Ca\(^{2+}\)], followed by a sustained phase (Fig. 1C), although only a subset of gustducin-expressing cells responded to denatonium (7 of 30 tested). These data indicate that only a fraction of gustducin-expressing taste cells express functional taste receptors for denatonium. In Ca\(^{2+}\)-free solution, the sustained phase disappeared, suggesting that the sustained phase requires Ca\(^{2+}\) influx. The sustained phase reappeared when Ca\(^{2+}\) was returned to the bath in the presence of denatonium (Fig. 1C).

Ca\(^{2+}\) store-operated channels are present in taste cells

As shown in Fig. 1, A and C, the sustained phase was generated slowly compared with the initial transient increases in [Ca\(^{2+}\)], due to release from Ca\(^{2+}\) stores. These data suggest that the sustained phase may be induced by Ca\(^{2+}\) entry through Ca\(^{2+}\)-store-operated channels, which are activated by Ca\(^{2+}\) store depletion, rather than by IP\(_3\) or other second messengers (Parekh and Penner 1997). To test this, we treated denatonium-responsive mudpuppy taste cells with thapsigargin (1 μM) in the absence of extracellular Ca\(^{2+}\) to deplete Ca\(^{2+}\) stores. After a transient elevation of intracellular Ca\(^{2+}\) due to passive leak from intracellular stores, intracellular Ca\(^{2+}\) levels returned to baseline. When extracellular Ca\(^{2+}\) was returned, a significant increase in [Ca\(^{2+}\)] occurred, due to Ca\(^{2+}\) influx (Fig. 2A; n = 19 cells tested). Denatonium stimulation following treatment with thapsigargin did not cause a further rise in intracellular Ca\(^{2+}\) (Fig. 2A), suggesting that Ca\(^{2+}\) influx is not enhanced further by receptor-mediated increases in DAG and IP\(_3\). These data indicate that store-operated channels are present in mudpuppy taste cells; however, they do not unequivocally prove that they mediate the Ca\(^{2+}\) influx in response to bitter stimulation. In addition to βγ-activation of PLC, α-gustducin activates PDE to reduce intracellular cAMP, and Ca\(^{2+}\) influx through a cyclic nucleotide-suppressible cation conductance has been suggested to result from activation of α-gustducin (Kolesnikov and Margolskee 1995). If the decrease in cAMP is responsible for the Ca\(^{2+}\) influx, then cAMP should decrease the Ca\(^{2+}\) influx in response to denatonium. However, prolonged stimulation with 100 μM 3-isobutyl-1-methylxanthine (IBMX), a phosphodiesterase inhibitor, had no effect on the sustained Ca\(^{2+}\) response (n = 3 cells; data not shown). In addition, treatment with the PLC inhibitor U73122 blocked both the transient and the sustained increases in Ca\(^{2+}\) in response to denatonium (Ogura et al. 1997), showing that

FIG. 1. Dependency of the Ca\(^{2+}\) responses to denatonium on external Ca\(^{2+}\). Stimulation with denatonium induced a transient increase in [Ca\(^{2+}\)], followed by a sustained (a) or oscillatory (b) response in mudpuppy taste cells, and a sustained response in gustducin-expressing taste cells of mouse (c). Concentration of denatonium was 2.5 mM for (a) and (b), and 1 mM for (c). The sustained and oscillatory responses disappeared in Ca\(^{2+}\)-free saline, suggesting that Ca\(^{2+}\) influx is required. In the presence of denatonium, changing the solution from Ca\(^{2+}\)-free to normal saline induced increases in [Ca\(^{2+}\)].
suggesting that depletion of Ca\(^{2+}\) stores induces Ca\(^{2+}\) influx in the denatonium response. Our data present the first evidence that responses of mouse and mudpuppy taste cells to the bitter stimulus denatonium involve Ca\(^{2+}\) influx in addition to release of Ca\(^{2+}\) from intracellular stores. Also, we show that \textit{Necturus} taste cells often generate oscillatory Ca\(^{2+}\) responses to denatonium, which also require Ca\(^{2+}\) influx. Although the Ca\(^{2+}\) influx is most apparent during the sustained phase, even the transient response is decreased in many taste cells in Ca\(^{2+}\)-free (i.e., Fig. 1), sug-

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Recent evidence suggests that store-operated Ca\(^{2+}\) influx is unlikely in mudpuppy taste cells, since denatonium hyperpolarizes these cells (Ogura et al. 1997). However, we cannot rule out the participation of other ion channels, particularly in mouse, since detailed pharmacological manipulation could not be performed due to the scarcity of bitter responses. One of the functions of store-operated Ca\(^{2+}\) channels is refilling the Ca\(^{2+}\) stores (Parekh and Penner 1997). Therefore Ca\(^{2+}\) influx in response to denatonium is likely also to be involved in long-term Ca\(^{2+}\) homeostasis. Rapid refilling of stores may be important for repetitive responses to bitter stimuli.

Recently, a specific transient receptor potential (TRP) channel, TRP-T, was identified in mammalian taste cells (Perez et al. 2002). TRP-T is co-expressed in taste cells with \(\alpha\)-gustducin, \(\gamma_{13}\), PLC\(\beta_{2}\); and Type III IP\(_3\) receptor (Clapp et al. 2001; Perez et al. 2001). Is TRP-T the store-operated channel that mediates bitter compound denatonium-stimulated Ca\(^{2+}\) influx? Recent evidence suggests that store-operated Ca\(^{2+}\) influx may be mediated by TRP channels (Birnbaumer et al. 2000). Although the mechanism of their activation is not clear, activation of PLC or Ca\(^{2+}\) store depletion appears to be required for their activation. Our data are consistent with these requirements. Further experiments will be required to demonstrate the precise role of TRP-T in Ca\(^{2+}\) influx following bitter stimulation.

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REFERENCES


