Making sense of chemicals

Timothy A Gilbertson[1] and Sue C Kinnamon[2]

The sensation of taste is initiated when chemicals interact with peripheral receptors in the oral cavity, activating a cascade of cellular events that lead to changes in neurotransmitter release onto afferent nerve fibers. The mechanisms of taste transduction are diverse and involve a rich array of signaling components.

Addresses: [1]Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA 70808, USA and [2]Department of Anatomy and Neurobiology, Colorado State University, Ft Collins, CO 80523, USA, and the Rocky Mountain Taste and Smell Center, University of Colorado Health Sciences Center, Denver, CO 80262, USA.

Chemistry & Biology April 1996, 3:233–237

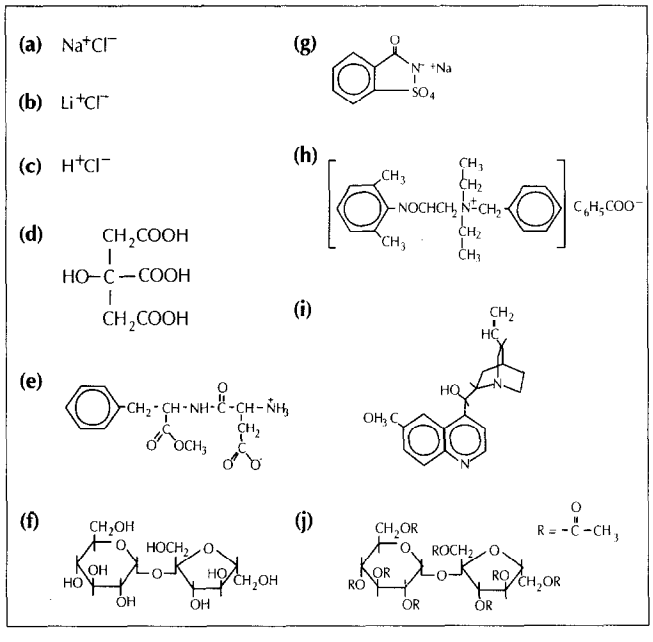
Peripheral taste receptors are situated at the outermost region of the enteric nervous system, and are critical in the selective acceptance or rejection of potential food sources. The ability of an organism to distinguish nutritional chemicals from those which are potentially harmful or toxic is crucial for survival. The mechanisms that have evolved to permit rapid identification of flavorful environmental chemicals by the receptor cells situated in the oral cavity range from the simple to the complex, and include examples from almost every known class of signaling pathway.

The cells responsible for flavor identification are arranged in pear-shaped groups of ~50–100 cells called taste buds. Taste-receptor cells are polarized, with their apical membrane (~3 % of the total membrane surface area) exposed in the taste pore. The remaining basolateral membrane, which is inaccessible to most sapid (or flavorful) chemicals, lies beneath the tight junctions that hold the taste receptor cells together.

Taste buds lie primarily in four areas of the oral cavity. The fungiform taste buds are found at the front of the tongue; these taste buds are found in groups of one or two on cup-shaped stalks of connective tissue called papillae. The foliate and vallate taste buds lie in the more posterior part of the tongue, along the sides and center, respectively. They are densely packed in deep crypts, with each crypt containing several hundred taste buds. The soft palate is the fourth chemosensitive area, although the palatine taste buds have not been extensively studied. Taste buds in the anterior oral cavity (fungiform, palatine) may be primarily involved in the initial identification and acceptance of appetitive stimuli, while those in the posterior region (vallate, foliate) are likely to be more important for the rejection of aversive chemicals. Most areas of the tongue respond to more than a single stimulus class, however, and the perceived signal is not a result of which bud type is triggered.

Although a vast array of chemicals elicit the sensation of taste, and a gourmet or wine taster will distinguish a wide variety of subjective tastes, the basic taste sensations identified by humans are classified into only four broad groups: salty, sour, sweet and bitter (Fig. 1). The typical response of a taste-receptor cell to a taste stimulus involves depolarization, leading to activation of voltage-dependent Na^+, K^+, and Ca^{2+} channels, a rise in intracellular free Ca^{2+}, and subsequent modulation of neurotransmitter release onto gustatory afferent nerve fibers (reviewed in [1,2]). The transduction mechanisms for sapid molecules have been the focus of considerable research. The consensus is that taste stimuli interact with receptor cells in one of three main ways (Fig. 2). Taste stimuli may interact directly with ion channels (Fig. 2a), bind to receptors linked either to ion channels or to G proteins involved in second messenger cascades (Fig. 2b,c), or may diffuse through the lipid phase of the membrane and bind to intracellular targets (Fig. 2d).

Figure 1



Examples of chemicals that humans perceive as salty, sour, sweet or bitter. **(a),(b)** Salty stimuli: sodium chloride and lithium chloride. **(c),(d)** Sour (acidic) stimuli: hydrochloric acid and citric acid. **(e)–(g)** Sweet stimuli: aspartame, sucrose and sodium saccharin, respectively. **(h)–(j)** Bitter stimuli: denatonium benzoate, quinine and sucrose octaacetate, respectively.

Ion channel interactions

There are two possible ways for taste stimuli to interact directly with ion channels to produce receptor-cell depolarization. They may themselves be charged, and may pass directly through the ion channel, or they may block the current through open, conducting ion channels, preventing the cell from maintaining a polarized state (Fig. 2a). Several types of ionic taste stimuli appear to be transduced through one or both of these mechanisms. For example, the predominant mechanism by which we taste sodium salts appears to be by the direct permeation of Na^+ ions through amiloride-sensitive sodium channels. This transfer of charge from the outside to the inside of the cell leads to the development of a depolarizing receptor potential. These channels (in mammals) are permeable to Na^+, Li^+ and H^+ ions, but not K^+ ions. These permeabilities may therefore account for the saltiness of NaCl and LiCl, and for some of the salty/sour confusion elicited by some acids (H^+). It does not explain the salty component of KCl, which humans describe partially as salty but primarily as bitter.

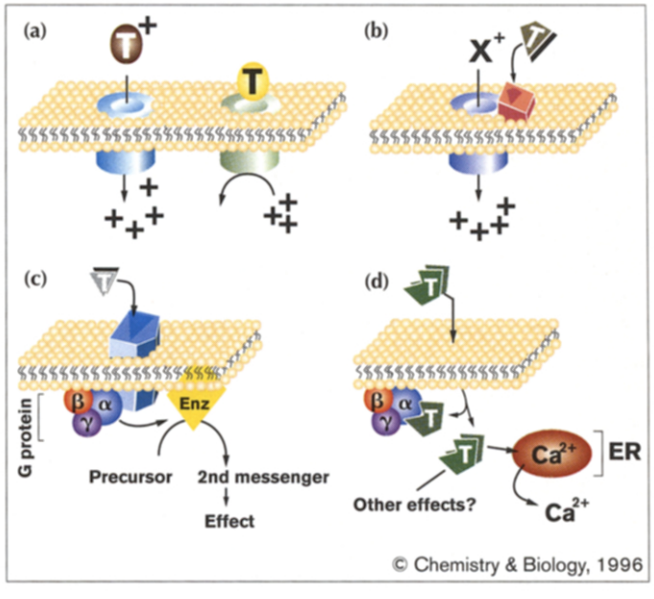
The involvement of amiloride-sensitive sodium channels in sodium salt transduction in numerous species has been confirmed in experiments recording from isolated taste receptor cells [3,4], intact taste buds [5,6], lingual epithelium [7], afferent nerve fibers [8,9] and brain gustatory centers [10,11]. But although these channels are undoubtedly important for signal transduction in response to NaCl, there is some doubt, as a result of recent human psychophysical studies, about whether signaling via amiloride-sensitive sodium channels is responsible for the perceived saltiness of NaCl or for some other taste quality (i.e. sourness) [12,13]. The response to sodium salts is not always transduced via amiloride-sensitive sodium channels, however. In mudpuppy [14] and certain mouse strains [15], sodium salts affect taste receptor cells through an undefined, amiloride-insensitive mechanism. These amiloride-insensitive mechanisms are also present in species with amiloride-sensitive sodium channels, are electrogenic, and probably contribute to sodium salt transduction [4,16].

The amiloride-sensitive sodium channels may also be involved in the response to protons, contributing to the perception of acids as sour-tasting. Although there is no direct evidence, this supports the idea that the permeation of salt through these channels is also perceived as sour. The proton permeability of amiloride-sensitive sodium channels has been well documented [17] and both behavioral and electrophysiological studies in hamster suggest that this mechanism contributes to acid taste [6,18–20]. Interestingly, in rats, proton permeability of amiloride-sensitive sodium channels appears less important for acid-taste transduction than other mechanisms [21].

Permeation of tastants is not limited to the ion channels that are found on the apical membrane of taste receptor cells. Small ionic taste stimuli (Na^+, H^+) also permeate the tight junctions between taste-receptor cells (the paracellular pathway) and are able to affect ion channels on the basolateral membrane [22,23]. The extent of this paracellular movement of Na^+ depends upon the identity of the anion, perhaps accounting for the distinct taste of various sodium salts [22].

Not all of the chemicals we can taste are small and positively charged, however, and not all of them can directly depolarize a receptor cell. Many chemical stimuli are instead detected via a block in the conductance of apical ion channels (Fig. 2a), such as the open, apically-localized K^+ channels, which also leads to receptor-cell depolarization. This mechanism has been clearly shown to operate in amphibia, where both acidic (i.e., protons) and bitter taste stimuli block apical K^+ channels without the involvement of second messenger systems. Using a variety of electrophysiological approaches at the single-channel and whole-cell level (reviewed in [1]), acids and the bitter compounds quinine and $CaCl_2$ have been shown to inhibit K^+ efflux at normal taste-receptor-cell resting potentials. Behavioral studies in amphibia indicate

Figure 2



General mechanisms of transduction in taste receptor cells. **(a)** Taste stimulus (T)–ion channel interactions produce receptor-cell depolarization either by direct permeation (left) or by blockage (right) of an open ion channel. The diagrammed blockage is only illustrative; protons probably do not block the channel directly but by binding to the selectivity filter of the channel. Alternatively, stimulus–receptor interactions can lead to depolarization by **(b)** activation of receptor-activated ion channels or **(c)** activation of receptors linked to G proteins and subsequent changes in intracellular second messenger concentrations. **(d)** Lipophilic taste stimuli may diffuse across the plasma membrane and affect intracellular targets such as G proteins or the endoplasmic reticulum (ER). See text for details and specific examples.

that any compound that inhibits apical K^+ channels in taste receptor cells may possess similar taste qualities [24]. It is not yet clear whether apical K^+ channels are involved in acidic or bitter taste in higher vertebrates.

Specific receptors

These general methods for detection of taste stimuli are thought to be supplemented by the use of specific membrane receptors for complex stimuli including sugars, amino acids, and many bitter-tasting compounds. Not a single taste receptor has yet been cloned or purified biochemically, however. In contrast, in the olfactory system a large multigene family has been discovered and shown to encode odorant receptors [25]. Specific taste receptors probably do exist but their identification may prove difficult given the low abundance of taste tissue and the low affinity of most taste stimuli for their receptors.

The best evidence for the existence of specific taste receptors comes from biochemical studies of catfish taste buds, found in high density on the barbels and over much of the body surface. Catfish taste buds are highly sensitive to amino acids, which bind to membrane preparations of taste buds with high affinity and specificity [26]. Sugars also bind to membrane preparations of taste buds, and the binding affinity of different compounds reflects their sweetness potency. Most sweet compounds share a common structural motif, a hydrogen bond donor (AH) located a short distance from a hydrogen bond acceptor (B); for example, the terminal carboxyl and amino groups of aspartame (Fig. 1e). This AH–B site is a predictor of sweetness, and has been used in the design of synthetic 'super-sweeteners' [27]. Sweet compounds with this structural motif probably all bind to receptors (cross-adaptation studies show that there are multiple receptors) with complementary hydrogen-bonding patterns. Bitter compounds, particularly hydrophilic molecules, may also recognize specific receptors, but the evidence for the existence of these receptors is more indirect. Congenic strains of mice have been developed that differ in their ability to taste the bitter acetylated sugar sucrose octaacetate (Fig. 1j; [28]). Similarly, humans differ in their ability to taste another bitter chemical, phenylthiocarbamide. Such genetic differences are most probably caused by differences in the expression of specific bitter receptors, but it is also possible that they result from changes in other steps in the signal transduction pathway.

Downstream signaling

The signaling systems used by receptors for taste chemicals are diverse. Some receptors are probably coupled directly to ion channels on the apical membrane of taste cells (Fig. 2b); when the taste stimulus binds to the receptor, it causes a conformational change in the receptor and subsequent opening of the ion channel. Influx of cations would lead to taste-cell depolarization, in a manner analogous to the action of acetylcholine on the neuromuscular junction. In some cases, the ion channel also acts as the receptor for the stimulus. Such a mechanism is thought to mediate detection of the amino acids L-arginine and L-proline in catfish taste cells [29]. Both L-arginine- and L-proline-gated cation channels have been partially purified and reconstituted in phospholipid bilayers, where they are directly activated by the amino acids.

Most taste receptors are thought to be coupled to G proteins and second messenger systems (Fig. 2c). Although the details of these mechanisms are not yet understood, the components of several pathways have been identified. Several G proteins are expressed in taste buds, including members of the G_q family (which is involved in the breakdown of phosphatidyl inositol bisphosphate, PIP_2), the G_i and G_s families (which inhibit and enhance the production of cyclic AMP, respectively), and the taste-cell-specific G protein gustducin [30,31]. Gustducin has considerable sequence homology to transducin, which activates phosphodiesterase in the phototransduction cascade. Recently, transducin was also shown to be expressed in taste buds. Although the specific roles of these G proteins in transduction have not been determined, clues are emerging from biochemical and molecular genetic studies of taste transduction.

There is now a great deal of evidence from several animal models that sucrose depolarizes taste cells via a cyclic AMP (cAMP)-dependent closure of K^+ channels. Biochemical studies have shown that sucrose increases cAMP levels; the increase requires GTP and is blocked by a specific sweet taste inhibitor [32]. The evidence that K^+ channels are involved comes from electrophysiological studies, which show that sucrose and cyclic nucleotides both inhibit K^+ channels in taste cells [33,34]. Recent studies suggest that synthetic sweeteners may use a different mechanism for signal transduction, however. Synthetic sweeteners stimulate the breakdown of PIP_2 to produce inositol triphosphate (IP_3) and diacylglycerol, but do not raise cAMP levels [35]; they thus appear to activate different G proteins from those activated by sucrose. But recent electrophysiological studies show that the synthetic sweeteners inhibit the same K^+ channels as does cAMP, suggesting that the two second messenger systems converge on the same target K^+ channels to mediate the transduction of the signal for the sweet taste [36].

There appear to be several mechanisms for the transduction of the signal for bitter taste, which is not surprising given the diversity of bitter compounds. One mechanism that has been studied extensively involves the potently bitter synthetic compound, denatonium (Fig. 1h). Studies using the Ca^{2+}-sensitive dye fura-2 have shown that denatonium elicits a release of Ca^{2+} from internal stores [37]. Biochemical studies support these findings, showing that

denatonium stimulates the production of IP_3 in membrane preparations of taste cells [38]. The IP_3 presumably binds to receptors that trigger the release of intracellular Ca^{2+}. These studies suggest that the denatonium receptor is coupled to a G_q-like protein that stimulates the production of both IP_3 and diacylglycerol. Other studies, however, suggest that the G protein coupled to the denatonium receptor is transducin-like. Denatonium was shown to activate transducin as well as gustducin in a membrane preparation of taste buds [39], and transgenic mice lacking the gustducin protein are less sensitive to bitter stimuli than control mice (R.F. Margolskee, personal communication). Curiously, these knockout mice are also less sensitive to sweet stimuli. Clearly, further studies will be necessary to evaluate the role of specific G proteins in taste transduction.

Intracellular targets

Several compounds that act as bitter or sweet taste stimuli have amphiphilic structures (Fig. 1) that allow them to penetrate the taste cell membrane. Amphiphilic peptides, such as bradykinin, have been shown to activate G proteins directly, bypassing the usual receptor-binding step [40]. Such molecules could, theoretically, activate G proteins in taste-cell membranes directly (Fig. 2d; [28]). A recent study by Naim *et al.* [41] lends support to this hypothesis. They found that some non-sugar sweeteners, as well as the bitter compound quinine, were able to activate a mixture of G_i/G_o proteins, as well as transducin, in an *in vitro* assay. Although these studies do not preclude the existence of a specific receptor for these compounds, they suggest that receptors may not always be required for the detection of the presence of amphiphilic compounds in taste cells.

The mechanisms used by taste cells to detect flavorful chemicals are many and varied, and the study of taste recognition provides perhaps the richest source of information available about how small molecules can be specifically recognized by living systems. A deeper chemical understanding of the mechanisms of taste transduction may give insight into a number of other signaling processes, such as those found in more complex and less accessible regions of the nervous system.

References

1. Kinnamon, S.C. & Cummings, T.A. (1992). Chemosensory transduction mechanisms in taste. *Annu. Rev. Physiol.* **54**, 715–731.
2. Gilbertson, T.A. (1993). The physiology of vertebrate taste reception. *Curr. Opin. Neurobiol.* **3**, 532–539.
3. Avenet, P. & Lindemann, B. (1988). Amiloride-blockable sodium currents in isolated taste receptor cells. *J. Membr. Biol.***105**, 245–255.
4. Doolin, R.E. & Gilbertson, T.A. (1996). Distribution and characterization of functional amiloride-sensitive sodium channels in rat tongue. *J. Gen. Physiol.*, in press.
5. Avenet, P. & Lindemann, B. (1991). Noninvasive recording of receptor cell action potentials and sustained currents from single taste buds maintained in the tongue: the response to mucosal NaCl and amiloride. *J. Membr. Biol.* **124**, 33–41.
6. Gilbertson, T.A., Avenet, P., Kinnamon, S.C. & Roper, S.D. (1992). Proton currents through amiloride-sensitive Na^+ channels in hamster taste cells: role in acid transduction. *J. Gen. Physiol.* **100**, 803–824.
7. Heck, G.L., Mierson, S. & DeSimone, J.A. (1984). Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway. *Science* **223**, 403–405.
8. Brand, J.G., Teeter, J.H. & Silver, W.H. (1985). Inhibition by amiloride of chorda tympani responses evoked by monovalent salts. *Brain Res.* **334**, 207–214.
9. Heck, G.L., Persuad, K.C. & DeSimone, J.A. (1989). Direct measurement of translingual epithelial NaCl and KCl current during the chorda tympani taste response. *Biophys. J.* **55**, 843–857.
10. Scott, T.R. & Giza, B.K. (1990). Coding channels in the taste system of the rat. *Science* **249**, 1585–1587.
11. Giza, B.K. & Scott, T.R. (1991). The effect of amiloride on taste-evoked activity in the nucleus tractus solitarius of the rat. *Brain Res.* **550**, 247–256.
12. Ossebaard, C.A. & Smith, D.V. (1995). Effect of amiloride on the taste of NaCl, Na-gluconate and KCl in humans: implications for Na^+ receptor mechanisms. *Chem. Senses* **20**, 37–46.
13. Smith, D.V. & Ossebaard, C.A. (1995). Amiloride suppression of the taste intensity of sodium chloride: evidence from direct magnitude scaling. *Physiol. Behav.* **57**, 773–777.
14. McPheeters, M. & Roper, S.D. (1985). Amiloride does not block taste transduction in the mudpuppy, *Necturus maculosus*. *Chem. Senses* **10**, 341–352.
15. Tonosaki, K. & Funakoshi, M. (1989). Amiloride does not block taste transduction in the mouse (Slc:ICR). *Comp. Biochem. Physiol. [A]* **94**, 659–661.
16. Formaker, B.K. & Hill, D.L. (1991). Lack of amiloride sensitivity in SHR and WKY glossopharyngeal taste responses to NaCl. *Physiol. Behav.* **50**, 765–769.
17. Garty, H. & Benos, D.J. (1988). Characteristics and regulatory mechanisms of the amiloride-blockable Na^+ channel. *Physiol. Rev.* **68**, 309–373.
18. Hettinger, T.P. & Frank, M.E. (1990). Specificity of amiloride inhibition of hamster taste responses. *Brain Res.* **513**, 24–34.
19. Gilbertson, T.A., Roper, S.D. & Kinnamon, S.C. (1993). Proton currents through amiloride-sensitive Na^+ channels in isolated hamster taste cells: enhancement by vasopressin and cAMP. *Neuron* **10**, 931–942.
20. Gilbertson, D.M. & Gilbertson, T.A. (1994). Amiloride reduces the aversiveness of acids in preference tests. *Physiol. Behav.* **56**, 649–654.
21. Harris, D.E., Gilbertson, D.M., Monroe, W.T., Kinnamon, S.C. & Gilbertson, T.A. (1994). Contribution of amiloride-sensitive pathways to acid transduction in rats. *Chem. Senses* **19**, 431–432.
22. Ye, Q., Heck, G.L. & DeSimone, J.A. (1991). The anion paradox in sodium taste reception: resolution by voltage-clamp studies. *Science* **254**, 724–726.
23. Simon, S.A., Holland, V.F., Benos, D.J. & Zamphigi, G.A. (1993). Transcellular and paracellular pathways in lingual epithelia and their influence in taste transduction. *Microsc. Res. Tech.* **26**, 196–208.
24. Bowerman, A.G. & Kinnamon, S.C. (1994). The significance of apical K^+ channels in mudpuppy feeding behavior. *Chem. Senses* **19**, 303–315.
25. Buck, L. & Axel, R. (1991). A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* **65**, 175–187.
26. Caprio, J., *et al.*, Wegert, S. (1993). The taste system of the channel catfish: from biophysics to behavior. *Trends Neurosci.* **16**, 192–197.
27. Walters, D.E. (1993). The rational discovery of sweeteners. In *Sweeteners: Discovery, Molecular Design and Chemoreception.* ACS Symposium Series **450**, 1–11.
28. Spielman, A.I., Huque, T., Whitney, G. & Brand, J.G. (1992). The diversity of bitter taste signal transduction mechanisms. In *Sensory Transduction.* (Corey, D.P. & Roper, S.D., eds), pp. 307–324, Rockefeller University Press, New York.
29. Teeter, J.H., Kumazawa, T., Brand, J.G., Kalinoski, D.L., Honda, E. & Smutzer, G. (1992). Amino acid receptor channels in taste cells. In *Sensory Transduction.* (Corey, D.P. & Roper, S.D., eds) pp. 291–306, Rockefeller University Press, New York.
30. McLaughlin, S.K., McKinnon, P.J. & Margolskee, R.F. (1992). Gustducin is a taste cell-specific G protein closely related to the transducins. *Nature* **357**, 563–569.
31. McLaughlin, S.K., McKinnon, P.J., Robichon, A., Spickofsky, N. & Margolskee, R.F. (1993). Gustducin and transducin: a tale of two G proteins. *Ciba Found. Symp.* **179**, 186–196.
32. Striem, B.J., Pace, U., Zehavi, U., Naim, M. & Lancet, D. (1989). Sweet

tastants stimulate adenylate cyclase coupled to GTP-binding protein in rat tongue membranes. *Biochem. J.* **260**, 121–126.
33. Tonosaki, K. & Funakoshi, M. (1988). Cyclic nucleotides may mediate taste transduction. *Nature* **331**, 354–356.
34. Avenet, P., Hofmann, F. & Lindemann, B. (1988). Transduction in taste receptor cells requires cAMP-dependent protein kinase. *Nature* **331**, 351–354.
35. Bernhardt, S.J., Naim, M., Zehavi, U. & Lindemann, B. (1996). Changes in IP_3 and cytosolic Ca^{2+} in response to sugars and non-sugar sweeteners in transduction of sweet taste in the rat. *J. Physiol.*, in press.
36. Cummings, T.A., Daniels, C. & Kinnamon, S.C. (1996). Sweet taste transduction in hamster: sweeteners and cyclic nucleotides depolarize taste cells by reducing a K^+ current. *J. Neurophysiol.*, in press.
37. Akabas, M.H., Dodd, J. & Al-Awqati, Q. (1988). A bitter substance induces a rise in intracellular calcium in a subpopulation of rat taste cells. *Science* **242**, 1047–1050.
38. Spielman, A.I., Huque, T., Nagai, H., Whitney, G. & Brand, J.G. (1994). Generation of inositol phosphates in bitter taste transduction. *Physiol. Behav.* **56**, 1149–1155.
39. Ruiz-Avila, L., *et al.*, & Margolskee, R.F. (1995). Coupling of bitter receptor to phosphodiesterase through transducin in taste receptor cells. *Nature* **376**, 80–85.
40. Mousli, M., Bueb, J.L., Bronner, C., Rouot, B. & Landry, Y. (1990). G protein activation: a receptor-independent mode of action for cationic amphiphilic neuropeptides and venom peptides. *Trends Pharmacol. Sci.* **11**, 358–362.
41. Naim, M., Seifert, R., Nurnberg, B., Grunbaum, L. & Schultz, G. (1994). Some taste substances are direct activators of G-proteins. *Biochem. J.* **297**, 451–454.