

# Receptors and transduction in taste

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Taste is the sensory system devoted primarily to a quality check of food to be ingested. Although aided by smell and visual inspection, the final recognition and selection relies on chemoreceptive events in the mouth. Emotional states of acute pleasure or displeasure guide the selection and contribute much to our quality of life. Membrane proteins that serve as receptors for the transduction of taste have for a long time remained elusive. But screening the mass of genome sequence data that have recently become available has provided a new means to identify key receptors for bitter and sweet taste. Molecular biology has also identified receptors for salty, sour and umami taste.

**T**here is no life form known between bacteria and mammals that would neglect to check its intake of matter by chemoreceptive scrutiny. A human baby, only a few days old, already can distinguish sweet and bitter and express pleasure for sweet taste but displeasure for bitter taste<sup>1</sup>. Inorganic ions, sugars and polysaccharides, amino acids and peptides, toxins and 'xenobiotics' are all subject to nutritional chemoreception followed by adaptive behaviour. But details differ widely, depending on the complexity of the organism and the ecological niche that it occupies. Even in closely related species, distinct differences in sensory performance may be noted, which seem to match the nutritional 'needs' of a species. To understand how such a match arose, that is, how receptor specificity changed with the availability of food ingredients, is perhaps the most fascinating of the future tasks of taste research.

## Taste buds and taste cells

Already in worms, like the model nematode *Caenorhabditis elegans*, a distinction can be made between olfaction and taste<sup>2</sup>. These two chemoreceptive senses are more clearly separate in arthropods and they are quite distinct in vertebrates. In the fruitfly *Drosophila melanogaster*, for example, taste sensations are mediated by nerve cells of characteristic topology. Their sensory dendrites are contained in 'hairs' found on the body surface. Other taste neurons, found on the labellum and clustered around the pharynx, express a family of G-protein-coupled receptors (GPCRs) named GR<sup>3</sup>. This family, however, contains candidate receptors for both taste and olfaction, as its genes are expressed in both gustatory and olfactory primary neurons<sup>4</sup>. In contrast, the taste receptor cells of vertebrates are not neurons, but originate from the epithelial covering of the body<sup>5</sup>.

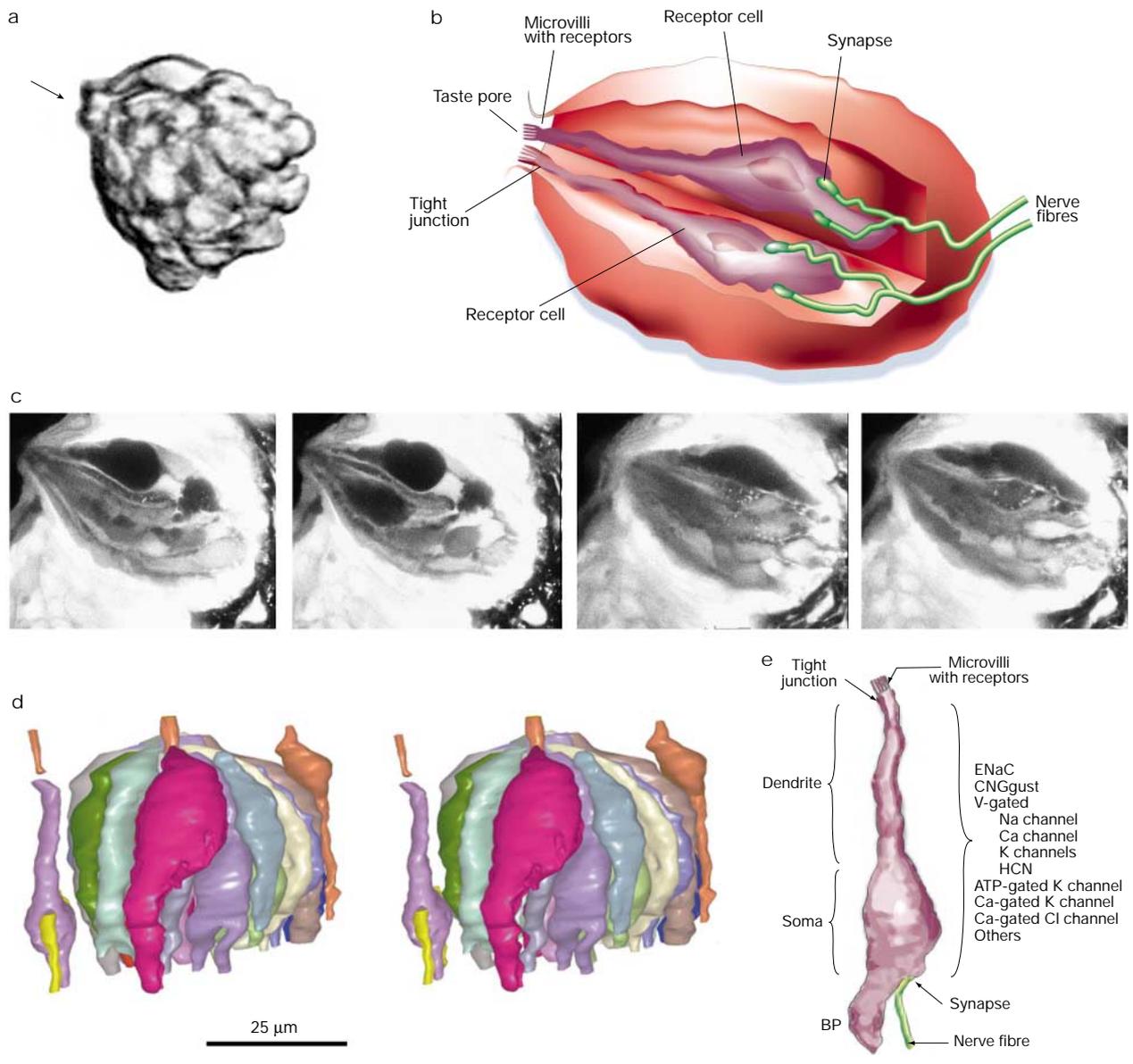
Vertebrate taste cells are small bipolar cells (Fig. 1). To connect to the oral space, they send a thin dendritic process to the epithelial surface. The cells occur either singly or densely packed in taste buds, where up to 100 form a functional unit (Fig. 1a, d). Although taste buds also occur abundantly on the body surface and barbels of some fish, all vertebrates have taste buds in the oral epithelium, typically on tongue, palate and pharynx. On the tongue, the taste buds are mounted in special folds and protrusions called papillae. The marker molecule gustducin, a taste-specific G protein<sup>6</sup>, shows additional 'taste cells' in the nasal mucosa<sup>7</sup> and in the stomach<sup>8</sup>.

A century ago it was determined that each chemoreceptive area of the human tongue responds to each of the qualities of sweet, sour, salty and bitter taste. Only minor differences in subjective thresholds were noted across areas<sup>9,10</sup>. May the time come soon when textbook authors wake up to this fact! In rodents, the differences in thresholds or sensitivities seem to be somewhat larger<sup>11</sup>. Here, morphological and functional differentiation between buds from the anterior and posterior tongue can be noted more easily, even though individual taste buds of all areas contain cells responding to several qualities.

## Lifespan, connectivity and coding

Mammalian taste-responsive cells age fast, their lifespan being about 10 days. This implies that any one nerve terminal in a taste bud frequently has to detach from an ageing cell, find a developing taste cell and form new synapses on its surface. The new cell has presumably to be of appropriate specificity such that a nerve fibre previously attached to a Na<sup>+</sup>-responsive cell, for instance, will again attach to a cell of this kind. As taste cells of different specificity occur together in one bud, one expects surface markers to guide a nerve fibre to the right cellular target. In fact, the receptor molecules themselves may possibly serve as markers, as they occur not only on the microvilli, but also on basolateral membrane areas of the receptor cells.

The basic rules for the developmental interactions that occur between taste cells and nerve fibres begin to be deduced using the methods of molecular biology<sup>12,13</sup>, and provide a foundation for a further analysis of the connectivity and the 'sensory code' by more function-oriented approaches such as electrophysiology and fluorescence imaging. Meanwhile, recordings from the sensory nerve fibres and from the soma of their neurons have consistently revealed the unexpected and striking feature that some nerve fibres are specialists, but many are generalists, carrying responses to more than one taste quality<sup>14</sup>. A simple 'labelled line' design, where each fibre responds to just one of the qualities, to bitter only or to sour only, is not evident, as many fibres are broadly tuned with respect to taste ligands. These generalist fibres carry responses to salty and sour, to glutamate and sucrose, and so on. Similarly, many taste receptor cells, too, are generalists, as responses to taste qualities are randomly and independently distributed, varying in intensity across cells<sup>15</sup>. Given such distributed responses, a part of the information about individual tastants must be buried in the quorum of the receptor cells and the 'across-fibre pattern' of the sensory nerve<sup>16</sup>. Retrieval



**Figure 1** Morphology of taste buds (rat). **a**, Viable bud isolated from the vallate papilla. Taste pore at the upper left (arrow). Length of bud is 30  $\mu\text{m}$ . **b**, Cut-open view of a bud (cartoon). Highlighted are two receptor cells with apical microvilli and basolateral synapses. **c**, Images of a viable bud from the vallate papilla, taken with a 2-photon microscope. The four optical planes depict multiple bipolar cells in different states of loading with a fluorescent dye, and nerve fibres. **d**, Three-dimensional reconstruction, from microscopic serial sections, of a bud from the foliate papilla, the taste pore facing upwards. On the left, a solitary bipolar cell with innervating nerve fibre is also visible. Scale bar, 25  $\mu\text{m}$ . (Image courtesy of V. I. Popov, Institute of Cell Biophysics, RAS, Pushchino, Russia.) **e**, Bipolar receptor cell with sensory nerve fibre attached. Some morphological details and the location of the main types of identified ion channels on the lateral membrane are indicated. BP, basal cell process.

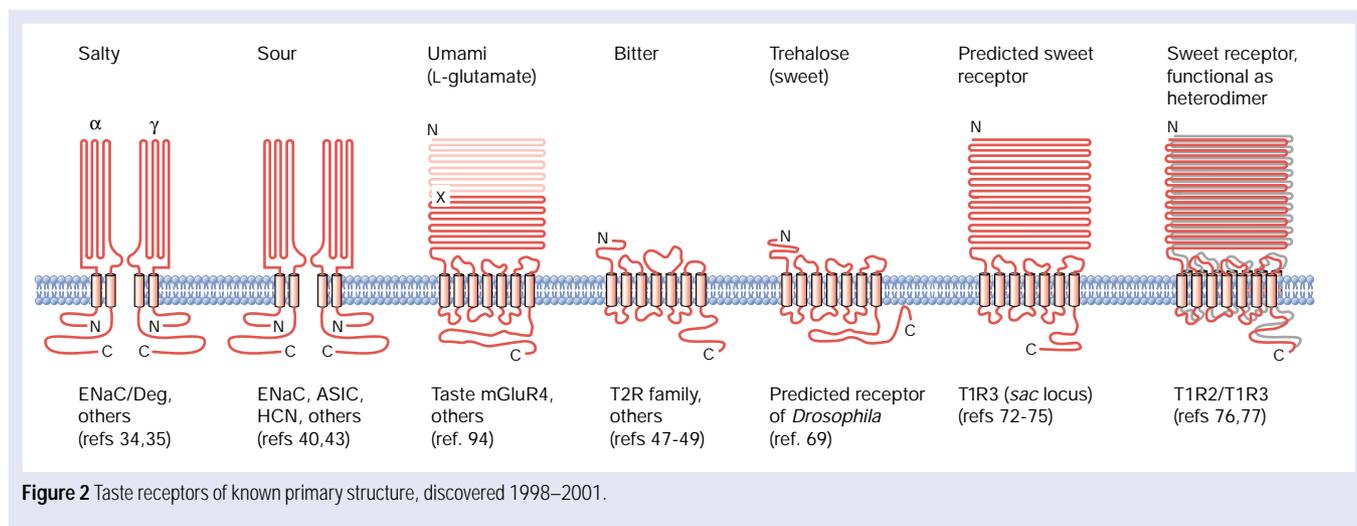
of this information, for instance by pattern discrimination, will be one of the main tasks of central taste processing.

**Receptors and transduction**

The bipolar taste cells have two specializations of obvious functional significance: microvilli in contact with the oral cavity and synapses with sensory nerve fibres. Taste receptor proteins are mounted on the microvilli, acting as molecular antennas listening into the chemical environment. On binding taste molecules, the receptors trigger transduction cascades that activate synapses and thus cause excitation of the nerve fibres. These carry the signal to the brain stem, where central taste processing begins, ultimately eliciting adaptive responses.

The first molecular encounter with tastants is made therefore by those membrane proteins — the ‘receptors’ — in the apical surface of

taste receptor cells (Fig. 1b, e). They provide the molecular specificity of the taste response. A plethora of proteins, including ion channels, ligand-gated channels, enzymes and GPCRs, serve as receptors for sensory qualities such as salty, sour, sweet, umami and bitter taste (Fig. 2), and trigger the downstream transduction events within taste cells. Included among these events is the firing of action potentials, which taste cells, like neurons, are able to generate by means of voltage-gated  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$  channels (Fig. 1e)<sup>17–19</sup>. A local increase in  $\text{Ca}^{2+}$  concentration is needed for synaptic activation (and hence nerve excitation), and transient rises in the cytosolic  $\text{Ca}^{2+}$  concentration were observed by fluorescence imaging (Fig. 1c) in taste cells responding to bitter and sweet agents<sup>20–22</sup>, while amino acids triggered either increases or decreases of the  $\text{Ca}^{2+}$  signal<sup>23,24</sup>.



**Figure 2** Taste receptors of known primary structure, discovered 1998–2001.

A number of transmitters have been found within taste buds, but those released by taste cell synapses have been difficult to identify. Noradrenaline and acetylcholine seem to be secreted by nerve fibres and modulate the responses of taste cells<sup>25,26</sup>. Serotonin is thought to act as a paracrine agent between taste cells. Secreted by one cell and modulating the taste response of a neighbouring cell, this agent mediates local signal processing within a taste bud<sup>27,28</sup>. Glutamate is a strong candidate for a mainstream afferent transmitter secreted by taste cell synapses<sup>29,30</sup>.

### Salt taste

Two taste qualities detect ions in the oral space: salt and sour taste. Salt taste guides the incorporation of NaCl and other required minerals, thus serving an essential function in ion and water homeostasis. It shows variations across animal species, depending on the ion content of the characteristic diet<sup>19</sup>.

Although salt taste is elicited by many ionic species, the component due to the presence of Na<sup>+</sup> ions may be the most relevant for mammals and is certainly the best studied<sup>31</sup>. The Na<sup>+</sup> taste, in turn, is composed of a Na<sup>+</sup>-specific and a nonspecific mechanism. It was long suspected that a sodium channel sensitive to the channel-blocker amiloride serves as a salt receptor<sup>32</sup>. This conjecture holds especially for rodents, where the Na<sup>+</sup>-specific salt taste is indeed mediated by a highly Na<sup>+</sup>-selective channel known as ENaC, the amiloride-sensitive epithelial sodium channel<sup>33</sup>. ENaC is a hetero-oligomeric complex comprising three homologous subunits (Fig. 2), which acts as a salt-taste receptor by providing a specific pathway for sodium current into taste cells, provided that Na<sup>+</sup> ions are present in the oral space in sufficient concentration<sup>34,35</sup>. The current triggers action potentials at the basolateral membrane of the taste cell<sup>36</sup>, followed by synaptic events.

Of the three essential subunits of ENaC, at least one is under inductive control by a steroid hormone, aldosterone<sup>35</sup>. Thus the sensitivity of sodium taste is increased in animals in sodium-need through induction of more ENaC channels. A systemic Na<sup>+</sup> deficiency, which leads to salt craving, occurs regularly in herbivores, but can be observed also in rodents and humans. The induction of ENaC subunits in vallate taste buds by circulating aldosterone provides an instructive example for adaptive tuning of taste acuity in a state of nutritional deficiency.

In humans, the amiloride sensitivity of salt taste is less pronounced<sup>37</sup>, suggesting the involvement of another, as yet unspecified channel. It is ironic that so little is known especially about the molecular foundation of human salt taste.

### Sour taste

Sour taste is acceptable or interesting when mild, thereby aiding the recognition of complex food, but it becomes increasingly unpleasant

when strong<sup>1</sup>. It serves to detect unripe fruits and spoiled food, and to avoid tissue damage by acids and problems of systemic acid–base regulation.

The receptors proposed for sour taste in mammals can be ordered in two groups. The first comprises channels that conduct an inward proton current if protons are available in the oral space. ENaC has this property<sup>38</sup>. The second group comprises H<sup>+</sup>-gated channels, including the apical K<sup>+</sup> channel of the mudpuppy *Necturus*<sup>39</sup>, MDEG1 of the ENaC/Deg family<sup>40</sup>, an unspecific H<sup>+</sup>-gated cation channel<sup>41,42</sup>, and HCN, the hyperpolarization-activated, cyclic nucleotide-gated cation channel<sup>43</sup>. The large variety of mechanisms found for sour taste highlights the complexity of taste transduction.

To some extent, the intracellular pH of taste cells follows extracellular changes in pH, especially when the extracellular changes extend over the basolateral membrane. This probably occurs because the tight junction, which closes the extracellular space of a taste bud towards the oral space (Fig. 1b), is permeable to H<sup>+</sup> ions<sup>44</sup>. H<sup>+</sup> ions may therefore invade the taste bud and initiate intracellular 'pH tracking', which is thought to contribute to sour transduction<sup>45</sup>.

### Bitter taste

Bitter taste is unpleasant though bearable when weak, but repulsive when strong. Many organic molecules, originating from plants and interfering with the internal signalling system of animals and humans, are bitter, including caffeine, nicotine and strychnine, and the same is true for many drugs produced by industry<sup>46</sup>. Bitter taste effectively warns us not to ingest potentially harmful compounds. One of the exciting challenges in taste research is to understand how bitter receptors were shaped by evolution to serve this task.

By scanning genomic databases near a promising bitter locus on mouse chromosome 6, a group of new GPCRs was discovered, the T2R family<sup>47,48</sup>. The receptors had short amino-terminal domains (NTDs) (Fig. 2) and were expressed specifically in a subset of taste cells. The large size of this family, with 40–80 members originally specified<sup>47</sup>, came as a surprise. Three of the receptors could be expressed in a cell line, where they responded to bitter tastants<sup>49</sup>. In humans, the T2R family comprises at least 24 genes coding for GPCRs, distributed over three chromosomes (B. Bufo and W. Meyerhof, personal communication).

It is likely but not yet certain that all 24 GPCRs of the T2R family respond to bitter agents. How are these GPCRs distributed across taste cells? Adler *et al.* concluded from *in situ* hybridization data<sup>47</sup> that "a single taste receptor cell expresses a large repertoire of T2Rs, suggesting that each cell may be capable of recognizing multiple tastants". Caicedo and Roper have probed this question differently, by using functional imaging experiments. They found that most bitter-responsive taste cells, which presumably expressed T2Rs, were

activated by only one out of five compounds tested. Thus, the tuning of receptor cells with respect to bitter compounds seems to be more focused than anticipated, and different bitter stimuli may activate, through the GPCRs expressed, different subpopulations of bitter-sensitive taste cells<sup>22</sup>. In agreement with this finding, single taste nerve fibres carry signals that discriminate between bitter compounds<sup>50</sup>.

It is not only GPCRs that act as bitter receptors. Some bitter peptides with amphiphilic properties interact directly with G proteins, probably by virtue of a structural similarity to the G-protein-binding site of the receptor<sup>46</sup>. Quinine, also an amphiphilic compound, permeates the cell membrane and activates G proteins, bypassing the receptor<sup>51</sup>. In *Necturus*, quinine blocks apical K<sup>+</sup> channels<sup>52</sup>, whereas in the bullfrog, it activates a cation conductance in taste cells<sup>53</sup>. Denatonium blocks voltage-gated delayed-rectifier K<sup>+</sup> channels<sup>54</sup>.

Caffeine and other methyl-xanthines also act without activating a GPCR (Fig. 3). After permeating the cell membrane they block an intracellular phosphodiesterase and cause activation of a soluble guanylate cyclase<sup>55</sup>. The latter effect may be under control of nitric oxide, as nitric oxide synthase was found in taste cells<sup>56</sup>. As the result of these complex events, a transient increase in guanine 3',5'-cyclic monophosphate (cGMP) was measured with stopped-flow methods<sup>55</sup>.

#### Bitter-taste transduction

Members of the T2R family were found co-expressed with the  $\alpha$ -subunit of the G protein gustducin<sup>47</sup>, a taste-specific signalling protein long known to have a prominent role in bitter taste<sup>6</sup>. Knockout mice lacking  $\alpha$ -gustducin show decreased sensitivity for bitter agents such as denatonium and quinine, and also for sweet agents such as saccharin and sucrose<sup>57</sup>.  $\alpha$ -Gustducin activates a taste-specific phosphodiesterase<sup>58</sup>, lowering the cellular concentration of cyclic nucleotides<sup>59</sup>.

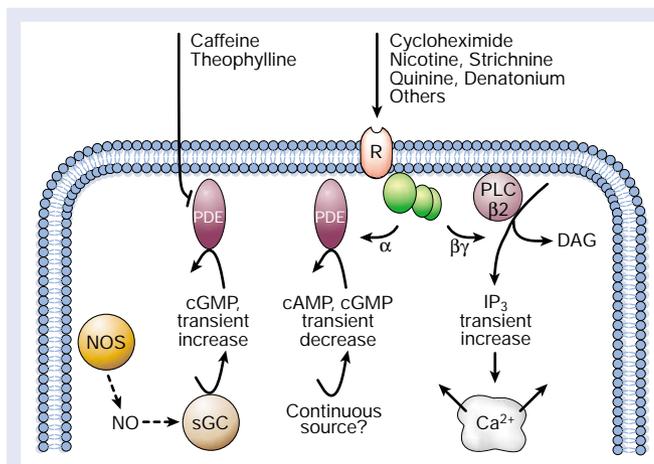
Another transduction cascade is also activated simultaneously by the GPCR-mediated bitter signal (Fig. 3). Of the  $\beta$ - and  $\gamma$ -subunits of heterotrimeric gustducin<sup>60</sup>, G $\gamma$ 13 and G $\beta$ 3 are able to activate phospholipase C $\beta$ 2 (PLC $\beta$ 2; refs 60–62), leading to the generation of inositol-1,4,5-trisphosphate (Ins(1,4,5)P<sub>3</sub>)<sup>63,64</sup>. This messenger activates Ins(1,4,5)P<sub>3</sub> receptors of intracellular Ca<sup>2+</sup> stores, of which the type III receptor, which may be modulated by calcium ions and by cAMP-dependent kinases, is the dominant form<sup>65</sup>. The receptor is co-expressed with PLC $\beta$ 2 (ref. 66). Concomitantly, Ca<sup>2+</sup> ions are released into the cytosol<sup>120,21,67</sup>. The subsequent elements of transduction, that is, the channels responsible for a change in membrane potential, remain to be identified.

Thus, the GPCR-mediated bitter signal triggers a transient decrease in cAMP and cGMP, accompanied by a transient increase in Ins(1,4,5)P<sub>3</sub> (refs 59, 64). Whether the two pathways, which are activated simultaneously, are connected, with the decrease in cyclic nucleotides enabling the increase in Ins(1,4,5)P<sub>3</sub>, has not yet been determined. Furthermore, it is unclear why dual signalling should be required for bitter taste, and whether or not it is more than parallel amplification. A similar design is not found in the receptor cells of other senses, and the other major chemoreceptive organ — the nose — seems to do well without it (see review in this issue by Firestein, pages 211–218).

#### Sweet taste

Sweet taste responds to soluble carbohydrates present in sufficient concentrations in the oral cavity, guiding high-calorie intake. Yet a wide diversity of non-carbohydrate molecules is also sweet. Sweet taste has a strong hedonic (pleasant) effect<sup>1</sup>.

Considerable efforts have been made by chemists and researchers in food-producing companies to deduce from hundreds of sweet-tasting compounds common structural features that were expected to capture characteristics of 'the' sweet molecule and, therefore, 'the' sweet receptor. The binding-site models obtained seemed realistic in that they could be used to design new high-potency sweeteners<sup>68</sup>. The time has come, however, to define sweet receptors more directly using the tools of genetics and molecular biology.



**Figure 3** Transduction of bitter taste as elicited by a variety of ligands. Rs, multiple GPCRs of the T2R family, coupled to the G protein gustducin<sup>47–49</sup>,  $\alpha$ ,  $\alpha$ -subunit of gustducin<sup>65,67</sup>;  $\beta\gamma$ , G-protein subunits  $\beta$ 3 and  $\gamma$ 13 (refs 60–62); PLC $\beta$ 2, phospholipase C subtype<sup>61</sup>; Ins(1,4,5)P<sub>3</sub>, inositol-1,4,5-trisphosphate<sup>59</sup>; PDE, taste-specific phosphodiesterase<sup>58</sup>; cAMP, cyclic adenosine monophosphate<sup>59</sup>; cGMP, cyclic guanosine monophosphate<sup>59</sup>; sGC, soluble guanylate cyclase<sup>55</sup>; NO, nitric oxide<sup>55</sup>; NOS, NO synthase<sup>56</sup>. For second-messenger kinetics, see refs 55, 59, 63, 64.

A first success was achieved by cloning a candidate trehalose receptor of *Drosophila*<sup>69</sup>. It is a GPCR that has only a short NTD (Fig. 2). In the mouse genome, sweet-receptor genes are most likely located on chromosome 4, where two sweet taste-related locations are found, the *Dpa* locus and the *Sac* locus<sup>70,71</sup>. Mutations in the *Dpa* locus resulted in a partial loss of taste acuity for the sweet amino acid D-phenylalanine, whereas mutations in the *Sac* locus caused a partial loss of taste acuity for sucrose, saccharin and other sweeteners. Searching the new genomic databases near these two loci may uncover genes for sweet receptors.

When applied recently to the *Sac* locus, this strategy proved remarkably successful. The receptor T1R3, found simultaneously by four laboratories<sup>72–75</sup>, has a large NTD (Fig. 2), just like the orphan receptors T1R1 and T1R2 described previously<sup>76</sup>. T1R3 was found in buds of the anterior, lateral and posterior tongue. It is expressed in many taste cells which also express the orphan T1R2, suggesting that the two receptors serve a common function. They might, for instance, form heterodimers, as is known from some other GPCRs with large NTDs<sup>72,73</sup>.

As the gene *Tas1r3* is the only GPCR-coding gene at the *Sac* locus, its product T1R3 is a strong candidate for a sweet receptor. This conjecture is supported by additional observations. First, strains of mice that differ in sweet-taste ability also differ by several point mutations in *Tas1r3*. These changes do not affect expression, but may result in a decline of function. Of course, owing to extended polymorphism, the strains also differ by many more mutations outside of *Tas1r3*. Second, inbreeding of taster and non-taster strains revealed a correlation between alleles received and sweet-taste acuity<sup>73</sup>. In the future, a more detailed proof might show that transgenic non-taster mice become tasters when *Tas1r3* is switched on. The ink was not yet dry on the candidate sweet receptor T1R3, when Charles Zuker and co-workers managed to express this GPCR in oocytes. They found that the receptor does not respond to sweeteners on its own. But responses were obtained after co-expressing T1R3 together with T1R2, showing that the first functional sweet receptor found in mammals is a dimer<sup>77</sup>.

#### Sweet-taste transduction

How is T1R3 coupled to the transduction machinery? This GPCR is expressed by about 20% of the taste cells, some of which also express  $\alpha$ -gustducin. The precise extent of co-expression is debatable, however, as one group found that less than 20% of the T1R3 cells have a

measurable gustducin signal<sup>73</sup>, whereas another specified a much larger percentage<sup>72</sup>. The co-expression is compatible with a role of  $\alpha$ -gustducin in sweet taste, as data obtained from knockout mice had suggested previously<sup>57</sup>. If the fraction of cells that co-express T1R3 with  $\alpha$ -gustducin is as low as 20%, then the co-expressing cells may be generalists, responding to both sweet and bitter signals. In this case,  $\alpha$ -gustducin would be a hallmark of bitter transduction, but would not be present in most sweet-responsive cells, which ignore the bitter signal. Many of the taste cells co-expressing T1R3 and  $\alpha$ -gustducin also express G $\beta$ 3, G $\gamma$ 13 and PLC $\beta$ 2 (ref. 72), all transduction elements of bitter taste (Fig. 3).

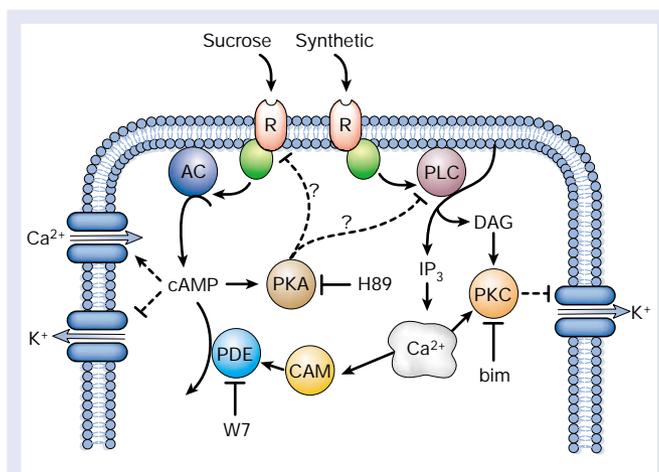
Like bitter-responsive cells, sweet-responsive cells use both cyclic nucleotides and Ins(1,4,5)P<sub>3</sub> as second messengers (Fig. 4). Ca<sup>2+</sup>-imaging experiments with isolated taste buds of rat vallate origin showed that stimulation with sugars or with forskolin caused Ca<sup>2+</sup> uptake from the extracellular space, whereas non-sugar sweeteners caused Ca<sup>2+</sup> release from intracellular stores<sup>21</sup>. This, together with other data, suggested that sugars activate a cyclic nucleotide cascade, leading to an increase of cAMP, membrane depolarization and Ca<sup>2+</sup> uptake, whereas non-sugar sweeteners activate the Ins(1,4,5)P<sub>3</sub> cascade in the same cell<sup>21</sup>. This notion was compatible with results from several laboratories, some of which involved inhibition of a K<sup>+</sup> conductance as the depolarizing step<sup>18,78–87</sup>. Inhibition of the K<sup>+</sup> conductance was thought originally to occur through protein kinase A (PKA)<sup>78</sup>, but a cyclic nucleotide-gated channel, the CNG<sub>gust</sub>, that was found in taste cells<sup>88</sup> might contribute to depolarization and Ca<sup>2+</sup> inflow when cAMP increases (Fig. 4). A co-localization of the channel with T1R3 or  $\alpha$ -gustducin has not yet been reported.

More recent evidence indicates modifications regarding the role of cAMP in sweet taste (Fig. 4). An inhibitor of PKA was found not to inhibit the sugar-sweet response in the hamster anterior tongue, but to enhance it<sup>89</sup>. This indicates that PKA is not involved directly in the response to sugars, but may be involved in adaptation. The response to artificial sweeteners was also enhanced. In contrast, inhibition of protein kinase C did not affect responses to sucrose, but inhibited responses to artificial sweeteners. This showed again that the transduction of the two kinds of sweeteners differs. Inhibition of the Ca<sup>2+</sup>/calmodulin-dependent cAMP-phosphodiesterase enhanced the responses to sucrose but not to synthetic sweeteners, indicating that the calcium ions released during stimulation with synthetic sweeteners may depress a simultaneous response to sucrose by activation of this enzyme<sup>89</sup>.

Furthermore, it was found with improved recording conditions that in the vallate papilla of the rat the second messenger cGMP rose transiently, with a peak-time of 150 ms, in response to sucrose. This signal was observed only as long as cAMP remained low, suggesting that cGMP is involved in the initial stage of sugar-taste transduction and may be more significant than cAMP at this stage<sup>90</sup>.

In conclusion, sweet-taste transduction is complex and our knowledge about it is far from complete. The data presently available suggest that sweet stimuli activate taste cells through at least two transduction pathways (Fig. 4), of which one involves an increase in cyclic nucleotides (cGMP or cAMP), the other an increase in Ins(1,4,5)P<sub>3</sub>. Membrane depolarization by inhibition of a K<sup>+</sup> conductance may be a common feature of the two pathways. An increase in the cytosolic Ca<sup>2+</sup> concentration occurs in both of the pathways, even though the source of the Ca<sup>2+</sup> ions is different. There seems to be variability in utilization of the pathways across the anterior and posterior regions of the tongue and across sweeteners and animal species. Now that candidates for sweet receptors are known, new biochemical experiments may soon identify the transduction elements downstream of the receptors.

Like other taste qualities, sweet taste is modified by circulating hormones. Recently, the effect of leptin on sweet-responding taste cells has generated much interest. Leptin, a protein hormone encoded by the *ob* gene, is secreted mainly by adipocytes and regulates body mass. The full-length leptin receptor Ob-Rb, a principal mediator of



**Figure 4** Molecules involved in the transduction of sweet taste. Two separate sweet receptors are shown, but the possibility that one receptor activates both of the transduction pathways<sup>100</sup> is not excluded at this stage. R, candidate receptor(s)<sup>72–75</sup>; AC, adenylate cyclase<sup>81,82,87</sup>; cAMP, cyclic adenosine monophosphate<sup>21</sup>; PDE, phosphodiesterase, inhibitor W7 (ref. 89); CAM, calmodulin<sup>89</sup>; PKA, protein kinase A, inhibitor H89 (ref. 89); PLC, phospholipase C<sup>89</sup>; DAG, diacylglycerol; Ins(1,4,5)P<sub>3</sub>, inositol-1,4,5-trisphosphate<sup>21</sup>; PKC, protein kinase C, inhibitor bim (bisindolylmaleimide)<sup>89</sup>. For crosstalk between pathways and effects of inhibitors (H89, bim and W7), see ref. 89.

the leptin signal, is expressed in various tissues including pancreatic  $\beta$ -cells and a subset of taste cells<sup>91,92</sup>. Leptin suppresses insulin secretion by the activation of ATP-sensitive K<sup>+</sup> channels. Its inhibitory effect on taste receptor cells also involves the activation of a K<sup>+</sup> conductance and membrane hyperpolarization<sup>91</sup>. Thereby the hormone partially blunts nerve signals indicating sweet taste, which, presumably, makes food less attractive. During starvation the production of leptin is decreased. The resulting disinhibition in the target tissues diminishes energy expenditure and leads to the motivational state of hunger. At the same time, disinhibition of sweet-responsive taste cells enhances sensitivity to sweet taste, making sweet food more attractive. Thus the effect of leptin on the taste system supports the general role of this hormone in regulating nutrition, body weight and energy balance<sup>92</sup>.

### Umami taste

The biological significance of this basic taste, discovered about 100 years ago, is high, comparable perhaps to that of sweet taste. 'Umami', a term derived from the Japanese *umai* (delicious), designates a pleasant taste sensation which is qualitatively different from sweet, salty, sour and bitter<sup>93</sup>. Umami is a dominant taste of food containing L-glutamate, like chicken-broth, meat extracts and ageing cheese. The rather common amino acid L-glutamate thus guides the intake of peptides and proteins, from which it is released by proteolysis (curing and decay). Characteristic taste-enhancing effects arise from the presence of purine 5'-ribonucleotides such as IMP and GMP, which are also present in decaying biological tissues.

A taste receptor for L-glutamate might possibly be related to one of the glutamate receptors well known from neuronal synapses. Starting with this hypothesis, it was discovered that a subset of taste cells contains a truncated form of brain mGluR4, a metabotropic GPCR abundant in the central nervous system. Although brain mGluR4 has a rather large NTD, the taste variant has a truncated NTD (Fig. 2) and this seems to adapt the receptor to the high glutamate concentrations occurring in food<sup>94</sup>. Synergism with ribonucleotides, a highlight of umami taste, was established<sup>95</sup>. Once again, the transduction is complex — one variant involves the sustained closure of an unspecific cation conductance, presumably causing hyperpolarization, even though transient inward currents, which would cause

depolarization, were also observed<sup>196,97</sup>. In addition to the taste mGluR4, other glutamate and amino-acid receptors were also found to function in taste cells<sup>23,24,98</sup>.

### Perspectives for taste research

Much effort is now being made to identify the receptors and other key molecules of transduction within taste receptor cells. The sequencing of the receptor genetic code in particular opens the way to study the corresponding proteins and map their structure with good spatial resolution. In this way, we should achieve a better understanding of how the protein machinery within taste GPCRs actually works.

The practical consequences of these efforts are considerable. Based on binding-site structure, advanced techniques of drug design are expected to allow the construction of taste ligands that activate or inhibit a receptor protein, thereby enhancing or inhibiting a specific taste. Thus it might become possible to expand the already huge commercial market for artificial sweeteners into other taste qualities. This will be beneficial in many ways. For example, aged people often have a general decline of taste function<sup>99</sup> and need taste enhancement to once again enjoy their food. And an organic enhancer of sodium taste would be a great help for patients on a low-sodium diet.

Other scientific challenges still wait to be tackled by taste research. In the evolution of animal species, adaptive changes of taste receptors have occurred, which supported or generated new food preferences, probably driven by changes in food availability. We do not yet understand these long-term changes. The future large-scale sequencing of animal genomes may enable us to shed light on this interesting aspect of evolution.

Finally, the perspective is likely to widen in the future, and taste-driven processing in the brain, today studied by few, will be a central theme of the field. Challenging topics such as the sensory code, short-term memory of taste (often exploited as conditioned taste acceptance and aversion), hormonal feedback systems serving nutritional needs, and the generation of motivational states which guide feeding behaviour will increasingly come into focus. A deeper understanding of our conscious and unconscious decisions in food selection and food intake may then have applications in diverse fields, from food processing to clinical medicine. □

- Ganchrow, J. R., Steiner, J. E. & Daher, M. Neonatal facial expressions in response to different qualities and intensities of gustatory stimuli. *Infant Behav. Dev.* **6**, 189–200 (1983).
- Pierce-Shimomura, J. T., Faumont, S., Gaston, M. R., Pearson, B. J. & Lockery, S. R. The homeobox gene *lim-6* is required for distinct chemosensory representations in *C. elegans*. *Nature* **410**, 694–698 (2001).
- Clyne, P. J., Warr, C. G. & Carlson, J. R. Candidate taste receptors in *Drosophila*. *Science* **287**, 1830–1834 (2000).
- Scott, K. et al. A chemosensory gene family encoding candidate gustatory and olfactory receptors in *Drosophila*. *Cell* **104**, 661–673 (2001).
- Stone, L. M., Finger, T. E., Tam, P. P. & Tan, S. S. Taste receptor cells arise from local epithelium, not neurogenic ectoderm. *Proc. Natl Acad. Sci. USA* **92**, 1916–1920 (1995).
- McLaughlin, S. K., McKinnon, P. J. & Margolskee, R. F. Gustducin is a taste-cell-specific G protein closely related to the transducins. *Nature* **357**, 563–569 (1992).
- Zancanaro, C., Caretta, C. M., Merigo, F., Cavaggoni, A. & Osculati, F.  $\alpha$ -Gustducin expression in the vomeronasal organ of the mouse. *Eur. J. Neurosci.* **11**, 4473–4475 (1999).
- Höfer, D., Püschel, B. & Drenckhahn, P. H. Taste receptor-like cells in the rat gut identified by expression of  $\alpha$ -gustducin. *Proc. Natl Acad. Sci. USA* **93**, 6631–6634 (1996).
- Hänig, D. P. Zur Psychophysik des Geschmackssinnes. *Phil. Stud.* **17**, 576–623 (1901).
- Lindemann, B. Receptor seeks ligand: on the way to cloning the molecular receptors for sweet and bitter taste. *Nature Med.* **5**, 381–382 (1999).
- Smith, D. V. & Margolskee, R. F. Making sense of taste. *Sci. Am.* **284**, 26–33 (2001).
- Mistretta, C. M., Goosens, K. A., Farnas, I. & Reichardt, L. F. Alterations in size, number, and morphology of gustatory papillae and taste buds in BDNF null mutant mice demonstrate neural dependence of developing taste organs. *J. Comp. Neurol.* **409**, 13–24 (1999).
- Krimm, R. F., Miller, K. K., Kitzman, P. H., Davis, B. M. & Albers, K. M. Epithelial overexpression of BDNF or NT4 disrupts targeting of taste neurons that innervate the anterior tongue. *Dev. Biol.* **232**, 508–521 (2001).
- Lundy, R. F. Jr & Contreras, R. J. Gustatory neuron types in rat geniculate ganglion. *J. Neurophysiol.* **82**, 2970–2988 (1999).
- Gilbertson, T. A., Boughter, J. D. Jr, Zhang, H. & Smith, D. V. Distribution of gustatory sensitivities in rat taste cells: whole-cell responses to apical chemical stimulation. *J. Neurosci.* **21**, 4931–4941 (2001).
- Erickson, R. P. The evolution of neural coding ideas in the chemical senses. *Physiol. Behav.* **69**, 3–13 (2000).
- Roper, S. D. Regenerative impulses in taste cells. *Science* **220**, 1311–1312 (1983).
- Avenet, P. & Lindemann, B. Patch-clamp study of isolated taste receptor cells of the frog. *J. Membr. Biol.* **97**, 223–240 (1987).
- Lindemann, B. Taste reception. *Physiol. Rev.* **76**, 719–766 (1996).
- Akabas, M. H., Dodd, J. & Al-Awqati, Q. A bitter substance induces a rise in intracellular calcium in a subpopulation of rat taste cells. *Science* **242**, 1047–1050 (1988).
- Bernhardt, S. J., Naim, M., Zehavi, U. & Lindemann, B. Changes in IP<sub>3</sub> and cytosolic Ca<sup>2+</sup> in response to sugars and non-sugar sweeteners in transduction of sweet taste in the rat. *J. Physiol.* **490**, 325–336 (1996).
- Caicedo, A. & Roper, S. D. Taste receptor cells that discriminate between bitter stimuli. *Science* **291**, 1557–1560 (2001).
- Zviman, M. M., Restrepo, D. & Teeter, J. H. Single taste stimuli elicit either increases and decreases in intracellular calcium in isolated catfish taste cells. *J. Membr. Biol.* **149**, 81–88 (1996).
- Hayashi, Y., Zviman, M. M., Brand, J. G., Teeter, J. H. & Restrepo, D. Measurement of membrane potential and [Ca<sup>2+</sup>]<sub>i</sub> in cell ensembles: application to the study of glutamate taste in mouse. *Biophys. J.* **71**, 1057–1070 (1996).
- Herness, M. S. & Sun, X. D. Characterization of chloride currents and their noradrenergic modulation in rat taste receptor cells. *J. Neurophysiol.* **82**, 260–271 (1999).
- Yamamoto, T., Nagai, T., Shimura, T. & Yasoshima, Y. Roles of chemical mediators in the taste system. *Jpn J. Pharmacol.* **76**, 325–348 (1998).
- Delay, R. J., Kinnamon, S. C. & Roper, S. D. Serotonin modulates voltage-dependent calcium currents in *Necturus* taste cells. *J. Neurophysiol.* **77**, 2515–2524 (1997).
- Herness, S. & Chen, Y. Serotonin inhibits calcium-activated K<sup>+</sup> current in rat taste receptor cells. *NeuroReport* **8**, 3257–3261 (1997).
- Caicedo, A., Kim, K. N. & Roper, S. D. Glutamate-induced cobalt uptake reveals non-NMDA receptors in rat taste cells. *J. Comp. Neurol.* **417**, 315–324 (2000).
- Lawton, D. M., Furness, D. N., Lindemann, B. & Hackney, C. M. Localization of the glutamate-aspartate transporter, GLAST, in rat taste buds. *Eur. J. Neurosci.* **12**, 3163–3171 (2000).
- Lindemann, B. Sodium taste. *Curr. Opin. Nephrol. Hypertension* **6**, 425–429 (1997).
- Heck, G. L., Mierson, S. & DeSimone, J. A. Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway. *Science* **223**, 403–405 (1984).
- Canessa, C. M. et al. Amiloride-sensitive epithelial Na<sup>+</sup> channel is made of three homologous subunits. *Nature* **367**, 463–467 (1994).
- Kretz, O., Barbry, P., Bock, R. & Lindemann, B. Differential expression of RNA and protein of the three pore-forming subunits of the amiloride-sensitive epithelial sodium channel in taste buds of the rat. *J. Histochem. Cytochem.* **47**, 51–64 (1999).
- Lin, W., Finger, T. E., Rossier, B. C. & Kinnamon, S. C. Epithelial Na<sup>+</sup> channel subunits in rat taste cells: localization and regulation by aldosterone. *J. Comp. Neurol.* **405**, 406–420 (1999).
- Avenet, P. & Lindemann, B. 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 (1991).
- Smith, D. V. & Ossebaard, C. A. Amiloride suppression of the taste intensity of sodium chloride: evidence from direct magnitude scaling. *Physiol. Behav.* **57**, 773–777 (1995).
- Gilbertson, T. A., Roper, S. D. & Kinnamon, S. C. Proton currents through amiloride-sensitive Na<sup>+</sup> channels in isolated hamster taste cells: enhancement by vasopressin and cAMP. *Neuron* **10**, 931–942 (1993).
- Kinnamon, S. C., Dionne, V. E. & Beam, K. G. Apical localization of K channels in taste cells provides the basis for sour taste transduction. *Proc. Natl Acad. Sci. USA* **85**, 7023–7027 (1988).
- Ugawa, S. et al. Receptor that leaves a sour taste in the mouth. *Nature* **395**, 555–556 (1998).
- Miyamoto, T., Fujiyama, R., Okada, Y. & Sato, T. Sour transduction involves activation of NPPB-sensitive conductance in mouse taste cells. *J. Neurophysiol.* **80**, 1852–1859 (1998).
- Miyamoto, T., Fujiyama, R., Okada, Y. & Sato, T. Acid and salt responses in mouse taste cells. *Prog. Neurobiol.* **62**, 135–157 (2000).
- Stevens, D. R. et al. The hyperpolarization-activated channels HCN1 and 4 mediate responses to sour stimuli. *Nature* (in the press).
- DeSimone, J. A., Callahan, E. M. & Heck, G. L. Chorda tympani taste response of rat to hydrochloric acid subject to voltage-clamped lingual receptive field. *Am. J. Physiol.* **268**, C1295–C1300 (1995).
- Stewart, R. E., Lyall, V., Feldman, G. M., Heck, G. L. & DeSimone, J. A. Acid-induced responses in hamster chorda tympani and intracellular pH tracking by taste receptor cells. *Am. J. Physiol.* **275**, C227–C238 (1998).
- Spielman, A. I., Huque, T., Whitney, G. & Brand, J. G. in *Sensory Transduction* (eds Corey, D. P. & Roper, S. D.) 307–324 (The Rockefeller University Press, New York, 1992).
- Adler, E. et al. A novel family of mammalian taste receptors. *Cell* **100**, 693–702 (2000).
- Matsunami, H., Montmayeur, J.-P. & Buck, L. A family of candidate taste receptors in human and mouse. *Nature* **404**, 601–604 (2000).
- Chandrasekar, J. et al. T2Rs function as bitter taste receptors. *Cell* **100**, 703–711 (2000).
- Dahl, M., Erickson, R. P. & Simon, S. A. Neural responses to bitter compounds in rats. *Brain Res.* **756**, 22–34 (1997).
- Naim, M., Seifert, R., Nürnberg, B., Grünbaum, L. & Schultz, G. Some taste substances are direct activators of G-proteins. *Biochem. J.* **297**, 451–454 (1994).
- Cummings, T. A. & Kinnamon, S. C. Apical K<sup>+</sup> channels in *Necturus* taste cells—modulation by intracellular factors and taste stimuli. *J. Gen. Physiol.* **99**, 591–613 (1992).
- Tsunenari, T. et al. A quinine-activated cationic conductance in vertebrate taste receptor cells. *J. Gen. Physiol.* **108**, 515–523 (1996).
- Spielman, A. I. et al. A method for isolating and patch-clamping single mammalian taste receptor cells. *Brain Res.* **503**, 326–329 (1989).
- Rosenzweig, S., Yan, W., Dasso, M. & Spielman, A. I. Possible novel mechanism for bitter taste mediated through cGMP. *J. Neurophysiol.* **81**, 1661–1665 (1999).
- Kretz, O., Bock, R. & Lindemann, B. Occurrence of nitric oxide synthase in taste buds of the rat vallate papilla. *Histochem. J.* **30**, 293–299 (1998).
- Wong, G. T., Gannon, K. S. & Margolskee, R. F. Transduction of bitter and sweet taste by gustducin. *Nature* **381**, 796–800 (1996).
- Spickofsky, N. et al. Biochemical analysis of the transducin-phosphodiesterase interaction. *Nature Struct. Biol.* **1**, 771–781 (1994).
- Yan, W. et al. Bitter taste transduced by PLC- $\beta_2$ -dependent rise in IP<sub>3</sub> and  $\alpha$ -gustducin-dependent fall in cyclic nucleotides. *Am. J. Physiol. Cell Physiol.* **280**, C742–C751 (2001).
- Huang, L. et al. G $\gamma$ 13 colocalizes with gustducin in taste receptor cells and mediates IP<sub>3</sub> responses to

- bitter denatonium. *Nature Neurosci.* **2**, 1055–1062 (1999).
61. Rössler, P., Kroner, C., Freitag, J., Noé, J. & Breer, H. Identification of a phospholipase c  $\beta$  subtype in rat taste cells. *Eur. J. Cell Biol.* **77**, 253–261 (1998).
  62. Rössler, P. *et al.* G protein betagamma complexes in circumvallate taste cells involved in bitter transduction. *Chem. Senses* **25**, 413–421 (2000).
  63. Spielman, A. I., Huque, T., Nagai, H., Whitney, G. & Brand, J. G. Generation of inositol phosphates in bitter taste transduction. *Physiol. Behav.* **56**, 1149–1155 (1994).
  64. Spielman, A. I. *et al.* Rapid kinetics of second messenger formation in bitter taste. *Am. J. Physiol. Cell Physiol.* **270**, C926–C931 (1996).
  65. Clapp, T. R., Stone, L. M., Margolskee, R. F. & Kinnamon, S. C. Immunocytochemical evidence for co-expression of Type III IP<sub>3</sub> receptor with signaling components of bitter taste transduction. *BMC Neurosci.* **2**, 6 (2001).
  66. Miyoshi, M. A., Abe, K. & Emori, Y. IP<sub>3</sub> receptor type 3 and PLC $\beta$ 2 are co-expressed with taste receptors T1R and T2R in rat taste bud cells. *Chem. Senses* **26**, 259–265 (2001).
  67. Ogura, T. & Kinnamon, S. C. IP<sub>3</sub>-Independent release of Ca<sup>2+</sup> from intracellular stores: a novel mechanism for transduction of bitter stimuli. *J. Neurophysiol.* **82**, 2657–2666 (1999).
  68. Nofre, C. & Tinti, J. M. Sweetness reception in man: the multipoint attachment theory. *Food Chem.* **56**, 263–274 (1996).
  69. Ishimoto, H., Matsumoto, A. & Tanimura, T. Molecular identification of a taste receptor gene for trehalose in *Drosophila*. *Science* **289**, 116–119 (2000).
  70. Ninomiya, Y., Sako, N. & Funakoshi, M. Selective effects of the dpa gene on the ability to taste D-phenylalanine in mice. *Proc. Jpn Symp. Taste Smell* **21**, 153–156 (1987).
  71. Lush, I. E. The genetics of tasting in mice. VI. Saccharin, acesulfame, dulcin and sucrose. *Genet. Res.* **53**, 95–99 (1989).
  72. Max, M. *et al.* *Tas1r3*, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus *Sac*. *Nature Genet.* **28**, 58–63 (2001).
  73. Montmayeur, J. P., Liberles, S. D., Matsunami, H. & Buck, L. B. A candidate taste receptor gene near a sweet taste locus. *Nature Neurosci.* **4**, 492–498 (2001).
  74. Kitagawa, M., Kusakabe, Y., Miura, H., Ninomiya, Y. & Hino, A. Molecular genetic identification of a candidate receptor gene for sweet taste. *Biochem. Biophys. Res. Commun.* **283**, 236–242 (2001).
  75. Sainz, E., Korley, J. N., Battey, J. F. & Sullivan, S. L. Identification of a novel member of the T1R family of putative taste receptors. *J. Neurochem.* **77**, 896–903 (2001).
  76. Hoon, M. A. *et al.* Putative mammalian taste receptors: a class of taste specific GPCRs with distinct topographic selectivity. *Cell* **96**, 541–551 (1999).
  77. Nelson, G. *et al.* Mammalian sweet taste receptors. *Cell* **106**, 381–390 (2001).
  78. Avenet, P., Hofmann, F. & Lindemann, B. Transduction in taste receptor cells requires cAMP-dependent protein kinase. *Nature* **331**, 351–354 (1988).
  79. Tonosaki, K. & Funakoshi, M. Cyclic nucleotides may mediate taste transduction. *Nature* **331**, 354–356 (1988).
  80. Béhé, P., DeSimone, J. A., Avenet, P. & Lindemann, B. Membrane currents in taste cells of the rat fungiform papilla: evidence for two types of Ca currents and inhibition of K currents by saccharin. *J. Gen. Physiol.* **96**, 1061–1084 (1990).
  81. Striemi, B., Pace, U., Zehavi, U., Naim, M. & Lancet, D. Sweet tastants stimulate adenylate cyclase coupled to GTP-binding protein in rat tongue membranes. *Biochem. J.* **260**, 121–126 (1989).
  82. Striemi, B. J., Naim, M. & Lindemann, B. Generation of cyclic AMP in taste buds of the rat circumvallate papilla in response to sucrose. *Cell. Physiol. Biochem.* **1**, 46–54 (1991).
  83. Cummings, T. A., Powell, J. & Kinnamon, S. C. Sweet taste transduction in hamster taste cells: evidence for the role of cyclic nucleotides. *J. Neurophysiol.* **70**, 2326–2336 (1993).
  84. Cummings, T. A., Daniels, C. & Kinnamon, S. C. Sweet taste transduction in hamster: sweeteners and cyclic nucleotides depolarize taste cells by reducing a K<sup>+</sup> current. *J. Neurophysiol.* **75**, 1256–1263 (1996).
  85. Uchida, Y. & Sato, T. Changes in outward K<sup>+</sup> currents in response to two types of sweeteners in sweet taste transduction of gerbil taste cells. *Chem. Senses* **22**, 163–169 (1997).
  86. Nakashima, K. & Ninomiya, Y. Transduction for sweet taste of saccharin may involve both inositol 1,4,5-trisphosphate and cAMP pathways in the fungiform taste buds in C57BL mice. *Cell. Physiol. Biochem.* **9**, 90–98 (1999).
  87. Ishimaru, Y., Yasuoka, A., Asano-Miyoshi, M., Abe, K. & Emori, Y. An actin-binding protein, CAP, is expressed in a subset of rat taste bud cells. *NeuroReport* **12**, 233–235 (2001).
  88. Misaka, T., Kusakabe, Y., Emori, Y., Arai, S. & Abe, K. Molecular cloning and taste bud-specific expression of a novel cyclic nucleotide-gated channel. *Ann. NY Acad. Sci.* **855**, 150–159 (1998).
  89. Varkevisser, B. & Kinnamon, S. C. Sweet taste transduction in hamster: role of protein kinases. *J. Neurophysiol.* **83**, 2526–2532 (2000).
  90. Krizhanovsky, V., Agamy, O. & Naim, M. Sucrose-stimulated subsecond transient increase in cGMP level in rat intact circumvallate taste bud cells. *Am. J. Physiol. Cell Physiol.* **279**, C120–C125 (2000).
  91. Kawai, K., Sugimoto, K., Nakashima, K., Miura, H. & Ninomiya, Y. Leptin as a modulator of sweet taste sensitivities in mice. *Proc. Natl Acad. Sci. USA* **97**, 11044–11049 (2000).
  92. Ninomiya, Y. *et al.* Leptin and sweet taste. *Vitam. Horm.* (in the press).
  93. Ikeda, K. On a new seasoning. *J. Tokyo Chem. Soc.* **30**, 820–836 (1909). [In Japanese.]
  94. Chaudhari, N., Landin, A. M. & Roper, S. D. A novel metabotropic glutamate receptor functions as a taste receptor. *Nature Neurosci.* **3**, 113–119 (2000).
  95. Delay, E. R. *et al.* Taste preference synergy between glutamate receptor agonists and inosine monophosphate in rats. *Chem. Senses* **25**, 507–515 (2000).
  96. Bigiani, A., Delay, R. J., Chaudhari, N., Kinnamon, S. C. & Roper, S. D. Responses to glutamate in rat taste cells. *J. Neurophysiol.* **77**, 3048–3059 (1997).
  97. Lin, W. & Kinnamon, S. C. Physiological evidence for ionotropic and metabotropic glutamate receptors in rat taste cells. *J. Neurophysiol.* **82**, 2061–2069 (1999).
  98. Brand, J. G. Receptor and transduction processes for umami taste. *J. Nutr.* **130**, 942S–945S (2000).
  99. Stevens, J. C., Cruz, L. A., Hoffman, J. M. & Patterson, M. Q. Taste sensitivity and aging: high incidence of decline revealed by repeated threshold measures. *Chem. Senses* **20**, 451–459 (1995).
  100. Zhu, X., Gilbert, S., Birnbaumer, M. & Birnbaumer, L. Dual signaling potential is common among G<sub>s</sub>-coupled receptors and dependent on receptor density. *Mol. Pharmacol.* **46**, 460–469 (1994).

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