# Chemoreception: **Tasting the sweet and the bitter**

Bernd Lindemann

**Our understanding of the molecular basis of taste transduction has lagged behind that of other senses, but now a signalling protein – the G protein α subunit gustducin – has been shown to be taste-tissue specific and essential for both bitter and sweet tastes.**

Address: Department of Physiology, Saarland University, D-66421 Homburg, Germany.

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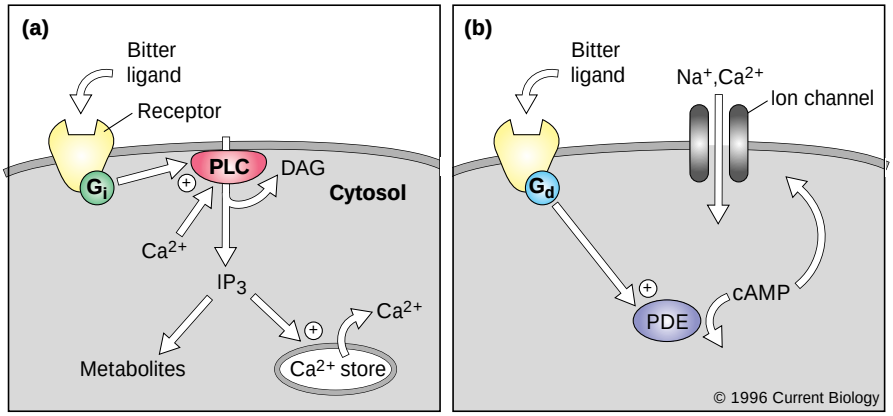
Taste has been the Cinderella of the senses, the one for which the transduction mechanism was, until recently, arguably the least well understood. If you were to ask a class of physiology students to speculate on the possible mechanism of taste transduction, it is likely that they would come up with a scheme along the following lines. The tasted substance, or tastant, is detected by receptors on the apical surface of a taste cell, which contacts the oral compartment. Receptor activation leads, *via* coupled G proteins, to activation of intracellular enzymes that generate second messengers. The second messengers, in turn, modulate a membrane ion channel, depolarizing the cell membrane potential and causing an inflow of calcium ions, and, perhaps, release of $Ca^{2+}$ from intracellular stores; together these result in the release of neurotransmitter molecules, which activate the postsynaptic sensory nerve. The scheme proposed is general enough that it might be correct as it stands, but essential details are unknown or debatable. One important detail has, however, recently been revealed: the involvement of transducin-like G proteins. The gene for a taste-cell specific G protein α subunit has been inactivated by targeted recombination, and the resulting 'knockout' mice have been found to lack the ability to sense both bitter and sweet tastes.

### What are the second messengers?

Because taste cells are small and scarce, it is difficult to determine unequivocally the nature of the second messengers that transduce the intracellular taste signal. Some progress has been made recently, however; in particular, Spielman and colleagues [1] have reported the successful use of the quenched-flow technique with taste-cell homogenates, in which second messenger signals were resolved on a millisecond time scale. They found a transient increase in the level of inositol 1,4,5-trisphosphate ($IP_3$) in response to the bitter agents denatonium and sucrose octaacetate; the response occurred with a delay of less than 50 milliseconds, and peaked near 100 milliseconds. The response was sensitive to pertussis toxin but not affected by cholera toxin, so it was probably mediated by a G protein of the $G_i$ type [2]. The physiological significance of the response is supported by the finding that taste tissue homogenates from mice genetically unable to respond to sucrose octaacetate, but still able to perceive denatonium as bitter, show the $IP_3$ response to denatonium but not to sucrose octaacetate [1].

These results suggested that $IP_3$ is at least one of the second messengers that transduce bitter taste (Fig. 1a). As $IP_3$ stimulates the release of $Ca^{2+}$ ions from intracellular stores, one would expect that $Ca^{2+}$ release occurs in response to denatonium. Indeed, in the rat, a small subset

**Figure 1**




Transduction of bitter taste following receptor activation by an agent such as denatonium. Two pathways, which may operate in parallel, are shown, based on evidence discussed in the text. **(a)** The active receptor turns on a G protein – probably $G_{\alpha i}$ [1,2] – which in turn activates $Ca^{2+}$-dependent phospholipase C (PLC), leading to transient generation of $IP_3$ and diacylglycerol (DAG). In response to the rising $IP_3$ concentration, $Ca^{2+}$ is released from intracellular stores [3]. **(b)** The receptor also turns on α-gustducin (Gd), and/or α-transducin, which activate(s) a phosphodiesterase (PDE). This model requires that cAMP is high under resting conditions. The stimulus-dependent decrease in the cAMP concentration may release cyclic-nucleotide suppressed channels from the closed state, thus causing membrane depolarization and inflow of $Ca^{2+}$ ions [6,7,10].

of taste cells was found to respond to denatonium with an increase in cytosolic $Ca^{2+}$ concentration which was not suppressed by the short-term removal of extracellular $Ca^{2+}$ [3,4]. In addition denatonium was found to trigger a similar $Ca^{2+}$ increase — in this case accompanied by membrane hyperpolarization — in taste cells of the mudpuppy *Necturus* [5]. Thus several findings, obtained with chemical assays and $Ca^{2+}$ imaging, point to $IP_3$ as a second messenger in bitter taste transduction. However, there was hardly time to celebrate this success before new data emerged which strongly implicated another messenger, cyclic (c)AMP, in bitter taste transduction.

### With and without α-gustducin

A few years ago, Margolskee and colleagues [6] discovered that taste tissue contains α subunits of trimeric G proteins, including those in the transducin family. Transducins — best known for their role in phototransduction where they are turned on by light-activated rhodopsin — activate phosphodiesterases (PDEs), enzymes which break down cyclic nucleotides such as cAMP and cGMP. In taste tissue, PDEs sensitive to transducins are present and can be activated by the bitter agent denatonium, presumably by means of a membrane receptor [7]. These discoveries suggested that cAMP and/or cGMP may play a role in the transduction of at least some taste qualities, including the bitter quality (Fig. 1b).

This notion was emphasized by the finding that taste tissue contains not only transducins of the kind found in photoreceptor cells, but in addition a related signalling protein, α-gustducin, which appears to be specific for taste cells of the tongue and some chemoreceptor cells of the intestine [8,9]. By analogy to the signalling pathway in vertebrate photoreceptors, it was suggested that receptor ligation causes, *via* α-gustducin and PDE, a decrease of free intracellular cyclic nucleotide concentrations. It was also suggested that the decreasing cAMP signal may open a novel cation channel (Fig. 1b) and thereby effect membrane depolarization [10].

Having cloned the gene for α-gustducin, Margolskee and colleagues [11] proceeded to use the technique of targeted gene inactivation to generate 'knockout' mice lacking a functional α-gustducin gene. The mutant mice were found to have taste buds that looked normal, but their behavioural and neuronal taste sensitivity to bitter agents, such as denatonium, was strongly impaired. (The taste sensitivity to sweet agents was also impaired, about which more later.) These results suggested a role for cyclic nucleotides in taste transduction, and for the G protein gustducin as a principal mediator of the response to bitter substances.

How can these findings be reconciled with the results mentioned above which indicated that $IP_3$ is the second messenger in cells that detect the bitter taste of denatonium? Is it possible that both cyclic nucleotides and $IP_3$ are involved in transducing this taste? In response to denatonium, $IP_3$ apparently goes up and cAMP down. This may mean that the increase in $IP_3$ can occur only if cAMP is first lowered. Yin–yang signalling of this kind is actually known from other systems, where it results from cross-inhibition between the cAMP and the $IP_3$ signalling pathways, established at the level of G proteins and of protein kinases [12]. It is not yet clear whether cross-inhibition occurs between cAMP and $IP_3$ pathways in bitter taste transduction, and, if so, which reactions mediate the inhibition. It is noteworthy, however, that cross-inhibition might explain why deletion of the gustducin gene abolishes bitter taste, even though $IP_3$ is a principal second messenger of bitter taste transduction. It therefore seems especially interesting to explore the possibility that elements of the cAMP signalling pathway interfere with the $IP_3$ pathway of taste cells.

### What about sweet taste transduction?

Working with membrane preparations of the rat tongue, Naim and colleagues [13–15] found an increase in cAMP production in response to sucrose; cAMP was similarly found to accumulate in response to sucrose in intact taste cells. To increase the sensitivity of the assays, a PDE inhibitor was added, so as to impair cAMP breakdown. The observed accumulation of cAMP turned out to be dependent on the presence of GTP, suggesting the involvement of a G protein that activates adenylate cyclase in response to extracellular sucrose [13].

In frog preparations, cAMP was found to inactivate a $K^+$ ion conductance, acting *via* the cAMP-regulated protein kinase A, and thereby to cause membrane depolarization [16,17]. In mouse taste cells, the injection of cyclic nucleotides also caused an increase in membrane resistance accompanied by depolarization, and the same response was elicited by sucrose applied to the surface of the tongue [18]. These results pointed to cAMP being the second messenger that transduces the sweet taste of sucrose, and indicated that the increase in cAMP was not due to stimulus-dependent inhibition of PDE (which was inhibited throughout some of the experiments [13,14]). The results further suggested that sweet transduction is accompanied by cAMP-dependent membrane depolarization, caused by closure of a $K^+$ conductance.

By recording action potentials from taste buds in the hamster tongue, Kinnamon and colleagues [19] found that a subset of such buds responded with excitation to sweet stimuli like sucrose and some non-sugar sweeteners. Interestingly, only this subset of buds also responded in the same way to membrane-permeant cyclic nucleotides and to a PDE blocker. Furthermore, in isolated hamster taste cells, non-sugar sweeteners and cyclic nucleotides elicited membrane depolarization and blockage of a resting $K^+$ conductance which was sensitive to tetraethyl ammonium

[20]. Taken together, these various results indicate that that cyclic nucleotides are second messengers that transduce sweet taste in mouse, rat and hamster, and that it is an increase in their intracellular concentration that mediates the response (Fig. 2a).

While these studies were being conducted, Naim and colleagues [4] attempted to measure a change in cAMP caused by artificial sweeteners. Much less cAMP accumulated in rat taste cells when stimulated with non-sugar sweeteners than when they were stimulated with sucrose. Instead, the level of another second messenger, $IP_3$, was increased in response to the sweeteners, but much less so in response to sucrose (Fig. 2b). Furthermore, it was shown by $Ca^{2+}$ imaging of single taste buds that identical cells responded to sucrose and to non-sugar sweeteners with an increase in free cytosolic $Ca^{2+}$, but only in the case of sweeteners was the $Ca^{2+}$ increase independent of the immediate presence of extracellular $Ca^{2+}$ — that is, due to $Ca^{2+}$ release from intracellular stores [4]. This suggests that different pathways transduce the response to sucrose and non-sugar sweeteners in rat taste cells. The sucrose response appeared to be mediated by cAMP, and the non-sugar response by $IP_3$ (Fig. 2b).

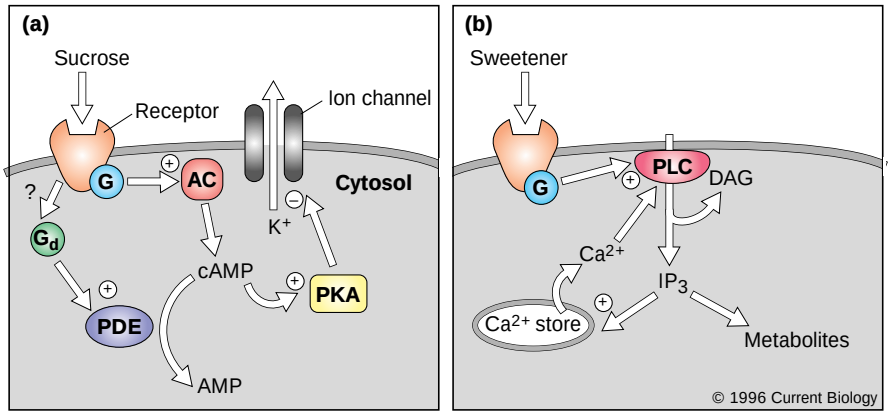
### Knocking out sweet taste

Let us now return to the challenging observation that α-gustducin-deficient mice are impaired in the detection of sweet, as well as bitter, tastes [11]. As α-gustducin is likely to activate a PDE, the immediate suggestion is that the PDE is a key mediator of sweet taste transduction and, by analogy with vertebrate phototransduction, one might expect it to work by lowering the intracellular concentration of a cyclic nucleotide. But this attractive scheme is contradicted by most of the other results on sweet taste transduction quoted above. Is it possible, then, that α-gustducin initiates a rise in cAMP by inhibiting, rather than activating, PDE? This, while compatible with the reported activation of sweet-tasting cells by PDE inhibitors [19], would not be compatible with the reported sucrose-stimulated increase in cAMP, because this increase was found to occur in the continuous presence of added PDE inhibitors [13,14].

There is, however, another possibility. Receptor-activated G-proteins might first cause an increase in cAMP mediated by adenylate cyclase and subsequently a decrease mediated by PDE. A rapid, but transient, elevation of cAMP would be quite compatible with the short bursts of action potentials elicited by sweet stimuli [19]. It would require that the cAMP level is kept low under resting conditions. In taste cells lacking α-gustducin, from the gene knockout mice, the resting cAMP concentration may be unusually large; the response to sucrose, which depends on an increase in cAMP, would be swamped in such cells and sweet taste consequently impaired. It is noteworthy that, in the α-gustducin knockout mice, the sweet taste elicited by non-sugar sweeteners was also impaired [11]. This observation can be reconciled with the reported finding that non-sugar sweeteners are transduced *via* $IP_3$ [4] if we postulate that an increase in $IP_3$ will occur only when the cAMP concentration is low, as discussed above in the context of bitter taste transduction.

The finding that a lack of α-gustducin impairs bitter and sweet taste perception has once more drawn attention to the role of cyclic nucleotides in taste transduction. It has indicated a key role for this taste-cell specific G protein, even though the nature of this role is at present not clear. One straightforward hypothesis is that the lack of α-gustducin

**Figure 2**




Transduction of sweet taste. **(a)** The sweet taste of sugar. Receptor occupation leads *via* adenylate cyclase (AC) to the generation of cAMP, which causes closure of $K^+$ channels and hence membrane depolarization; channel closure may be effected through protein kinase A. The depolarization triggers current flow through voltage-gated $Na^+$ and $K^+$ channels, resulting in presynaptic action potentials [15]. It is likely, but has not yet been shown, that the action potentials cause significant $Ca^{2+}$ inflow which, in turn, triggers synaptic exocytosis of neurotransmitters. Breakdown of cAMP is catalyzed by a phosphodiesterase (PDE), probably activated by α-gustducin [7,11]. **(b)** The sweet taste of a non-sugar sweetener. In rat taste cells, the sweeteners saccharin and SC-45647 induce the production of $IP_3$ and the release of $Ca^{2+}$ ions from intracellular stores. The responding receptor cells also respond to sucrose, in the way shown on the left; they do not respond to denatonium [4]. The two non-gustducin G proteins in (a) and (b) are likely to be different.

causes an increase in the resting levels of cyclic nucleotides, which inhibits both bitter and sweet taste transduction. Further experimentation, including the measurement of resting cAMP levels in taste cells of the α-gustducin knock-out mice and the exploration of cross-inhibition between cAMP and $IP_3$ signalling pathways, will be required before the role of α-gustducin can be fully understood.

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