The molecular physiology of taste transduction

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Taste receptor cells use a variety of mechanisms to transduce chemical information into cellular signals. Seven-transmembrane-helix receptors initiate signaling cascades by coupling to G proteins, effector enzymes, second messengers and ion channels. Apical ion channels pass ions, leading to depolarizing and/or hyperpolarizing responses. New insights into the mechanisms of taste sensation have been gained from molecular cloning of the transduction elements, biochemical elucidation of the transduction pathways, and electrophysiological analysis of the function of taste cell ion channels.

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Abbreviations

AC	adenylyl cyclase
ASIC	acid-sensing ion channel
BNaC	brain-type Na^+ channel
cNMP	cyclic nucleotide monophosphate
CV	circumvallate
deg	degenerin channel
dpa	D-phenylalanine taste sensitivity locus
DRASIC	dorsal root acid-sensing ion channel
ENaC	epithelial-type Na^+ channel
GC	guanylyl cyclase
GPCR	G-protein-coupled receptor
IP_3	inositol trisphosphate
MDEG1	mammalian degenerin-1 channel
mGluR	metabotropic glutamate receptor
NCBI	National Center for Biotechnology Information
NPPB	5-nitro-2-(3-phenylpropylamino)-benzoic acid
PDE	phosphodiesterase
PLC	phospholipase C
PROP	6-n-propyl-2-thiouracil
RT–PCR	reverse transcriptase polymerase chain reaction
sac	saccharin taste sensitivity locus
SOA	sucrose octaacetate
TRC	taste receptor cell

Introduction

The sensation of taste is initiated by the interaction of sapid molecules ('tastants') with receptors and ion channels in the apical microvilli of taste receptor cells (TRCs). Some taste transduction pathways convert chemical information into a cellular second-messenger code (e.g. cyclic nucleotide monophosphates [cNMPs] and inositol trisphosphate [IP_3]); these messengers are typically part of a signaling cascade that leads to TRC depolarization and Ca^{2+} release. In other cases, the tastant itself may constitute all or part of the initial cellular signal (e.g. Na^+, K^+, H^+). The structural and chemical diversity of tastants necessitates multiple transduction mechanisms. This contrasts with the senses of vision and olfaction where one general type of stimulus (photons or small volatile molecules) is transduced in each sensory system by one basic mechanism.

This review focuses on recent molecular-biological, biochemical and electrophysiological studies of vertebrate taste transduction components and pathways. Additional background is provided by earlier reviews of this topic [1–3] and of chemosensory responses of invertebrates [4].

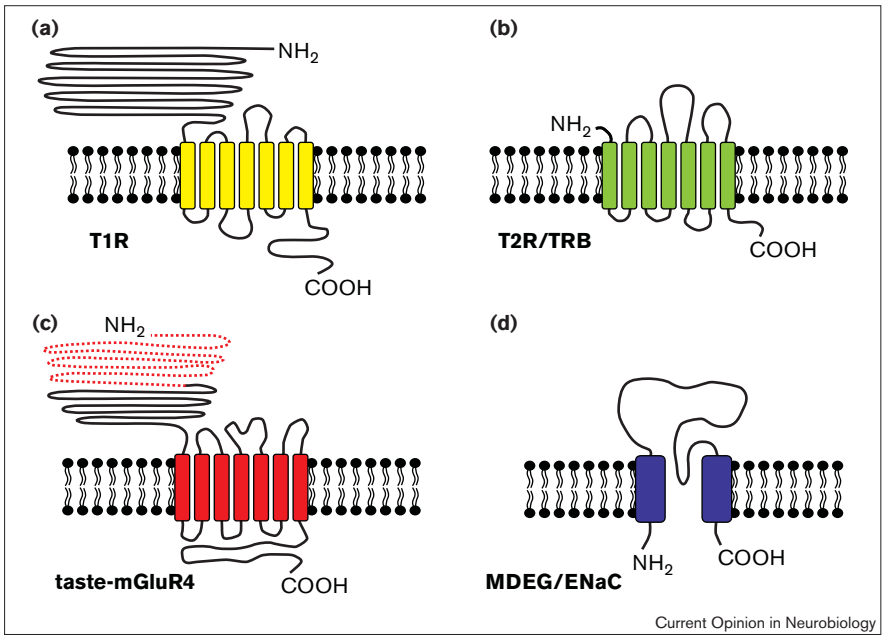
Peripheral anatomy of the taste system

TRCs are modified epithelial cells, but with many neuronal properties (e.g. the ability to depolarize) and neuronal markers. Based on light- and electron-microscopy, as well as molecular characterization, TRCs can be categorized as light, dark or intermediate types; all three types of TRCs are electrically excitable and form synapses with afferent gustatory nerve fibers. Taste buds are focal collections of ~100 TRCs, clustered within onion-shaped structures. The taste buds of the tongue (lingual buds) are found within three types of papillae: fungiform, foliate and vallate, found, respectively, at the front, sides and rear of the tongue. Human fungiform papillae each typically contain 3–5 taste buds at the top of a mushroom-shaped structure; foliate and vallate papillae contain many hundreds of taste buds lining the walls of troughs. Taste buds are also found in the soft palate, uvula, epiglottis, pharynx, larynx and esophagus. Innervation of taste buds is by the glossopharyngeal (IX) nerve (posterior tongue), the chorda tympani branch of the facial (VII) nerve (anterior tongue), and the vagal (X) nerve (laryngeal buds).

Transduction versus coding

Although central taste coding depends fundamentally on inputs from TRCs via afferent nerve fibers, the information encoded as taste 'quality' depends on comparative 'across-fiber' patterns [5]. Individual taste nerve fibers may be classified as, for example, 'sweet-best' or 'salt-best', yet these fibers typically respond to multiple types of tastants and in some cases to tactile and temperature stimuli. The four or five primary taste qualities (sweet, sour, salty, bitter and umami [glutamate]) are perceptual and psychophysical descriptors such as are red and green. Just as the perception of red and green depends on red-sensitive and green-sensitive cone opsins, but the perceptual code is disrupted by the loss of either the red or green opsin, so too can we infer that the perception of bitter, sweet and other taste qualities depends on coding of input from TRCs expressing 'bitter-sensitive receptors', 'sweet-sensitive receptors' and 'salt-sensitive ion channels'.

Figure 1



Predicted membrane topology of receptors and channels present in taste receptor cells. **(a)** The orphan receptor family, T1R, **(b)** the bitter receptor family, T2R/TRB, and **(c)** the candidate umami receptor, taste-mGluR4 (t-mGluR4) contain seven transmembrane helices, typical of GPCRs. The dotted line in (c) indicates the extended amino terminus of the brain form of mGluR4, which is lacking in t-mGluR4. **(d)** In contrast to the GPCRs, the MDEG/ENaC superfamily of ion channels, whose members include ENaC, ASIC, DRASIC and MDEG, are the simplest of ion channels, with only two transmembrane domains. This family of channels has been implicated in both Na^+ salt and acid taste transduction.

Taste transduction elements

Both physiological and molecular biological/biochemical approaches have been used during the past several years to identify candidate taste transduction elements. The progression from candidate status to acceptance as a transduction element requires a confluence of the following types of evidence: a demonstrated presence in TRCs; an involvement in biochemical and/or physiological taste transduction pathways; and the development of genetic or transgenic models followed by physiological and/or biochemical studies. On the basis of other signal and sensory transduction pathways, as well as many years of study of the taste system, it is expected (and in some cases demonstrated) that taste transduction pathways will depend on receptors, G proteins, ion channels, and effector enzymes.

Candidates and orphans

In the absence of corroborating evidence, TRC-expressed ion channels, G proteins and G-protein-coupled receptors (GPCRs) must be considered candidate transduction elements. Among the more intriguing candidates are T1R1 and T1R2 (formerly TR1 and TR2, so-called 'orphan' receptors because they lack known ligands). These two GPCRs are predicted to have a large amino-terminal extracellular domain (see Figure 1). They share 40% identity and are most closely related to the calcium-sensing receptors, V2R-type vomeronasal receptors and metabotropic glutamate receptors (mGluRs) [6•]. T1R1 is preferentially expressed in TRCs of the fungiform papillae and the geschmacksstreifen ('taste stripe'), to a lesser extent in foliate TRCs, and rarely in circumvallate (CV) TRCs. In contrast, T1R2 is commonly expressed in TRCs of the CV and foliate papillae, but rarely in fungiform or geschmacksstreifen TRCs. That T1R1 and T1R2 do not colocalize with gustducin, a G protein expressed in bitter- and sweet-responsive TRCs and implicated in taste transduction (see below), argues against involvement of these receptors in transducing TRC responses to bitter or sweet compounds. The topographic pattern of expression of T1R1 and T1R2 is inconsistent with the known pattern of taste sensitivity of rats, which differs from that of mice (there are no such pronounced regional differences in human taste sensitivity, despite the omnipresent tongue maps in standard textbooks) [7,8]. Other TRC-expressed orphan GPCRs have been described previously (reviewed in [1]).

Transduction mechanisms

Bitter

Gustducin-mediated pathways

Gustducin is a transducin-like G protein selectively expressed in 20–30% of TRCs in palate and all taste papillae [9,10], as well as in apparent chemosensory cells in the gut and vomeronasal organ [11,12]. *In vitro* biochemical assays with crude taste receptors and *in vivo* results from α-gustducin knockout mice have demonstrated clearly that gustducin plays a key role in TRC responses to numerous bitter compounds [13,14]. α-Gustducin knockout mice display markedly reduced behavioral and nerve responses to the bitter compounds denatonium benzoate and quinine sulfate, and to the sweet compounds sucrose and SC45647 (see below; [14]). It has been proposed that α-gustducin activates a taste PDE (phosphodiesterase; reviewed in [1]). Consistent with this proposal, PDE from bovine taste tissue is activated *in vitro* by gustducin and transducin [15]. Several bitter compounds elicit a decrease in cNMP levels in taste tissue that can be selectively blocked by antibodies against

α-gustducin (W Yan, S Rosenzweig, JG Brand, AI Spielman, abstract 320, 22nd Meeting of the Association for Chemoreception Sciences, Sarasota FL, April 2000). The subsequent steps in this transduction pathway are presently unclear (reviewed in [16]): decreased cNMPs may act on protein kinases which in turn may regulate TRC ion channel activity, or cNMP levels may directly regulate the activity of cNMP-gated and cNMP-inhibited ion channels expressed in TRCs (see Figure 2; MS Herness, abstract in *Soc Neurosci Abstr* 1993, 19:1428; K Sugimoto, abstract 120, 12th International Symposium on Olfaction and Taste, San Diego CA, July 1997; [17–20]).

A previously puzzling observation was that many bitter compounds thought to be transduced by the gustducin-mediated pathway lead to pertussis-toxin-sensitive generation of IP_3 [21,22], yet antibodies directed against α-gustducin do not block the IP_3 response (W Yan, S Rosenzweig, JG Brand, AI Spielman, abstract 320, 22nd Meeting of the Association for Chemoreception Sciences, Sarasota FL, April 2000). The resolution comes from two recent studies. First, rat TRCs express phospholipase Cβ2 (PLCβ2), and denatonium-benzoate-stimulated taste receptor activation of this PLC isoform is required for generation of IP_3 in taste tissue [23]. Second, a novel Gγ subunit, Gγ13, has been shown to colocalize absolutely with α-gustducin and Gβ3 [24••]. Gγ13 interacts with α-gustducin *in vitro*, and α-gustducin–Gβ1–Gγ13 heterotrimers are activated by crude taste receptors plus denatonium benzoate. Quench flow experiments, in which rapid sub-second measurements of second messengers can be made, show that Gγ13, like PLCβ2, is required to mediate the IP_3 response to denatonium benzoate. Thus, it appears that heterotrimeric gustducin mediates two responses in TRCs: a decrease in cNMPs via its α-subunit and a rise in IP_3 via its βγ moiety. The subsequent steps in the βγ/PLC/IP_3 pathway are apparently activation of IP_3 receptors and release of Ca^{2+} from internal stores followed by neurotransmitter release [25].

Gustducin-coupled receptors

The long-sought gustducin-coupled taste receptors were cloned recently [26••,27••], and two of these expressed GPCRs were shown to be activated by bitter compounds [28••]. On the basis of genetic mapping of bitter-response loci of humans [26••] and of mice, extended by synteny to humans [27••], two research groups independently searched the NCBI (National Center for Biotechnology Information) DNA sequence databases and identified the same novel multigene family of candidate GPCRs, named T2Rs or TRBs. The T2R/TRB family of GPCRs maps to regions of three human chromosomes (5p15, 12p13 and 7q31) and the syntenic regions of two mouse chromosomes (6 and 15). The proposal that T2R/TRB receptors are bitter-responsive is consistent with the genetic data: 6-n-propyl-2-thiouracil (PROP) sensitivity maps to human 5p15 and 7q31 [29], whereas sucrose octaacetate (SOA) sensitivity maps to the distal region of mouse chromosome 6 (syntenic with human 12p13) [30,31].

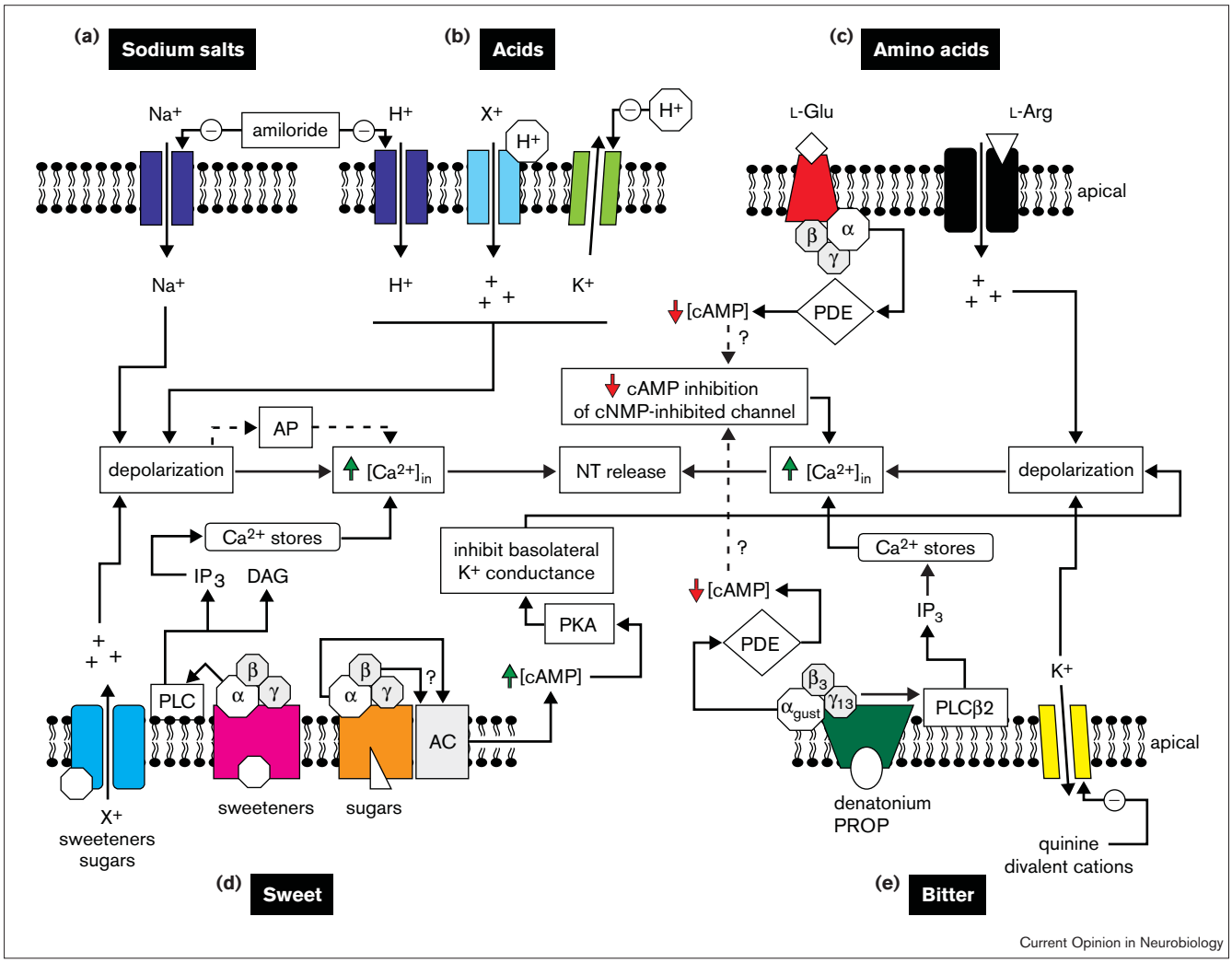
T2R/TRB genes are typically arranged in multigene clusters, with an estimated 40–80 total family members in humans [26••]. T2R/TRB receptors are only distantly related to other known GPCRs (e.g. opsins and V1R vomeronasal receptors). T2R/TRB receptors share 30–70% identity, with the greatest conservation in the three cytoplasmic loops (predicted sites of G-protein interaction) and their adjacent transmembrane segments, and the greatest divergence in the extracellular regions (predicted regions of ligand binding).

The pattern of expression of several T2R/TRB clones has been examined in rat and mouse TRCs [26••,27••]: approximately 15–20% of TRCs in all taste buds of CV, foliate, geschmacksstreifen and epiglottis have been found to be T2R/TRB-positive. In contrast, fewer than 10% of fungiform taste buds contain T2R/TRB-positive TRCs, although in those particular buds approximately 15% of the TRCs are positive. *In situ* hybridization with mixed versus individual T2R/TRB probes has identified largely the same TRCs, suggesting that most or even all T2R/TRB receptors are expressed in the same TRCs. The T2R/TRB receptors are expressed in gustducin-positive TRCs only. About two-thirds of the gustducin-positive TRCs in the CV, foliate or palate are also T2R/TRB-positive; however, most gustducin-positive TRCs in the fungiform papillae are T2R/TRB-negative, suggesting that other receptors may be expressed in these particular cells — for example, the still-elusive sweet taste receptors (see below).

Heterologous expression of chimeric T2R/TRB receptors containing the amino-terminal 39 amino acids of rhodopsin has demonstrated that one mouse taste receptor, mT2R-5, responds only to cycloheximide from among 55 tastants tested. The cycloheximide concentration needed to elicit a half-maximal response in mT2R-5-transfected cells is comparable to the murine threshold for aversion. Five amino acid differences have been noted in mT2R-5 variants isolated from strains of mice that are sensitive (CBA/Ca, BALB/c and C3H/He) versus insensitive (C57BL/6 and 129/Sv) to the taste of cycloheximide. Cells expressing mT2R-5 from the nontaster required an approximately eight-fold higher concentration of cycloheximide to elicit a response, similar to the difference in *in vivo* responses between the taster and nontaster strains. The findings that mT2R-5 selectively couples to α-gustducin versus the other α-subunits, α_i, α_s, α_o, or α_q, and that like other T2R/TRB receptors it is expressed in the α-gustducin-positive TRCs, add support to the proposal that it is a cycloheximide-responsive bitter receptor. Final proof that mT2R-5 encodes the cycloheximide-responsive taste receptor will require generation and testing of the appropriate mouse knockout or transgenic strain.

Taste responses have been obtained from only one other T2R/TRB-transfected cell: a human/mouse orthologous pair, hT2R-4/mT2R-8 apparently encodes a denatonium-/PROP-responsive receptor. However, the concentration of denatonium required is more than three orders of magnitude higher than the human threshold for detection.

Figure 2



Proposed transduction mechanisms in vertebrate taste receptor cells. All taste pathways are proposed to converge on common elements (center of diagram) that mediate a rise in intracellular Ca^{2+} ($[Ca^{2+}]_{in}$) followed by neurotransmitter (NT) release. **(a)** Sodium salts depolarize taste cells directly via Na^+ influx through amiloride-sensitive ENaC (dark blue). **(b)** Acids, in the form of protons (H^+), also permeate ENaC, activate H^+-activated cation (X^+) channels (MDEG and, perhaps, ASIC [pale blue]) and inhibit apical K^+ channels (light green). **(c)** L-glutamate (L-Glu), which elicits umami taste, activates the taste form of mGluR4 (t-mGluR4; red) (see Figure 1), a GPCR that decreases cAMP levels via PDE activation. The decrease in cAMP may disinhibit cNMP-inhibited channels to elevate $[Ca^{2+}]_{in}$. Other amino acids, such as arginine (L-Arg), activate ionotropic glutamate receptors (black), causing TRC depolarization. **(d)** Artificial sweeteners activate both ionotropic receptors (cyan) linked to cation channels, and GPCRs (magenta) linked via PLC to IP_3 production and release of Ca^{2+} from intracellular stores. Natural sugars apparently activate GPCRs (orange) linked via AC to cAMP production which, in turn, may inhibit basolateral K^+ channels through phosphorylation by cAMP-activated protein kinase A (PKA). **(e)** Bitter compounds, such as denatonium and PROP, activate particular T2R/TRB isoforms (dark green), which activate gustducin heterotrimers. Activated α-gustducin stimulates PDE to hydrolyze cAMP, whereas βγ subunits (e.g. $\beta_3\gamma_{13}$) released from activated α-gustducin activate PLCβ2 to generate IP_3, which leads to release of Ca^{2+} from internal stores. Other bitter compounds, including quinine and divalent cations, have been demonstrated to inhibit apical K^+ channels (yellow) in some species. AP, action potentials; DAG, diacylglycerol. See text for additional details.

Other pathways

Compared to wild-type mice, the responses of α-gustducin-knockout mice to bitter (and sweet) compounds are reduced but not totally abolished, suggesting that other G proteins and/or pathways may also be involved [14]. Transgenic expression of a dominant-negative form of α-gustducin from the gustducin promoter further decreases the residual responses of α-gustducin-knockout mice, consistent with their mediation by another G protein expressed in the gustducin lineage of TRCs (GT Wong, L Ruiz-Avila, RF Margolskee, abstract 59, 21st Meeting of the Association for Chemoreception Sciences, Sarasota FL, April 1999). $G\alpha_{i-3}$, $G\alpha_{14}$, $G\alpha_{15}$, $G\alpha_q$, $G\alpha_s$ and α-transducin are possible candidates to mediate these responses because they are expressed in TRCs [9,15,32].

Quench flow studies with murine taste tissue indicate that caffeine and theophylline inhibit PDE to raise TRC cGMP levels [33•]. Soluble guanylyl cyclase (GC) is the presumed source of the cGMP; both GC and nitric oxide synthase (NOS) are known to be present in TRCs [34,35]. Other bitter compounds are known to block K^+ channels and to cause TRC depolarization [1–3]. Bullfrog TRC membrane patches contain a cation channel that is apparently gated directly by quinine and denatonium benzoate and other compounds that humans find bitter [36]. Mudpuppy (*Necturus maculosus*) TRCs respond to denatonium benzoate with an increase in intracellular Ca^{2+}, derived predominantly from internal stores [37]. This response is inhibited by thapsigargin, GDPβS and a PLC inhibitor, but is not affected by IBMX (3-isobutyl-1-methylxanthine), membrane permeant cNMPs, or pertussis toxin, arguing against involvement of gustducin, transducin, or G_i. Dextromethorphan also releases Ca^{2+} from mudpuppy TRCs, but this response is independent of G proteins and is not blocked by the PLC inhibitor, suggesting a direct action on Ca^{2+} stores [38]. Dextromethorphan also blocks K^+, Na^+ and Ca^{2+} currents. The various pathways that mediate bitter transduction are presented in Figure 2.

Sweet

GPCR-mediated pathways

Current working models implicate adenylyl cyclase (AC)-generated cAMP and PLCβ2-generated IP_3 as second messengers in sweet transduction (see Figure 2; for reviews, see [1–3]). Biochemical studies with taste tissue have shown that sucrose causes a GTP-dependent elevation in cAMP, whereas certain artificial sweeteners lead to the generation of IP_3 [1–3]. In rat CV TRCs, sucrose leads to Ca^{2+} influx, whereas the artificial sweeteners saccharin and SC45647 elevate Ca^{2+} via release from internal stores [25]. The cAMP generated by sucrose and other sweeteners has been proposed to depolarize TRCs via protein kinase A (PKA) phosphorylation and closure of apical K^+ channels, or via cation influx through cNMP gated channels. Electrophysiological and Ca^{2+} imaging studies have shown that hamster and rat TRCs respond to multiple sweeteners (for reviews, see [1–3]); there is apparent segregation of cell types because sweet-responsive TRCs do not also respond to bitter stimuli [25].

Gustducin may be involved in both sweet and bitter responses because α-gustducin-knockout mice display diminished behavioral and electrophysiological responses to sucrose and SC45647. In contrast to the robust activation of gustducin by bitter-stimulated taste receptors, neither sucrose nor artificial sweeteners activate gustducin in the presence of taste-receptor-containing membranes [13,15]. However, a number of artificial sweeteners competitively inhibit bitter-receptor activation of gustducin suggesting that these compounds may act as bitter antagonists [39]. It has been noted that quinine suppresses chorda tympani nerve responses to sucrose [40], but the potential of artificial sweeteners to inhibit nerve responses to bitter has not been investigated.

Sweet response loci

Although gustducin, G_s, AC and PLCβ2 are potential components of the sweet transduction pathways, the sweet-responsive receptors have not been purified or cloned. A genomics-based approach such as has been used to clone the mammalian T2R/TRB receptors may yield the elusive sweet taste receptors. The *sac* and *dpa* loci in mouse are major contributors to differences between sweet-sensitive and sweet-insensitive strains [31]. The *sac* locus maps to the distal arm of mouse chromosome 4, whereas *dpa* maps to the proximal arm of chromosome 4 [41,42]. Although the candidate taste receptor T1R1 locus maps to distal 4, it is sufficiently distant from *sac* (~5 centiMorgans) such that the receptor cannot be coded by *sac* (X Li *et al.*, abstract 112, 22nd Meeting of the Association for Chemoreception Sciences, Sarasota FL, April 2000).

Sodium salts

Though several mechanisms have been proposed for the transduction of Na^+ salts, the prototypical salty stimuli, the only molecular components that have been identified unequivocally as contributing to this transduction mechanism are the family of epithelial-type sodium channels (ENaCs). Over the past decade, evidence from numerous studies has demonstrated that these channels are present in TRCs and contribute to the detection of Na^+ salts via an influx of Na^+ ions that leads directly to taste cell activation (see Figure 2). Electrophysiological recordings from single taste cells, lingual epithelial preparations, gustatory afferent nerve fibers and central gustatory neurons are all consistent with amiloride-sensitive pathways contributing to the detection of Na^+ salts (for reviews, see [1–3]). Functionally, the ENaC found in taste cells is similar to that reported in other Na^+-transporting epithelia. These channels have a small unitary conductance (~ 5 pS) [43], are inhibited by amiloride in the submicromolar range (EC_{50} ~0.1 μM) [44–46], display self-inhibition [47] and are regulated by natriferic hormones [48,49•].

In most tissues, ENaC comprises three homologous subunits: α, β and γ (see Figure 1). Whereas amiloride-sensitive Na^+ currents may be produced by expression of α-ENaC alone, coexpression with β- and γ-ENaC enhances these currents more than 100-fold [50,51]. Recently, the use of specific antibodies has shown that taste buds from all three lingual papillae contain α-, β- and γ-ENaC subunits [49•,52•]. These studies may help to resolve an on-going controversy in salt taste transduction. Immunocytochemical and *in situ* hybridization studies demonstrate that rat CV TRCs contain α-ENaC [53,54], yet electrophysiological studies fail to demonstrate any amiloride sensitivity of the salt responses in these TRCs [46,55,56]. Immunocytochemical and molecular (reverse transcriptase PCR [RT–PCR]) approaches have shown that whereas the α-ENaC subunit is expressed similarly in the anterior and posterior tongue, the β and γ subunits are expressed to a much greater extent in the fungiform than in the CV papillae [49•,52•]. The relative expression of the β- and γ-ENaC subunits is enhanced in TRCs from all lingual

papillae by aldosterone, leading to an increase in the amplitude of the amiloride-sensitive Na^+ current [49•]. The level of ENaC expression may also account for differences in the amiloride-sensitivity of salt responses in different mouse strains [57].

Acids

Proton (H^+) concentration is directly related to the sensation of sour taste in humans. Because many ion channels, transport proteins, and intracellular signaling components are pH-sensitive, it might be expected that acidic stimuli act through a wide range of transduction mechanisms. This is apparently the case, as acids have been shown in TRCs to block ion channels, permeate ion channels, activate ion channels, alter transporter function and change intracellular pH (see Figure 2; [1–3]). However, despite the variety of effects and potential targets, until recently there has been little success at identifying the molecular components of the acid transduction machinery.

Interestingly, similar to Na^+ salt transduction, members of the degenerin (deg)/ENaC superfamily of ion channels [58] apparently play a role in acid transduction. Several years ago, depolarization of taste cells by H^+ permeation of ENaC was shown to contribute to acid taste transduction under conditions of low mucosal Na^+ [59,60], as is the case in hamster. Recently, other members of the deg/ENaC family have been implicated in acid sensing in taste cells. The three identified members of this subfamily, MDEG1 (mammalian degenerin-1 channel, also called BNaC1 [brain-type Na^+ channel-1]), ASIC (acid-sensing ion channel, also referred to as BNaC2) and DRASIC (dorsal root acid-sensing ion channel) all function as cation channels that are activated by H^+ and are sensitive to the diuretic amiloride [58]. Thus, acid-stimulated activation of these channels would be predicted to lead to depolarization (from resting potential) in cells in which they are present.

As a result of its biophysical and pharmacological properties, the original report detailing the cloning and functional expression of ASICs [61] suggested that they may be involved in mediating TRC responses to acids. The attempts to date to identify ASICs in taste tissue have been equivocal. Kretz *et al.* [52•] failed to find PCR products corresponding to either ASIC or MDEG using specific primers. However, in a recent preliminary report (L Liu, SA Simon, abstract in *Soc Neurosci Abstr* 1999, 25:2183), RT–PCR was used to demonstrate that rat fungiform papillae TRCs lack both ASIC-α and DRASIC, but contain the acid-activated vanilloid receptor (VR1) and ASIC-β (which may play a specialized role in sensory neurons [62]). On the other hand, BNaC1/MDEG1 has been cloned from taste tissue and localized by *in situ* hybridization to both the apical and basolateral membranes of CV papillae TRCs [63]. Moreover, large, rapidly activating amiloride-sensitive acid-induced currents have been recorded following expression of BNaC1 in a heterologous system. Ugawa *et al.* [63] have demonstrated that acetic acid, which is perceived as more intensely sour by humans than HCl, produces larger responses than does HCl at an equivalent pH in *Xenopus* oocytes expressing BNaC1. Thus, BNaC1 has been implicated as a candidate transduction element in acid taste.

Recently, acidic stimuli have also been suggested to activate an NPPB (5-nitro-2-[3-phenylpropylamino]-benzoic acid)-sensitive conductance in mouse taste cells [64]. On the basis of the NPPB-sensitivity of the acid response, the authors concluded that this was reflective of a chloride conductance. However, the reversal potential of the acid-induced currents did not match the chloride equilibrium potential in this study, suggesting that there may be multiple mechanisms for acid responses or that NPPB may be affecting other channel types. Consistent with the latter interpretation, a preliminary report has shown that acids induce currents in rat taste cells that are similar to currents through ASICs (W Lin, T Ogura, SC Kinnamon, abstract 312, 22nd Meeting of the Association for Chemoreception Sciences, Sarasota FL, April 2000). These currents are carried by cations, primarily Na^+, are not dependent upon Cl^- and are partially inhibited by NPPB. Clearly, more research will be needed to clarify the transduction elements involved in acid sensing in taste cells.

Amino acids: umami

Umami is a fifth basic taste quality primarily stimulated by L-glutamate, typically in the form of monosodium glutamate in the diet [65]. In contrast to acids, which operate through a number of transduction pathways, the response to L-glutamate is probably much more limited in its mechanism of action. In TRCs, L-glutamate has been shown to have two modes of action, acting at both ionotropic and metabotropic (G-protein-coupled) glutamate receptors [66]. The picture that is emerging suggests that the ionotropic (i.e. non-NMDA) glutamate receptors represent synaptic receptors or autoreceptors [67•], whereas a metabotropic glutamate receptor (mGluR) mediates the umami taste sensation [68]. Patch clamp recordings are consistent with there being two types of glutamate responses. Rat taste cells exhibit both depolarizations (presumably via ionotropic receptors) and hyperpolarizations (via mGluR activation) to glutamate [69•]. The latter response is mimicked by the glutamate analog NMDA and the mGluR agonist, L-AP4 (L-2-amino-4-phosphonobutyric acid).

Chaudhari *et al.* [70••] have recently identified a unique variant of a subtype of mGluR that has all the properties of the umami receptor. The truncated form of mGluR4 (called taste-mGluR4; see Figure 1) is expressed in taste cells and binds glutamate in the millimolar range, consistent with concentrations known to elicit the sensation of umami. This receptor is activated by L-AP4, which elicits a taste similar to L-glutamate in rats, and is potentiated by 5′-ribonucleotides — another hallmark of the umami response. Furthermore, ligand binding studies have demonstrated that peptides that elicit the umami sensation

bind effectively to heterologously expressed mGluR4, whereas those peptides that do not elicit the same taste in humans bind less well [71]. The activation of the taste-mGluR4 leads to decreased production of cAMP [70••]. The effect the net decrease in cAMP has in taste cells is presently unclear, although interactions downstream with a cyclic-nucleotide-gated channel have been hypothesized (see Figure 2) [65]. The final proof of taste-mGluR4's role in umami taste sensation awaits the generation of a knock-out mouse specifically lacking the taste glutamate receptor isoform; however, earlier studies of mGluR4-knockout mice have been complicated by the broad distribution of these receptors.

Conclusions

Recent efforts in molecular cloning have identified a number of candidate taste transduction elements. Studies combining molecular biology, biochemistry, and electrophysiology have characterized the function in TRCs of these candidate molecules and have provided new insights into taste transduction pathways. We anticipate that this is just a tempting appetizer of the post-genomic functional studies to come in the new millennium.

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- • of special interest
- •• of outstanding interest

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