HUMAN TASTE RECEPTORS

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Abstract

Taste research has made large progress during the last decade. This article summarises the recent developments in the field of gustation with particular emphasis on those aspects that have attracted greatest attention, i.e., the molecular biology of taste receptors, taste genetics and the functional organisation of taste buds.

Functional organisation of the taste bud

In order to produce a perception, taste stimuli must interact with oral taste receptor cells. These specialised epithelial cells assemble with other cell types into groups of ~50 to 100 cells, called taste bud (1). Several thousand of these small organs are embedded in the oral epithelium, yet on the tongue taste buds are organised in fungiform, foliate, or circumvallate papillae. Taste buds are innervated by afferent nerve fibers that convey gustatory information to the brain. Depending on their localisation on the anterior tongue, posterior tongue, and pharynx, taste buds are innervated by the 7th, 9th, and 10th cranial nerves, respectively. Tastants interact with taste receptor cells in the pore region that they form with their apical aspects, whereas their basolateral parts are shielded from external stimuli by tight junctions.

We can distinguish two principle cell types engaged in taste signaling. One has been referred to as type II cells (2,2a,3). Morphologically, this cell type is characterised by a large, electron-lucent nucleus and several short apical microvilli. Functionally, type II cells are characterised by the receptor proteins they contain. In a mutually exclusive manner type II cells express the receptors for sweet, bitter or umami compounds (4,4a,5); thus, they form at least three different subpopulations dedicated exclusively to the detection of sweet compounds, amino acids/ribonucleotides, or bitter substances. Evidence has been presented showing that receptor cells dedicated to bitter detection are not functionally equivalent but form a heterogeneous population of cells (6). This is due to the fact that they express, on average, only seven out of 25 bitter taste receptors. It remains to be elucidated whether this property of bitter taste cells allows us to discriminate among bitter stimuli. Type II cells also express a variety of downstream signaling molecules, including the γ13 subunit of heterotrimeric G proteins, the G protein subunit α-gustducin, phospholipase C-β2, type III inositol trisphosphate receptor, transient receptor potential channel M5 (Trpm5), pannexin 6 and connexin 30 (7). They lack however, voltage-gated calcium channels and other proteins engaged in synaptic release of neurotransmitter (3,8). In accordance with these observations, type II cells can only be excited by tastants but not by depolarisation with potassium chloride. Moreover, although type II cells are in close proximity to the afferent nerves they cannot form synapses. When exited, they release the neurotransmitter ATP through
so called hemichannels (9,10). Evidence shows that gustatory neurotransmission requires ionotropic P2X2/P2X3 ATP receptors be present on the afferent nerves (11).

The other principle type of taste signaling cells is the type III cell (2,2a,3). Morphological characteristics of type III cells are an elongated invaginated nucleus and a single large microvillus. These cells express a number of neuronal marker proteins such as neuronal cell adhesion molecule, protein gene product 9.5, synaptosome associated protein 25, and glutamate decarboxylase. Type III cells form synapses with the afferent nerves indicating that excitation of these cells induces gustatory nerve transmission. Type III cells cannot be activated by sweet, bitter or umami stimuli, they are, however, activated by potassium depolarization or the transmitter compound ATP. Functional and histochemical experiments indicate that activated type III cells release serotonin as transmitter substance (9). Type III cells also express the polycystic kidney disease gene 1L3 (PKD1L3) and the polycystic kidney disease gene 2L1 (PKD2L1) that encode transient receptor potential channels thought to act as heterodimers (7,12-14). Mice genetically ablated for PKD2L1 cells selectively lost their sensitivity to sour stimuli arguing that PKD2L1-expressing cells are dedicated to detecting acidic stimuli (14). Moreover, data from functional expression studies with PKD1L3/PKD2L1 in heterologous cells proposed, but by far did not prove, that these channels may be part of a sour transduction mechanism (13).

A population of cells dedicated to salt taste detection has not been precisely identified. However, electrophysiological data suggest that salt sensitive cells lack voltage-gated calcium channels and therefore do not belong to the population of type III cells (15). Based on their electrical response profile it has been proposed that the salt receptor cells belong to so called type I cells, a population of cells originally been thought of as supportive cells for the taste signaling cells (16).

The cellular organization of the taste bud raises questions about how taste stimulation is coupled to nerve excitation and how taste qualities are encoded. Originally, researchers assumed that the taste buds function unidirectionally, i.e. signal detection at the apical side is linked to neurotransmitter release at the basolateral side. Now, we learn that these small organs not only detect but also likely process gustatory information (2). Stimulation of type II cells by tastants could possibly be propagated to excitation of the afferent nerves via two pathways. In one, type II cells would directly stimulate afferent nerves through P2X2/P2X3 receptors. The other pathway would involve activation of type III cells through type II cells and the type III cells would finally excite the afferent fibers. At present, experimental data argue in support of both pathways. Future work will reveal the details of taste bud function and the rules that govern the innervation of taste buds.

Two hypotheses have been put forward to solve the longstanding question of how taste qualities are encoded (17). The labeled line theory proposes that the basic taste qualities are conveyed to the cerebral cortex by separate pathways, i.e. taste cells in the periphery and central gustatory neurons are dedicated to only one quality. In other words, these cells would respond either to sweet, umami, bitter, sour, or salty stimuli but never to two or more stimuli. In marked contrast, the pattern theory assumes that peripheral taste cells and central gustatory neurons respond variably to stimuli across modalities, i.e. they are excited by several different taste stimuli. In the pattern generated by a given taste stimulus, information is contained in excited neurons and in silent neurons alike. The generated patterns have to be decoded by the cerebral cortex. Neurophysiological and behavioural work with gene-targeted animals and data from calcium-imaging experiments using ex vivo taste bud
preparations clearly showed that the type II receptor cells are dedicated to only one taste quality (4,18). However, the latter experiments also demonstrated that presynaptic type III cells, which, as mentioned above, cannot respond directly to sweet, umami, and bitter stimuli, were activated by stimuli of different modalities. This led to the conclusion that type II cells converge on type III cells generating a pattern of activity. This activity pattern of taste bud cells is likely transmitted by a small number of afferent fibers to the gustatory neurons of the nucleus tractus solitarius (19), from where gustatory information is sent to the respective thalamic relay nuclei, and finally to the somatosensory cortex. This is supported by neurophysiological recordings that revealed similar activity patterns also in these regions (17).

Taste bud cells express an impressive number of hormones and/or hormone receptors. The list includes neuropeptide Y, cholecystokinin, glucagon-like peptide 1, and leptin (20-22). Data suggest that these hormones regulate signal processing within the taste buds and allow cross talk among the various taste bud cells. Another very attractive idea is that these hormones adapt taste signaling to metabolic requirements (21,22). This assumption is based on the well-known function of these hormones in appetite regulation or energy balance. For instance, it has been shown that sweet taste cells carry OBRB, the functional form of the leptin receptor and respond to leptin with reduced sensitivity to sweet stimulation (22). Thus, when satiation is reached sweets may be less attractive resulting in reduced consumption.

**Receptors for salt stimuli**

Minerals are essential for body fluid composition, cell physiology, and function of the nervous system. As land living animals, we continually excrete and thereby loose minerals. Salt taste is considered part of a homeostatic system that maintains appropriate mineral levels in the body (23). It functions as a mineral sensor that governs the intake of minerals, in particular sodium, and in this manner compensates for the loss of salts in sweat, urine and feces. Humans have a taste for various salts, but only sodium chloride possesses a pure salty taste. The taste of almost all other mineral ions is associated with numerous other descriptors.

Two salt transduction pathways have been described in rodents. One is sensitive to the drug amiloride and specific for sodium ions. Various lines of evidence suggest that the epithelial sodium channel, ENaC, likely composed of two alpha, one beta, and one gamma subunit, is a *bona fide* rodent “receptor” candidate for tasting sodium ions (23). In humans, we found that alpha, beta, and gamma-ENaC subunits are expressed in taste bud cells (24). We also found the delta-ENaC subunit, for which a gene is missing in the rodent genome, is expressed in human taste buds. This subunit is exclusively seen in taste pores, the site at which taste stimuli contact the receptor cells, whereas the other three subunits are found at the basolateral part of taste bud cells. When alpha-, beta-, gamma-ENaC or delta-, beta-, gamma ENaC were functionally expressed in *Xenopus laevis* oocytes, we found that several substances that enhanced saltiness of sodium chloride solutions in sensory experiments with human subjects increased ENaC-mediated sodium membrane currents in an amiloride-sensitive manner. Together, the data suggest that ENaC may have a role in taste, particularly the delta- subunit. This channel may even play a role in salt taste, although final proof is missing. The data also raise questions about the subunit composition of lingual ENaC. Based on the histochemical staining pattern of the delta-subunit that resembles that of tight junction proteins (25), it may also be that the delta subunit either alone or in association with other proteins allows sodium
ions to access the intragemmal fluid, while alpha-, beta, and gamma ENaC could mediate sodium influx though the basolateral membrane of taste cells. Future work will reveal these details.

The other salt transduction pathway is insensitive to amiloride and not specific for sodium ions. This transduction pathway is sensitive to cetylpyridinium chloride (26). It has been proposed that a variant of the transient receptor potential channel V1 is involved in this pathway (27). In humans, such a pathway has not been identified yet.

**Sour transduction**

Sour taste detects acids. Strong sourness is aversive and potentially associated with the rejection of unripe or spoiled food. Mild sourness, however, is considered to be interesting, fully consistent with the fact that most of our beverages are slightly acidic (23). Sourness is only loosely correlated to the pH of the extracellular fluid (2, 28). This is evident, for instance, from the observation that the strong inorganic acid, HCl, elicits almost no sour taste at pH 2, whereas weak organic acid such as acetic acid or citric acid taste already sour at higher pH values. Sourness correlates much better to the concentration of the protonated acids. Recently, Lyall et al. showed that intracellular acidification is the proximate sour stimulus (29). The undissociated organic acids readily diffuse through the plasma membrane into the cytosol and then dissociate leading to intracellular acidification. At high concentrations also protons permeate to limited extent through ion channels or transport systems into the cytosol explaining the slight sourness of inorganic acids at very low pH (2). Recently, Roper and colleagues showed that presynaptic type III taste cells responded with calcium influx and release of the neurotransmitter serotonin to exposure with acetic acid (30). In line with the finding that intracellular acidification is the proximate sour stimulus these authors further demonstrated that the response amplitudes were correlated with concentration changes of acetic acid at constant pH but not with acetic acid at a fixed concentration titrated to various pH values.

In the past, a number of transmembrane ion channels have been proposed to function as the sour sensors based on their gating properties by extracellular protons (2). The challenge now is to verify or falsify these candidates by proving or disproving that they are gated by intracellular acidification. Evidence for gating by intracellular protons is also missing for the PKD1L3-PKD2L1 channel that is expressed in taste cells dedicated to sour detection. Other candidates for a sour receptor are the two-pore domain potassium channels. Some of these channels are sensitive to intracellular acidification and are expressed in taste cells (31). However, their specific role in (sour) taste transduction still needs to be demonstrated.

**Sweet and umami taste receptors**

Both of these tastes are considered to function as detectors of nutritive calories in form of carbohydrates or meat (32). Consistent with this conjecture sweet taste is stimulated by mono- and disaccharides that may be released in situ by lingual amylase. However, a number of other substances taste sweet as well including but not limited to the amino acids L-glycine, L-alanine, and D-tryptophane, aliphatic alcohols such as glycerol, sorbitol, and xylitol, secondary plant metabolites such as stevioside, proteins of tropical plants such as monellin or thaumatin, the synthetic sulfonlamide sweeteners saccharin and acesulfame-K, synthetic peptide sweeteners such as aspartame or alitame, metal salts of lead and beryllium, whereas
umami taste is elicited by amino acids and ribonucleotides. Sweet and umami taste are associated with attraction and promote ingestion of the respective food.

Three genes play a major role in umami and sweet taste, referred to as TAS1R1, TAS1R2 and TAS1R3 (4). The encoded polypeptides, also referred to as T1R1, T1R2, and T1R3, belong to the class C of G protein-coupled receptors characterised by large N-terminal ectodomains. Evidence suggests that they dimerize to form functional taste receptors. In heterologous expression assays T1R1-T1R3 responds to various L-amino acids (for the mouse form) and to L-glutamate (for the human form), the responses being enhanced by the simultaneous presence of ribonucleotides. This property makes this dimer a strong umami receptor candidate, although it should be mentioned that other glutamate receptors have also been implicated in the umami response.

Strong evidence from experiments with gene-targeted mice, heterologous expression assays, and human sensory studies, indicates that T1R2-T1R3 functions as a general sweet taste receptor. Numerous compounds known to taste sweet activate this heterodimer in-vitro (33). In addition, the T1R2-T1R2 and T1R3-T1R3 homodimers have been suggested to function as sweet taste receptors for perillartine or high concentrations of sucrose [Jay Slack, personal communication]. In fish, T1Rs have only been activated by amino acids but not by sugars (34) suggesting that these receptors originally functioned as amino acid sensors. Only later in evolution the T1R2 gene developed to encode sensors for sugars.

An important question that emerged is how sweet compounds bind to and activate their cognate receptor. Based on truncated receptor subunits that have been functionally expressed and analyzed it has been concluded that the T1R1 and T1R2 subunits of the dimeric sweet or umami receptors signal through G proteins. In addition, various efforts have been made to investigate how sweeteners interact with T1R2-T1R3 including biochemical and biophysical analysis of the recombinantly produced N-terminal ectodomains, functional expression of interspecies mixtures of T1R2 and T1R3 subunits, mutational analysis of heterologously expressed subunits and molecular modeling (33). The data that emerged from these studies indicated that the T1R2-T1R3 heterodimer contains several binding sites for sweeteners explaining the ability of mammals to sense so many structurally different sweet tasting chemicals with only one receptor (33). Based on homologies to other class C GPCRs, the ectodomains of T1Rs have been shown to form so called venus fly trap binding motifs. It appears that glucose, sucrose and the halogenated sucrose derivative sucralose binds to or at these motifs in both subunits, while the peptide sweeteners aspartame and neotame have been shown to bind the venus fly trap motif of T1R2 only. Brazzein, an intensely sweet tasting protein, binds to a cysteine-rich region in T1R3 that connects the ectodomain to the heptahelical part of the receptor subunit. Finally, the sweet substances cyclamate and neohesperidin dihydrochalcone bind to sites formed within the transmembrane segments of T1R3. Interestingly, the sweet taste inhibitor lactisole also binds to this site. Mutational analyses clearly showed that the three chemicals interacted with an overlapping set of amino acids of T1R3’s transmembrane domains. Consistent with this finding is the observation that lactisole inhibited T1R2-T1R3 activation by cyclamate or neohesperidin dihydrochalcone competitively, whereas it allosterically blocked T1R2-T1R3 activation by aspartame, a compound that binds, as mentioned above, to the venus fly trap motif of T1R2.
Bitter taste receptor genes

Bitter compounds are even more numerous and more chemically diverse than sweet molecules. Estimates go into thousands (35). Usually, bitter substances elicit aversion leading to rejection of bitter food. It has been argued that bitter taste prevents us from ingesting potentially harmful or poisonous compounds. In fact, many bitter compounds, including food-borne substances, are toxic, although a relation of bitterness and toxicity has not been established. Moreover, certain toxins, such as α-amanitin, do not taste bitter at all, whereas some compounds, such as salicin, a compound that has successfully been used in human medicine for several thousand years, exhibit an offending bitter taste. Bitter taste is innate and the rejection response is particularly prominent in neonates and children, i.e. at times when our other defense systems are not fully developed. Later in life with aging, we accept and even appreciate a moderate bitterness of some compounds in our food and beverages. The reasons for and the mechanisms underlying this change are unknown. However, it is not correlated to altered bitterness sensitivity measured as threshold values of recognizing bitter compounds (36). It could be that the reduced bitterness rejection reflects only learned eating behaviours. Alternatively, in light of the fact that plants use bitter compounds as protective agents against infections, oxidative stress, etc., it could also be that uptake of certain bitter chemicals with our food transfers chemical protection to our own bodies – particularly at ages when we are beginning to suffer again from greater vulnerability. The fact that health beneficial effects have been assigned to various bitter compounds supports this assumption.

In vertebrates, bitter compounds are detected by means of a family of specific receptors, the TAS2Rs. However, the number of genes in different species varies enormously. In the chicken genome, researchers found only three TAS2R genes, whereas they surprisingly discovered 49 genes in frogs. These findings are difficult to explain. Possibly chicken feed very selectively on few non-toxic items making a defense system based on the bitterness of fed-contained chemicals superfluous. Frogs which predominantly feed on insects are apparently exposed to astonishingly numerous bitter toxins. Insects may frequently use bitter chemicals as weapons against predators or for hunting prey. Another property of TAS2R genes is that they fall into subgroups (37). One subgroup shows a clear one-to-one orthology among species as most other genes do. The other displays lineage-specific expansions. It has been argued that the former allows animals to detect chemicals present in common food whereas the latter allows recognition of potentially harmful substances in food encountered specifically by only one species.

Lessons from functional expression of TAS2R genes

The human genome contains 25 TAS2R genes at four loci on chromosomes 5, 7, and 12 (35). Based on a lack of sequence conservation with other G protein-coupled receptor genes they encode an own subfamily of G protein-coupled receptors. These receptors are glycosylated at a strictly conserved Asn-linked glycosylation sites in the second extracellular loop (38). During biosynthesis TAS2Rs interact with various chaperones (39). Both phenomena, glycosylation and interaction with auxiliary proteins, are required for functional cell surface expression of TAS2Rs.

For the majority of TAS2Rs cognate compounds have been identified by functional expression of TAS2Rs in heterologous cells (40). Intriguingly, all compounds that activate TAS2Rs taste bitter. This observation suggests that all members of the TAS2R family serve as oral bitter receptors, although they likely
have additional functions based on the observation that the TAS2R genes are expressed at various extra-oral sites. Various TAS2Rs show quite specific agonist-profiles, in a sense that the agonists have certain chemical substructures in common. Prototypical receptors are TAS2R16 and TAS2R38 (41,42). TAS2R16 is activated by β-gluco, and mannopyranosides with small hydrophobic aliphatic or phenolic aglycons. TAS2R16 does, however, not respond to galactosides. Increasing sizes and hydrophilicity of the aglycons renders compounds less potent to stimulate TAS2R16. Good agonists are the above mentioned substance salicin and the toxin of bitter almonds amygdalin. As estimates count some ten thousand plant species that produce toxic glycosides the number of chemicals to which TAS2R16 responds is numerous and could be in the range of hundreds to thousand. Similarly, TAS2R38 is sensitive to numerous chemicals that contain –N=C=S groups. In marked contrast, some TAS2Rs are very broadly tuned to chemicals that do not necessarily share common substructures. For instance, TAS2R14 was activated in heterologous expression systems by a quarter of all bitter compounds tested (43). Another example is given by TAS2R46, a receptor, sensitive to sesquiterpene lactones, clerodane and labdane diterpenoids, strychnine, sucrose octaacetate, and denatonium benzoate (44). The data indicate that the broad tuning of TAS2Rs fully explains the ability of humans to recognize Thousands of compounds as bitter with only 25 receptors. Other mechanisms may not or not substantially contribute.

Bitter taste and nutrition

An important issue that emerges is whether bitter taste sensitivity is related to intake behaviour and eventually with health and disease risk. Previous studies conducted before the TAS2Rs have been cloned were biased by the facts that (i) subjects have only phenotypically been classified based on their tasting abilities and that (ii) only bitterness in general has been studied. Now the challenge is to design studies with defined genotyped cohorts to be tested with specific cognate bitter compounds for known TAS2Rs.

The threshold values of activation of TAS2Rs determined in functional expression assays agreed well with the recognition threshold values for the same compounds determined in sensory studies with human subjects (41,42,45). In some cases the threshold values measured for the recombinant receptor in cell lines were somewhat lower than those recorded in sensory studies. These differences have been explained by the fact that the cellular assays use chimeric G proteins designed for specifically for that purpose (42), whereas TAS2R-G protein-coupling in taste receptor cell membranes involves natural G proteins, including α-gustducin, Gβ1, Gβ3, and Gγ13 (35). From these studies it has been concluded that the biochemical properties of taste receptors determine our sensitivity of tasting bitter compounds.

Above conclusion is further supported by the genetics of bitter taste. Recently, the genetic basis has been uncovered for perceiving the bitterness of –N=C=S compounds, such as phenyl thiocarbamide and propyl thiouracil, or not (46). Three non-synonymous single nucleotide polymorphisms in the TAS2R38 gene define two major haplotypes that are referred to as PAV or AVI depending on the amino acid residues encoded by the affected triplets. When the two variants are expressed in heterologous cells the PAV variant of TAS2R38 was sensitive to low concentrations of compounds with the –N=C=S group, whereas the AVI variant did not respond at all (42). Moreover, subjects homozygous for the PAV haplotype readily taste low concentrations of –N=C=S compounds whereas subjects homozygous for the AVI haplotype are taste "blind" for these compounds. A linkage of genotype, receptor
properties and sensitivity for bitterness has also been demonstrated for the TAS2R43 and TAS2R44 genes and their agonists aloin and saccharin (47). Based on the observation of the pronounced genetic variability among TAS2R genes we might expect that in the future additional genetically determined perceptual differences in the population will be uncovered. Many of the detected SNPs are not equally present in the genomes of the world populations suggesting that genetic variations in the TAS2R genes likely account for ethnic differences in bitter taste sensitivities as well (48).

Above data suggested that TAS2R gene sequences determine the biochemical properties of the encoded receptor, which, in turn, defines the sensitivity of subjects to taste certain bitter compounds. Moreover, they indicate that the sensitivity for bitter compounds varies in the population. This raises the question whether or not the genetically determined perceptual differences in the population cause individual likes or dislikes of food rich in such substances and eventually result in personal eating habits. Due to a lack of data, we do not have the answer yet. However, it is clear that, for instance, in the case of TAS2R38 cognate bitter compounds are present in various frequently consumed legumes in concentrations in the perceptual range of PAV-tasters and out of the perceptual range of AVI-non-tasters (49). Consequently, bitterness scores for edible plants containing –N=C=S compounds segregate according to TAS2R38 genotype of subjects, while bitterness scores for plants not containing –N=C=S compounds do not (50).

To date, we have only circumstantial evidence in support of impact of taste receptors on nutrition and health. One example stems from the molecular evolution of the TAS2R16 gene (51). A mutation in this gene at a site corresponding to the putative agonist binding site occurred in the Paleolithic some 80,000 to 800,000 years ago. The novel allele encodes a receptor with higher potency for certain glycopyranosides, many of which are cyanogenic and therefore highly toxic. This allele shows many signs of positive selection. Therefore, the novel high-potency-allele became rapidly fixed in the genomes of our ancestors and, with the migration of humans out of Africa, distributed all over the planet. In contrast, the ancestral low-potency-variant survived in central Africa with a relatively low frequency of ~ 14%. This observation strongly suggests that carriers of the novel allele could establish healthier diets low in the content of toxic glycosides posing a selective advantage on them allowing them to more effectively reproduce and pass on their genes to the next generations. The ancestral gene variant shows a distribution similar to that of anti-malaria genes. Its survival may be explained by assuming that high threshold values for tasting glycosides, including cyanogenic glycosides, are causing elevated intake of plants containing such compounds, leading to chronic cyan intoxication. The resulting sickle cell anemia protects subjects from deadly Malaria infections. Thus the ancestral allele likely was of advantage for humans living in areas contaminated by mosquitoes infected with Plasmodium falciparum.

Another example that illustrates the connection of taste and nutrition comes from the animal kingdom. All kinds of cats from tigers to our domestic cats are indifferent to sweets. They cannot be trained through the awards of sugar lumps to perform certain tricks or stunts. This correlates to the fact that cats do not possess a functional sweet taste receptor due to various mutations in the gene encoding the T1R2 subunit of the sweet receptor (52). This distinguishes cats from other carnivores, including dogs and bears, which have a functional sweet receptor and are well-known to be attracted by sweets. These pieces of circumstantial evidence are in support of a strong impact of taste on nutrition and health and let us optimistically expect more direct evidence in the near future.
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