Cell. Mol. Life Sci. 63 (2006) 1501–1509
1420-682X/06/131501-9
DOI 10.1007/s00018-006-6113-8

Cellular and Molecular Life Sciences

Bitter taste receptors and human bitter taste perception

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Online First 29 May 2006

Abstract. A vast number of structurally diverse bitter compounds need to be detected by a subfamily of only ~25 human bitter receptors. Failure in detecting them might be lethal, since some naturally occurring bitter compounds, such as strychnine, are very toxic. This review presents an overview about the enormous progress in the field of mammalian bitter taste research with special emphasis on humans, if data were available. It summarizes the current knowledge about the anatomical basis for bitter taste perception, intracellular signal transduction, evolution, expression and polymorphisms of *hTAS2R* genes, and the molecular basis for the recognition of bitter compounds.

Keywords. Bitter taste receptor, gustatory system, G-protein-coupled receptor, single-nucleotide polymorphisms, tongue, sensory physiology.

Introduction

Humans can detect and distinguish the five basic taste qualities salty, sour, sweet, bitter and umami. The sense of taste is very important for the well-being and even survival of an individual, since it provides the last checkpoint before the ingestion of potentially harmful substances. This is especially true for bitter taste, as many toxic plant metabolites taste bitter and, thus, the corresponding receptor molecules serve an indispensable role as warning sensors. On the other hand, a number of moderately bitter beverages and food items are well-accepted by individuals, and therefore considering bitter taste receptors solely as triggers for avoiding behavior is, at least in humans, not justified. This review summarizes our current knowledge on mammalian bitter taste, with special emphasis on human bitter taste. However, as different areas of taste research progressed from different model systems, it is necessary to include the plethora of data obtained in other vertebrate systems.

Anatomical organization of the gustatory system

Within the oral cavity of mammals taste receptor cells (TRCs) reside in several specialized zones, the tongue, part of the palate, epiglottis, larynx and pharynx [1]. TRCs exhibit a rapid turnover; their average lifespan in rat is about 10 days [2, 3]. On the tongue, TRCs are organized into groups of cells, called taste buds. Based on their morphology the cells within a taste bud can be subdivided into four different groups, referred to as type I, II, III and IV cells [4]. More details are presented in the chapter contributed by S. Roper (this issue). Every taste bud consists of a single apical porus where microvilli of TRCs come into contact with tastants present within the oral cavity. On the tongue, taste buds are embedded in three types of epidermal specializations. The fungiform papillae are distributed over the anterior two-thirds of the tongue. The foliate papillae, which are transient in humans as they are well developed at birth but rudimentary in adults, are found on the sides of the posterior one-third of the tongue. Seven to nine circumvallate papillae are located far back on the posterior tongue close to the terminal sulcus [1]. In addition to the 'classical' TRCs organized in taste buds, chemosensory cell clusters or solitary chemosensory cells are found in non-lingual epithelia of endodermal origin. This system of chemosensory cells has been termed the diffuse chemosensory system and shares signal transduction components typical for classical TRCs [5]. Since TRCs are sensory cells of epidermal origin, they require afferent innervation to transmit signals to the brain. Three cranial nerves send fibers to different gustatory structures. The fungiform papillae re-

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ceive afferent input from the chorda tympani. The glossopharyngeal nerve contacts foliate and circumvallate papillae. The greater superficial petrosal branch of cranial nerve VII sends fibers to the palate, whereas epiglottis and larynx are innervated by cranial nerve X. The first relay station for gustatory information is the gustatory division of the nucleus tractus solitarius (NTS). Here, basic somatic acceptance and rejection reflexes in response to gustatory stimulation are thought to be generated [6]. Importantly, parasympathetic reflexes like salivation, and gastric reflexes are controlled via the NTS. From the NTS neurons project towards the ventroposteromedial nucleus of the thalamus and further into the insular-opercular cortex, the primary gustatory cortex and finally the orbitofrontal cortex, the secondary gustatory cortex. It should be noted here that species-specific differences have been observed with regard to their processing of gustatory information. In rodents, gustatory information from the NTS converges on the medial parabrachial nucleus before reaching the thalamic taste area [7].

Because the lifespan of TRCs is restricted to just a few days, the peripheral part of the gustatory system is persistently required to differentiate. During embryonic development signaling molecules form specific patterns, which are thought to establish competency for epithelial cells to develop into papillary structures or suppress this competency in the interplacodal epithelium [8]. Whereas the initial development of fungiform papillae, including the formation of taste buds, is independent of innervation [8, 9], the maintenance of mature, functional fungiform papillae requires communication between gustatory epithelia and nerve afferents [10].

An important constituent of the gustatory system is saliva secreted from different salivary glands resulting in the formation of a specific peri-receptor milieu (for a review see [11]). For the taste qualities sour and salty this peri-receptor milieu has direct consequences, such as the buffering of acids by saliva [12] or the modulation of sodium taste sensitivity by the salivary sodium concentration [13]. For other taste qualities the mechanism for their modulation by saliva is less understood. Especially for bitter substances, which are often rather hydrophobic molecules, an interaction with salivary proteins has been discussed. One such molecule is the von Ebner's gland protein (VEGP), which belongs to a family of lipophilic-ligand carrier proteins [14]. Another group of proteins found in saliva are the proline-rich proteins (PRPs), which have been genetically linked to sensitivity for certain bitter substances in mice [15].

Evolution of hTAS2Rs

Since their identification bitter taste receptor genes have drawn increasing attention from evolutionary biologists, due to the pivotal role of bitter taste receptors as warning sensors against the ingestion of bitter toxic plant metabolites [16]. Therefore, the analysis of the human bitter taste receptor gene repertoire and comparison with the corresponding gene sets in primates and other vertebrates is likely to unravel fascinating details on human evolution, lifestyle, regional individualization and socialization.

The ~25 human *TAS2R* genes are located on chromosomes 5, 7 and 12. Except for *hTAS2R1*, which is localized on chromosome 5, all other *hTAS2R* genes are organized in dense clusters on chromosomes 7 and 12 [17]. Sequence comparison revealed that *hTAS2R* genes located on the same chromosome are more closely related to each other than to *hTAS2Rs* located on different chromosomes. This could be explained by tandem duplication of ancestral genes leading to a diversification of the bitter taste receptor repertoire [18, 19]. The *hTAS2R* containing chromosomal loci exhibit synteny with *TAS2R*-gene-positive regions on mouse chromosomes, indicating that major duplication events occurred prior to the separation of primates and rodents. Based on sequence homology, vertebrate *TAS2R* genes have been divided into a variable number of groups [18–20]. Within these groups, putative orthologs are generally recognizable, although, due to species-specific gene duplications, deviations from a simple one-to-one orthology are common. In fact, Shi and colleagues propose that a one-to-one orthology might indicate that the function of the corresponding *TAS2R* genes is conserved, e.g. the

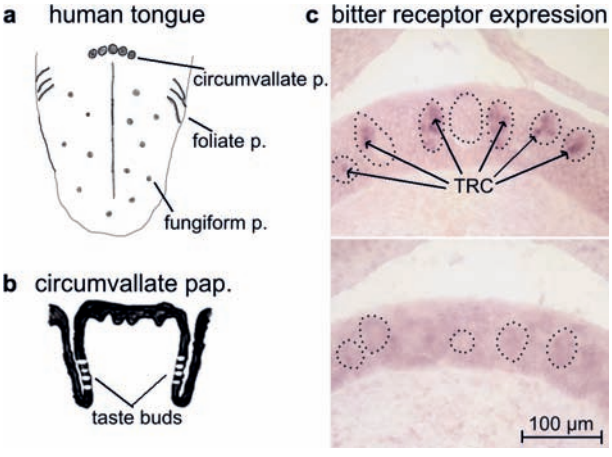

Figure 1. Anatomical organization of bitter taste. (*a*) Scheme of the human tongue showing the localization of fungiform, foliate, and circumvallate papillae. (*b*) Drawing of a cross-section of a circumvallate papillae with taste buds. (*c*) *In situ* hybridization of a cross-section through a human circumvallate papilla. Dark signals (upper panel) demonstrate the expression of the PROP/PTC-receptor hTAS2R38 in a subpopulation of taste receptor cells (TRC). Absence of signals using the sense probe for hTAS2R38 demonstrates the specificity of the hybridization reaction (lower panel). The taste buds are circled. Scale bar, 100 µm.

detection of toxic bitter compounds important for both species. On the other hand, *TAS2R* genes exhibiting a multiple-to-one ratio seem to be products of duplication events which occurred after divergence of the species. As they appear amplified in each species separately, they might reflect important constituents for species-specific adaptation to habitats [19].

The rate of *TAS2R* gene pseudogenization in humans (11 pseudogenes/36 total) is higher than in mice (6 pseudogenes/41 total), and since no orthologous pseudogenes are found in human and mouse, pseudogenization seems to be species-specific [20]. Several studies compared the repertoire of *TAS2R* genes between different primate species [20–23]. It appears that, based on the rate between nonsynonymous to synonymous exchanges, primate *TAS2R* genes generally underlie relaxed selective constraints [20, 21, 23]. Evolutionary constraints on the open reading frames of *TAS2R* genes differ also depending on their location within the polypeptide chain. Nonsynonymous mutations are more frequent within the extracellular loops, whereas synonymous mutations are predominant within the transmembrane domains and intracellular loops [19–21, 23]. This fact indicates that the extracellular loops might be subjected to positive selection because they increase receptor diversification, whereas mutations within intracellular loops and transmembrane domains are under negative selection to maintain the structural integrity of the receptors.

Currently, the discussion about the extent and direction of the evolutionary forces shaping the *hTAS2R* genome is controversial, ranging from relaxed functional constraints [20, 23] to even positive selection for a particular receptor variant [24]. Changes in the environment might result in opposing selective pressure on certain bitter taste receptors. A receptor specific for a group of bitter compounds might be very important in a habitat where poisonous plants containing such substances are abundant or during exploratory phases in an unfamiliar environment. However, many bitter substances encountered in nature are not toxic, and bitterness and toxicity are not always strictly correlated [25]. In this case, a functional receptor could limit the nutritional resources available for that individual and therefore might even be detrimental during periods of limited food supply. Also, changes in nutritional behavior and civilization need to be taken into account [23]. The growing number of reports on non-gustatory expression of *TAS2R* genes [26–28] imply that this gene family has functions beyond gustation, perhaps even beyond chemosensation of xenobiotica.

TAS2R gene expression

Several studies have investigated the expression patterns of different rodent and human *TAS2R* genes in the different taste buds of the oral cavity by *in situ* hybridization of TAS2R messenger RNA (mRNA). In rodents, it has been demonstrated that similar numbers of TAS2R mRNA-positive taste receptor cells (~15%) can be found in taste buds of fungiform, vallate and foliate papillae, palate and epiglottis. However, the fraction of TAS2R mRNA-positive fungiform papillae/taste buds is with less than 10% substantially lower than in the other regions where almost all taste buds contain *TAS2R* gene-expressing cells [26]. *In situ* hybridization experiments using mixtures of probes for different TAS2R mRNAs showed that the number of cells only slightly increased with the number of probes in the mixture (~20%/bud) but the staining intensity increased. This finding argues for an overlapping expression pattern of most *TAS2R* genes within the same subset of cells. For one pair of receptors cellular co-expression has been directly demonstrated by double-labeling *in situ* hybridization [26]. The largely overlapping expression of *TAS2R* genes within individual bitter taste receptor cells has elegantly been proven by the re-introduction of PLCβ2 under the control of three different *TAS2R* gene promoters into bitter taste-deficient PLCβ2 null mice. The use of every single gene-targeting construct completely restored normal bitter taste for all engineered transgenic mouse lines [29].

For humans the data on *hTAS2R* gene expression patterns is less extensive. However, *in situ* hybridization analyses of human circumvallate papillae for *hTAS2R14*, *-16*, *-38*, *-43* and *-44* gene expression [30–33] resulted in the labeling of similar numbers of TRCs with the individual probes. The data suggest the presence of ~20% hTAS2R-mRNA-positive cells per taste bud, thus paralleling the findings in rodents [17]. Since all or most *TAS2R* genes are expressed within the same subset of bitter taste cells, discrimination between different bitter stimuli should be barely possible. Indeed, behavioral studies in rats [34] and rhesus monkeys [35] demonstrated that both species failed to discriminate among a variety of bitter compounds. However, in functional experiments on isolated mouse taste buds contradictory results have been obtained. Here, most TRCs responded only to a single substance of five widely used bitter compounds, indicating that, on a cellular level, discrimination between bitter compounds is possible [36]. It appears that bitter TRCs behave physiologically less uniformly than one would predict from *in situ* hybridization data and therefore provide some discriminatory capacity. The ability to differentiate between bitter stimuli has been observed in glossopharyngeal and chorda tympani neurons of the rat as well, suggesting a conservation of some discriminatory power within first-order neurons [37].

Also non-gustatory tissues express *TAS2R* genes, indicating that these receptors have additional roles apart form taste. Within the respiratory epithelium of the nasal cav-

ity chemosensory cells expressing *TAS2R* genes together with α-gustducin were identified. Intranasal stimulation with bitter compounds led to activation of the trigeminal nerve, resulting in changes of the respiratory rate [27]. Another site of *TAS2R* gene expression is the gastrointestinal tract. By RT-PCR *TAS2R* genes were detected in stomach and duodenum of mice and rats. Along with *TAS2R* gene expression α-gustducin and α-transducin were detected in these tissues, implying the presence of a functional signaling cascade for the detection of bitter substances. Moreover, the enteroendocrine cell line STC-1 shows rapid transient increases of intracellular calcium levels after stimulation with five different bitter compounds, indicating that these cells might represent true sensors for bitter compounds [28]. Both studies dealing with extragustatory expression of *TAS2R* genes, however, failed to detect the complete set of bitter taste receptors expressed in gustatory tissue. This raises the intriguing possibility that only the gustatory system expresses the entire set of *TAS2R* genes to fulfill an exploratory warning function, whereas non-gustatory tissues only express some *TAS2R* genes.

Signal transduction

Nearly a decade before the molecular identification of the receptor molecules involved in the transduction of bitter taste stimuli, the G protein α-subunit α-gustducin was discovered, and its expression was localized to a subset of TRCs [38]. The role of α-gustducin within the signaling cascade evoked by bitter tastants is firmly established by a variety of independent approaches. Whereas genetic ablation of α-gustducin in mice led to impaired responses to stimulation with bitter compounds [39], the transgenic expression of rat α-gustducin fully rescued the bitter tasting ability in α-gustducin null mice [40]. Fine mapping of the carboxy terminus of α-gustducin revealed that the last 37 (44) amino acids are sufficient for the interaction with recombinantly expressed bitter taste receptors [41]. This led to the generation of chimeric Gα subunits that efficiently couple to bitter taste receptors in heterologous expression systems and hence facilitated the functional characterization of those receptors [30, 32, 33, 41]. The direct activation of α-gustducin by recombinantly expressed mouse bitter taste receptor mT2R5 in response to stimulation with its agonist cycloheximide resulted in the incorporation of radiolabeled GTP, providing further proof for the proposed interactions of TAS2Rs with α-gustducin [42].

The observation that genetic ablation of α-gustducin reduces but does not abolish responses of mice challenged with bitter stimuli [40] and the identification of a residual population of bitter-responsive TRCs in taste buds of gustducin null mice [43] suggested that additional G protein α-subunits might supplement α-gustducin's role in bitter taste transduction. Another G protein which might be involved in bitter taste transduction is the gustducin-related rod transducin. Transducin is also expressed in TRCs and its overexpression in α-gustducin null mice leads to a partial rescue of their sensitivity to bitter stimulation [44]. Additionally, it has been shown that bitter taste receptors can functionally couple to transducin *in vivo* [45] as well as other Gi/Go proteins *in vitro* [46].

In addition to α-gustducin, the other subunits of a functional heterotrimeric G protein have been identified too. By differential screening of complementary DNA (cDNA) of single, α-gustducin-positive TRCs, the G protein subunits β1, β3 and γ13 were identified [47]. Consequently, antibodies raised against Gγ13 [47], β1, or β3 [48] suppress the responses of mouse taste tissue to the bitter substance denatonium benzoate. As this blocking effect was more robust using an anti-Gβ3 than an anti-Gβ1 antibody, the predominant G protein composition in bitter TRCs appears to be Gα-gustducin/Gβ3/Gγ13.

Tentatively, TAS2R-mediated activation of heterotrimeric gustducin in bitter TRCs should stimulate phosphodiesterase and phospholipase C (PLC) activities via α-gustducin and the βγ-subunits, respectively. The former should lower cyclic nucleotide levels, while the latter should generate diacyglycerol and inositol trisphosphate (IP_3) as second messenger molecules. Indeed, two phosphodiesterases [49] and PLCβ2 [50] were identified in taste tissue. Subsequently, in mouse taste tissue the α-gustducin-dependent rapid decrease in cyclic AMP concentration after stimulation with bitter substances has been demonstrated [51]. Although the presence of a cyclic-nucleotide-gated channel in taste receptor cells was shown [52], the exact

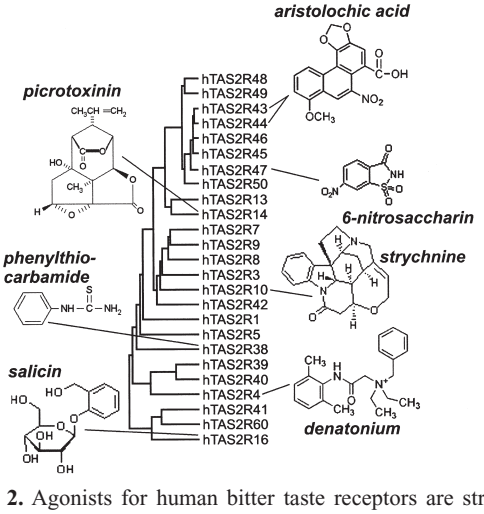

Figure 2. Agonists for human bitter taste receptors are structurally diverse. The peptide sequence relationship of 25 human TAS2R genes is shown by the phylogenetic tree in the center. The selection of bitter compounds that activate certain hTAS2Rs indicates their enormous structural diversity.

role of cyclic-nucleotide signaling in TRCs remains to be determined.

Whereas the physiological consequences of α-gustducin activation remain to be determined, the consequences of β1/3/γ13 activation are well established. They lead *via* PLCβ2 to the generation of IP_3. The type-III IP_3 receptor is expressed in taste receptor cells that are positive for α-gustducin, PLCβ2 and Gγ13 [53], accounting for the concomitant increase in intracellular calcium concentration. Antibodies and inhibitors specific for PLCβ2 blocked the denatonium-stimulated increase in IP_3 [50] or the release of calcium from intracellular stores [54], confirming the importance of PLCβ2 in bitter taste sensation. These findings are confirmed by the genetic ablation of PLCβ2 in mice. PLCβ2 knockout mice lose their responsiveness to bitter stimulation completely [55] or, at least, substantially [56].

Recently, an additional signaling component, the transient receptor potential channel M5 (TRPM5), was identified [57]. *TRPM5* is expressed in the same subset of TRCs of mice that express α-gustducin, Gβ3, Gγ13 and PLCβ2. The activity of this channel is influenced by several parameters, including rapid changes in intracellular calcium ion levels [58–61]. The importance of *TRPM5* was shown by its targeted disruption in mice resulting in the loss of responses to various bitter compounds [55], although an independently generated knockout model demonstrates residual responses to umami, sweet and bitter stimuli, indicating the presence of TRPM5-independent transduction mechanisms [62, 63].

Agonist-receptor interactions

Humans have only ~25 bitter taste receptor genes for the detection of an enormous number of structurally very different bitter compounds [17]. It is therefore necessary that a given receptor responds to more than one substance, implying that the TAS2Rs are broadly tuned or that other mechanisms exist which increase the number of agonists stimulating a bitter taste receptor.

By heterologous expression a number of hTAS2Rs have been deorphanized. The first human receptor to be deorphanized was hTAS2R4. This receptor is activated by high concentrations of the synthetic compounds denatonium and 6-n-propyl-thiouracil (PROP), already indicating some promiscuity of hTAS2Rs for bitter substances [42]. The first human bitter taste receptor that was identified to be activated by natural bitter compounds was hTAS2R16 [31]. This receptor combines high specificity, even stereoselectivity for the β-D-conformation at the C1 position of the pyranose moiety, with flexibility for other positions within the group of agonists. These features allow detection of a large number of chemically related bitter β-D-glucopyranosides. It is worth noting that, the receptor properties measured in calcium-imaging experiments closely matched data obtained in human psychophysical studies, indicating that data relevant for human bitter taste can be obtained *in vitro*. The same study identified hTAS2R10 as a receptor for the toxic substance strychnine, which is structurally very different from β-D-glucopyranosides, denatonium and PROP, indicating the enormous capacity of the ~25 hTAS2Rs to detect structurally diverse bitter compounds. An even higher level of promiscuity for structurally different bitter compounds has been observed for hTAS2R14. Of 33 compounds tested, approximately one quarter were able to activate the receptor. This, so far, uniquely broad tuning of hTAS2R14 suggests that the family of bitter taste receptors might consist of 'generalist' receptors tuned to detect a large variety of different substances encountered in nature, and more specifically tuned receptors, perhaps for the detection of the most important toxins in a given habitat [30]. Unlike the other receptors mentioned here, the two very closely related receptors hTAS2R43 and hTAS2R44 show an overlapping agonist spectrum for the two artificial sulfonyl amides saccharin and acesulfame K, and the plant bitter substance aristolochic acid. This demonstrates that evolution of *hTAS2R* genes has not only created diversity but also, to some degree, redundancy [33]. The recently identified PROP/PTC receptor hTAS2R38 [32, 64] currently has the smallest chemical motif responsible for its activation, the N–C=S moiety, which was first proposed to stimulate bitter taste some 50 years ago [65]. Thus, TAS2R38 can be activated by a number of different bitter compounds sharing this chemical group. This activation behavior could be a general mechanism for more broadly tuned receptors to respond to a multitude of bitter compounds. The study by Pronin et al. [66] is the first investigation of motifs involved in agonist activation of hTAS2Rs. Using heterologous expression in insect cells combined with measurement of G protein activation by the incorporation of radioactively labeled GTP-γS hTAS2R43, 44 and 47 were characterized. In this study hTAS2R47 was identified to respond to 6-nitrosaccharin, denatonium and derivatives of denatonium. Additionally, further agonists for hTAS2R43 were identified [6-nitrosaccharin and IMNB (N-isopropyl-2-methyl-5-nitrobenzenesulfonamide)]. Moreover, by domain swapping between hTAS2R43, hTAS2R44 and hTAS2R47, extracellular loop 1 (EC1) of TAS2R43 was demonstrated to be most important for agonist activation by IMNB and 6-nitrosaccharin. In the case of 6-nitrosaccharin also extracellular loop 2 (EC2) was also involved in responsiveness of the receptor, while the third extracellular loop (EC3) had no effect. However, the authors also noticed but did not examine a contribution of the transmembrane segments to agonist activation.

Processing of gustatory cues

The question of how gustatory information is encoded has resulted in the formulation of two, seemingly opposing theories, the across-fiber pattern theory and the labeled-line theory [67–69]. The across-fiber pattern theory states that all gustatory neurons, excited or not, contribute to acitivity patterns that encode taste qualities and that are decoded by the brain. This theory is supported by electrophysiological recordings, which revealed broad tuning of gustatory neurons and TRCs across modalities. On the level of TRCs it has been shown that a considerable number of rodent TRCs can respond to stimulation with more than one taste quality by whole-cell patch clamp [70] and calcium imaging on isolated intact taste buds [71]. Similar observations were made by recordings from sensory afferent axons to higher gustatory centers (reviewed in [69]). The labeled-line theory assumes that taste qualities are transmitted independently of each other by separate pathways or labeled lines. This theory is supported by molecular biology. It has been shown that *TAS1R* and *TAS2R* expressing cells form separate populations, although co-expression of the two receptor types has been observed in some cells [72]. A clear demonstration for the separation of different taste modalities comes from experiments with transgenic animals. Whereas mice with a deletion of either *TRPM5* or *PLCβ2* lose their responsiveness to bitter, sweet and umami stimulation [55], the reintroduction of *PLCβ2* under the control of bitter taste receptor gene promoters selectively restores the animals' responsiveness to bitter compounds but not to sweet stimuli or glutamate [29]. An interesting question is how taste aversion or attraction is constituted. Two different studies used transgenic mice that express a receptor specific for a synthetic agonist (RASSL receptor) in either *TAS1R* [73] or *TAS2R* gene expressing cells [29]. Both studies clearly demonstrated that the targeted TRC type is predetermined to transmit, in case of *TAS1R* gene expressing cells, attractive stimuli or, in case of *TAS2R* gene expressing cells, aversive stimuli. The receptor type itself does not influence this predetermination. This is indicated by the observation that salicin is attractive rather than aversive to mice which have been engineered to express the cognate human bitter taste receptor hTAS2R16 in sweet taste receptor cells [29].

In a very elegant approach using different labeled transneuronal tracer molecules, Sugita et al. elucidated the wiring and segregation of gustatory cells transmitting bitter and sweet/umami taste in mice [74]. They observed that labeled wheat germ agglutinin (WGA) under the control of the *mT2R5* gene promoter is expressed in selected TRCs within taste buds and that the protein is transsynaptically transported to the brain via the primary afferent nerve fibers. The transsynaptic tracer was found in the posterior part of the NTS, the so-called gustatory nucleus. In this structure a distinct area has been shown to respond to various orally applied bitter substances with a highly similar pattern of Fos-like immunoreactivity [75]. Sugita et al. [74] found the transsynaptic tracer in the same central nervous system (CNS) areas also when it has been expressed under control of the gene promoter of *mT1R3*, the common subunit of the sweet (mT1R2/3) and umami taste (mT1R1/3) receptor. This time, however, the anterior part of the NTS was labeled. Labeled cells remain segregated throughout NTS, parabrachial nuclei, thalamic gustatory area and gustatory cortex. In general, the T1R-directed tracer was localized more rostral, whereas the T2R-directed tracer was found predominantly caudal. For both transgenes labeled neurons were also observed within the amygdala, piriform cortex and somatosensory cortex [74]. These findings clearly argue for a spatial separation of neurons transmitting different taste qualities from the level of TRCs to that of gustatory neurons in all examined brain areas. They do not, however, rule out that cross-communication can occur between neurons simultaneously stimulated by different taste qualities.

Polymorphisms in bitter taste receptor genes

An important question for human bitter taste research is how individualized the perception of different bitter compounds is. It appears that humans vary greatly with regard to bitterness perception of some bitter compounds [76]. The analyses of 25 *hTAS2R* genes for polymorphic sites within the coding region in a large number of humans revealed 151 haplotypes. The majority of the observed single-nucleotide polymorphisms (SNPs) are non-synonymous, leading to altered amino acid sequences [77, 78]. Whereas about one-third of the observed SNPs have only been detected once, two-thirds of the SNPs show a considerable frequency of 1–50%. If only part of the polymorphic alleles that occur with high frequency result in functional changes of the corresponding receptor, a remarkable degree of individuality among humans is possible with respect to their bitter taste perception. This might affect dietary habits profoundly and ultimately lead to consequences for an individual's health. The first *hTAS2R* gene with experimentally proven functional polymorphisms is *hTAS2R38*. After its chromosomal localization has been narrowed down by a large linkage study to a small area on chromosome 7 [79], the major responsible gene for the bimodal distribution of different sensitivities to phenylthiocarbamide (PTC) was subsequently demonstrated to be *hTAS2R38* [64]. Five *hTAS2R38* haplotypes specify variant receptors that differ in their sensitivity to the chemicals PROP and PTC. The resulting taster and non-taster phenotypes are about equally abundant, usually with small regional differences [64]. The taster variant of

hTAS2R38 recognizes the chemical group N–C=S, a constituent of isothiocyanates, which are present in *Brassica* vegetables. In regions of low iodine overingestion of isothiocyanates, present in *Brassica* vegetables, is associated with thyroid disease and goiter [80], while, on the other hand, these vegetables display potent anti-cancer effects [81]. Depending on regional influences (over)ingestion of such vegetables can have positive as well as negative health effects for individuals which might lead to balancing selection for *hTAS2R38* gene variants [32].

Another example for genetically determined bitter taste receptor variants is hTAS2R16, the β-glucopyranoside receptor [31]. The less sensitive ancestral allele of *hTAS2R16* [24] occurs with a high frequency in African populations living in malaria-contaminated areas. The more sensitive derived *hTAS2R16* allele shows signs of positive selection and is almost exclusively found in all other human populations. The selecting constraint likely was exposure to bitter cyanogenic glycosides, a large group of toxic plant metabolites. Although these substances are toxic in general, chronic ingestion of sublethal doses can lead to increased resistance to malaria infection by various putative mechanisms ([24], and references therein).

It appears very likely that a number of SNPs found in *hTAS2R* genes will affect receptor characteristics. For the three nonsense alleles detected within *hTAS2R* genes (one observed in *hTAS2R7* and two in *hTAS2R46*) [77] a loss of function is highly probable, whereas other polymorphisms might result in altered sensitivities or even different agonist selectivities. As more and more hTAS2Rs are being deorphanized and therefore become accessible for functional analyses of receptor variants, the number of known functional polymorphisms will increase considerably in the future. It will then be very interesting to see how personal bitter taste perception might influence dietary habits and, ultimately, health.

Perspectives

Although bitter taste research has seen enormous advances during the last several years, many fundamental questions are still left unanswered. The deorphanization of TAS2Rs is a critical prerequisite for some of these future goals to be reached. An important question in view of the large number of bitter receptor haplotypes within the human population is how individual our bitter taste perception is. This will clearly affect the efforts necessary to develop bitter taste reducing compounds for improving popular acceptance of bitter foodstuffs and drugs. The elucidation of structure-function relationships of hTAS2Rs will allow understanding of how such a limited number of hTAS2Rs might be able to detect thousands of structurally diverse bitter compounds. How bitter taste sensation is transmitted into the brain and how the signal is processed within the brain to result in behavioral responses is another key question. Techniques that made the recent advances concerning taste quality coding in rodents possible [29, 74] are not applicable in human bitter taste research. However, functional magnetic resonance studies might be a useful tool to pinpoint similarities and differences between rodents' and humans' bitter taste perception in a non-invasive way.

Acknowledgment. The work was supported by the German Science Foundation (DFG).

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