Cell, Vol. 106, 381–390, August 10, 2001, Copyright ©2001 by Cell Press

Mammalian Sweet Taste Receptors

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Summary

The sense of taste provides animals with valuable information about the quality and nutritional value of food. Previously, we identified a large family of mammalian taste receptors involved in bitter taste perception (the T2Rs). We now report the characterization of mammalian sweet taste receptors. First, transgenic rescue experiments prove that the *Sac* locus encodes T1R3, a member of the T1R family of candidate taste receptors. Second, using a heterologous expression system, we demonstrate that T1R2 and T1R3 combine to function as a sweet receptor, recognizing sweet-tasting molecules as diverse as sucrose, saccharin, dulcin, and acesulfame-K. Finally, we present a detailed analysis of the patterns of expression of T1Rs and T2Rs, thus providing a view of the representation of sweet and bitter taste at the periphery.

Introduction

Our sense of taste is capable of detecting and responding to sweet, bitter, sour, salty, and umami stimuli (reviewed by Lindemann, 1996). It is also responsible for distinguishing between these various taste modalities, for instance, the sweetness of honey from the bitterness of tonic water; the sourness of unripe fruit from the saltiness of the ocean. This discriminatory power provides valuable sensory input: bitter receptors elicit aversive behavioral reactions to noxious substances, while sweet receptors allow recognition of high-caloric food sources.

We have been interested in basic questions of taste signal detection and information coding, and have focused on the isolation and characterization of genes encoding sweet and bitter taste receptors. The identification of taste receptors generates powerful molecular tools to investigate not only the function of taste receptor cells, but also the logic of taste coding. For example, defining the size and diversity of the receptor repertoire provides evidence for how a large number of chemosensory ligands may be recognized, while analysis of the patterns of receptor expression contributes important insight to our understanding of chemosensory discrimination and coding. Recently, we described the isolation of two novel families of G protein-coupled receptors (GPCRs) expressed in subsets of taste receptor cells of the tongue and palate (T1Rs and T2Rs; Hoon et al., 1999; Adler et al., 2000). One of these, the T2Rs, is a family of ~30 different genes that include several functionally validated mammalian bitter taste receptors (Adler et al., 2000; Chandrashekar et al., 2000; Matsunami et al., 2000). Nearly all of the T2R genes are clustered in regions of the genome that have been genetically implicated in controlling responses to diverse bitter tastants in humans and mice, consistent with their proposed role as bitter taste receptors (Adler et al., 2000). Notably, most T2Rs are coexpressed in the same subset of taste receptor cells (Adler et al., 2000), suggesting that these cells are capable of responding to a broad array of bitter compounds, but not discriminating between them. This is logical for a sensory modality like bitter, in which the animal needs to recognize and react to many noxious tastants, but not necessarily discriminate between them. This interpretation is consistent with behavioral and psychophysical findings in rodents and humans demonstrating limited discrimination between various bitter tastants (McBurney and Gent, 1979).

How is sweet taste specified? There is considerable evidence that G protein-coupled receptors are also involved in this taste modality (Lindemann, 1996). In contrast to bitter taste, the number of biologically relevant sweet tastants is modest. Thus, we might expect the sweet receptor family to be quite small. Interestingly, psychophysical, behavioral, and electrophysiological studies suggest that animals distinguish between various sweet tastants (Schiffman et al., 1981; Ninomiya et al., 1984, 1997), perhaps reflecting (and predicting) the organization of the sweet taste system into distinct types of sweet receptor cells and pathways.

Genetic studies of sweet tasting have identified a single principal locus in mice influencing responses to several sweet substances (Fuller, 1974; Lush, 1989). This locus, named *Sac*, determines threshold differences in the ability of some strains to distinguish saccharin-containing solutions from water (Fuller, 1974). Sac tasters respond to ~5-fold lower concentrations of saccharin than "sweet-insensitive" Sac nontaster mice (Fuller, 1974; Capeless and Whitney, 1995); additionally, *Sac* influences preferences to sucrose, acesulfame-K and dulcin (Lush, 1989). Recently, several groups reported that a T1R-related gene, *T1R3*, might encode Sac (Kitagawa et al., 2001; Max et al., 2001; Montmayeur et al., 2001; Sainz et al., 2001). We also isolated T1R3 and noted its association with Sac, but sought to obtain proof that *Sac* encodes T1R3, especially since there is no clear loss-of-function allele for *T1R3.*

We now demonstrate that transgenic expression of T1R3 from a taster strain transforms sweet-insensitive animals to tasters, affirming *T1R3* as the *Sac* gene. We then developed a cell-based reporter system to prove that T1Rs encode functional sweet taste receptors. Lastly, we show that the patterns of T1R expression

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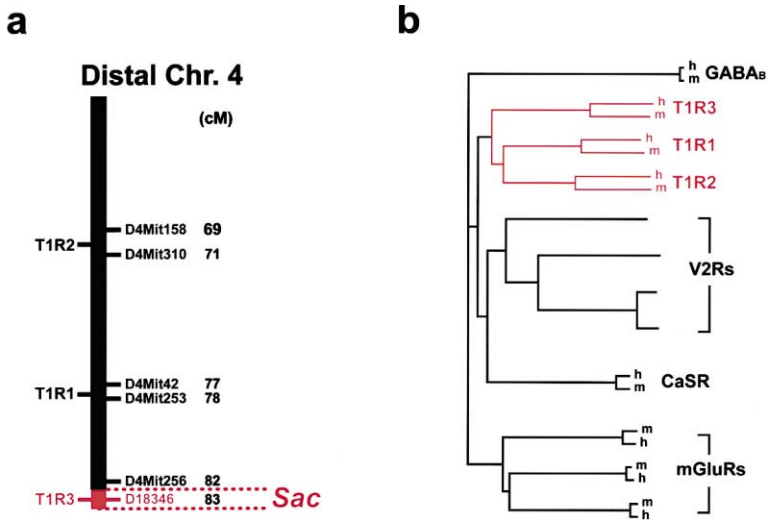

Figure 1. The T1R Family

(a) Radiation hybrid and STS mapping localized all three T1R genes to the distal end of chromosome 4. The *T1R3* gene is closely linked to *D18346*, an STS marker within the *Sac* genetic interval (Kitagawa et al., 2001; Li et al., 2001; Max et al., 2001; Montmayeur et al., 2001; Sainz et al., 2001; see Experimental Procedures for details on our cloning, mapping, and characterization of T1R3). (b) Cladogram showing sequence similarity between human (h) and mouse (m) T1Rs and related receptors (Nakanishi, 1992; Brown et al., 1993; Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997; Kaupmann et al., 1997; Hoon et al., 1999); mouse V2Rs do not have human counterparts.

define at least three distinct cell types, and that sweet and bitter receptors are tightly segregated at the periphery.

Results

T1R3 Is Encoded by the *Sac* Locus

In previous studies, we identified two novel G protein-coupled receptors, T1R1 and T1R2, that are selectively expressed in subsets of taste receptor cells of the tongue and palate epithelium (Hoon et al., 1999). Both *T1R1* and *T1R2* were initially mapped to the distal end of chromosome 4, in the proximity of *Sac* (Hoon et al., 1999). However, radiation hybrid analysis and high-resolution genetic mapping separated these receptors from the *Sac* genetic interval (Li et al., 2001), thus eliminating them as candidate *Sac* genes (Figure 1). Recently, six independent groups reported that a related receptor gene, *T1R3*, is tightly linked to the *Sac* locus (Kitagawa et al., 2001; Max et al., 2001; Montmayeur et al., 2001; Sainz et al., 2001; Senomyx, La Jolla, CA; Li et al., 2001 Achems XXIII, Sarasota FL), and that polymorphic variants of *T1R3* cosegregate with *Sac* taster and non-taster alleles. This genetic linkage was used to hypothesize that *T1R3* corresponds to the *Sac* gene. We also isolated and characterized T1R3 (see Experimental Procedures for details), and reasoned that if *Sac* in fact encodes T1R3, then introduction of a taster allele of this candidate receptor should rescue the taste deficit of Sac non-taster mice.

A 16 kb genomic clone containing the *T1R3* sequence from a Sac taster strain (C57 BL/6) was used to engineer a transgenic rescue construct (Figure 2a). In order to follow the presence and expression of the transgene versus the endogenous *T1R3* allele, we replaced its 3′-UTR and polyadenylation signal with that of bovine growth hormone. Our strategy was to produce progeny that were homozygous for the *T1R3* non-taster allele, but carried the taster-derived transgene. We obtained 4 founder mice, and two independent lines were examined for appropriate expression of the transgene and assayed for behavioral rescue of sucrose and saccharin tasting (Fuller, 1974). Age- and sex-matched siblings that lacked the transgene were used as controls in all experiments. Figure 2b illustrates that all the cells expressing the endogenous T1R3 receptor, and only these cells, also express the transgene (identical results were obtained in taster and non-taster genetic backgrounds; data not shown).

If the *T1R3* taster allele rescues the taste deficiency of Sac non-tasters, their saccharin and sucrose dose responses should be shifted to recapitulate the sensitivity seen in Sac taster animals (Fuller, 1974; Bachmanov et al., 1997). Figure 2 demonstrates that the T1R3 transgene fully rescues the taste defect of Sac non-tasters. Animals without a transgene are indistinguishable from non-taster 129/Sv control mice (Figures 2c and 2d, open black circles). In contrast, siblings with the same *Sac* non-taster background but expressing the transgene are now equivalent to taster C57BL/6 control mice (Figures 2c and 2d, red traces). The presence of the transgene did not influence other taste modalities (Figures 2e–h), nor did it alter the sweet sensitivity of taster strains (data not shown). Equivalent results were obtained with the two independent transgenic lines. These results validate *T1R3* as the *Sac* locus, and suggest that T1R3 may function as a sweet taste receptor.

Expression of T1Rs

Where is T1R3 expressed? Recently, T1R3 was shown to be expressed in subsets of taste receptor cells in various taste papillae (Kitagawa et al., 2001; Max et al., 2001; Montmayeur et al., 2001; Sainz et al., 2001). We examined the expression of T1R3 in circumvallate, foliate, fungiform, and palate taste buds and show that T1R3 is expressed in ~30% of cells from all types of taste buds (Figure 3; see also Kitagawa et al., 2001 and Montmayeur et al., 2001). This topographic pattern of expression closely approximates the aggregate of T1R1 and T1R2 expression (Figure 3; Hoon et al., 1999), and suggests possible coexpression of T1R1 with T1R3 and of T1R2 with T1R3. The coexpression of T1R2 and T1R3 in circumvallate (Max et al., 2001; Montmayeur et al., 2001) and foliate papillae (Montmayeur et al., 2001) was recently examined by RT-PCR and by in situ hybridization, but a comprehensive study of all three T1Rs in the

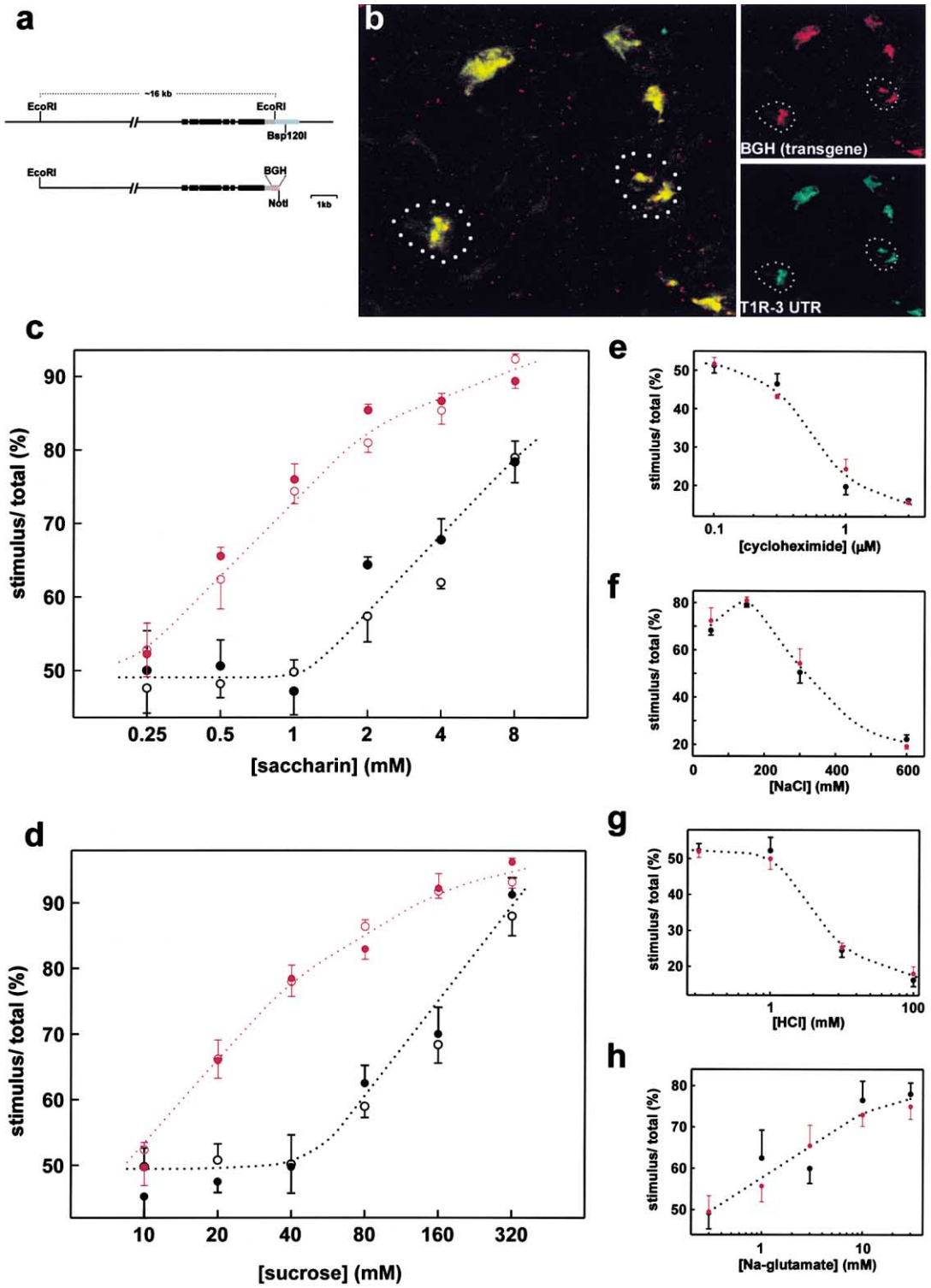

Figure 2. *T1R3* Encodes *Sac*

(a) Schematic diagram indicating structure of the *T1R3* gene and transgenic construct. The alternate 3′-UTRs used for genotyping and in situ hybridization are highlighted in green and red. (b) In situ hybridization demonstrated perfect concordance in the expression pattern of the T1R3 transgene (red) and the endogenous gene (green). The dotted lines illustrate the outline of selected taste buds; sections were cut perpendicular to the planes shown in Figure 3. (c–h) Taste preferences of control and transgenic animals (solid red circles) were measured using standard two-bottle preference tests. The behavioral responses of mice expressing the T1R3 transgene to saccharin and sucrose (c and d) were indistinguishable from those of the control taster mice (C57BL/6; open red circles). Siblings without the transgene (solid black circles) behaved like 129/Sv non-taster control mice (open black circles). Responses to bitter, salty, sour, and umami stimuli (e–h) were not affected by presence of the transgene.

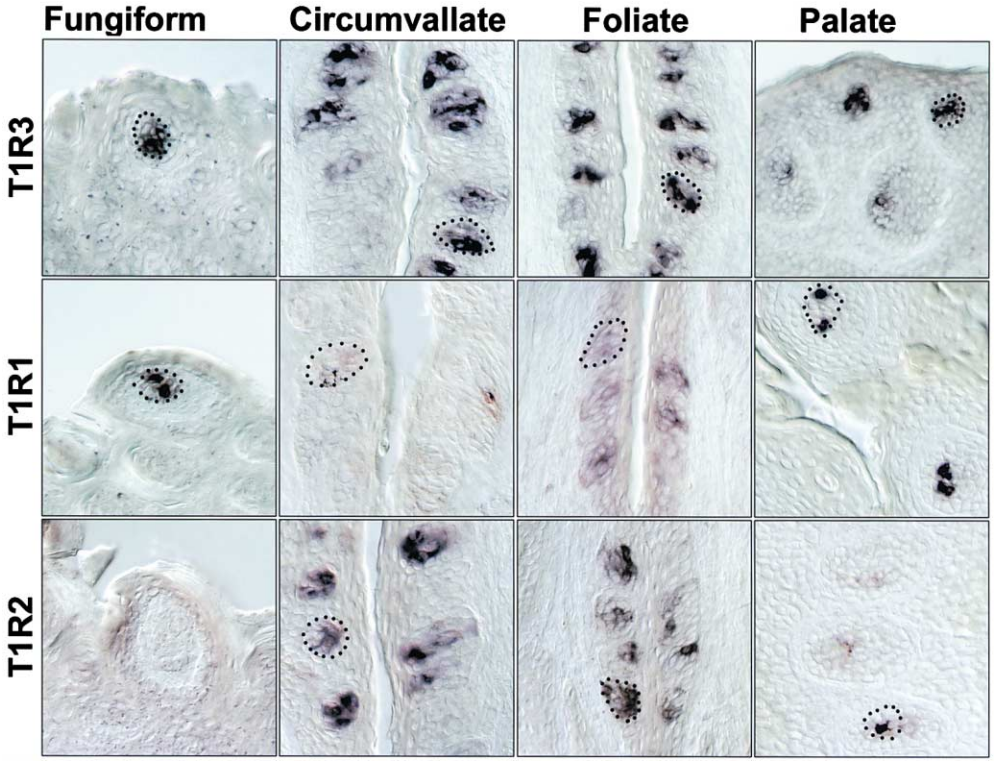

Figure 3. Expression of T1Rs in Subsets of Taste Receptor Cells

In situ hybridizations with digoxigenin-labeled antisense RNA probes demonstrated that T1R3 is expressed in subsets of mouse taste receptor cells (upper panels). Approx. 30% of cells in fungiform, circumvallate, foliate, and palate taste buds express T1R3. Shown for comparison are similar, but not serial, sections labeled with T1R1 and T1R2 (middle and lower panels; see also Hoon et al., 1999 and Figure 4). The dotted lines illustrate the outline of a sample taste bud. Note that the selectivity of T1R3 expression closely resembles that of T1R1 plus T1R2.

different classes of taste buds was lacking. Thus, we performed double labeling experiments using two-color fluorescent in situ hybridization. Our results demonstrated that T1R3 is coexpressed with T1R2 in all circumvallate, foliate, and palate taste buds, with every T1R2-positive cell also expressing T1R3 (Figure 4). Similarly, T1R1 is coexpressed with T1R3 in fungiform and palate taste receptor cells. However, there is also a fraction of cells with nonoverlapping expression of T1R3 in fungiform and palate taste buds. Therefore, we can define three major classes of cell types based on their T1R expression profiles: T1R1 and T1R3 (T1R1+3), T1R2 and

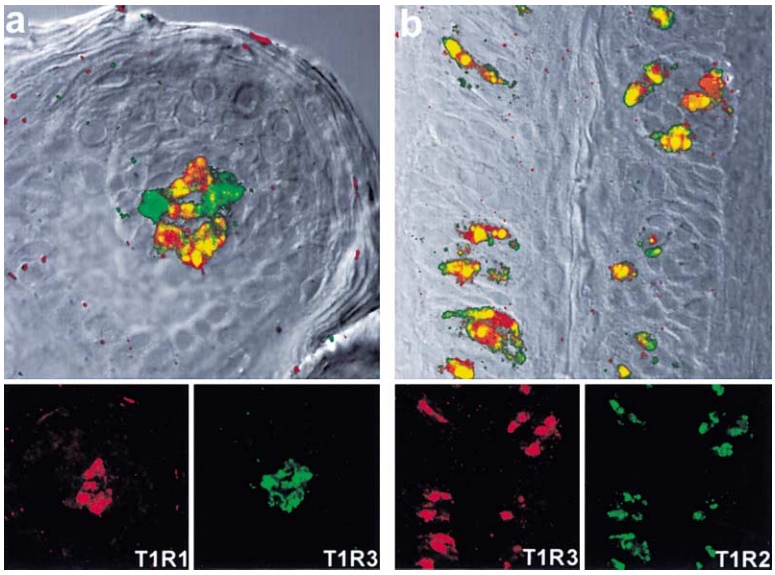

Figure 4. T1R Expression Patterns Define Three Cell Types

Double-label fluorescent in situ hybridization was used to directly examine the overlap in cellular expression of T1Rs. Two-channel fluorescent images (1–2 μm optical sections) are overlaid on difference interference contrast images. (a) Fungiform papillae illustrating coexpression of T1R1 (red) and T1R3 (green). At least 90% of the cells expressing T1R1 also express T1R3; similar results were observed in the palate. Note the presence of some T1R3-positive but T1R1-negative cells. (b) Circumvallate papillae illustrating coexpression of T1R2 (green) and T1R3 (red). Every T1R2 positive cell expresses T1R3.

T1R3 (T1R2+3) and T1R3 (perhaps plus an additional yet undiscovered receptor, see Discussion).

T1Rs Encode Functional Sweet Taste Receptors

Demonstration that T1Rs encode sweet receptors requires functional validation. We faced two challenges in generating a reporter system to detect T1R receptor activity: first, a poor understanding of the native signaling pathway (i.e., G protein and effector system); and second, the recognized difficulty of targeting chemosensory receptors to the plasma membrane of heterologous cells (Baker et al., 1994; Dwyer et al., 1998; Krautwurst et al., 1998; Chandrashekar et al., 2000). To monitor translocation of receptors to the plasma membrane, we raised antibodies against T1R1, T1R2 and T1R3, and tested expression of native and epitope-tagged mouse and rat receptors in various tissue culture cell lines. We observed that rat T1Rs were expressed significantly more efficiently than the mouse counterparts (data not shown); we therefore used the rat genes in all heterologous expression studies. To assay function, we expressed T1Rs with a Gα16-Gz chimera and Gα15, two G protein α-subunits that together efficiently couple Gs, Gi, Gq and gustducin-linked receptors to phospholipase Cβ (Offermanns and Simon, 1995; Krautwurst et al., 1998; Chandrashekar et al., 2000; Mody et al., 2000). In this system, receptor activation leads to increases in intracellular calcium $[Ca^{2+}]_i$, which can be monitored at the single cell level using the FURA-2 calcium indicator dye (Tsien et al., 1985).

Because of the apparent obligatory coexpression of T1R1 or T1R2 with T1R3, we transfected various rat T1Rs singly and in combinations into HEK-293 cells expressing the promiscuous Gα15 and Gα16-Gz proteins. After loading the cells with FURA-2, we assayed for responses to a wide range of sweet tastants, including sugars, amino acids, and artificial sweeteners; we also tested several bitter tastants (see Experimental Procedures). Cells expressing rat T1R2 and T1R3 (T1R2+3) robustly responded to a subset of sweet compounds including sucrose, fructose, saccharin (but not to N-methyl-saccharin, a nonsweet saccharin derivative), acesulfame-K, dulcin, and two novel intensely sweet compounds (Nagarajan et al., 1996, guanidinoacetic acid 1 and 2, referred to as GA-1 and GA-2; Figures 5 and 6a). The responses were receptor- and Gα-dependent, because cells lacking either of these components did not trigger $[Ca^{2+}]_i$ changes, even at vastly higher concentrations of tastants (Figure 5). Notably, the activation of T1R2+3 is extremely selective. On the one hand, this receptor combination did not respond to a large number of mono- and disaccharides and artificial sweeteners, including glucose, galactose, maltose and aspartame (Figure 6a). On the other hand, the response was strictly dependent on the presence of both T1R2 and T1R3; either receptor alone did not respond to any of the compounds assayed in these studies, even at concentrations that far exceeded their biologically relevant range of action (data not shown). These results strongly suggest that T1R2 and T1R3 combine to function as a heteromeric receptor. Additional evidence that heteromerization is required for the formation of a functional T1R receptor was obtained by coimmunoprecipitations of differentially tagged T1R receptors (G. Zhao, N.R. and C.Z., unpublished data), and by coexpression of a dominant negative T1R. Cotransfection of wild-type T1R2 and T1R3 with a T1R2 receptor harboring a C-terminal truncation (Salahpour et al., 2000) nearly abolished the T1R2+3 responses (>85% reduction, data not shown).

If the responses of T1R2+3 reflect the function of the native sweet receptor, we reasoned that the rank order seen in the cell-based assays should parallel, and the sensitivity may approximate, the behavioral thresholds for detection of these sweet tastants in vivo. Indeed, Figure 6b shows dose-responses for GA-2 (in vivo threshold ~2 μM), saccharin (in vivo threshold ~0.5 mM), acesulfame-K (in vivo threshold ~0.5 mM), and sucrose (in vivo threshold ~20 mM), demonstrating a good match between the cell-based responses and their biological threshold. No responses were ever detected against a panel of bitter tastants, or umami stimuli.

To examine the sweet taste responses in detail, cells transfected with T1R2+3 were placed on a microperfusion chamber and superfused with test solutions under various conditions. Figure 6c shows that responses to the sweet tastants closely follow application of the stimulus (latency <1 s). As expected, when the tastant was removed, $[Ca^{2+}]_i$ returned to baseline. A prolonged exposure to the sweet compound (>10 s) resulted in adaptation: a fast increase of $[Ca^{2+}]_i$ followed by a rapid, but incomplete decline to the resting level. Similarly, successive applications of the tastant led to significantly reduced responses, indicative of desensitization (Lefkowitz et al., 1992), while a prolonged period of rest (>5 min) was required for full response recovery. As would be expected if T1R2+3 mediate the responses to the various sweet compounds (i.e., GA-2, sucrose, and acesulfame-K), successive application of different tastants from this panel led to full cross-desensitization (Figure 6c), while sweet tastants that did not activate this receptor complex (e.g., glucose and cyclamate) had no effect on the kinetics, amplitude, or time course of the responses. Taken together, these results validate T1R2+3 as a sweet taste receptor.

We have attempted to determine the ligand/tastant specificity of T1R1+3 using a variety of strategies, but have been hampered by the difficulty of functionally expressing this receptor combination in heterologous cells (data not shown). However, we propose that all T1Rs encode sweet receptors: first, they are all members of the same receptor family; second, T1R1, T1R2, and T1R3 are tightly coexpressed in distinct subsets of cells; third, two of the three T1Rs combine to function as a validated sweet receptor.

Spatial Map of T1R and T2R Expression

Studying the expression of T1Rs in the context of other taste modalities may provide a view of the representation of sweet taste coding at the periphery. Recently, we showed that members of the T2R family of bitter taste receptors are rarely expressed in fungiform taste buds, but are present in 15%–20% of the cells of all circumvallate, foliate, and palate taste buds. Given that T1Rs are also expressed in the same taste buds, we examined whether there is overlap between T1R- and T2R-expressing cells. Double-labeling experiments us-

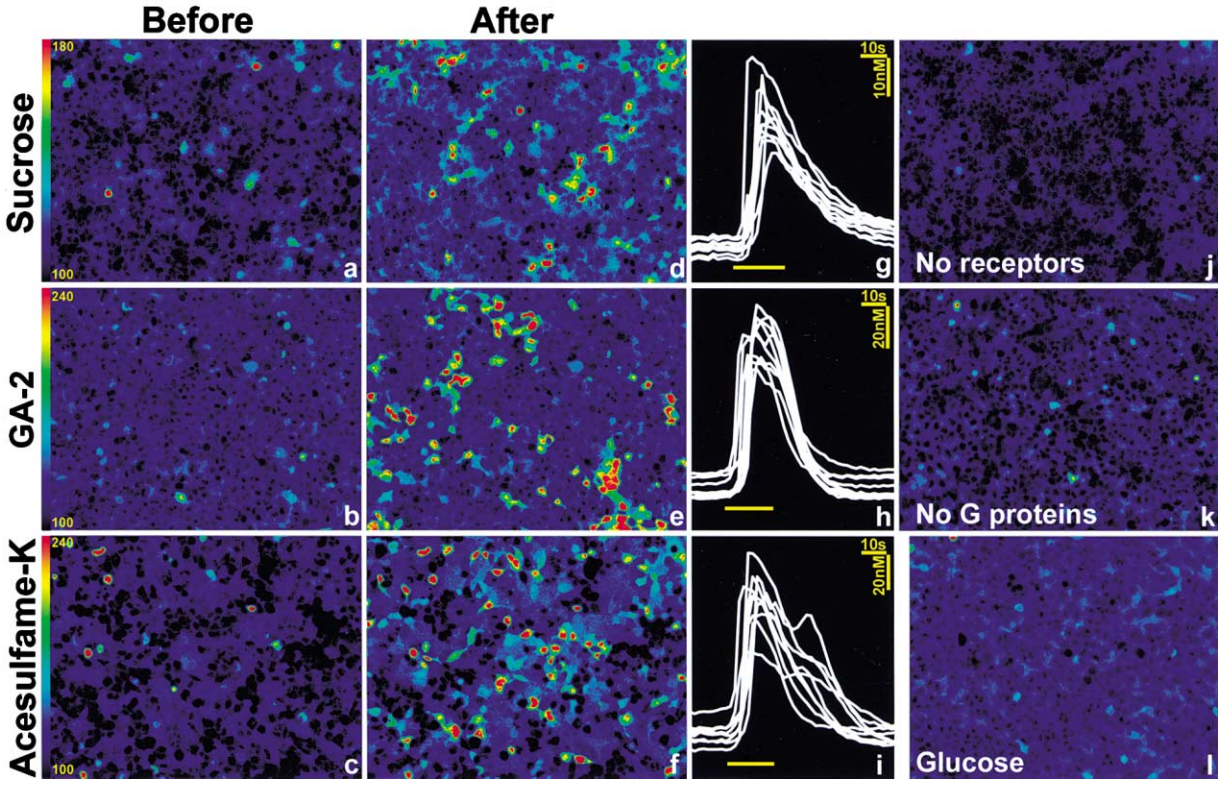

Figure 5. T1R2+3 Responds to Sweet Tastants

HEK-293 cells coexpressing promiscuous G proteins and rat T1R2 and T1R3 were stimulated with various sweet compounds. Robust increases in $[Ca^{2+}]_i$ were observed upon addition of 250 mM sucrose (d and g), 180 μM GA-2 (e and h) and 10 mM acesulfame-K (f and i). (a)–(c) show cells prior to stimulation. No responses were detected without receptors (panel j) or promiscuous G proteins (panel k). Glucose and several other sweet tastants (see figure 6) did not activate this receptor combination (l); scales indicate $[Ca^{2+}]_i$ (nM) determined from FURA-2 F_{340}/F_{380} ratios. Line traces (g–i) show the kinetics of the $[Ca^{2+}]_i$ changes for representative cells from panels (d)–(f). The bar indicates the time and duration of the stimulus.

ing mixes of T1Rs and T2R probes demonstrated that T2Rs are not coexpressed with any of the T1R family members (Figure 7, see also Adler et al., 2000). This was seen in all taste buds, and with mixes that included as many as 20 T2Rs. The strong segregation in the expression profile of these two receptor families makes an important prediction about the logic of taste coding and discrimination at the taste bud level: sweet and bitter are encoded by the activation of different cell types.

Discussion

The physiological basis of sweet taste perception has captured the imagination of many philosophers, cooks and scientists. In the early 1800s the great French gastronomer Jean Anthelme Brilliat-Savarin wrote:

"Taste seems to posses two functions: (1) It invites us, by arousing our pleasure, to repair the constant losses which we suffer though our physical existence. (2) It helps to choose from the variety of substances which Nature presents to us those which are best adapted to nourish us."

While taste signaling involves many steps and events, it all begins at the periphery, with the recognition of tastant molecules by taste receptors. Indeed, understanding how taste discrimination is accomplished, defining the topographic organization of sweet and bitter responding cells in the various taste buds and papillae, and elucidating how the information is transmitted and encoded in the afferent nerves would be greatly aided by the isolation of taste receptor genes that can be used to mark the cells, dissect receptor specificity, generate topographic maps, and trace the respective neuronal connectivity circuits. Our recent identification of functional bitter taste receptor genes (Adler et al., 2000; Chandrashekar et al., 2000), and our demonstration that T1Rs function as sweet taste receptors (this study), afford a new view of the organization of the mammalian taste system.

The T1R Family

How many receptors does an animal need to sample the sweet world? It is often argued that the size of the sweet taste receptor family should be significantly smaller than that of bitter receptors. This is because of the small repertoire of biologically pertinent sweet compounds (which of course do not include artificial sweeteners). We suggest that T1Rs are all sweet receptors and, based on the patterns of T1R receptor coexpression, predict a minimum of three (T1R1+3, T1R2+3, T1R3) and a maximum of 5 receptor varieties (T1R1, -2 and -3, T1R1+3, and T1R2+3). We have screened

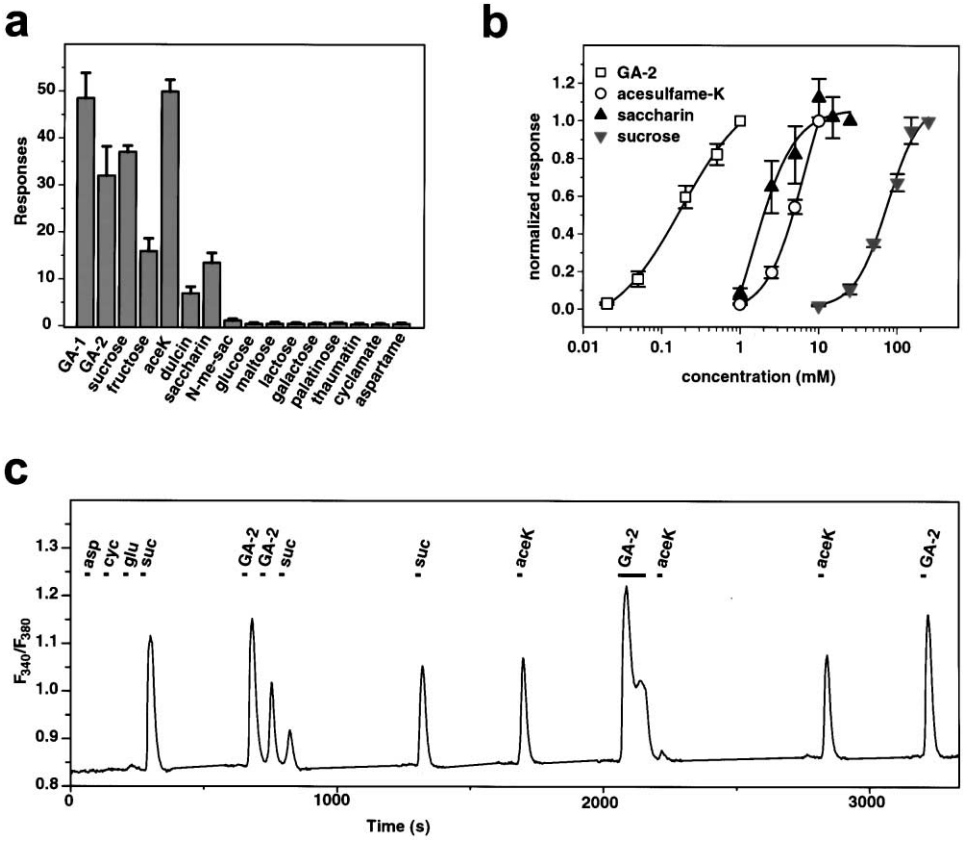

Figure 6. T1R2+3 Selectively Responds to a Broad Range of Sweet Compounds

(a) The responses of the T1R2+3 receptor combination were specific to sucrose, fructose, and five artificial sweeteners. Concentrations used are: GA-1 (500 μM); GA-2 (500 μM); sucrose (250 mM); fructose (250 mM); acesulfame-K (10 mM); dulcin (2 mM); sodium saccharin (5 mM); N-methyl saccharin (5 mM); glucose (250 mM); maltose (250 mM); lactose (250 mM); galactose (250 mM); palatinose (250 mM); thaumatin (0.1%); sodium cyclamate (15 mM); aspartame (2 mM). Columns represent the mean ± SEM of a minimum of 16 independent determinations. (b) Dose response of T1R2+3 to sucrose, saccharin, acesulfame-K and GA-2. The relative changes in $[Ca^{2+}]_i$ are shown as FURA-2 (F_{340}/F_{380}) ratios normalized to the responses obtained for the highest concentration of each compound. Each point represents the mean ± SEM of a minimum of 20 assays. (c) Kinetics and desensitization of T1R2+3 sweet responses. Cells expressing T1R2+3 were stimulated with multiple pulses of sweet tastants: GA-2 (360 μM), sucrose, (suc: 250 mM), acesulfame-K (aceK: 10 mM), cyclamate (cyc: 15 mM), glucose (glu: 250 mM), and aspartame (asp: 2 mM). Dots and horizontal bars indicate the time and duration of the stimulus. Sucrose, GA-2, and acesulfame-K elicit robust responses; repeated or prolonged stimulation with any one of these tastants (e.g., GA-2) results in a decreased response indicative of desensitization. Stimulation with sucrose or acesulfame-K immediately after GA-2 results in an attenuated response, suggesting cross-desensitization. The trace was derived from 80 responding cells in the field of view. See text for additional details.

genomic and cDNA libraries using a variety of strategies, and examined the draft of the assembled human and mouse genomes for homologous genes (NCBI and Celera databases), but have not identified additional T1Rs.

Are there more than three sweet receptor genes? Our functional studies demonstrated that the T1R2+3 receptor combination displays a very broad selectivity spectrum, responding to at least 7 of 24 structurally

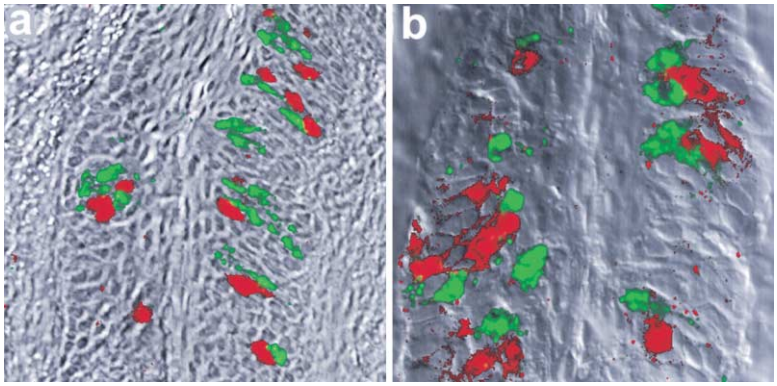

Figure 7. T1Rs and T2Rs Are Segregated in Distinct Populations of Taste Receptor Cells

Double-label fluorescent in situ hybridization was used to examine the degree of overlap between the T1R and T2R families of sweet and bitter taste receptors. (a) T1R3 (green) and T2Rs (a mixture of 20 receptors, red) are never coexpressed. A section through a circumvallate papilla is shown (b) like panel (a), but with a mixture of all three T1Rs (green) versus 20 T2Rs in a foliate papilla.

distinct sweet molecules tested in our assays. Thus, we propose that a combinatorial arrangement of three receptors that may include homomeric as well as heteromeric receptor complexes may be sufficient to accommodate the sweet taste repertoire. However, we cannot rule out that additional divergent receptors exist in the genome. The notion that there could be many receptors with similar function, but little sequence homology, has been elegantly validated in *C. elegans* (Troemel et al., 1995) and *Drosophila* (Scott et al., 2001), where hundreds of highly divergent chemosensory receptors have been identified.

Humans and rodents exhibit some notable differences in their ability to detect certain artificial sweeteners and intensely sweet proteins. For instance, rodents do not taste aspartame, monellin, or thaumatin, while humans have submillimolar to micromollar sensitivities to these compounds (Danilova et al., 1998). We have sequenced the human, rat, and mouse T1R receptors, and find that human and rodent T1Rs are only 70% identical (see Figure 1; GenBank accession numbers: AY032620–AY032623). In contrast, nonchemosensory GPCRs, even those within the same superfamily as T1Rs (e.g., CaSR and mGluRs), are significantly more closely related across species (>90% identity; Brown et al., 1993; Nakanishi, 1992). We suggest that this high level of T1R receptor variability underlies the differences in sweet perception between humans and rodents. Interestingly, this high level of interspecies variability is also seen in other candidate chemoreceptors. For example, the closest human and rodent T2R pairs share only ~70% sequence identity (Adler et al., 2000). Thus, it appears that chemosensory receptors can tolerate greater variation than receptors for other modalities, or perhaps such differences reflect the evolutionary tuning of chemosensory systems to distinct ecological niches.

Implications for Taste Coding

Coding in the periphery of the taste system could occur at two levels: taste receptor cells and afferent fibers. In principle, a taste receptor cell could be tuned to a single modality (e.g., sweet, sour, bitter, or salty), or it could be tuned to more than one modality. Likewise, subsets of cells having similar response profiles could be innervated by a common fiber (i.e., labeled lines), or single fibers may carry information from different types of cells (i.e., mixed lines).

Previously, we showed that a large repertoire of T2Rs is present in each T2R-expressing cell (Adler et al., 2000). In this paper, we have shown that cells expressing T2Rs are distinct from those that express the T1R receptors. Our finding of complete nonoverlap between T1R- and T2R-expressing cells strongly suggests that sweet and bitter tastes are encoded by activation of different subsets of taste receptor cells. Indeed, we suggest that since bitter and sweet taste modalities influence such opposite behaviors (aversion versus attraction), it would be important to segregate the cell types and pathways that mediate these responses.

What about the different types of T1R cells? A prediction of this study is that taste buds in all taste papillae contain sweet receptor cells, and that the anatomical representation of sweet sensitivity in the oral cavity should match the topographic distribution of T1R receptor expression. For instance, the back of the tongue and palate contain all of the T1R2+3 expressing cells, and so they would display high sensitivity for ligands of this receptor combination. Conversely, the front of the tongue would respond to the T1R1+3 combination, but poorly to the repertoire specific for T1R2+3. Moreover, since the front and back of the tongue are innervated by nerves originating in different ganglia (Mistretta and Hill, 1995), we conclude that T1R2+3 sweet cells must exhibit connectivity pathways that differ from those of T1R1+3 cells. Interestingly, the rat is known to be more sensitive to sucrose applied to the back of the tongue and palate than to stimulation of the front of the tongue (Smith and Frank, 1993). Our expression and functional studies now provide a molecular explanation to these findings.

A critical next step in defining the logic of sweet taste coding would be to examine the physiology and connectivity pathways of T1R-expressing cell in the various taste buds, and to study the impact of genetic ablation, or knockouts, of the different cells and receptor combinations. Ultimately, the study of sweet taste perception should help us explore the hedonic aspects of taste transduction, and perhaps understand why a spoonful-of-sugar helps the medicine go down.

Experimental Procedures

Molecular Cloning of T1R3

Human T1R3 was identified in the draft sequence of BAC clone RP5-890O3 by homology to T1R1. A fragment of rat T1R3 was amplified from genomic DNA using degenerate PCR primers designed on the basis of the human sequence. The PCR derived probe was used to identify full-length rat T1R3 from a circumvallate cDNA library (Hoon et al., 1999) and to probe mouse BAC filter arrays (Incyte Genomics and Research Genetics). The sequences of T1R3 in Sac-taster and non-taster mouse strains (C57BL/6 and 129/Sv) were determined from the genomic clones. The sequence of the entire coding region of the gene of other mouse strains that are sweet sensitive (SWR, ST, C57L, FVB/N) and sweet insensitive (DBA/1Lac, DBA/2, C3H, AKR, BALB/c) was determined from amplified genomic DNA (Jackson Laboratory). For SWR mice, T1R3 was also sequenced from amplified taste tissue cDNA. Amongst the 11 inbred strains, we found two taster alleles (taster 1: C57BL/6, C57L, and taster 2: SWR, ST, FVB/N) and a single non-taster allele (DBA/1Lac, DBA/2, C3H, AKR, BALB/c, 129/Sv). Taster 1 and taster 2 alleles differ from each other in six amino acid positions (P61L, C261R, R371Q, S692L, I706T, G855E; one of these, G855E, was missed by Kitagawa et al. (2001) and Max et al. (2001), likely due to its inclusion in the primers used in their amplifications reactions). Non-tasters differ from taster 1 allele in six residues (A55T, T60I, L61P, Q371R, T706I, E855G), and from taster 2 in 4 amino acid positions (A55T, T60I, R261C, L692S).

Mouse T1Rs were mapped using a mouse/hamster radiation hybrid panel (Research Genetics and Whitehead Institute/MIT Center for Genome Research). Physical mapping of T1R3 involved PCR-based typing of T1R3 positive BAC clones for the presence of STS markers.

In Situ Hybridization

Tissue was obtained from adult mice. No sex-specific differences of expression patterns were observed. Therefore, male and female animals were used interchangeably. For foliate sections, no differences in expression pattern were observed between the papillae. Fresh frozen sections (16 μm/section) were attached to silanized slides and prepared for in situ hybridization as described previously (Hoon et al., 1999). All in situ hybridizations were carried out at high stringency (hybridization, 5 X SSC, 50% formamide, 65°C–72°C;

washing, 0.2 X SSC, 72°C). For single-label detection, signals were developed using alkaline-phosphatase conjugated antibodies to digoxigenin and standard chromogenic substrates (Boehringer Mannheim). Control hybridizations with sense probes produced no specific signals in any of the taste papillae. Cells were counted based on the position of their nucleus as previously described (Boughter et al., 1997). For double-label fluorescent detection, probes were labeled either with fluorescein or with digoxigenin. At least 50 taste buds from at least 3 different animals were analyzed with any combination of probes. An alkaline-phosphatase conjugated anti-fluorescein antibody (Amersham) and a horseradish-peroxidase conjugated anti-digoxigenin antibody were used in combination with fast red and tyramide fluorogenic substrates (Boehringer Mannheim and New England Nuclear). Confocal images were obtained with a Leica TSC confocal microscope using an argon-krypton laser; 1–2 μm optical sections were recorded to ensure that any overlapping signal originated from single cells.

Generation of T1R3 Transgenic Mice and Behavioral Assays

An approximately 16 kb EcoRI fragment including the 6 coding exons of T1R3 and about 12 kb upstream of the starting ATG was isolated from a C57BL/6 BAC clone. This fragment contains the stop codon of the T1R3 coding sequence, but lacks much of the 3′-UTR. The sequence of the entire ~16 kb clone was determined from a taster and a non-taster strain. This fragment also contains the full sequence for a glycolipid transferase-like gene ~3 kb upstream of *T1R3*, but there are neither expression nor amino acid sequence differences in this gene between Sac taster (SWR) and non-taster (129/Sv) strains. In the transgenic construct, the bovine growth hormone polyadenylation (BGH) signal from pCDNA3.0 (Invitrogen) was ligated to the 3′ end of the *T1R3* gene. This modification allowed PCR-based genotyping of mice and permitted direct comparison of the expression of T1R3 from the transgene with that from the normal gene. Transgenic mice were generated by pronuclear injection of FVB/N oocytes. Since we determined that FVB/N mice are sensitive to sweet tastants, and carry a *T1R3* taster allele, transgenic founders were crossed to 129/SvJ. F1 mice carrying the transgene were then backcrossed to 129/SvJ. F2 mice were typed for the presence of the transgene using the BGH tag, and for homozygosity of the endogenous non-taster *T1R3* allele using a Bsp120I restriction polymorphism between FVB/N and 129/SvJ (see Figure 2a). All four genetic groups were tested behaviorally. Mice were weaned at 3 weeks and trained for 7–10 days to drinking from two bottles of water prior to initiating testing.

For behavioral assays, 2 or 3 mice were housed per cage; mice derived from different transgenic founders (and males and females) were kept separate to allow comparison of the raw data. The group sizes used for assays consisted of 4 or more cages, each with a minimum of 2 animals. Mice were always assayed at the low concentrations first (Fuller, 1974). In all cases, animals were given at least 2 days of water between concentration series. Each test consisted of a two-bottle choice assay over a 48 hr period; the positions of the bottles were switched after 24 hr. Preference ratios were calculated by dividing the consumption of the test solution by total intake. Data from each cage were individually analyzed to prevent systematic bias. The same assay was used to analyze the taste preferences of 129/Sv, C57BL/6, and FVB/N control mice.

Heterologous Expression of T1Rs

All receptors were cloned into a pEAK10 mammalian expression vector (Edge Biosystems, MD). Modified HEK-293 cells (PEAK^{rapid} cells; Edge BioSystems, MD) were grown and maintained at 37°C in UltraCulture medium (Bio Whittaker) supplemented with 5% fetal bovine serum, 100 μg/ml gentamycin sulfate (Fisher), 1 μg/ml amphotericin B and 2 mM GlutaMax I (Lifetechnologies). For transfection, cells were seeded onto matrigel coated 6-well culture plates, 24-well culture plates, or 35 mm recording chambers. After 24 hr at 37°C, cells were washed in OptiMEM medium (Lifetechnologies) and transfected using LipofectAMINE reagent (Lifetechnologies). Transfection efficiencies were estimated by cotransfection of a GFP reporter plasmid, and were typically >70%. Activity assays were performed 36–48 hr after transfection for cells transfected in 24-well culture plates and 35 mm recording chambers; cells transfected in 6-well culture plates were grown overnight, trypsinized, transferred to 96-well culture plates, and assayed 36–48 hr following reseeding. Transfections involving dominant negative receptors were carried at approximately 1:1 ratios of wild-type and mutant receptors

Calcium Imaging

Transfected cells were washed once in Hank's balanced salt solution containing 1 mM sodium pyruvate and 10 mM HEPES, pH 7.4 (assay buffer), and loaded with 2 μM FURA-2 AM (Molecular Probes) for 1 hr at room temperature. The loading solution was removed and cells in 24-well plates were incubated with 250 μl of assay buffer (cells in 96-well plates were incubated with 50 μl) for 1 hr to allow the cleavage of the AM ester. Cells expressing T1Rs and G proteins (Offermanns and Simon, 1995; Chandrashekar et al., 2000; Mody et al., 2000) in 24-well tissue culture plates were stimulated with 250 μl of a 2× tastant solution (cells in 96-well plates were stimulated with 50 μl of a 2× tastant solution). As a control for Gα15 and Gα16-Gz signaling, a set of plates was cotransfected with mGluR1 and the μ-opioid receptor and assayed for responses to ACPD and DAMGO.

One of two imaging stations was used to measure $[Ca^{2+}]_i$ changes. One system comprises of a Nikon Diaphot 200 microscope equipped with a 10×/0.5 fluor objective, the TILL imaging system (T.I.L.L Photonics GmbH), and a cooled CCD camera. Acquisition and analysis of these fluorescence images used TILL-Vision software. Also, an Olympus IX-70/FLA microscope equipped with a 10×/0.5 fluor objective, a variable filter wheel (Sutter Instruments), and an intensified CCD camera (Sutter Instruments) was utilized. VideoProbe software (Instrutech) was used for acquisition and analysis of these fluorescence images. Generally, individual responses were measured for 60 s. The F_{340}/F_{380} ratio was analyzed to measure $[Ca^{2+}]_i$.

Kinetics of activation and deactivation were measured using a bath perfusion system. Cells were seeded onto a 150 μl microperfusion chamber, and test solutions were pressure-ejected with a picospritzer apparatus (General Valve, Inc.). Flow rate was adjusted to ensure complete exchange of the bath solution within 4 s. Responses were measured from 80 individual responding cells.

List of Tastants

The following tastants were tested, with the following typical maximal concentrations: sucrose (250 mM), sodium saccharin (25 mM), N-methyl saccharin (5 mM), dulcin (2 mM), aspartame (2 mM), palatinose (250 mM), sodium cyclamate (15 mM), guanidinoacetic acid-1 (1 mM), guanidinoacetic acid-2 (1 mM), guanidinoacetic acid-3 (1 mM), acesulfame-K (10 mM), glucose (250 mM), maltose (250 mM), lactose (250 mM), fructose (250 mM), galactose (250 mM), xylitol (250 mM), raffinose (250 mM), sorbitol (250 mM), trehalose (250 mM), thaumatin (0.1%), monellin (0.1%), alanine (20 mM), glycine (20 mM), arginine (20 mM), monosodium glutamate (20 mM), cycloheximide (5 μM), denatonium (10 mM), phenyl-thiocarbamide (2.5 mM)

Acknowledgments

We thank Wei Guo for expert help generating various T1R expression constructs and Paul Steinbach for software development and imaging expertise. We are also grateful to Ann Leslie for preparation of T1R antibodies, D. Cowan for help with DNA sequencing, Laramiya Phillips for expert technical assistance with cells and cultures, and Andrew Cho and Sreenath Taduru for outstanding help in generating transgenic animals. We thank Lubert Stryer, Roger Tsien, Reuben Siraganian, and members of the Zuker lab for valuable help and advice, and Ricardo Dreyfuss for photography. Finally, we thank the UCSD graphics design unit for help with the cover photograph, and G.N. (left) and J.C. (right) for expert modeling. This work was supported in part by a grant from the National Institute on Deafness and Other Communication Disorders to C.Z. C.Z. is an investigator of the Howard Hughes Medical Institute.

Received June 19, 2001; revised July 9, 2001

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Accession Numbers

Rat and mouse T1R receptor sequences have been deposited in GenBank under accession numbers AY032620-AY032623.