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Putative Mammalian Taste Receptors: A Class of Taste-Specific GPCRs with Distinct Topographic Selectivity

Mark A. Hoon,* Elliot Adler,‡ Jürgen Lindemeier,‡ James F. Battey,† Nicholas J. P. Ryba,*§ and Charles S. Zuker‡§

*National Institute of Dental and Craniofacial Research
†National Institute on Deafness and Other Communication Disorders
National Institutes of Health
Bethesda, Maryland 20892
‡Howard Hughes Medical Institute and
Departments of Biology and Neurosciences
University of California at San Diego
La Jolla, California 92093-0649

Summary

Taste represents a major form of sensory input in the animal kingdom. In mammals, taste perception begins with the recognition of tastant molecules by unknown membrane receptors localized on the apical surface of receptor cells of the tongue and palate epithelium. We report the cloning and characterization of two novel seven–transmembrane domain proteins expressed in topographically distinct subpopulations of taste receptor cells and taste buds. These proteins are specifically localized to the taste pore and are members of a new group of G protein–coupled receptors distantly related to putative mammalian pheromone receptors. We propose that these genes encode taste receptors.

Introduction

The senses of vision, hearing, touch, olfaction, and taste have the critical roles of providing the organism with a faithful representation of the external world. In its simplest form, taste perception is responsible for basic food appraisal and bestows the organism with valuable discriminatory power. For example, sweet receptors allow recognition of high-caloric food sources, while signaling through bitter receptors may stimulate behavioral aversion to noxious substances. Although much is known about mechanisms of signal transduction and information processing in photoreceptors, mechanoreceptors, and olfactory neurons (Corey and Zuker, 1996), little is known about the molecular basis of taste perception.

Mammals are believed to have five basic taste modalities: sweet, bitter, sour, salty, and umami (the taste of monosodium glutamate) (Kawamura and Kare, 1987; Kinnamon and Cummings, 1992; Lindemann, 1996a; Stewart et al., 1997). Extensive psychophysical studies in humans have reported that different regions of the tongue display different gustatory preferences (Figure 1; Hoffmann, 1875; Bradley et al., 1985; Miller and Reedy, 1990), and numerous physiological studies in animals have shown that taste receptor cells may selectively respond to different tastants (Akabas et al., 1988; Gilbertson et al., 1992; Bernhardt et al., 1996; Cummings et al., 1996). In mammals, taste receptor cells are assembled into taste buds that are distributed into different papillae in the tongue epithelium (Figure 1). Circumvallate papillae are found at the very back of the tongue, contain hundreds (mice) to thousands (human) of taste buds, and are particularly sensitive to bitter substances. Foliate papillae localize to the posterior lateral edge of the tongue, contain dozens to hundreds of taste buds, and are particularly sensitive to sour and bitter. Fungiform papillae contain a single or a few taste buds, are at the front of the tongue, and are thought to mediate much of the sweet taste modality.

How are the different taste modalities specified? Each taste bud, depending on the species, contains 50–150 cells, including precursor cells, support cells, and taste receptor cells (Lindemann, 1996a). Receptor cells are innervated at their base by afferent nerve endings that transmit information to the taste centers of the cortex through synapses in the brain stem and thalamus. In the simplest model, different taste modalities could be encoded by different cells expressing different receptors (and perhaps transduction pathways). In this scenario, the topographic sensitivity of the tongue would directly reflect the receptor cell composition of different taste buds and papillae. Alternatively, taste receptor cells could be broadly tuned within or between taste modalities. In this case, decoding strategies would be required to allow tastant discrimination.

What are the intracellular signaling pathways involved in taste transduction? Electrophysiological studies suggest that sour and salty tastants modulate taste cell function by direct entry of H^+ and Na^+ ions through specialized membrane channels on the apical surface of the cell. In the case of sour compounds, taste cell depolarization is hypothesized to result from H^+ blockage of K^+ channels (Kinnamon et al., 1988a) or activation of pH-sensitive channels (Gilbertson et al., 1992); salt transduction may be partly mediated by the entry of Na^+ via amiloride-sensitive Na^+ channels (Heck et al., 1984; Brand et al., 1985; Avenet et al., 1988). Most of the molecular components of the sour or salty pathways have not been identified. Sweet, bitter, and umami transduction are believed to be mediated by G protein–coupled receptor (GPCR) signaling pathways (Striem et al., 1989; Chaudhari et al., 1996; Wong et al., 1996). Confusingly, there are almost as many models of signaling pathways for sweet and bitter transduction as there are effector enzymes for GPCR cascades (e.g., cGMP phosphodiesterase, phospholipase C, adenylate cyclase; see Kinnamon and Margolskee, 1996). The identification of genes encoding taste receptors would provide a critical step in a comprehensive dissection of taste biology.

We have used subtractive and differential single-cell screening techniques to isolate two novel GPCR genes expressed in subsets of taste receptor cells. These genes encode putative taste receptors with appropriate tissue specificity and distinct topographic distributions. We

§To whom correspondence should be addressed: (e-mail: czuker@flyeye.ucsd.edu [C. S. Z.], ryba@yoda.nidr.nih.gov [N. J. P. R.]).

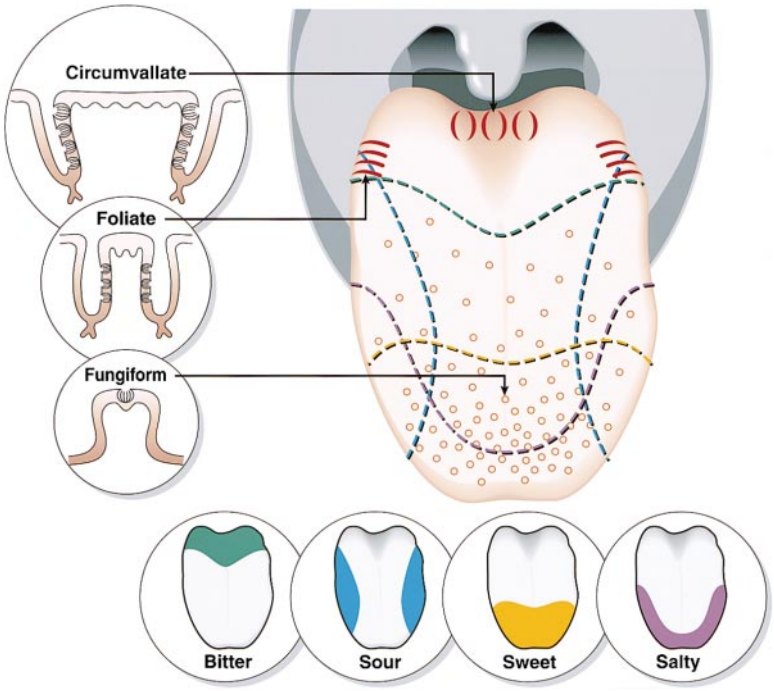

Figure 1. Functional Anatomy of the Human Tongue

Diagram of a human tongue, highlighting the regional preferences to sweet, sour, bitter, and salty stimuli. Note that, while different areas of the tongue display strong preferences to certain taste modalities, there is significant overlap between the various regions. Also shown, in expanded scale, are the three different types of taste papillae and their corresponding topographic distribution (for simplicity, taste buds were only drawn in one side of the papillae folds). We thank MH for posing for this diagram.

examined the subcellular localization of these receptors and show they are optimally positioned to recognize and respond to taste stimuli.

Results and Discussion

Isolation of GPCRs Expressed in Taste Receptor Cells

Sweet and bitter taste transduction are thought to be mediated by different signaling mechanisms employing distinct membrane receptors. We hypothesized that such receptors are likely to be tissue and cell specific, and we designed a screening strategy to identify molecules expressed in subsets of taste receptor cells. In essence, we generated a subtracted cDNA library from rat circumvallate papillae (Hoon and Ryba, 1997) and constructed a collection of single-cell cDNA libraries using RNA isolated from individual rat taste receptor cells (Dulac and Axel, 1995). The libraries were screened for sequences preferentially expressed in taste cells by differential hybridizations with cDNA probes prepared from taste and non–taste tissue; candidate clones were assayed for taste cell specificity by in situ hybridization to tissue sections of rat tongue. These screens identified a number of genes specifically expressed in taste receptor cells (data not shown), including a novel G protein–coupled receptor expressed in a small subset of receptor cells in taste buds of rat and mouse circumvallate papillae (clone TR1, Figures 2a and 2b). This receptor defines a new subgroup of GPCRs (Figure 3) most closely related to the Ca^{2+} sensing receptor (CaSR; Brown et al., 1993), a family of putative pheromone receptors (V2R; Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997), and metabotropic glutamate receptors (mGluRs) (Nakanishi, 1992). All of these receptor families share low but striking sequence similarity and are distinguished from other GPCRs by the presence of a very long N-terminal extracellular domain proposed to encode the ligand-binding site (see Figure 2b). Because TR1 is expressed in very few circumvallate taste receptor cells, we presumed that additional receptor(s) may be found in circumvallate taste buds. We therefore carried out PCR and low-stringency screens on rat circumvallate, foliate, and fungiform cDNAs to isolate possible members of a gene family. A single additional receptor (TR2, Figures 2 and 3) was isolated following exhaustive screens (see Experimental Procedures). TR1 and TR2 share ~40% amino acid identity with each other, and each displays ~30% identity with the CaSR and 22%–30% amino acid identity with V2R pheromone receptors and mGluRs. As expected, TR1 and TR2 contain all the hallmarks of this superfamily of membrane receptors, including seven putative transmembrane segments, a series of conserved cysteine residues in the extracellular domain, and several conserved short sequence motifs scattered throughout the molecule. In situ hybridizations to a variety of non–taste tissues, including the main olfactory epithelium, vomeronasal organ, and brain, did not detect any TR1 or TR2 signals.

In efforts to further define the sequence relationships between TR1 and TR2 and to identify additional related GPCRs, we screened genomic and cDNA libraries for homologous sequences in mouse and human DNA. We isolated the mouse and human orthologs of TR1 and TR2 (see Experimental Procedures) but did not identify additional members using a variety of screening strategies, including degenerate PCR reactions from single taste bud cDNA (see Concluding Remarks). Southern blot analysis with genomic DNA at low stringency (54°C, 0.75 M NaCl) further validates these genes as single family members (data not shown).

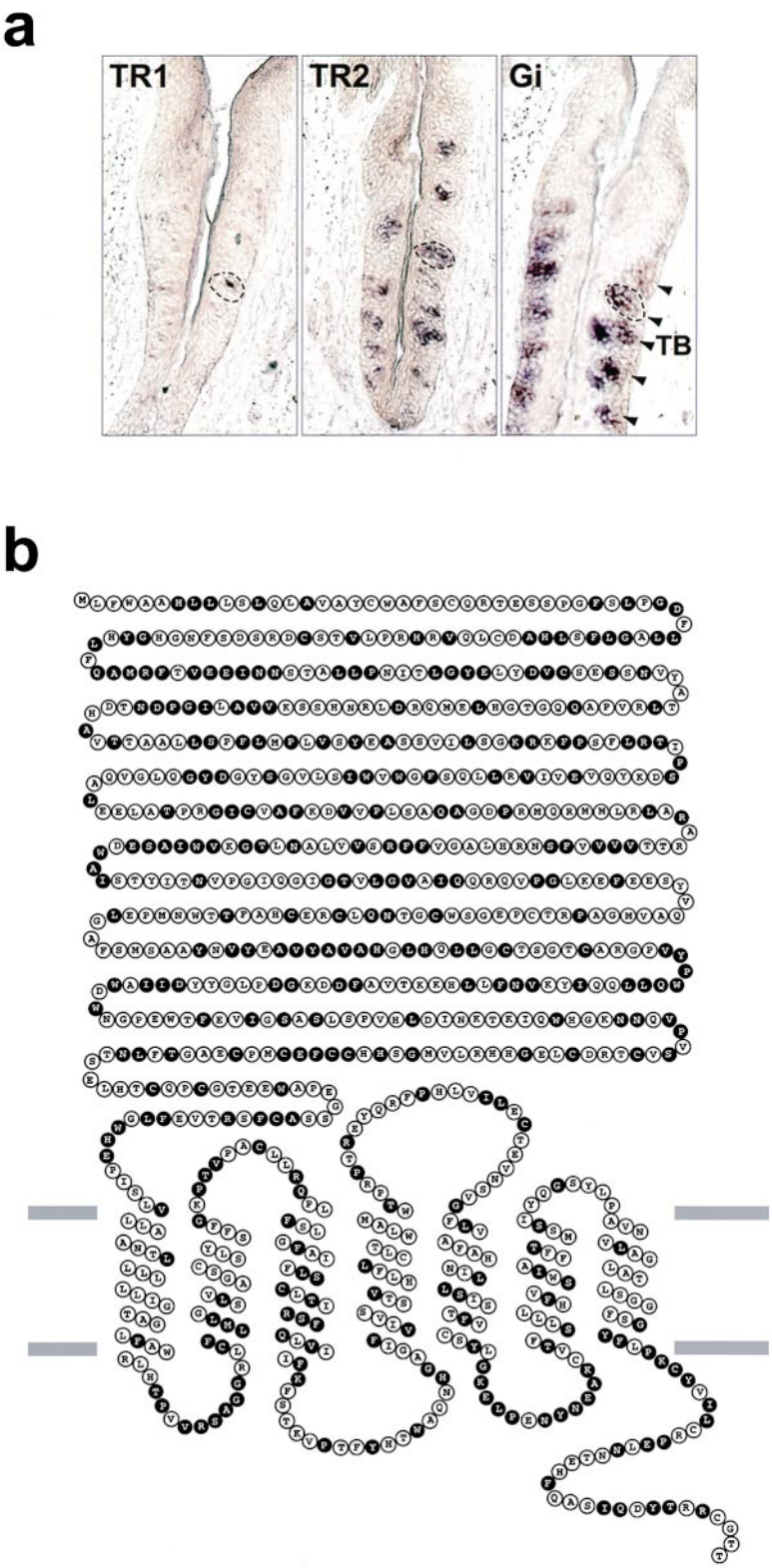

Figure 2. TR1 and TR2 Encode Novel G Protein–Coupled Receptors

(a) Shown are in situ hybridizations to sections of rat circumvallate papillae hybridized with digoxigenin-labeled antisense probes for TR1, TR2, and a rat Gαi G protein subunit. Note the dramatic differences in the representation of TR1- and TR2-expressing cells. Control hybridizations with sense probes did not produce signals, while hybridization with the control Gαi probe specifically labeled all receptor cells in all circumvallate taste buds (TB).

(b) Predicted transmembrane topology of TR1. Amino acids conserved between TR1 and TR2 are shown as filled circles. The rat genes are 92% identical to the corresponding mouse genes, except that mouse TR1 contains a two amino acid insertion at position 15 (data not shown); the human sequences are ~70% identical to the corresponding mouse sequences (see Figure 3 for the full alignment of TR1 and TR2).

TR1 and TR2 Are Expressed in Distinct Subsets of Taste Receptor Cells

As illustrated for the human tongue in Figure 1, taste buds are organized into three types of papillae in the lingual epithelium: circumvallate, foliate, and fungiform. The different papillae display a marked bias in their sensitivity to different tastants (Hoffmann, 1875; Frank et al., 1983; Bradley et al., 1985; Frank, 1991). For example, psychophysical studies of topographic preferences to different tastants in humans and electrophysiological

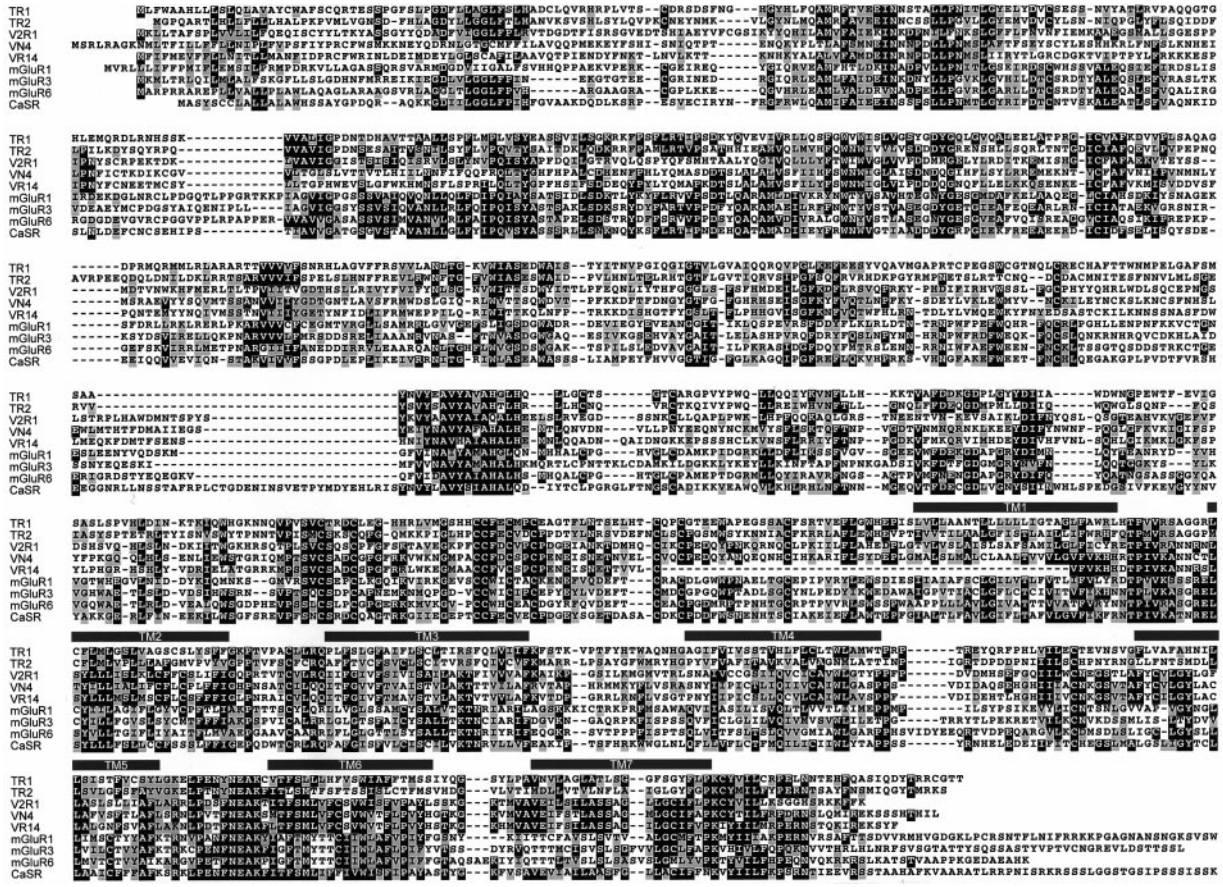

Figure 3. TR1 and TR2 Are Distantly Related to the Calcium-Sensing Receptor, V2R Pheromone Receptors, and Metabotropic Glutamate Receptors

ClustalW alignment between the mouse TR1 and TR2 proteins, three different V2R putative pheromone receptors (Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997), three mGluRs (Nakanishi, 1992), and the CaSR (Brown et al., 1993). The V2Rs and mGluRs used in the alignment were chosen because they represent distinct subfamilies within the VR and mGluR families. Black boxes denote identity between at least four of the nine sequences. The mGluR1 and CaSR sequences were truncated at residues 896 and 918, respectively.

recordings of nerve activity in rodents indicate that circumvallate papillae are particularly sensitive to bitter compounds, while fungiform papillae are strongly biased to salty and sweet stimuli. In addition to the tongue, other parts of the oral cavity also have taste buds; these are particularly concentrated in the palate epithelium in an area known as the geschmackstreifen ("taste stripe"). These taste buds have strong sweet responses and poor bitter sensitivity (Nejad, 1986; Krimm et al., 1987). If TR1 and TR2 function as specific taste receptors, we reasoned they should be expressed in subpopulations of taste receptor cells, with distinctive topographic distribution. To examine the patterns of expression of TR1 and TR2 in detail, we performed in situ hybridizations to sections of various taste papillae. Figure 4 and Table 1 illustrate the marked topographic selectivity of TR1 and TR2. TR1 is rare in the taste buds of circumvallate papillae (less than 10% of circumvallate taste buds contain a TR1-positive cell) but is expressed in all fungiform taste buds (a, c, and d). In contrast, TR2 is almost undetectable in fungiform papillae (we have observed a single positive receptor cell in over 200 fungiform taste buds examined) but is expressed in all circumvallate taste buds (b, e, and f). This differential expression profile is also found in the palate. TR1 is expressed in all geschmackstreifen taste buds, each with many TR1-positive cells. However, TR2 is found in only a small number of the geschmackstreifen buds, and these have only a few TR2-positive cells (j–l). In situ hybridizations to foliate papillae showed that both TR1 and TR2 are expressed in foliate taste buds, but TR2-positive cells are considerably more abundant than TR1-expressing cells (g–i; also note that TR1 signals are much weaker than TR2). Control hybridizations with sense probes produced no specific signals in any of the taste papillae, while hybridization with a cDNA encoding a $G\alpha i$ subunit demonstrated uniform labeling in all taste cells of all taste buds (Figures 2a and 4c).

The high degree of topographic discrimination between TR1 and TR2 was independently examined by performing RT–PCR reactions with RNA from rat and mouse circumvallate papillae. We designed PCR oligonucleotide primers that could selectively amplify TR1 and TR2 sequences and used them for profiling TR1

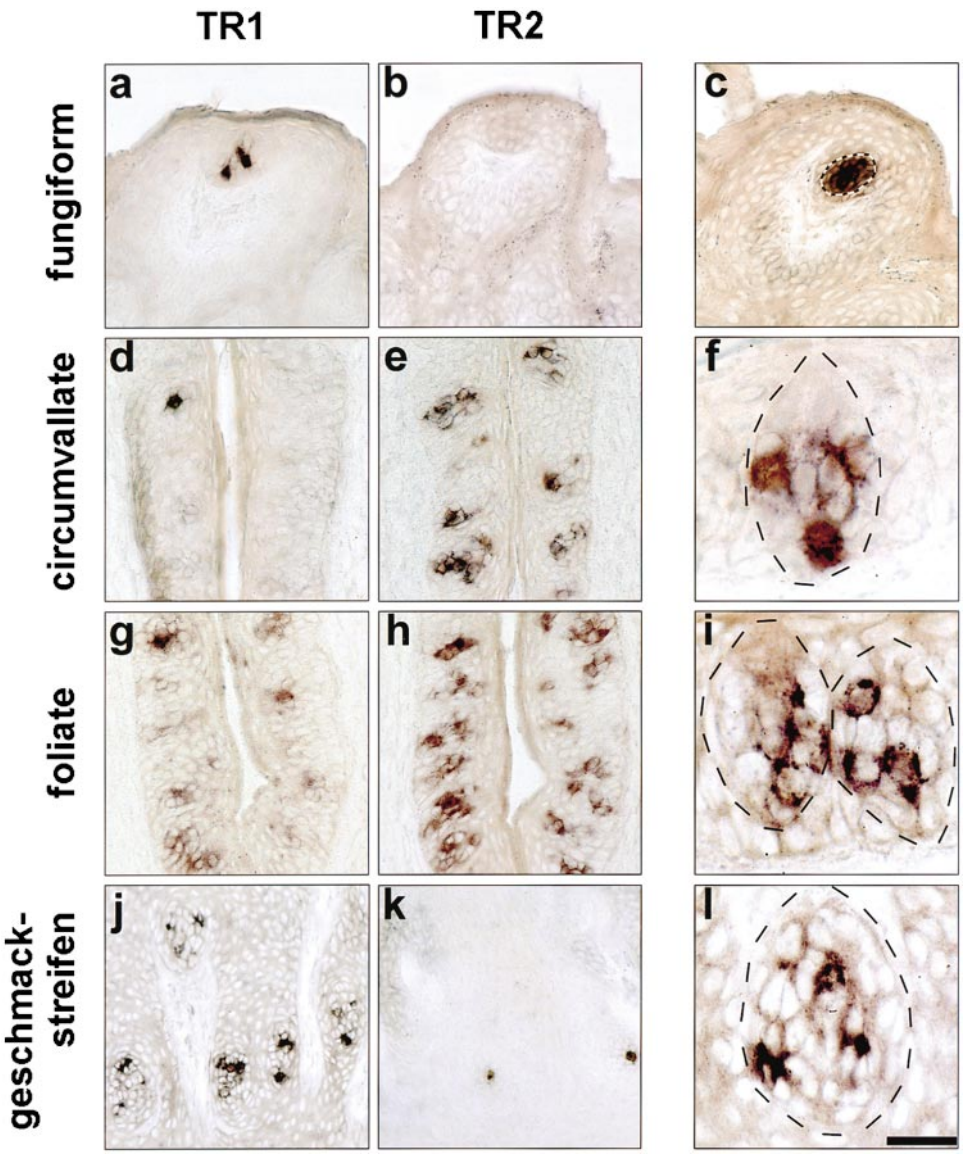

Figure 4. TR1 and TR2 GPCRs Display Rich Topographic Selectivity in the Tongue and Palate Epithelium

In situ hybridizations with TR1 and TR2 digoxigenin-labeled antisense RNA probes demonstrated that TR1 is expressed in many cells in the fungiform and geschmackstreifen taste buds, while TR2 is abundantly expressed in circumvallate and foliate papillae. All fungiform taste buds express TR1 in 20%–30% of the receptor cells (a), while TR2 is not detectable in most fungiform taste papillae (b). As a control, (c) shows hybridization of a fungiform papilla with a Gαi probe that labels all receptor cells. In the circumvallate papilla, only about 10% of the taste buds contain a TR1-expressing cell (see also Figure 2a). In contrast, all circumvallate taste buds express TR2 ([e]; the presence of a TR2-negative region is due to the plane of section. Serial sections demonstrated that all taste buds of mouse and rat circumvallate papillae contain TR2-positive cells). A higher magnification image of TR2 in a circumvallate taste bud demonstrates that only a subpopulation of receptor cells (20%–30%) express this GPCR (f). Both TR1 (g) and TR2 (h and i) are expressed in foliate taste buds; approximately three times as many cells express TR2 as TR1. In the geschmackstreifen, TR1 is expressed in 20%–30% of receptor cells of all taste buds (j and l), while less than half of these taste buds contain a single TR2-expressing cell (k). Equivalent results were obtained in rat and mouse taste papillae. Bar, 20 μm.

and TR2 expression. (To prevent amplification of genomic DNA, we used primers that span an intron sequence in TR1 and TR2.) Of 470 RT-PCR cDNAs isolated from circumvallate taste buds, 430 encoded TR2 and 40 encoded TR1. Together, these results demonstrate refined specificity in the anatomical sites of expression of TR1 and TR2 GPCRs and strengthen the postulate that regional tastant sensitivities reflect regional selectivity in the expression of taste signaling molecules.

TR1 and TR2 Are Expressed in Gustducin-Positive and Gustducin-Negative Cells

The distinctive topographic distribution of TR1 and TR2 and the behavioral representation of sweet and bitter transduction suggest a correlation between the sites of TR1 expression and sweet sensitivity, and the sites of TR2 expression with bitter sensitivity. Gustducin is a Gα subunit abundantly expressed in subpopulations of taste receptor cells of all taste buds (McLaughlin et al.,

Table 1. Summary of TR1 and TR2 Expression Data

	% Taste Buds (% Cells/Bud)			
	Fungiform	Circumvallate	Geschmackstreifen	Foliate
TR1	**100** (20–30)	<**10** (<5)	**100** (20–30)	~**30**[a] (~10[a])
TR2	<**1** (<5)	**100** (20–30)	~**20** (<5)	**100** (20–30)

These data are derived from in situ hybridizations with TR1 and TR2 cRNAs to multiple sections of circumvallate, fungiform, and foliate papillae from at least 20 different mouse and rat tongues. Similar studies were performed on taste buds of mice and rat palates. The (% cells/bud) refers to the percent of taste cells labeled in a standard 14 μm thick section.

[a]The majority of these cells have a much weaker signal than the corresponding TR2-positive cells, or TR1 cells in fungiform papillae or geschmackstreifen taste buds (see Figure 4).

1992). It has been proposed that gustducin is involved in bitter and sweet transduction, since gustducin knockout mice show decreased sensitivity to some sweet and bitter tastants (Wong et al., 1996). However, the precise role of gustducin in taste transduction, and the relationship between gustducin-expressing cells and sweet and bitter responses, is unknown (see for example Lindemann, 1996b; Ogura et al., 1997).

To investigate the correlation between gustducin-expressing cells and TR1 and TR2 expression, we performed double-labeling experiments using differentially labeled TR1, TR2, and gustducin riboprobes (Figure 5). Our experimental strategy was to use fluorescein- and digoxigenin-labeled probes in combination with alkaline phosphatase–conjugated anti-fluorescein antibodies, and horseradish peroxidase–conjugated anti-digoxigenin antibodies with different fluorogenic substrates to distinguish between the various transcripts (see Experimental Procedures). Because TR1, TR2, and gustducin are expressed in foliate papillae, we first focused our studies on foliate tissue and then examined TR1 and gustducin in fungiform papillae, and TR2 and gustducin in circumvallate papillae. Our results (Figure 5) demonstrated no correlation between TR1 or TR2 expression and gustducin-positive cells. For example, in the foliate papillae, only ~1/5 of TR1-positive and ~1/10 of TR2-positive cells also express gustducin, and only ~15% of all gustducin-positive cells express either TR1 or TR2. Thus, it is unlikely that gustducin functions as the Gα subunit that couples TR1 and TR2 receptors to downstream effectors.

Numerous physiological studies suggest that taste buds contain receptor cells for various taste modalities (e.g., sweet, salty, and sour responses in fungiform taste buds; Béhé et al., 1990; Gilbertson et al., 1992; Cummings et al., 1996; Doolin and Gilbertson, 1996). Yet, there is very limited data proving that the very same taste bud responds to multiple modalities (Bernhardt et al., 1996). To directly demonstrate that TR1 and TR2 may be expressed in the same taste buds, we carried out double labeling of foliate papillae using differentially labeled TR1 and TR2 riboprobes. Our studies showed that TR1 and TR2 are indeed expressed in the same taste buds (Figure 5e) but are not, for the most part, expressed in the same cells. Interestingly, we also found a small population of double-positive cells (Figure 5e; right panel). These results demonstrate that a given taste cell may express more than one receptor, and they are consistent with experimental observations suggesting that some taste receptor cells may respond to more than one modality (Lindemann, 1996a; Furue and Yoshii, 1997, 1998).

TR1 and TR2 GPCRs Localize to the Transducing Membrane of Taste Receptor Cells

Sensory cells of all modalities, including vision, olfaction, and taste, have evolved highly specialized subcellular compartments to house their transduction machinery. This compartmentalization enhances receptor–ligand interactions and increases the sensitivity and specificity of the response. Mammalian taste receptor cells contain apical microvilli that are exposed to the oral cavity through a narrow opening known as the taste pore. This structure, ~5 μm in diameter in mouse taste buds, is formed by the juxtaposition of taste receptor cells and the convergence of their apical ends at the lingual surface (Cummings et al., 1987; see diagram in Figure 6b). The taste pore functions as the conduit for the presentation of tastants to receptor cells and is hypothesized to be the site of tastant recognition by membrane receptors. Therefore, we reasoned that bona fide taste receptors should be specifically localized to this area. We generated antibodies to TR1 and TR2 proteins and used them in immunofluorescence staining of tongue tissue sections. Examination of foliate and fungiform papillae demonstrated that TR1 specifically localizes to the apical surface of taste receptor cells, with the antibodies specifically and selectively labeling the taste pore region (Figure 6a). Similar studies with TR2 demonstrated specific labeling of the taste pore of circumvallate and foliate taste buds (Figure 6c). Labeling with a control N-CAM antibody (Nolte and Martini, 1992) showed labeling throughout the surface of taste bud cells (Figure 6d). These results strongly implicate TR1 and TR2 in the transduction process and substantiate their potential role as taste receptors.

Concluding Remarks

We have identified two novel seven–transmembrane domain proteins that are likely to represent mammalian taste receptors. First, at the primary sequence level, TRs are GPCRs most closely related to mammalian (Brown et al., 1993; Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997) and fish (Cao et al., 1998; Naito et al., 1998) candidate sensory receptors. Second, TRs are specifically expressed in subsets of taste receptor cells of the tongue and palate epithelium. Third, these receptors show high topographic selectivity among different taste papillae. Finally, TR1 and TR2 localize to the taste pore of taste buds; this is the only

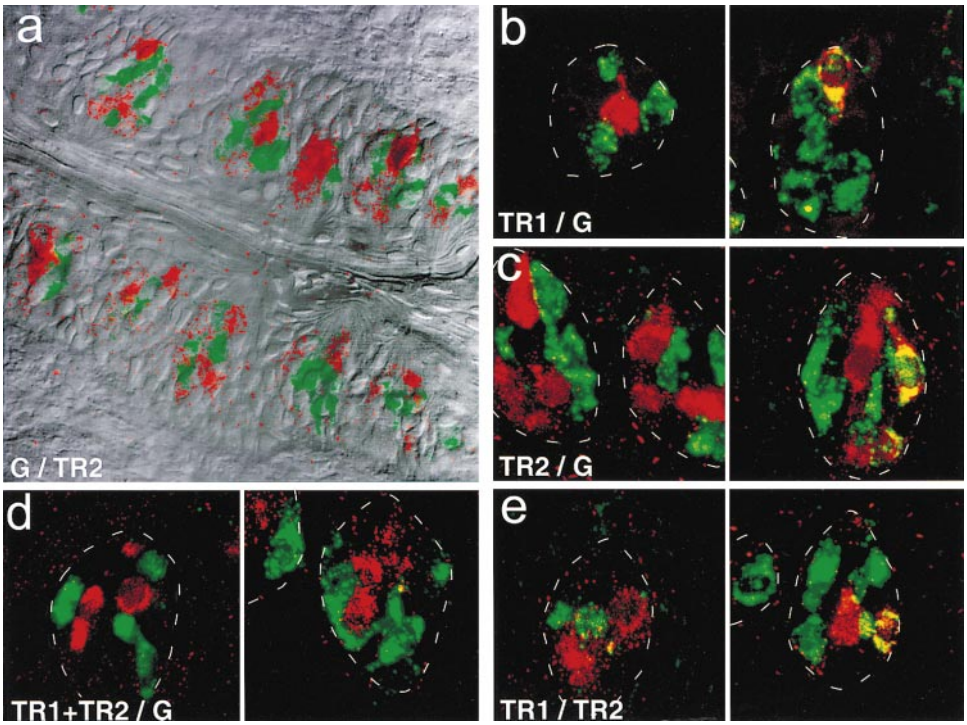

Figure 5. TR1 and TR2 Do Not Colocalize with Gustducin

Double-label fluorescent in situ hybridizations were used to examine the coexpression profiles of TR1, TR2, and gustducin (G). The dotted lines outline the approximate area of the sectioned taste buds. For each panel, the probe detected with fast red is listed first in the figure (e.g., G/TR2 refers to gustducin in red and TR2 in green). (a) A low magnification image of a foliate papillae probed with gustducin (red) and TR2 (green), overlaid on a difference interference contrast image, shows that taste receptor cells that express TR2 generally do not express gustducin. However, there are a few (b) TR1- or (c) TR2-positive cells that also express gustducin (see text for details). (d) To further demonstrate that most gustducin-positive cells do not express either TR1 or TR2, we also used a mixed TR1 and TR2 probe (TR1+TR2) with a gustducin probe. To determine whether TR1 and TR2 could be expressed in the same cells, we examined foliate papillae with differentially labeled TR1 and TR2 probes. (e) demonstrates that that there is a rare population of cells that coexpress TR1 and TR2. All images were obtained with a Leica TSC confocal microscope using an argon-krypton laser. (b)–(e) are 3–5 μm optical sections of 14 μm thick frozen sections all presented at the same magnification; each taste bud displays 9–15 cells on the plane of section (approximate number of cells/bud: [b], 9 and 11 cells; [c], 11, 10, and 12 cells; [d], 14 and 15 cells; [e], 9 and 11 cells).

region of taste receptor cells exposed to taste stimuli on the tongue surface.

TR1 and TR2 share only ~40% sequence identity, highlighting the likelihood of distinct ligand selectivity. What sensory modality may these receptors encode? The similarity of TR1 and TR2 with mGluRs suggested a role in amino acid, or umami, taste. However, the receptor cell selectivity of TR1 and TR2 expression and the anatomical representation of umami taste do not support this notion (Kawamura and Kare, 1987; Chaudhari et al., 1996). Instead, the topographic distribution of TR1-expressing receptor cells suggests a strong correlation with sweet sensitivity (fungiform papillae and geschmackstreifen) and TR2 expression with bitter taste (circumvallate papillae). We have attempted to determine the ligand/tastant specificity for TR1 and TR2 using a variety of strategies but have been hampered by the difficulty of functionally expressing these molecules in heterologous systems (J. Chandrashekar et al., unpublished data). Novel expression systems (Krautwurst et al., 1998), comprehensive genetic studies, and in situ physiological recordings from TR1- and TR2-expressing cells tagged with fluorescent reporters may help define the specificity of TR1 and TR2 GPCRs.

Are there more than two TR genes? Southern blot analysis of mouse and rat genomic DNA probed with TR1 and TR2 at a variety of stringencies did not detect cross-hybridization between these two GPCRs or with additional genomic fragments (data not shown). However, a number of psychophysical and physiological studies insinuate the presence of multiple receptors for a given taste modality. For instance, a number of genetic and cross-desensitization studies suggest different receptors for different types of bitter compounds (see for example Lush, 1981, 1984; Spielman et al., 1992; Bartoshuk et al., 1994). Thus, we presume that additional divergent receptors exist in the genome. The concept that there could be many receptors with similar function, but little if any sequence homology, has been amply demonstrated in *C. elegans* where hundreds of highly divergent chemosensory receptors have been identified (Troemel et al., 1995; Bargmann, 1998). Expression cloning strategies and advances in the human and mouse genome projects may help identify additional receptors. However, if there are many additional TR genes in the genome, we infer that a given taste cell would have to express many receptors, since TR1 is expressed in

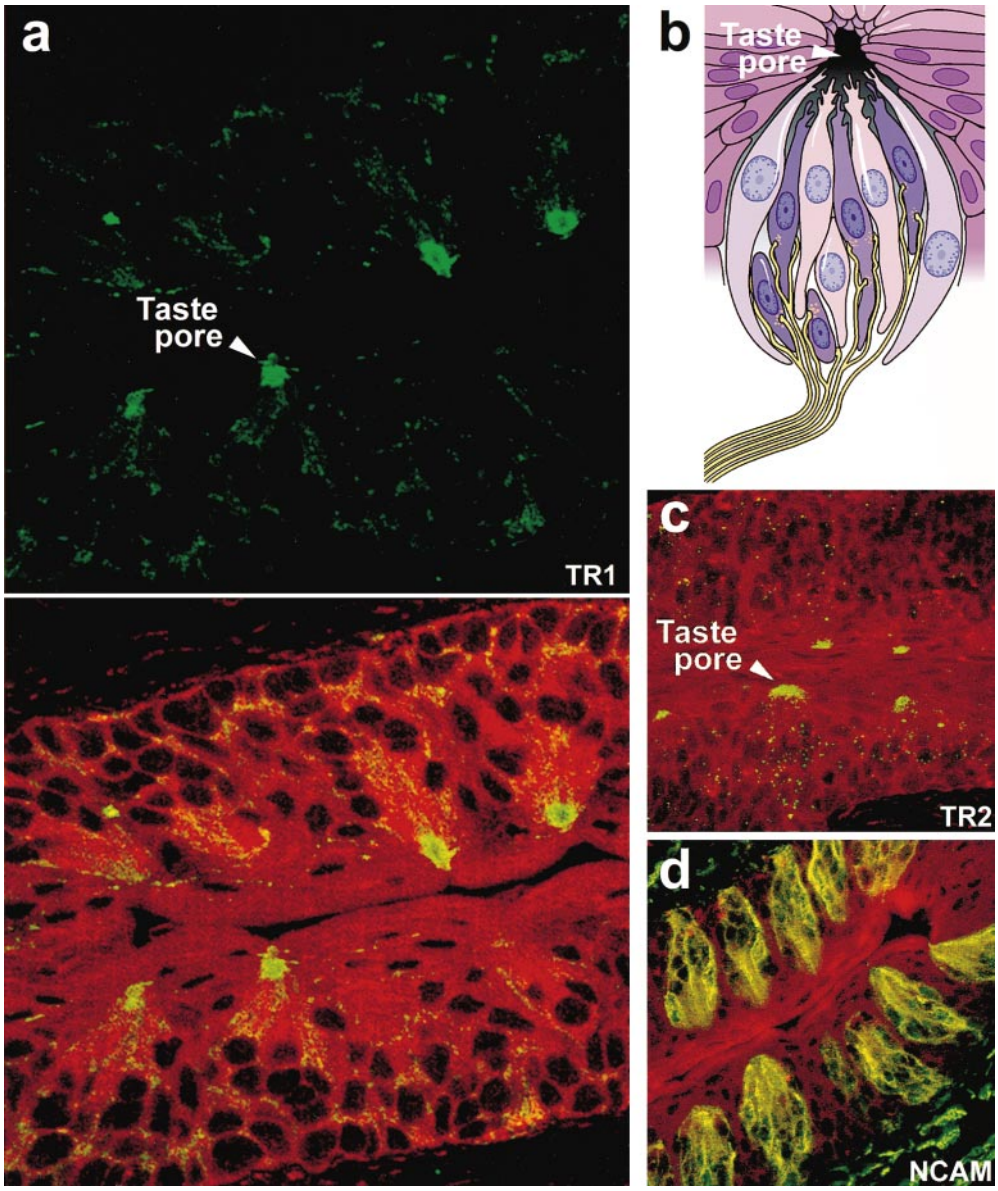

Figure 6. TR GPCRs Localize to the Taste Pore of Taste Buds

(a) Immunofluorescent stainings of TR1 in rat foliate taste buds. The upper panel shows that TR1 is concentrated in the taste pore; the lower panel shows colabeling with a fluorescent F-actin probe (BODIPY TR-X phallacidin; Molecular Probes) to illustrate the outline of the taste buds and the structure of the papillae.

(b) Diagram of a taste bud, highlighting the location of the taste pore.

(c) Immunofluorescent and F-actin staining of rat circumvallate taste buds, demonstrating TR2 localization to the taste pore like TR1. In contrast, (d) a control integral membrane protein (N-CAM) is distributed throughout the plasma membrane of taste cells.

20%–30% of the cells of all fungiform and geschmackstreifen taste buds, and TR2 is expressed in 20%–30% of the cells of all circumvallate and foliate taste buds.

How many different receptors does it take to sample the sweet and bitter world? The answer to this simple question has challenged researchers for well over a century (Brillat-Savarin, 1826). At a fundamental level, the evolution of sweet and bitter responses should primarily reflect the need to detect and measure caloric content and noxious stimuli. As such, it is easy to envision the need for high discrimination between these two modalities but greater promiscuity within a modality. Under this premise, the sweet and bitter receptor family would be far less diverse than the family of olfactory receptors (Mombaerts et al., 1996). Notably, genetic data in mice strongly argue for a single prominent "sweet locus" (*sac*, saccharin; Lush, 1989). It would be of great interest to determine the relationship between TR1 and *sac* (both of which map to the distal end of mouse chromosome 4; unpublished data).

How are taste signals encoded? There are a number of different strategies that organisms use to encode chemosensory information. For example, individual mammalian olfactory neurons express only 1 of ~1000 different olfactory receptors, and all neurons expressing a common receptor project to the same glomerulus (reviewed by Mombaerts et al., 1996). Thus, decoding of complex olfactory stimuli is reduced to identifying the appropriately activated glomeruli. In contrast, *C. elegans* chemosensory neurons express a large number of different receptors in a given sensory cell (Troemel et al., 1995). Therefore, these neurons either function as on-off switches, reporting only the presence of a stimulus with no regard to "quality" (Troemel et al., 1997), or the nervous system must devise strategies to deconvolute the compound signal by using differential encoding and decoding paradigms. In the case of mammalian taste, it is well known that individual nerve fibers in the mammalian tongue receive synaptic input from multiple taste receptor cells (see for example, Kinnamon et al., 1985, 1988b; Royer and Kinnamon, 1988). Yet, it is not known how those taste receptor cells are functionally related to each other. The isolation of taste receptor genes makes it possible to identify individual receptor cells and determine their relationship to specific nerve fibers; this will be particularly revealing in foliate papillae, where a significant number of taste buds contain both TR1- and TR2-positive cells (Table 1 and Figure 5e). These studies will help define the logic of synaptic connectivity and information processing in taste perception. Finally, the availability of taste receptors will also provide a rational strategy for identifying high-potency agonists and antagonists of taste cell function, and offer a sensible approach for modulating taste perception.

Experimental Procedures

Molecular Cloning of Taste Receptors

Construction and initial analysis of a taste receptor cell–subtracted cDNA library was as described by Hoon and Ryba (1997). Further enrichment of taste-specific transcripts was achieved by dot blot screening of cDNA clones with non–taste cDNA probes. Taste buds and single taste receptor cells were isolated by enzymatic digestion and microdissection of lingual epithelium from adult rats and mice according to Béhé et al. (1990). Single-cell libraries were generated from rat circumvallate cells using the method of Dulac and Axel (1995); a similar approach was used to generate cDNA libraries from whole taste buds isolated from circumvallate, fungiform, and foliate papillae, except that the lysis volumes were increased 10-fold.

TR1 was isolated as a partial cDNA clone from the subtracted circumvallate cDNA library. This clone shared sequence homology with the CaSR, and in situ hybridizations demonstrated that the transcript was specifically expressed in a small subpopulation of cells in taste buds of the circumvallate papilla. Full-length TR1 cDNA clones were isolated from a rat λZAPII circumvallate cDNA library. Multiple clones were isolated and their DNA sequence determined by fluorescent-based sequencing methods (Applied Biosystems). TR2 was isolated by PCR amplification of single circumvallate taste bud cDNAs (Dulac and Axel, 1995) using degenerate primers encoding the highly conserved NY/FNEAK and PKCYI/VIL motifs in TR1, CaSR, mGluRs, and V2Rs (see Figure 3). Low-stringency cDNA and genomic screens with TR1 or TR2 (see Results) did not produce additional sequences. The mouse TR1 and TR2 clones were isolated by 5′-end and 3′-end RACE reactions (Marathon kit, Clontech) using first-strand cDNA prepared from mouse circumvallate and foliate papillae, and the corresponding genomic clones were isolated from mouse Bac and λ libraries. The human TR1 cDNA was isolated from a human testis cDNA library; this was prompted by the observation that a number of sensory receptors, including rhodopsin and olfactory receptors, are expressed in testis. The human TR2 fragment was isolated by PCR amplification using degenerate primers encoding the conserved VWIASE and VYPWQL motifs in TR1 and TR2.

In Situ Hybridization and Immunolocalization

Tissue was obtained from adult rats and mice. No sex-specific differences in 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 (14 μm) were attached to silanized slides and prepared for in situ hybridization as described previously (Ryba and Tirindelli, 1997). All in situ hybridizations were carried out at high stringency (5× SSC, 50% formamide, 72°C). For single-label detection, signals were developed using alkaline phosphatase–conjugated antibodies to digoxigenin and standard chromogenic substrates (Boehringer Mannheim). For double-label fluorescent detection, 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).

Anti-peptide antibodies to TR1 (C-terminal tail) and anti-fusion protein antibodies to TR2 (extracellular domain) were generated in rabbits and purified as described by Cassill et al. (1991). The antibodies were assayed by Western blot analysis of protein homogenates from circumvallate (TR2) or fungiform (TR1) papillae and by in vitro translation of in vitro transcribed TR1 and TR2 RNA (A. Leslie et al., unpublished data). The rabbit polyclonal antibody to N-CAM was obtained from Chemicon. For immunohistochemistry, frozen sections were cut as described above for in situ hybridization. For TR1, sections were prepared as for in situ hybridization except that no acetylation was carried out and blocking reactions used 10% donkey immunoglobulin, 1% bovine serum albumin, 0.3% Triton X-100. For TR2 and N-CAM, sections were fixed in 4% paraformaldehyde in methanol and blocked using 10% donkey immunoglobulin, 1% bovine serum albumin. Sections were incubated in the appropriate dilution of anti-TR1 (1:100), anti-TR2 (1:50/100), or anti-NCAM (1:100) in blocking buffer for 12–18 hr and detected using fluorescein-conjugated donkey anti-rabbit secondary antibodies (Jackson Immunolaboratory). Taste buds were counterstained with the F-actin marker BODIPY TR-X phallacidin (Molecular Probes). Fluorescent images were obtained using a Leica TSC confocal microscope with an argon-krypton laser. Pretreatment of the antibodies with the peptide immunogen abolished staining (data not shown).

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