Chem. Senses 32: 255–262, 2007

doi:10.1093/chemse/bjl053
Advance Access publication January 17, 2007

Expression of T1Rs and Gustducin in Palatal Taste Buds of Mice

Leslie M. Stone[1,2], Jennell Barrows[1,3], Thomas E. Finger[1,3] and Sue C. Kinnamon[1,2]

[1]Rocky Mountain Taste and Smell Center, Aurora, CO 80045, USA, [2]Department of Biomedical Sciences, Colorado State University, Fort Collins, CO 80523, USA and [3]Department of Cell and Developmental Biology University of Colorado School of Medicine, Aurora, CO 80045, USA

Correspondence to be sent to: Leslie M. Stone, Department of Biomedical Sciences, Colorado State University, Fort Collins, Colorado, USA. e-mail: leslie.stone-roy@colostate.edu

Abstract

The palatal region of the oral cavity in rodents houses 100–300 taste buds and is particularly sensitive to sweet and umami compounds; yet, few studies have examined the expression patterns of transduction-related molecules in this taste field. We investigated the interrelationships between members of the T1R family and between each T1R and gustducin in palatal taste buds. Similar to lingual taste buds, T1R1 and T1R2 are generally expressed in separate palatal taste cells. In contrast to lingual taste buds, however, T1R2 and T1R3-positive palatal taste cells almost always coexpress gustducin, suggesting that sweet taste transduction in the palate is almost entirely dependent on gustducin. T1R1-positive palate taste cells coexpress gustducin about half the time, suggesting that other G proteins may contribute to the transduction of umami stimuli in this taste field.

Key words: coexpression, gustatory, immunocytochemistry, taste transduction, umami

Introduction

Taste buds, the transducing elements of gustatory sensation, are distributed throughout the oral cavity including the palate as well as the tongue. Lingual taste buds lie in 3 different types of connective tissue papillae residing in different areas of the tongue. Fungiform papillae, small, raised papillae on the anterior two-thirds of the tongue, each contain one or two taste buds that are innervated by the chorda tympani branch of the facial nerve. Vallate and foliate papillae are invaginations on the posterior and lateral surfaces of the tongue. These papillae contain hundreds of taste buds that are innervated primarily by the glossopharyngeal nerve. The taste buds of the palate are most plentiful in 3 locations: near and within the nasoincisor ducts, in a discrete Geschmacksstreifen, a stripe of taste buds located at the juncture of the soft and hard palate, and scattered within the soft palate just posterior to the Geschmacksstreifen. Palatal taste buds are innervated by the greater superficial petrosal branch of the facial nerve.

The chemical sensitivity of taste buds varies according to their location in the oral cavity (Frank et al. 1983). The greater superficial petrosal branch of the facial nerve, which innervates taste buds on the palate, is highly responsive to sweet and umami taste stimuli (Krimm et al. 1987; Travers and Norgren 1991; Harada et al. 1997; Sako et al. 2000; Sollars and Hill 2005). In contrast, the glossopharyngeal nerve responds best to acidic and bitter stimuli (Danilova and Hellekant 2003), whereas the chorda tympani nerve responds to salty and acidic tastants and to a lesser extent to sweet and umami compounds (Frank et al. 1983; Sako et al. 2000; Danilova and Hellekant 2003).

Bitter, sweet, and umami taste stimuli are detected by G protein–coupled receptors, leading to activation of intracellular signaling cascades and transmission of taste information to associated nerve fibers. The T2Rs represent a family of about 30 receptors, which are activated by bitter compounds (Adler et al. 2000; Chandrashekar et al. 2000; Matsunami et al. 2000). Consistent with taste field specificity, T2Rs are more prevalent in the bitter-sensitive vallate papilla (Adler et al. 2000), although T2R expression in the palatal Geschmacksstreifen is similar to vallate and foliate indicating that the palate is also important for bitter taste transduction (Adler et al. 2000). Sweet and umami transduction are mediated through T1R receptors, a family consisting of 3 members, which combine to form primarily heteromeric receptors. T1R1 + T1R3 form an amino acid (umami) receptor, whereas T1R2 + T1R3 form a broadly tuned sweet receptor. The distributions of the T1Rs vary with location. T1R3 is expressed in all lingual papillae, as well as in taste buds of the palate (Kitagawa et al. 2001; Max et al. 2001; Montmayeur et al. 2001; Nelson et al. 2001; Sainz et al. 2001). In contrast,

some reports indicate that T1R1 is expressed primarily in fungiform taste buds, whereas T1R2 is expressed primarily in vallate and foliate papillae (Hoon et al. 1999), but see Kim et al. (2003) who report more widespread expression of T1R1 and T1R2. The palate is the only taste field where both T1R1 and T1R2 are abundantly expressed, and it is also an area responsive to sweet and umami stimuli.

In addition to receptor proteins, downstream signaling effectors play important roles in taste transduction. One of these effectors is the G protein α-gustducin, which is present in some taste cells in all gustatory fields (Boughter et al. 1997). Mice lacking the α-gustducin protein are defective in their ability to detect bitter, sweet, and umami tastants (Wong, Gannon, et al. 1996; Wong, Ruiz-Avila, et al. 1996; Ruiz-Avila et al. 2001; Caicedo et al. 2003; Ruiz et al. 2003; Glendinning et al. 2005), suggesting that gustducin impacts on both the T2R and T1R transduction pathways. T2Rs and gustducin are coexpressed in many taste cells of the bitter-sensitive vallate region as expected. In contrast, T1Rs and gustducin are rarely coexpressed in this region, although some coexpression of T1Rs and gustducin was recently reported in fungiform taste buds (Kim et al. 2003). Palatal taste buds have not been examined for coexpression of gustducin and T1Rs, despite being the most sweet-sensitive region of the oral cavity.

To better understand sweet and umami taste transduction, the current study examines the expression patterns of each member of the T1R family and the relationship of each T1R with gustducin in palate taste buds. In addition, to facilitate comparisons between palatal and lingual taste buds, and because there is controversy in the literature, we reexamined the expression of T1Rs and gustducin in fungiform and circumvallate taste buds. A portion of these data was presented previously in abstract form (Stone et al. 2003).

Materials and methods

Animals

The tongues and palates from adult male and female C57/BL mice were used for these studies. Animals were housed and handled according to Animal Care and Use Committees at Colorado State University and the University of Colorado Health Sciences Center. For some experiments, mice were anesthetized with an overdose of sodium pentobarbital (or chloral hydrate) injection (55 mg/ml) and perfused transcardially with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer. For the remaining experiments, animals were first euthanized with carbon dioxide, followed by cervical dislocation. Tongues and palates were removed from the mice and fixed by immersion in 4% PFA.

In situ hybridization

In situ hybridization was carried out as previously described (Finger et al. 2003). Briefly, sense and antisense riboprobes were synthesized from cDNA plasmids for mouse T1R1 and T1R2 (gifts from C. Zuker, University of California at San Diego and N. Ryba, National Institutes of Health) incorporating digoxigenin or fluorescein-labeled uridine triphosphate (UTP) and unlabeled UTP and hydrolyzed to ≈500 bp. Tongues and palates from perfused mice were postfixed in PFA with 20% sucrose overnight. Frozen cryostat sections (12–14 microns) were collected onto Fisher Superfrost Plus slides maintained at room temperature and stored at −20 °C until ready for hybridization. Sections were treated with proteinase K (20 μg/ml), washed, and treated with 0.5% H_2O_2 to quench endogenous peroxidase activity. After being incubated at 37–45 °C in prehybridization buffer for 30–60 min, the slides were incubated at 59 °C overnight in hybridization solution (same as prehybridization buffer with the addition of 5% dextran sulfate and 40–200 ng/ml of digoxigenin or fluorescein-labeled probe). Digoxigenin label was detected using biotin-labeled mouse antidigoxin antibody (Jackson Immunoresearch, West Grove, PA) diluted 1:200. After washing, avidin–biotin complex (Vector Laboratories, Burlingame, CA) was applied to the slides, followed by Alexa Fluor 568 tyramide (TSA, Molecular Probes, Eugene, OR) and reacted with 0.0015% H_2O_2. The slides then were washed, blocked with 1% normal serum (Jackson), and incubated overnight with rabbit anti-gustducin (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:1000 or rabbit anti-T1R3 (gift from Robert Margolskee) at 1:500. The gustducin and T1R3 antisera were detected with Alexa Fluor 488 goat anti-rabbit antibody (Molecular Probes). The slides were washed and coverslipped with Fluoromount-G. Controls in which the probe or the primary antiserum was omitted showed no cross-reactivity.

For double in situ hybridization labeling, one probe was labeled with digoxigenin, the other with fluorescein. The tissue was prepared in the same manner as the single in situ labeled tissue except that the fluorescein-labeled probe was detected with rabbit anti-fluorescein (Zymed Laboratories, San Francisco, CA) before the digoxigenin-labeled probe was detected. The fluorescein antibody was detected using biotin donkey anti-rabbit (Jackson), avidin–biotin complex, and Alexa Fluor 488 TSA. The sections were then retreated with 0.5% H_2O_2 and blocked with avidin–biotin blocking reagent (Vector), and the digoxigenin-detecting procedure described above was performed for detection of the second probe. Controls in which one of the two probes was omitted showed no cross-reactivity.

Immunocytochemistry

Tissue was collected and postfixed for 1–2 h in 4% PFA. Both immersion-fixed and perfused tissues were transferred into 20% sucrose in 0.1 M phosphate buffer and put at 4 °C overnight. Next, tissue was trimmed and frozen in O.C.T. Compound (Electron Microscopy Sciences, Fort Washington, PA). Fifteen-micron cryosections were collected

on Superfrost Plus slides maintained at room temperature (VWR, West Chester, PA) and washed in 0.1 M phosphate-buffered saline (PBS).

To evaluate the relationship between T1R3 and gustducin expression, an immunocytochemical protocol similar to that developed by Shindler and Roth (1996) was used. This technique allows immunocytochemical evaluation of 2 antigens using antibodies raised in the same species. To prepare taste tissue for immunolabeling, endogenous peroxidases were inactivated by incubating tissue sections in 0.5% hydrogen peroxide in 0.1 M phosphate buffer for 15 min. This was followed by washes in 0.1 M PBS, and then the slides were incubated in blocking solution (0.3% triton X-100 [US Biochemical Corporation, Cleveland, OH], 1% bovine serum albumin, and 1% normal goat serum [Jackson Immunoresearch Laboratories] in 0.1 M PBS), for 1–2 h at room temperature. The sections then were incubated in rabbit anti-gustducin antibodies (Santa Cruz #sc-395; 1:20 000 to 1:30 000) overnight. Due to the dilute concentration of primary antibody, subsequent application of fluorescent anti-rabbit secondary antibodies using standard protocols produces no visible label. However, amplification protocols result in detectable labeling. Tyramide amplification of the signal was used for the current studies. For this protocol, first the sections were washed in 0.1 M PBS and incubated in biotinylated goat anti-rabbit antibodies (Jackson; 1:1000 in blocking solution) for 2–3 h. Next, the slides were washed in PBS and incubated with streptavidin–horseradish peroxidase (Molecular Probes, TSA kit, component C) for 1 h at room temperature. Following one 5-min wash in PBS, the slides were washed twice in wash buffer (0.1 M Tris, 150 mM NaCl, 0.1% Tween20). Tyramide working solution was then applied to the slides. This consisted of the labeled tyramide (with Alexa Fluor 488 or Alexa Fluor 647, Molecular Probes kit component A) dissolved in amplification buffer (kit component E) with 0.0015% hydrogen peroxide (made from kit component F). The labeled tyramide solution was left on the slide for 5–10 min, then rinsed off with PBS plus 0.1% tween (3 washes of 15 min each), resulting in tyramide precipitate indicative of gustducin. Labeling for the second protein was done according to standard indirect immunofluorescence procedures. The tyramide-labeled sections were incubated in blocking solution for 1 h, and the second primary antibody was added at a normal dilution (rabbit anti-T1R3 1:200 to 1:300). The second primary antibody was left on overnight, and then the slides were washed in PBS and the second secondary antibody applied (Cy5 [1:400] or fluorescein isothiocyanate [1:100] goat anti-rabbit). After 2 h, the slides were washed in PBS and coverslipped with fluoromount-G. For each experiment, 2 controls were done: one lacking primary antibodies and one omitting the first secondary antibody and the second primary antibody to insure that the second secondary antibody did not cross-react with the first primary antibody. The controls indicated that there was no cross-reactivity. Unless noted, chemicals were purchased from Sigma.

Imaging and analysis

Processed tissue was viewed with an Olympus Fluoview FV300 confocal microscope or a Zeiss LSM 510 meta confocal microscope. *Z* stacks of images from labeled taste buds were collected with each channel being acquired separately to avoid bleedthrough of signal from one channel to the other. Acquisition settings were kept constant for experimental samples and control samples. The individual images of each Z series were combined prior to counting T1R3-immunoreactive (IR) cells, gustducin-IR, and double-labeled cells. Only labeled cells with visible nuclei were included in the cell counts. Images were saved as tif files, and identifying labels were applied using Photoshop 6.0 or 7.0 software. If necessary, brightness and contrast were adjusted using Photoshop 6.0 or 7.0 software.

Results

The distribution of T1R family members and the relationships between specific T1Rs and between each T1R and α-gustducin in palate and lingual taste buds was examined using immunocytochemistry and in situ hybridization. The primary focus of the current study was the evaluation of palatal taste buds. Examination of lingual taste buds was done to allow comparisons between different taste cell types and because there are differing reports in the literature concerning the distributions of T1Rs in lingual taste buds. We found that all 3 T1R subunits are expressed in palate taste buds, as reported by Nelson et al. (2001). In addition, and in agreement with previous work, T1Rs also were expressed in taste buds from fungiform, vallate, and foliate papillae (Hoon et al. 1999; Kitagawa et al. 2001; Max et al. 2001; Montmayeur et al. 2001; Nelson et al. 2001; Sainz et al. 2001; Kim et al. 2003).

Of all palatal taste cells labeled by T1R1 or T1R2 probes, 40% expressed T1R1 only, 50% expressed T1R2 only, and 10% expressed both subunits (Figures 1 and 2A, Table 1). Similar to previous studies on lingual taste buds (Hoon et al. 1999), we found that about 10% of circumvallate and foliate taste cells coexpress T1R1 and T1R2. Thus, palatal taste buds are similar to taste buds in other regions in that T1R1 and T1R2 are generally expressed in different taste cells. Although the percentage of cells coexpressing both T1R subunits was similar in all taste fields, the proportions of cells expressing T1R1 only or T1R2 only differed in some regions. Palate and fungiform taste buds were similar to each other but differed from taste buds in vallate and foliate fields. Palate and fungiform taste buds expressed roughly equal numbers of T1R1 and T1R2 cells, whereas in circumvallate and foliate taste buds, T1R2 expression predominated, with T1R2-only cells representing over 70% of the T1R-expressing population (Figure 1, Table 1).

Both T1R receptors and the G protein α-gustducin play roles in sweet and umami transduction (Wong et al. 1996; Nelson et al. 2001; Ruiz-Avila et al. 2001; Li et al. 2002;

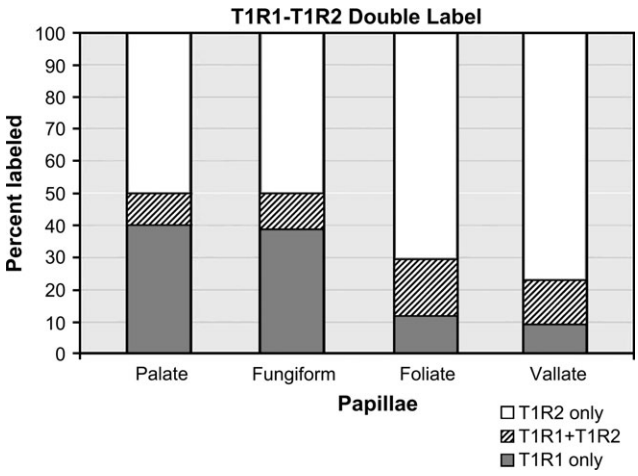

Figure 1 Histogram showing the relationship between T1R1 and T1R2 expression in palatal, fungiform, foliate, and vallate taste buds. Note that in all taste fields, less than 20% of labeled taste cells coexpress T1R1 and T1R2.

Caicedo et al. 2003; Damak et al. 2003; Ruiz et al. 2003; Zhao et al. 2003; Glendinning et al. 2005); yet, T1R receptors and gustducin are usually not coexpressed in the same taste cells in vallate or foliate papillae (Hoon et al. 1999; Kim et al. 2003). To test whether T1Rs and gustducin are coexpressed in taste buds of the sweet-sensitive palate, we examined the expression of each T1R relative to α-gustducin in these taste buds. We found that T1Rs and gustducin were commonly expressed in the same cells in palate taste buds (Figure 2B,C and Table 2). The highest incidence of coexpression was with T1R3 and gustducin; 100% of gustducin-IR cells also expressed T1R3 (Figures 3 and 4); 38% of gustducin cells expressed T1R2, and 12% expressed T1R1 (Figure 5). In contrast to the palate, the foliate and vallate papillae contained a large percentage of gustducin-positive cells that did not express a member of the T1R family (Figures 3 and 5). Presumably the non-T1R gustducin-positive cells in these lingual fields express T2R receptors as reported elsewhere (Adler et al. 2000; Kim et al. 2003).

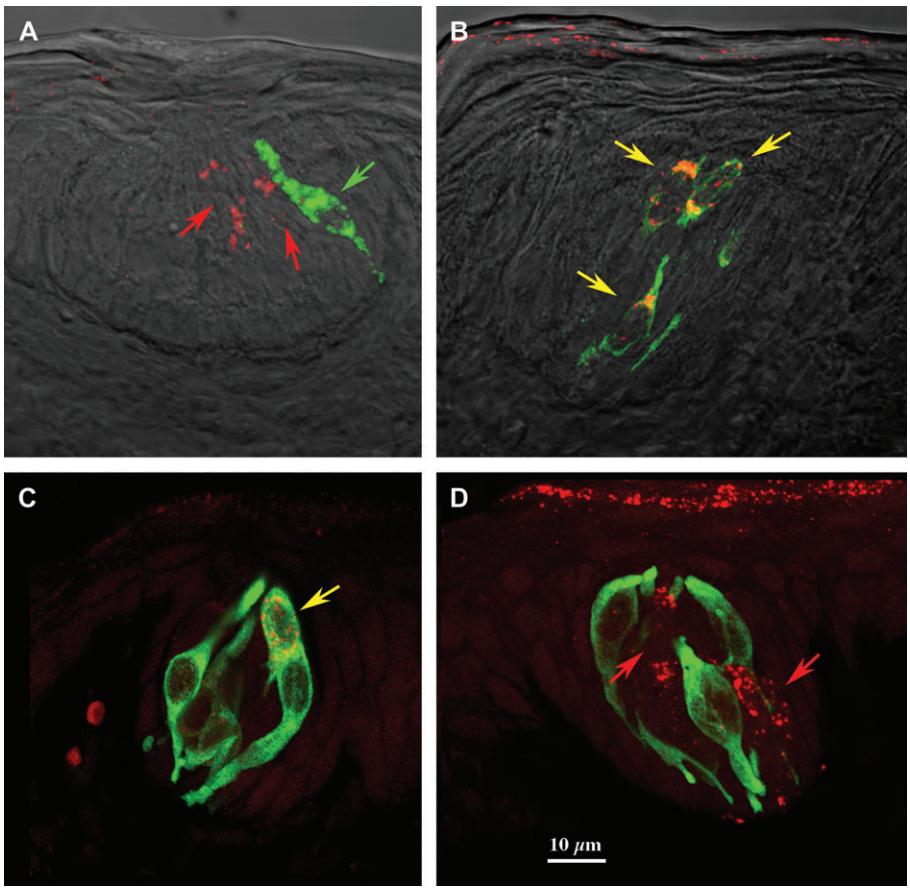

Figure 2 **(A)** Section through a palatal taste bud showing dual label in situ hybridization for T1R1 (green) and T1R2 (red). These probes label largely separate populations of taste cells. Two T1R2-positive (red arrows) cells are visible compared with a single T1R1-positive cell (green arrow). **(B)** Dual labeling of a palatal taste bud showing in situ hybridization (red) for T1R2 and immunocytochemical localization for gustducin (green). Nearly all T1R2-positive cells are double labeled for gustducin (yellow arrows) unlike the situation in vallate taste buds (compare with panel D below). **(C)** Dual labeling of a palatal taste bud showing in situ hybridization (red) for T1R1 and immunocytochemical localization for gustducin (green). Virtually all T1R1-positive cells also react for gustducin (yellow arrow). **(D)** Dual labeling of a vallate taste bud showing in situ hybridization (red) for T1R2 and immunocytochemical localization for gustducin (green). Most T1R2-positive cells (red arrows) do not exhibit gustducin immunoreactivity (compare with panel B above).

Evaluation of the data in terms of the percentage of T1R cells coexpressing gustducin illustrates that in the palate, T1R3 and T1R2 cells almost always express gustducin and half of T1R1 cells express gustducin (Figure 6, Table 2). Specifically, 100% of T1R3+ palate cells and 91% of T1R2+ palate cells coexpressed gustducin with T1R1 cells coexpressing gustducin 50% of the time (Figure 6). Examination of fungiform taste buds revealed the expression of T1Rs relative to gustducin was similar to palate taste buds. However, fewer T1R2-expressing cells were present in the fungiform papillae (Figure 6). Taste buds located in the vallate and foliate papillae contained few cells expressing both a known T1R member and gustducin (Figures 2D and 6) as reported previously (Hoon et al. 1999; Adler et al. 2000; Montmayeur et al. 2001; Kim et al. 2003).

Discussion

The palate is the most sweet-sensitive region of the oral cavity and is also responsive to umami stimuli. Despite this, few studies have focused on palatal taste buds in terms of the expression patterns of molecules implicated in sweet and umami taste transduction. The principal finding in this study is that T1Rs are expressed only by gustducin-containing cells in the palate. This contrasts with circumvallate and foliate taste buds where these molecules are rarely found in the same cells and helps explain behavioral and physiological data that indicate that both T1Rs and gustducin are involved in the transduction of sweet and umami stimuli.

Our data also confirm previous studies showing largely independent expression of T1R1 and T1R2 in lingual taste buds. Previous reports indicated that T1R1 and T1R2 are not only expressed in different taste cells but also mostly expressed in different taste fields; T1R1 expression is prevalent in fungiform taste buds but rare in vallate and the reverse for T1R2 (Hoon et al. 1999; Adler et al. 2000; Nelson et al. 2001). However, a more recent report by Kim et al. (2003) suggests that the expression of T1R1 and T1R2 are more widespread and that T1R1 and T1R2 are coexpressed in some taste cells. In the current study, we find that T1R1 and T1R2 are expressed in vallate and fungiform taste fields, although we did not find T1R expression to be as widespread as that reported by Kim et al. (2003). Furthermore, similar to studies by Hoon, Adler, and Nelson, we found that the amino acid–sensitive subunit T1R1 and the sweet-sensitive subunit T1R2 generally occur in separate cells in both lingual and palatal taste buds.

This is interesting in light of data from single fiber recordings, which show that gustatory afferents that respond best to sucrose often also respond to umami stimuli (Formaker et al. 2004). Also, behavioral data suggest mice have difficulty distinguishing sucrose from monosodium glutamate

Table 1 T1R1 and T1R2 expression in palate and lingual taste buds

T1R1 versus T1R2	Total cells	T1R1-only cells	T1R2-only cells	Double-labeled cells
Palate	10	4	5	1
Fungiform	18	7	9	2
Circumvallate	22	2	17	3
Foliate	17	2	12	3

Table 2 T1Rs and gustducin expression in palate and lingual taste buds

	Total labeled cells	T1R-only cells	Gustducin-only cells	Double-labeled cells	Percent gustducin cells expressing T1R	Percent T1R cells expressing gustducin
T1R3 versus gustducin						
Palate	28	0	0	28	100	100
Fungiform	25	0	8	17	68	100
Circumvallate	56	39	14	3	18	7
T1R2 versus gustducin						
Palate	81	3	50	31	38	91
Fungiform	29	0	22	7	24	100
Circumvallate	47	12	34	1	2.9	8
Foliate	72	20	47	5	9.6	20
T1R1 versus gustducin						
Palate	37	4	29	4	12	50
Fungiform	31	3	21	8	28	73
Circumvallate	23	10	11	2	15	17
Foliate	32	12	17	3	15	20

(MSG) when amiloride is added to block the sodium component of MSG taste (Yamamoto et al. 1991; Chaudhari et al. 1996; Stapleton et al. 1999; Heyer et al. 2003, 2004). The single fiber and behavioral data are consonant with the hypothesis that a subset of taste receptor cells responds to both sweet and umami stimuli. However, because T1R1 and T1R2 are usually not expressed in the same taste cells, the behavioral and electrophysiological overlap between sweet and umami qualities may indicate that umami-sensitive receptors other than the T1R1/T1R3 heteromer may be expressed in T1R2 (sweet responsive) taste cells. Consistent with this hypothesis, previously published data indicate the possibility of additional umami receptors including a truncated form of mGluR4 (Chaudhari et al. 2000; Maruyama et al. 2006). Functional studies of T1R3 knockout mice have produced different conclusions about whether T1R1/T1R3 is the only umami receptor. Damak et al. (2003) and Maruyama et al. (2006) noted residual responses to umami stimuli in T1R3 knockout mice and concluded that receptors other than T1R1/T1R3 must be responsible for the remaining response. In contrast, studies by Zhao et al. (2003) indicated that T1R1 and T1R3 knockout mice completely lose their ability to detect umami tastants, and therefore, the T1R1/T1R3 receptor is the only receptor responsible for mediating umami taste. Our data are consonant with the hypothesis of multiple umami receptors, some of which are expressed in T1R2-expressing, sweet-sensitive receptor cells.

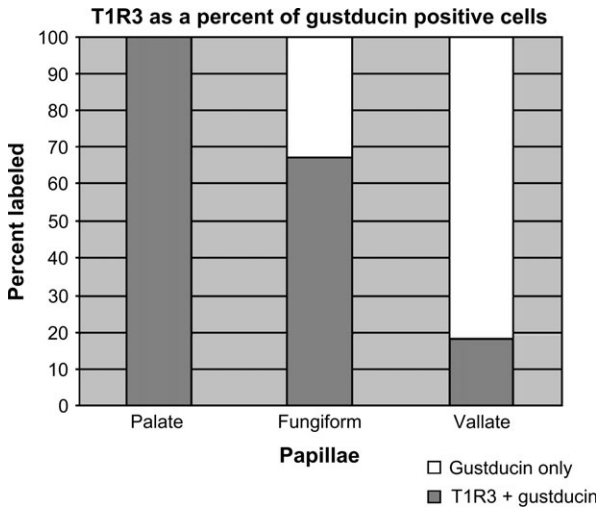

Figure 3 Histogram showing the percentage of gustducin cells that express T1R3 in palate, fungiform, and vallate taste cells. Note that in palate taste buds, all gustducin-positive cells coexpress T1R3. In contrast, less than 20% of gustducin-expressing cells in vallate taste buds also express T1R3.

The idea that G proteins other than gustducin play a role in umami taste is also supported by the present study. Previous studies of vallate and foliate taste buds indicated that the sweet-sensitive T1R2/T1R3 receptor is expressed in different taste cells than gustducin (Hoon et al. 1999; Adler et al. 2000; Montmayeur et al. 2001; Kim et al. 2003). Yet, gustducin knockout mice are compromised in their ability to detect sweet compounds (Wong et al. 1996). Thus, it was unclear how gustducin could impact on the sweet transduction pathway as mediated by the T1R2/T1R3 heteromer. We found that in the palate, most T1R2 and all T1R3 taste cells coexpress gustducin. Our data from palate and fungiform taste buds expand on the description of gustducin expression in T1R2-expressing cells of fungiform taste buds (Kim et al. 2003), suggesting that an α-gustducin–mediated pathway does exist in T1R2/T1R3-positive cells in these highly sweet-responsive taste fields. This is consistent with the behavioral data that support a role for gustducin in sweet taste transduction. Furthermore, because T1R2 and T1R3 are present in vallate and foliate taste buds, but are not expressed in gustducin cells, another G protein, perhaps Gαi or Gαs,

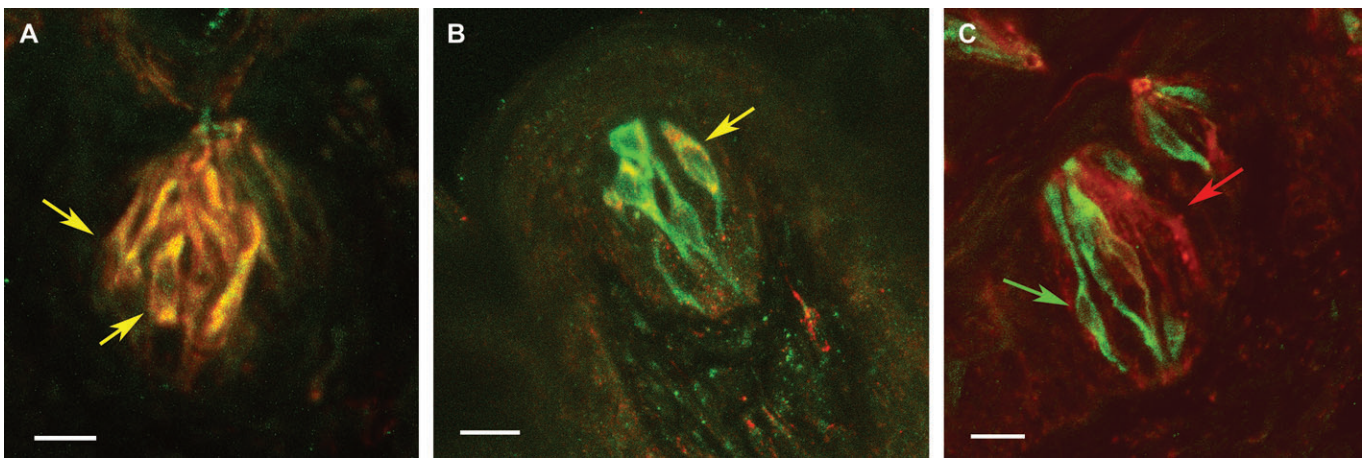

Figure 4 Confocal images of taste buds labeled with T1R3 (green) and gustducin (red) antibodies. **(A)** Palatal taste bud shows mostly double-labeled cells (yellow arrows). **(B)** Fungiform taste bud containing mostly double-labeled cells (yellow arrow). **(C)** Vallate taste bud where T1R3 and gustducin are mostly expressed in separate cells (red arrow—gustducin-IR cell, green arrow T1R3-IR cell). Scale bars represent 10 μm.

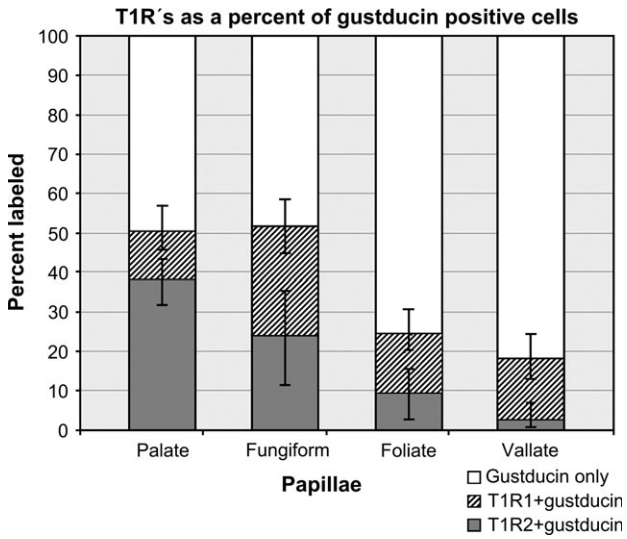

Figure 5 Histogram showing the percentage of gustducin-IR cells that express T1R1 or T1R2 (in situ hybridization) in palate, fungiform, foliate, and vallate taste buds. Note that the percentage of T1R2/gustducin (sweet sensitive) cells is much higher in the palate compared with other taste fields. Note that the gustducin-only expressing cells likely include the T2R-expressing population.

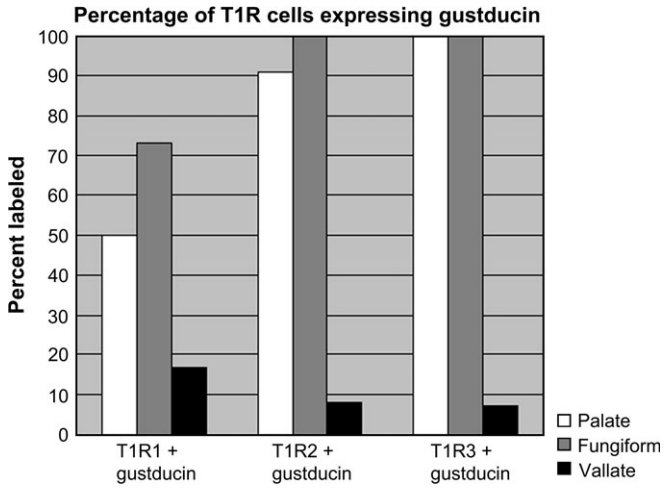

Figure 6 Histogram showing the percentage of T1R cells that coexpress gustducin. T1R2 and T1R3 cells almost always coexpress gustducin in the sweet-sensitive palate and fungiform taste buds. In contrast, few T1R cells coexpress gustducin in vallate taste buds.

may be involved in the limited sweet responsiveness in those taste fields. We also found that T1R1 is coexpressed with gustducin only half of the time, even in the palate. This is consistent with the idea that additional G proteins are involved in umami transduction as reported by Ruiz et al. (2003) and He et al. (2004).

Acknowledgements

This work was supported by National Institutes of Health grants RO1 DC006021 and P30 DC04657. The authors thank Dr Nicholas Ryba and Dr Charles Zuker for the cDNA plasmids for mouse T1R1 and T1R2 and Dr Robert Margolskee for the T1R3 antibody. In addition, we thank Elodie Chaplain for technical assistance.

References

Adler E, Hoon MA, Mueller KL, Chandrashekar J, Ryba NJ, Zuker CS. 2000. A novel family of mammalian taste receptors. Cell. 100:693–702.

Boughter JD Jr, Pumplin DW, Yu C, Christy RC, Smith DV. 1997. Differential expression of alpha-gustducin in taste bud populations of the rat and hamster. J Neurosci. 17:2852–2858.

Caicedo A, Pereira E, Margolskee RF, Roper SD. 2003. Role of the G-protein subunit α-gustducin in taste cell responses to bitter stimuli. J Neurosci. 23(30):9947–9952.

Chandrashekar J, Mueller KL, Hoon MA, Adler E, Feng L, Guo W, Zuker CS, Ryba NJ. 2000. T2Rs function as bitter taste receptors. Cell. 100:703–711.

Chaudhari N, Landin AM, Roper SD. 2000. A metabotropic glutamate receptor variant functions as a taste receptor. Nat Neurosci. 3:113–119.

Chaudhari N, Yang H, Lamp C, Delay E, Cartford C, Than T, Roper S. 1996. The taste of monosodium glutamate: membrane receptors in taste buds. J Neurosci. 16:3817–3826.

Damak S, Rong M, Yasumatsu K, Kokrashvill Z, Varadarajan V, Zou S, Jiang P, Ninomiya Y, Margolskee RF. 2003. Detection of sweet and umami taste in the absence of taste receptor T1r3. Science. 301(5634):850–853.

Danilova V, Hellekant G. 2003. Comparison of the responses of the chorda tympani and glossopharyngeal nerves to taste stimuli in C57BL/6J mice. BMC Neurosci. 4:5.

Finger TE, Bonger B, Hansen A, Anderson KT, Hessamedin A, Silver W. 2003. Solitary chemoreceptor cells in the nasal cavity serve as sentinels of respiration. Proc Natl Acad Sci USA. 100(15):8981–8986.

Formaker BK, Stapleton JR, Roper SD, Frank ME. 2004. Responses of the rat chorda tympani nerve to glutamate-sucrose mixtures. Chem Senses. 29:473–482.

Frank ME, Contreras RJ, Hettinger TP. 1983. Nerve fibers sensitive to ionic taste stimuli in chorda tympani of the rat. J Neurophysiol. 50:941–960.

Glendinning JI, Bloom LD, Onishi M, Zheng KH, Damak S, Margolskee RF, Spector AC. 2005. Contribution of alpha-gustducin to taste-guided licking responses of mice. Chem Senses. 30(4):299–316.

Harada S, Yamamoto T, Yamaguchi K, Kasahara Y. 1997. Different characteristics of gustatory responses between the greater superficial petrosal and chorda tympani nerves in the rat. Chem Senses. 22(2):133–140.

He W, Yasumatsu K, Varadarajan V, Yamada A, Lem J, Ninomiya Y, Margolskee RF, Damak S. 2004. Umami taste responses are mediated by alpha-transducin and alpha-gustducin. J Neurosci. 24:7674–7680.

Heyer BR, Taylor-Burds CC, Mitzelfelt JD, Delay ER. 2004. Monosodium glutamate and sweet taste: discrimination between the tastes of sweet stimuli and glutamate in rats. Chem Senses. 29:721–729.

Heyer BR, Taylor-Burds CC, Tran LH, Delay ER. 2003. Monosodium glutamate and sweet taste: generalization of conditioned taste aversion between glutamate and sweet stimuli in rats. Chem Senses. 28:631–641.

Hoon MA, Adler E, Lindemeier J, Battey JF, Ryba NJ, Zuker CS. 1999. Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. Cell. 96:541–551.

Kim MR, Kusakabe Y, Miura H, Shindo Y, Ninomiya Y, Hino A. 2003. Regional expression patterns of taste receptors and gustducin in the mouse tongue. Biochem Biophys Res Commun. 312:500–506.

Kitagawa M, Kusakabe Y, Miura H, Ninomiya Y, Hino A. 2001. Molecular genetic identification of a candidate receptor gene for sweet taste. Biochem Biophys Res Commun. 283:236–242.

Krimm RF, Nejad MS, Smith JC, Miller IJ Jr, Beidler LM. 1987. The effect of bilateral sectioning of the chorda tympani and the greater superficial petrosal nerves on the sweet taste in the rat. Physiol Behav. 41:495–501.

Li X, Staszewski L, Xu H, Durick K, Zoller M, Adler E. 2002. Human receptors for sweet and umami taste. Proc Natl Acad Sci USA. 99(7):4692–4696.

Maruyama Y, Pereira E, Margolskee RF, Chaudhari N, Roper SD. 2006. Umami responses in mouse taste cells indicate more than one receptor. J Neurosci. 26:2227–2234.

Matsunami H, Montmayeur JP, Buck LB. 2000. A family of candidate taste receptors in human and mouse. Nature. 404:601–604.

Max M, Shanker YG, Huang L, Rong M, Liu Z, Campagne F, Weinstein H, Damak S, Margolskee RF. 2001. Tas1r3, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus Sac. Nat Genet. 28:58–63.

Montmayeur JP, Liberles SD, Matsunami H, Buck LB. 2001. A candidate taste receptor gene near a sweet taste locus. Nat Neurosci. 4:492–498.

Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, Zuker CS. 2001. Mammalian sweet taste receptors. Cell. 106:381–390.

Ruiz CJ, Wray K, Delay E, Margolskee RF, Kinnamon SC. 2003. Behavioral evidence for a role of alpha-gustducin in glutamate taste. Chem Senses. 28:573–579.

Ruiz-Avila L, Wong G, Damak S, Margolskee RF. 2001. Dominant loss of responsiveness to sweet and bitter compounds caused by a single mutation in α-gustducin. Proc Natl Acad Sci USA. 98:8868–8873.

Sainz E, Korley JN, Battey JF, Sullivan SL. 2001. Identification of a novel member of the T1R family of putative taste receptors. J Neurochem. 77:896–903.

Sako N, Harada S, Yamamoto T. 2000. Gustatory information of umami substances in three major taste nerves. Physiol Behav. 71:193–198.

Shindler KS, Roth KA. 1996. Double immunofluorescent staining using two unconjugated primary antisera raised in the same species. J Histochem Cytochem. 44(11):1331–1335.

Sollars SI, Hill DL. 2005. In vivo recordings from rat geniculate ganglia: taste response properties of individual greater superficial petrosal and chorda tympani neurones. J Physiol. 564:877–893.

Stapleton JR, Roper SD, Delay ER. 1999. The taste of monosodium glutamate (MSG), L-aspartic acid, and N-methyl-D-aspartate (NMDA) in rats: are NMDA receptors involved in MSG taste? Chem Senses. 24:449–457.

Stone LM, Baker LS, Margolskee RF, Kinnamon SC. 2003. Immunocytochemical characterization of palate taste buds in mice. Chem Senses. 28:A110.

Travers SP, Norgren R. 1991. Coding the sweet taste in the nucleus of the solitary tract: differential roles for anterior tongue and nasoincisor duct gustatory receptors in the rat. J Neurophysiol. 65:1372–1380.

Wong GT, Gannon KS, Margolskee RF. 1996. Transduction of bitter and sweet taste by gustducin. Nature. 381:796–800.

Wong GT, Ruiz-Avila L, Ming D, Gannon KS, Margolskee RF. 1996. Biochemical and transgenic analysis of gustducin's role in bitter and sweet transduction. Cold Spring Harbor Symp Quant Biol. 61:173–184.

Yamamoto T, Matsuo R, Fujimoto Y, Fukunaga I, Miyasaka A, Imoto T. 1991. Electrophysiological and behavioral studies on the taste of umami substances in the rat. Physiol Behav. 49:919–925.

Zhao GQ, Zhang Y, Hoon MA, Chandrashekar J, Erlenbach I, Ryba NJ, Zuker CS. 2003. The receptors for mammalian sweet and umami taste. Cell. 115(3):255–266.

Accepted December 8, 2006