Nasal Solitary Chemoreceptor Cell Responses to Bitter and Trigeminal Stimulants In Vitro

Brian D. Gulbransen,1,2 Tod R. Clapp,1,3 Thomas E. Finger,1,2 and Sue C. Kinnamon1,3

1Rocky Mountain Taste and Smell Center; 2Department of Cell and Developmental Biology, Neuroscience Program, University of Colorado Denver School of Medicine, Aurora; and 3Department of Biomedical Sciences, Colorado State University, Fort Collins, Colorado

Submitted 18 January 2008; accepted in final form 4 April 2008

INTRODUCTION

The nose is a complex organ that functions to monitor, condition, and filter inspired air (Baranikut and Kaliner 1991; Harkema et al. 2006). Intranasal trigeminal fibers not only mediate somatosensory sensations such as touch, temperature, and pain but are also responsive to a wide variety of chemical stimuli (Bryant and Silver 2000). Stimulation of nasal trigeminal fibers by noxious chemical substances elicits respiratory, cardiovascular, and hormonal responses that may protect the airways from further exposure (Silver et al. 1995). Therefore the primary function of this intranasal trigeminal system is to act as a sentinel of the airways, guarding the respiratory system from inhalation of noxious substances (Silver 1990).

Nasal detection of irritants has classically been thought to be mediated by trigeminal free nerve endings that reside in the nasal mucosa and terminate within a few micra of the epithelial surface, just below the level of tight junctions (Bryant and Silver 2000; Finger et al. 1990). Therefore detection of chemical stimuli in the nasal cavity was thought to necessitate diffusion across this barrier to gain access to free nerve endings. However, a newly discovered population of modified epithelial cells termed solitary chemoreceptor (or chemosensory) cells (SCCs) also contributes to nasal trigeminal sensitivity (Finger et al. 2003; Sbarbati and Osculati 2005). SCCs form synaptic contacts with afferent trigeminal nerve fibers and possess a transduction and signaling cascade similar to type II taste cells that includes T2R “bitter” taste receptors, the G protein α-gustducin, phospholipase C isoform β2 (PLCβ2), and the transient receptor potential melastatin channel subtype 5 (TRPM5) (Finger et al. 2003; Lin et al. 2008). In mice, SCCs are scattered throughout the nasal respiratory epithelium with the highest numbers positioned just posterior to the nasal vestibule (Finger et al. 2003). Cells with signal transduction cascades similar to SCCs occur throughout the respiratory and digestive tracts (Bezençon et al. 2006; Finger et al. 2003; Hofer et al. 1996; Mergo et al. 2005, 2007; Sbarbati and Osculati 2005; Sbarbati et al. 2004a,b; Tizzano et al. 2006; Wu et al. 2002).

This bitter-taste signaling cascade is usually associated with the detection of potentially noxious substances in the oral cavity. Bitter tastants are detected by a large, highly divergent family of G-protein–coupled receptors called T2Rs (Adler et al. 2000; Chandrashekar et al. 2000). There are about 25 human and 35 mouse T2Rs (Chandrashekar et al. 2006). Of the 35 mouse T2Rs, nasal SCCs are known to express two, mT2Rs 8 and 19 (Finger et al. 2003). To date, relatively few murine T2Rs have been deorphanized (i.e., ligands for the receptors are not known). The only mouse T2Rs where ligands are known are mT2Rs 5 and 8. mT2R5 binds cycloheximide, whereas mT2R8 binds denatonium and 6-n-propyl-2-thiouracil (Chandrashekar et al. 2000). Several human T2Rs have been deorphanized. Heterologous expression assays demonstrate human T2R16 binds salicin, human T2Rs 4 and 44 bind denatonium, human T2R38 binds PTC, and human T2Rs 43 and 44 bind saccharin just to name a few (Buze et al. 2002; Chandrashekar et al. 2006; Kuhn et al. 2004; Pronin et al. 2004). However, this leaves ligands for the majority of T2Rs unknown.

Since the discovery of SCCs in mammals, their functional significance has remained speculative. Expression of bitter-taste transduction machinery, coupled with the finding by

Address for reprint requests and other correspondence: B. D. Gulbransen, University of Colorado Denver School of Medicine, Department of Cell and Developmental Biology, Neuroscience Program, Aurora, CO 80045 (E-mail: bgulbran@ucalgary.ca).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Finger et al. (2003) that bitter compounds in the nasal cavity cause respiratory depression and ethmoid nerve responses, led to speculation that SCCs broaden trigeminal chemosensitivity by detecting bitter compounds. But do SCCs actually mediate trigeminal responses to bitter compounds or are the trigeminal responses to these compounds mediated by other mechanisms? Liu and Simon (1998) demonstrated that cultured trigeminal ganglion neurons are capable of responding to bitter substances so SCCs may not mediate the trigeminal response to these compounds. Only one study to date has attempted to isolate and record from nasal SCCs. Lin et al. (2008) observed that TRPM5-expressing cells isolated from the mouse nasal cavity respond to high concentrations of odorants, which may or may not act via T2R receptors. To date, no study has investigated whether isolated nasal SCCs actually respond to bitter-tasting compounds.

To determine which component of nasal trigeminal chemoreception is mediated by SCCs, we used calcium imaging to examine the response of isolated SCCs to an array of bitter-tasting and trigeminal irritant stimuli. We find that SCCs respond robustly and specifically to the bitter compound d-nitrophenyl benzoate through a PLC-mediated signaling cascade, but do not respond to other tested bitter compounds or classical trigeminal stimuli.

**Methods**

**Animals**

All experiments referred to in this report were carried out in accordance with approved UCSD or Colorado State University IACUC animal protocols.

TRPM5-GFP mice were developed by R. F. Margolskee (Mt. Sinai School of Medicine, New York) and contained S5′ to S3′: 11 kb of mouse TRPM5 S5′ flanking sequence, TRPM5 Exon 1 (untranslated), Intron 1, and the untranslated part of Exon 2, and eGFP (Clapp et al. 2006). Immunolabeling with an antibody against the TRPM5 protein by Lin et al. (2008) demonstrates nearly complete colocalization between green fluorescent protein (GFP) and the TRPM5 protein. Gustducin-GFP mice were also developed by R. F. Margolskee and the construct contained an 8.4-kb segment from the upstream region of the mouse α-gustducin gene (Huang et al. 1999). Expression of GFP for both mouse strains has been verified by comparison with immunocytochemistry for the relevant proteins (Huang et al. 1999; Lin et al. 2008).

**Isolation of SCCs**

SCCs were isolated from transgenic mice expressing GFP under control of promoters upstream of either the TRPM5 or α-gustducin using an adaptation of the protocol described by Rawson et al. (1998). Briefly, mice were killed by cervical dislocation and rapid exsanguination, the heads were split down the midline, and the anterior respiratory nasal epithelium was dissected in Tyrode’s solution (pH 7.2–7.4). Tissue was transferred to calcium-free Tyrode’s and minced by 10.220.33.2 on May 27, 2017 http://jn.physiology.org/ Downloaded from

---

**Ca**\(^{2+}\) imaging

**Ca**\(^{2+}\) imaging was carried out using the same experimental setup and protocol as in Clapp et al. (2006). Briefly, images were acquired every 5 s through the \( \times 40 \) oil-immersion objective of an inverted Nikon Diaphot TMD microscope using Imaging Workbench 5.2 software (Indec Biosystems, Santa Clara, CA) and a CCD Sensicam QE camera (Cooke, Romulus, MI). Excitation wavelengths of 350 and 380 nm were used with an emission wavelength of about 510 nm. Stimuli were bath-applied using a gravity-flow perfusion system (Automate Scientific, San Francisco, CA) and laminar-flow perfusion chambers (RC-25F, Warner Scientific, Hamden, CT). Data were graphed using OriginPro 7.5 software with intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) levels being reported as F350/F380 versus time.

**Solutions**

Tyrode’s solution contained (in mM): 140 NaCl, 5 KCl, 1 MgCl\(_2\), 1 CaCl\(_2\), 10 HEPES, 10 glucose, and 1 pyruvic acid, adjusted to pH 7.4 with NaOH. All test stimuli were diluted in Tyrode’s solution. Capsaicin and menthol were first suspended in a small amount (0.1% of final volume) of 95% ethanol vehicle to allow them to go into solution. The final pH of stimulus solutions was 7.4. All chemicals were purchased from Sigma–Aldrich (St. Louis, MO) with the exception of U73122 (1-[6-[(17β)-3-methoxyoestra-1,3,5(10)-tri-en-17-y]-amino]hexyl)-1H-pyrrole-2,5-dione), which was purchased from Calbiochem (EMD Chemicals, San Diego, CA).

**Histology**

**TISSUE PREPARATION.** TRPM5-GFP mice were deeply anesthetized with 20% chloral hydrate and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Heads were postfixed for 30 min and then rinsed in 0.1 M PB before being split down the midline to expose the nasal epithelium, which was then dissected and stored in 0.1 M PB. Tissue was cryoprotected overnight in 0.1 M PB containing 20% sucrose before being cut on a cryostat. The 16-μm sections were mounted on Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA) and stored at −20°C before being analyzed by immunocytochemistry.

**IMMUNOCYTOCHEMISTRY.** Sections from TRPM5-GFP mice were rinsed in phosphate-buffered saline (PBS), pH 7.2, and then blocked for 1 h with 10% normal goat serum (NGS). Primary antibodies to gustducin [rabbit (Rb) anti-α-gustducin (1:1,000, Santa Cruz Biotechnology, Santa Cruz, CA)] were then applied overnight at 4°C. The following day, sections were rinsed in PBS and then incubated with goat anti-Rb Alexa 568 (Molecular Probes/Invitrogen) secondary antibodies for 2 h. Sections were then rinsed in PBS and mounted with Fluoromount G (Southern Biotech, Birmingham, AL).

**WHOLE-MOUNT IMMUNOCYTOCHEMISTRY.** Mice expressing GFP under control of the TRPM5 promoter were perfused with 4% PFA as described earlier and the heads postfixed for 30 min. The head was then split down the midline and the anterior nasal epithelium dissected in 0.1 M PB. Nasal tissue was then rinsed well with PBS, blocked for 45 min with 10% NGS, and incubated with the primary antibody [Rb anti-α-GPP95 (1:1,000, AbD Serotec, Raleigh, NC)] overnight at room temperature on a shaker table. The following day tissue was rinsed well with PBS and then incubated with the secondary antibody goat anti-Rb Alexa 568 (1:400, Molecular Probes/Invitrogen). Tissue was then rinsed well in PBS, spread flat on slides, and mounted with Fluoromount G (Southern Biotech).

**Confocal microscopy**

Immunofluorescence of labeled SCCs was imaged on an Olympus Fluoview laser scanning confocal microscope (Olympus America,
Melville, NY) using ×20 (0.80 n.a.) and ×60 (1.4 n.a.) oil-immersion lenses. Optical sections (0.8 μm) were acquired through each field of view and then compiled into a z-stack. Sequential scanning of the two separate fluorescence channels avoided “bleed through” of signal from the inappropriate channel.

Analysis

Analysis and generation of traces were performed using OriginPro 7.5 software. Responses were defined as increases in [Ca$^{2+}$]i >2SDs from baseline (calculated as the average ratio during the 50–100 s preceding stimulus application) during stimulus presentation. Off responses were defined as responses elicited on removal of the stimulus. Responses were considered completely blocked if they were reduced to <2SDs from baseline. Responses reduced by the block but still >2SDs from baseline are reported as the percentage of the original response left after block.

For the most part, traces show all points and are unaltered. However, in some traces single points >2SDs from baseline due to fluctuations in the light source were omitted. To be considered for omission, omitted points had to be noted as light fluctuation during the experiment, occur during a nonstimulation time, and be ≥2SDs from baseline. Only points that significantly detracted from the visual quality of the trace were omitted. No points were omitted during stimulus application.

To assess the specificity of responses, non-GFP–labeled as well as GFP-labeled cells were measured for stimulus-induced changes in [Ca$^{2+}$]i. The non-GFP–labeled cells were either adjacent to or nearby GFP+ cells and were identified under bright field and by being loaded with fura-2. To assess whether the number of GFP+ cells responding
to a particular stimulus was significantly different from the number of non-GFP cells responding to that stimulus, a chi-square test was used. The GraphPad on-line statistical calculator (http://www.graphpad.com/quickcalc/index.cfm) was used to perform this analysis.

The dose–response curve for denatonium was generated by measuring responses to 0.3, 0.998, 3.16, 9.97, and 31.5 mM denatonium in five TRPM5-GFP+ cells. The response to 31.5 mM denatonium was classified as the maximal response in each cell. The change in the ratio (340/380) in response to each concentration of denatonium is represented as a percentage of the maximal response (to 31.5 mM denatonium).

R E S U L T S

Identification of nasal SCCs

To identify SCCs, we utilized two strains of transgenic mice in which GFP expression is driven by promoters upstream of two components of the transduction cascade: gustducin or TRPM5. Dissociation of the anterior nasal epithelium (area inside dotted line in Fig. 1A) from either strain yielded numerous isolated non-GFP epithelial cells and a smaller number of GFP+ cells. Acutely isolated GFP+ cells were located either in small clumps of tissue or were completely isolated (Fig. 2). In agreement with Lin et al. (2008) and Kaske et al. (2007) we found numerous TRPM5-GFP+ cells in the nasal epithelium of mice (Fig. 1B). Also in agreement with Lin et al. (2008) we found that gustducin-expressing cells constitute a subset of the TRPM5-expressing population of cells in the nasal cavity. In sections of the area of nasal epithelium from the vestibule to the posterior end of the vomeronasal organ (the area of respiratory epithelium isolated in this study), we found that approximately two thirds (417/624) of the TRPM5-GFP+ cells were immunoreactive for α-gustducin, whereas >90% (417/446) of α-gustducin immunoreactive cells were TRPM5-GFP+ (Fig. 1, C–E). Lin et al. (2008) reported that in the nasal cavity as a whole, about 20% of TRPM5 cells express gustducin. Accordingly, the population at the anterior end of the nasal cavity that we sampled for physiological recordings represents a specialized subset of the entire TRPM5+ population of SCCs. In keeping with these findings, more GFP+ cells could be isolated from TRPM5-GFP mice than from gustducin-GFP mice, reflecting the larger population of TRPM5+ chemosensory cells in the nasal cavity (Kaske et al. 2007; Lin et al. 2008).

**Ca**²⁺ responses in TRPM5-GFP cells

To investigate which component of nasal trigeminal chemosensitivity is mediated through SCCs we applied a variety of classic bitter and trigeminal stimulants and recorded relative [Ca**²⁺]** levels in TRPM5- and gustducin-GFP+ cells (Tables 1 and 2). For most stimuli tested, TRPM5-GFP+ cells did not increase [Ca**²⁺]** by >2SDs above baseline (see Table 1). Rare TRPM5-GFP+ cells responded to the bitter compounds cycloheximide (1/38 GFP+ cells) and salicin (1/18 GFP+ cells).

Likewise, rare TRPM5-GFP+ cells responded to the trigeminal stimulants capsaicin (1/15 GFP+ cells) (see Fig. 3) and menthol (2/15 GFP+ cells). These rare responses were small (=2SD from baseline), slow (gradual increase in Ca**²⁺**), and cells did not usually recover (return to the baseline level before stimulus application). The classic bitter compound phenylthiocarbamide (PTC) elicited larger increases in [Ca**²⁺]**, in TRPM5-GFP+ cells (Fig. 3). However, these increases in Ca**²⁺** were relatively gradual and were not blocked by the PLC inhibitor U73122 (2 μM) (Fig. 3), suggesting a non-T2R mechanism. The number of TRPM5-GFP+ cells responding to the above-cited stimuli was not significantly different from the number of non-GFP cells responding (P > 0.05, χ² test). Therefore responses to these compounds are not specific to TRPM5-GFP+ cells and do not act through a PLC cascade. TRPM5-GFP+ cells also responded to acetylcholine (ACh) (8/30 GFP+ cells) and adenosine 5'-triphosphate (ATP) (12/41 GFP+ cells) (Fig. 4). However, the fraction of TRPM5-GFP+ cells responding to ATP and ACh was not significantly different from the fraction of non-GFP cells responding (P > 0.05, χ² test), indicating that ATP and ACh are not specifically detected by SCCs.
When exposed to the bitter compound denatonium benzoate, TRPM5-GFP cells rapidly (sharp increase in \([\text{Ca}^{2+}]_i\) within 5 s of application) and robustly increased \([\text{Ca}^{2+}]_i\) levels (66/125 GFP cells) (Fig. 5). Denatonium-evoked \([\text{Ca}^{2+}]_i\) responses reached a peak more rapidly (\(P < 0.05\), \(t\)-test) than PTC-evoked \([\text{Ca}^{2+}]_i\) responses in SCCs [11.52 ± 2.22 s for denatonium vs. 61.88 ± 10.68 s for PTC, when time of response was measured for the two compounds in the same cell (\(n = 4\))]. Denatonium-evoked \([\text{Ca}^{2+}]_i\) responses in TRPM5-GFP cells range in size from 4 to 50% increases in relative \([\text{Ca}^{2+}]_i\) levels over baseline. The fraction of TRPM5-GFP cells responding to denatonium is significantly higher than the fraction of non-GFP cells responding (9/56) (\(P < 0.0001\), \(\chi^2\) test). Denatonium increases \([\text{Ca}^{2+}]_i\) in TRPM5-GFP+ cells in a dose-dependent manner with an \(EC_{50}\) value of about 5.15 mM (Fig. 5C).

**Ca\(^{2+}\) responses in gustducin-GFP cells**

Gustducin-GFP+ cells also respond robustly and specifically to denatonium (Fig. 6). Since gustducin-GFP+ cells express T2R receptors (Finger et al. 2003) and are a subset of the TRPM5-expressing population of nasal cells (Lin et al. 2008), it was expected that a larger percentage of gustducin-GFP cells would respond to denatonium than TRPM5-GFP+ cells. In agreement, 46.4% (66/125) of TRPM5-GFP cells responded to denatonium, whereas 76.6% (36/47) gustducin-GFP cells responded to denatonium (Table 2). The number of gustducin-GFP cells responding to denatonium (36/47) is significantly higher (\(P < 0.001\), \(\chi^2\) test) than the number of non-GFP cells responding to denatonium (0/7). Denatonium responses in gustducin-GFP+ cells, like those in TRPM5-GFP+ cells, are blocked by the PLC inhibitor U73122 (2 \(\mu\)M) (Fig. 6). In 9 of 13 GFP cells, the denatonium response was blocked by U73122, indicating that denatonium is most likely acting through a T2R-PLC signaling cascade. The inactive analog of this drug (U73343) did not block denatonium responses (data not shown).

---

**FIG. 3.** Responses to 15 mM phenylthiocarbamide (PTC) in TRPM5-GFP+ cells are more gradual than responses to 15 mM denatonium. A: a TRPM5-GFP+ cell responds robustly and quickly to 15 mM denatonium and, although the cell also responds to 15 mM PTC, the response appears slower and more gradual. In this cell the first denatonium response peaked within 6.21 s after application of denatonium. However, the PTC response in this cell took 50.68 s to peak. This particular cell was a rare case since it also gave a small response to 100 \(\mu\)M capsaicin. The capsaicin response is also more gradual than the response to denatonium. B: PTC responses are not blocked by the phospholipase C (PLC) inhibitor U73122.

**FIG. 4.** Adenosine 5'-triphosphate (ATP) responses in TRPM5-GFP+ cells. A: a TRPM5-GFP+ cell responds with increased intracellular \([\text{Ca}^{2+}]_i\) to 10 \(\mu\)M acetylcholine (ACh) and 10 \(\mu\)M ATP. B: a TRPM5-GFP+ cell responsive to 15 mM denatonium also responds to 10 \(\mu\)M ATP but not to 500 \(\mu\)M (an irritatingly high concentration) liliac. The adjacent cell (GFP, gray trace) responded only to ATP. The PLC inhibitor U73122 (2 \(\mu\)M) blocked the denatonium response in the GFP+ cell completely but did not block the ATP responses in either cell.

*J Neurophysiol • VOL 99 • JUNE 2008 • www.jn.org*
completely blocked. On average (all denatonium-responsive gustducin-GFP cells where U73122 was tested), U73122 reduced the denatonium response to 5.31 ± 4.04% of the original peak value. Thus denatonium activates SCCs via a PLC-mediated signaling cascade.

**DISCUSSION**

SCCs were first characterized in the nasal cavity of rodents by Finger et al. (2003) who demonstrated that SCCs express T2R “bitter”-taste receptors and that the ethmoid nerve responds when the bitter stimuli (cycloheximide, quinine, or denatonium) are presented in the nasal cavity. Therefore it was hypothesized that SCCs mediate trigeminal responses to bitter-tasting compounds. Our results partially support this hypothesis by demonstrating that isolated TRPM5-GFP+ or gustducin-GFP+ cells in the nasal cavity respond to the bitter compound denatonium. However, out of all the bitter stimuli tested, SCCs reliably responded to only denatonium through a PLC cascade. The ethmoid responses to denatonium in the rat nasal cavity recorded by Finger et al. (2003) were not as large as those for cycloheximide and quinine so it was surprising that denatonium evoked the best responses in SCCs. It is possible that trigeminal responses to cycloheximide and quinine are mediated by activation of trigeminal nerve fibers themselves. Liu and Simon (1998) demonstrated that cultured rat trigeminal ganglion cells are able to respond to several bitter stimuli; quinine and strychnine elicited calcium increases in, respectively, 22 and 18% of cultured trigeminal ganglion cells, whereas only 5% responded to denatonium. Therefore denatonium responses may be more specific to SCCs and not mediated by broadly responsive nerve fibers.

Lin et al. (2008) recently demonstrated that the anterior nasal epithelium and TRPM5-expressing nasal SCCs respond to high concentrations of several odorants. We did not observe broad responsiveness of SCCs to multiple irritating stimuli. One possible explanation for the apparent discrepancy between the findings of Lin et al. (2008) and the current study is that the larger population of TRPM5-expressing SCCs exhibits a broad detection range for irritants, whereas the gustducin-expressing subpopulation of SCCs is specifically tuned to detect a narrow range of relevant stimuli.

Our results demonstrate that SCCs are sensitive to denatonium benzoate in the same concentration range as taste recep-

---

**FIG. 5.** TRPM5-GFP+ cells from the anterior nasal cavity respond specifically to denatonium benzoate through a PLC-mediated pathway. A: TRPM5-GFP+ cells respond with a rapid increase in [Ca2+]i to 15 mM denatonium (den) but not to the trigeminal stimulants menthol (men; 0.1 mM) and caffeine (caff; 50 mM) (although this cell gave an off response to caff). Denatonium responses in TRPM5-GFP+ cells are completely blocked by the PLC inhibitor U73122 (2 μM). B: TRPM5-GFP+ cells respond to den but not to the bitter compounds salicin (sal; 15 mM) or cycloheximide (cyx; 1 mM). TRPM5-GFP+ cells also do not respond to capsaicin (caps; 10 μM). C: dose–response curve for denatonium in TRPM5-GFP+ cells. For each point n = 5 and error bars show SE. D: the detection of denatonium is specific to TRPM5-GFP+ cells because adjacent non-GFP epithelial cells do not respond.
tor cells in mice (Boughter Jr et al. 2005; Caicedo et al. 2003; Damak et al. 2006; Finger et al. 2005; Ruiz et al. 2003) and hamsters (Frank et al. 2004). Since mT2R8 is a denatonium receptor (Chandrashekar et al. 2000) and is expressed by SCCs (Finger et al. 2003), our results support the hypothesis that mT2R8 is mediating the response to denatonium in SCCs.

An alternative explanation for our results is that the concentration of denatonium was activating nonspecific pathways in SCCs. High concentrations of denatonium are capable of activating G-protein–independent pathways (Sawano et al. 2005) and nonspecifically activating cells in culture (Liu and Simon 1998). Furthermore, gustducin-transducin double knockout mice are able to detect high concentrations (>5 mM) of denatonium (He et al. 2004). However, we believe that the denatonium responses we observed in SCCs are not acting through nonspecific pathways for several reasons.

First, when chorda tympani (CT) and glossopharyngeal (NG) taste nerve responses to three concentrations of denatonium were compared in C57BL/6J mice by Daniilova and Hellekant (2003), they found that, in contrast to the NG, the CT was unresponsive to 20 mM denatonium. If 20 mM denatonium was activating G proteins or PLC directly, the CT would be unresponsive to 20 mM denatonium as well. If denatonium was activating nonspecific pathways in the CT, as we observed in SCCs, the nerve recordings were made in rat and in situ hybridization performed on mouse tissue by Finger et al. (2003) is not known whether mice and rats should be expected to express similar nasal T2Rs.

Second, the denatonium responses observed were very fast. This is in contrast to what we believe to be nonspecific PTC responses (see responses in Figs. 3 and 5 for comparison). In our experience, receptor-mediated events elicit responses with fast response kinetics, whereas nonreceptor-mediated effects show slower, more prolonged, increases in $[\text{Ca}^{2+}]_{i}$. Nonreceptor-mediated pathways also might be expected to have the same coincident, nonspecific effects on adjacent epithelial cells, a phenomenon that we did not observe during denatonium presentations.

Finally, denatonium responses in SCCs are almost always completely blocked by the PLC inhibitor U73122. If denatonium were activating G-protein–independent pathways as described by Sawano et al. (2005), these pathways would not have been blocked by U73122. Therefore our evidence supports a specific detection of denatonium by T2R receptors expressed in SCCs rather than a nonspecific mechanism.

We find that cycloheximide does not stimulate most SCCs. Cycloheximide was ineffective at stimulating most SCCs with concentrations ranging from 0.1 mM to as high as 20 mM. At this extremely high concentration, cells showed slow, large decreases in $[\text{Ca}^{2+}]_{i}$, or died. This correlates with in situ hybridization results by Finger et al. (2003), which failed to demonstrate the mouse T2R responsible for detecting cycloheximide (mT2R5) in nasal SCCs. Therefore it is possible that cycloheximide acts through some nonspecific mechanism that activates nerve fibers in the rat nasal cavity. It is also possible that rat SCCs express different T2R receptors than mice. Since the nerve recordings were made in rat and in situ hybridization performed on mouse tissue by Finger et al. (2003) it is not known whether mice and rats should be expected to express similar nasal T2Rs.

Other T2R-mediated compounds tested are also ineffective at stimulating SCC receptor pathways. Phenylthiocarbamide (PTC) and the related 6-propyl-2-thiouracil (PROP) cause increases in intracellular calcium in SCCs, although this effect appears to be through some nonspecific mechanism since all epithelial cells respond in a similar manner and the response is not blocked by a PLC inhibitor. Responses to PTC and PROP are also slower than what is usually reported for receptor-mediated events. Salicin, which is known to activate a human T2R, did not stimulate SCCs.

Since SCCs do not respond to many bitter compounds and seem to be relatively narrowly tuned, they may express a limited number of T2Rs. It is possible the T2Rs expressed by SCCs are not activated by many classical bitter tastants because they have evolved to detect other classes of compounds. For example, hTAS2R46—a human T2R—responds to a broad array of substances including lactones and diterpenoids, as well as denatonium (Brockhoff et al. 2007) but does not respond to unrelated bitter-tasting substances such as ouabain or salicin.

Lactones and diterpenoids are known to be produced by bacteria, including the respiratory pathogen *Pseudomonas aeruginosa* (Fuqua et al. 1994). Therefore the physiological significance of bitter detection by nasal SCCs may be to detect other classes of compounds expressed by bacteria.
bacteria rather than spoiled food as in the oral cavity, a hypothesis put forth by Sbarbati and Osculati (2006). Stimulation of SCCs by bacterial metabolites would lead to trigeminal nerve activation eliciting protective responses such as sneezing to expel bacteria from the nasal cavity or apnea to prevent inhalation of bacteria further into the respiratory system (Baraniuk and Kim 2007). SCCs could also activate local protective mechanisms to inactivate bacteria metabolites (Chun et al. 2004) or to accelerate mucociliary activity to dilute or expel bacteria (Lindberg et al. 1987). Therefore the nasal SCC system could represent a novel early-detection system to prevent bacterial colonization of the respiratory system.

In conclusion, the principal finding of our study is that not all nasal SCCs are broad detectors of trigeminal irritants, but rather may be “tuned” to detect a narrow range of noxious compounds in the nasal cavity. The only tested compound that both TRPM5-GFP and gustducin-GFP cells responded to through a PLC-mediated signaling cascade was denatonium benzoate. Although SCCs likely mediate trigeminal nerve responses to denatonium, the mechanisms by which the trigeminal nerve responds to other bitter compounds, such as cycloheximide, remain enigmatic.

ACKNOWLEDGMENTS

We thank Dr. Robert F. Margolskee, who generously permitted use of the transgenic mouse strains used in this study, and Dr. Tatsuya Ogura, who developed and demonstrated many of the techniques used in this study. Present address of B. D. Gulbransen: University of Calgary, Department of Physiology and Biophysics, 3330 Hospital Dr. NW, Calgary, Alberta, T2N 4N1, Canada.

GRANTS

This work was supported by National Institute on Deafness and Other Communication Disorders Grants National Research Service Award DC-008275-01, R01 DC-006070, and P30 DC-04657.

REFERENCES


Damak S, Rong M, Yasumatsu K, Kocrashvili Z, Perez CA, Shigemura N, Yoshida R, Mosinger B Jr, Glendingin JJ, Ninomiya Y, Margolskee RF. Trpm5 null mice respond to bitter, sweet, and umami compounds. Chem Senses 31: 253–264, 2006.


Sbarbati A, Mergo F, Benati D, Tizzano M, Bernardi P, Crescimanno C, Osculati F. Identification and characterization of a specific sensory epithe-


