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Solitary chemosensory cells and bitter taste receptor signaling in human sinonasal mucosa

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Abstract

Background—Solitary chemosensory cells (SCCs) are specialized cells in the respiratory epithelium that respond to noxious chemicals including bacterial signaling molecules. SCCs express components of bitter taste transduction including the TAS2R bitter taste receptors and downstream signaling effectors: α -Gustducin, PLC β 2, and TRPM5. When activated, SCCs evoke neurogenic reflexes, resulting in local inflammation. The purpose of this study was to test for the presence SCCs in human sinonasal epithelium, and to test for a correlation with inflammatory disease processes such as allergic rhinitis and chronic rhinosinusitis.

Methods—Patient demographics and biopsies of human sinonasal mucosa were obtained from control patients (n=7) and those with allergic rhinitis and/or chronic rhinosinusitis (n=15). RT-PCR, qPCR and immunohistochemistry were used to determine whether expression of signaling effectors was altered in diseased patients.

Results—RT-PCR demonstrated that bitter taste receptors TAS2R4, TAS2R14 and TAS2R46 and downstream signaling effectors α -Gustducin, PLC β 2, and TRPM5 are expressed in the inferior turbinate, middle turbinate, septum and uncinate of both control and diseased patients. PLC β 2/TRPM5-immunoreactive SCCs were identified in the sinonasal mucosa of both control and diseased patients. qPCR showed similar expression of α -Gustducin and TRPM5 in the uncinate process of control and diseased groups, and there was no correlation between level of expression and SNOT-22 or pain scores.

Conclusion—SCCs are present in human sinonasal mucosa in functionally relevant areas. Expression level of signaling effectors was similar in control and diseased patients and did not correlate with measures of pain and inflammation. Further study into these pathways may provide insight into nasal inflammatory diseases and may offer potential therapeutic targets.

Keywords

Brush cell; respiratory epithelium; rhinosinusitis; rhinitis; inflammation; SNOT-22

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INTRODUCTION

Inflammatory diseases of the nose and sinuses—including, but not limited to rhinosinusitis, allergic rhinitis, and nonallergic rhinitis—are among the most prevalent and present a significant health and economic burden to society. These diseases are responsible for approximately 250 million annual office and emergency room visits in the United States ⁽¹⁾, and account for over \$1500 per patient in overall annual costs ⁽²⁾. These inflammatory conditions may be associated with symptoms of pain and pressure to such a degree in chronic rhinosinusitis for instance; that disease related quality of life is comparable to congestive heart failure, angina, or chronic obstructive pulmonary disease ⁽³⁻⁵⁾. Mechanisms of the inflammatory state are under study, but at this point are poorly understood and thought to be multifactorial ^(3,6).

In the last decade, a population of cells termed solitary chemosensory cells (SCCs) has been studied for their possible role in detection of noxious stimuli within the nasal and respiratory epithelia ⁽⁷⁾. SCCs are chemoresponsive cells in the respiratory epithelium that respond to diverse irritants to activate polymodal nociceptors of the trigeminal nerve thereby inducing local inflammatory responses in the surrounding epithelium ⁽⁸⁾. SCCs express all of the components of the bitter taste transduction pathway, a signaling cascade in taste buds of the tongue presumed to warn against ingestion of potentially toxic compounds ⁽⁹⁻¹¹⁾. This pathway includes the TAS2R family of G protein coupled (bitter) taste receptors on the apical surface, and their downstream intracellular signaling effectors, including the G protein α -Gustducin, phospholipase C β 2 (PLC β 2), and the transduction channel TRPM5 ^(12,13). In murine models, these cells are scattered in the nasal respiratory mucosa ⁽⁷⁾, the laryngeal and tracheal epithelium ⁽¹⁰⁾, and at the entrance to the vomeronasal ducts ⁽¹⁴⁾. They are most abundant in the upper airways, where they are densely innervated by pain fibers of the trigeminal nerve ⁽⁷⁾. These SCCs respond to a variety of irritants including bacterial quorum sensing molecules such as acyl-homoserine lactones produced by *Pseudomonas aeruginosa*. Activation of SCCs elicits trigeminally-mediated protective airway reflexes, including changes in respiration and local neurogenic inflammation ^(7-10,15).

Although these cells may initiate a protective response to avoid and eliminate toxins or bacteria, activation of the trigeminal nociceptors results in pain and inflammation, two common symptoms of highly prevalent human diseases such as rhinitis and rhinosinusitis. Although SCCs are now well-characterized in animal models, their presence in the human upper airway is not well documented ^(7,14,16). We hypothesize that SCCs are present in human upper airway mucosa in regions associated with high airflow and particulate deposition, and that activation of these cells may induce pain and inflammation commonly associated with pathologic states such as rhinitis and rhinosinusitis. The purpose of this study was to test for the presence of solitary chemosensory cells and bitter taste transduction pathways in human sinonasal mucosa, and to test whether there is an association between density of these cells and degree of inflammation and pain in allergic rhinitis and chronic rhinosinusitis.

MATERIALS AND METHODS

Patient Recruitment

This prospective study was approved by the Colorado Multi-Institutional Review Board (COMIRB #HS-11-1134). Informed consent was obtained from all patients. Patients were recruited from a tertiary care rhinology practice at the University of Colorado Department of Otolaryngology. Patients with allergic rhinitis, chronic rhinosinusitis, nasal obstruction, and skull base disorders who were scheduled to undergo sinonasal surgery were invited to participate in the study. Patient demographics, disease characteristics, and tissue biopsies

were obtained from each patient. The experimental group consisted of patients undergoing endoscopic sinonasal surgery for chronic rhinosinusitis (CRS) or nasal obstruction associated with allergic rhinitis (AR). The control group consisted of patients undergoing endoscopic transnasal surgery for orbital or intracranial pathology who did not have AR or CRS. The diagnosis of CRS was made based on 2004 Sinusitis Task Force criteria⁽¹⁷⁾. The diagnosis of AR was made by classic history and physical exam findings with confirmation by positive skin prick testing.

Patient Demographics and Clinical Data Collection

At the time of biopsy, information was collected regarding age, gender, race, and residence. Subjective patient data was recorded, including visual analog pain score and symptom score using the SNOT-22 questionnaire. Objective data, including Lund-Mackay CT score and Lund-Kennedy endoscopy score, were also recorded^(18,19). Study data were collected and managed using REDCap electronic data capture tools hosted at the University of Colorado.⁽²⁰⁾ REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

Biopsy Protocol

After the induction of general anesthesia, the nasal cavity was prepared with topical oxymetazoline-soaked cottonoids and submucosal injection of 1% lidocaine with 1:100,000 epinephrine. Mucosal biopsies (5mm diameter) were obtained with a smooth grasping forceps under endoscopic visualization from the anterior aspect of the inferior turbinate and middle turbinates, the adjacent septum, and uncinate process. Forceps were rinsed in saline between each biopsy to minimize cross-contamination. These specific areas were chosen because inferior turbinates are both implicated and affected in AR^(21,22), uncinate processes are thought to be affected early in the CRS disease process⁽²³⁾, and middle turbinates and septums are also predicted to have a high likelihood of having SCCs based on mapping of previous animal (rodent) studies. Additionally, all of these areas are subject to significant airflow, airflow turbulence and particle deposition⁽²⁴⁻²⁶⁾. SCCs function to detect environmental pathogens and allergens, and it was therefore hypothesized that these cells would cluster in areas of high airflow and particle deposition, where such irritants would be most likely to come into contact with the mucosal surface. Biopsies were either immediately placed in RNAlater (Qiagen, Valencia, CA) and stored at 4°C until RNA extraction, or fixed with paraformaldehyde (PFA) or periodate-lysine-paraformaldehyde (PLP) for immunohistochemistry. For measurement of mRNA, reverse transcriptase PCR (RT-PCR) and quantitative PCR (qPCR) were used. Additionally, immunohistochemistry was used to visualize these cells.

RT-PCR

RNA from human tissue was extracted according to manufacturer's instructions using the RNeasy Mini kit from Qiagen (Valencia, CA), including a 30 minute DNase I treatment at room temperature to remove genomic DNA. Reverse transcription (cDNA synthesis) was performed using the iScript kit from Biorad (Hercules, CA). Reactions were set up in which the reverse transcriptase enzyme was omitted as a control to detect for genomic DNA contamination. Two microliters of cDNA were added to the PCR reaction (Qiagen Taq PCR Core kit, Valencia, CA). RT-PCR Primer sequences and annealing temperatures are located in Table 1. When possible, primers were designed to span at least one intron, however in the case of the bitter taste receptors TAS2R4, TAS2R14 and TAS2R46, no introns were present in the gene. PCR conditions included an initial 5 minute denaturation step followed by 35

cycles of 30 second denaturation at 95°C, 30 second annealing and 45 second extension at 72°C; concluding with a 7 minute final extension step. To validate the PCR, we included cDNA from human tongue (Clontech, Mountain View, CA) and a no template control (water). Amplified sequences were visualized by gel electrophoresis in 2% agarose gels stained with GelRed (Biotium, Hayward, CA). Experiments were repeated for a total of six samples per experiment. Representative PCR products of the correct size were purified with the Qiagen QIAquick PCR Purification kit or QIAEX II Gel Extraction kit (Valencia, CA) and sequences were determined using an ABI (Foster City, CA) 3130 Genetic Sequencer. Over the regions sequenced, there was 100% homology between our PCR product and NCBI published sequences for TRPM5, α -Gustducin, PLC β 2, TAS2R4, TAS2R14 and TAS2R46.

Immunohistochemistry

Immunohistochemistry was performed using similar methodology to that used in prior studies of taste signaling proteins expressed in human taste tissues and the rodent airway^(9,28). Nasal tissue was fixed in buffered 4% paraformaldehyde (PFA) or PLP (periodate-lysine-1.6% PFA) and cryoprotected overnight in 20% sucrose/0.1M phosphate buffer solution. Most samples were sectioned at 16 μ M with a cryostat, and collected on glass slides for immunohistochemistry. For sections, a rabbit anti-PLC β 2 antibody (synthetic peptide from amino acids 1170-1181 of human origin; 1:500; Santa Cruz Biotechnology, USA) was used to identify immunoreactive SCCs and taste receptor cells. One specimen of the middle turbinate from a control patient was reacted as a whole mount for TRPM5 (1:500 dilution; anti-TRPM5 antibody generated against the terminal 70 amino acids of mouse TRPM5, amino acids 1088–1158)⁽²⁹⁾. Some of the whole mount tissue was sectioned after being imaged to better assess the morphology of the labeled cells. Both primary antisera were validated for human proteins using fixed human taste buds, obtained from human patients undergoing sleep surgery.

The secondary antibody used was a fluorescent donkey anti-rabbit Alexa fluor 568 (Molecular Probe, USA) at the concentration of 1:400. All experiments involved a negative control in which the primary antibody was omitted to ensure specificity of the secondary antibody. A Fluoview Olympus confocal microscope was used for imaging. Post-acquisition modification of the images using brightness, contrast and gamma adjustments, was achieved with the software Photoshop CS2™.

qPCR

Based on the RT-PCR results, we chose to further examine the potential differential expression of bitter taste transduction signaling effectors found in the uncinate region. Quantitative PCR was used to compare expression levels of TRPM5 and α -Gustducin between diseased and control subjects in the uncinate samples. All qPCR assays were run in triplicates and experiments were performed on seven control and eight diseased uncinate samples. The eight diseased samples chosen were those with the most severe subjective disease based on SNOT-22 scores, and highest quality of extracted RNA.

Procedures were the same as previously described for RNA extraction and cDNA synthesis, except reverse transcription experiments were performed to obtain 1 μ g of cDNA for each sample. Two microliters of cDNA were used in each singleplex PCR reaction using the ABI SYBR Green PCR Master Mix (Foster City, CA). Primers (10 μ M) were designed for TRPM5, α -Gustducin and the reference gene, GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and purchased from Integrated DNA Technologies (Coralville, IA). Primer sequences are located in Table 1. A two-step PCR was used to amplify TRPM5, α -Gustducin and GAPDH (initial 10 minute denaturation at 95°C followed by 40 cycles of 15

second denaturation at 95°C and 60 second annealing and extension at 60°C) in the Biorad CFX Real-Time PCR Detection System (Hercules, CA). We utilized the SYBR green dye qPCR technique to detect double-stranded DNA/PCR products as they accumulated during PCR cycles.

For quantitative analysis, we adapted the Comparative C_T relative expression method (or $\Delta\Delta C_T$) in which the cycle threshold (C_T) values for our reference gene, GAPDH were subtracted from the C_T of our target genes (TRPM5 and α -Gustducin) and compared to a calibrator. The calibrator for the TRPM5 and α -Gustducin experiments was the mean ΔC_T value (C_T target gene - C_T GAPDH) for all control subjects. C_T for each sample was set to occur during the exponential growth phase of the curve.

Statistics

Mean values of relative cycling threshold for uncinate expression of TRPM5 and α -Gustducin were compared to patient demographic information, visual analog pain score, SNOT-22 score, Lund-Mackay CT score, and Lund-Kennedy endoscopy score. To compare expression levels of signaling effectors in diseased and control patients, an unpaired t test was used. Linear regression analysis was used to correlate the expression level of signaling effectors with the patient's pain and SNOT-22 scores. Differences were considered significant at $p=0.05$.

RESULTS

A total of twenty-five patients were included in the study; twenty-two were examined by RT-PCR, consisting of 15 diseased and 7 control patients. qPCR was performed on fifteen patients. The mean age was 52.4 yrs (range 29 to 77 years), with a male:female ratio of 1.2:1. Average age of the control patients was 54.4 years (range 30-71) and consisted of three females and four males. Average age of the diseased patients was 51.5 years (range 29-77) and consisted of seven females and eight males. Average SNOT-22 of the diseased and control patients were 51.93 (range 10-87) and 14.71 (range 7-32), respectively. Average VAS pain scores of the diseased and control patients were 3.47 (range 0-8) and 0.14 (range 0-1), respectively. Average preoperative Lund-Mackay CT scores of the diseased and control patients were 7.27 (range 1-17) and 1.14 (range 0-2), respectively. Average Lund-Kennedy endoscopy scores (bilateral) of the diseased and control patients were 2.9 (range 1-6), and 0.57 (range 0-1), respectively (see Supplementary Table).

RT-PCR

RT-PCR revealed expression of the bitter taste receptors TAS2R4, TAS2R14, and TAS2R46 and downstream taste signaling effectors PLC β 2, α -Gustducin, and TRPM5 in the epithelium of both control and diseased patients, suggesting the presence of SCCs in all biopsy regions (Figure 1, Table 2). Expression of PLC β 2 occurred in nearly every sample, while detection of α -Gustducin and TRPM5 was somewhat more variable, with some patients failing to show detectable expression in both control and diseased groups. Expression in the uncinate was the most variable, with some control patients (3/7) lacking detectable expression of all three downstream signaling effectors, but demonstrating expression in the other three biopsy areas ($p=0.02$). The three TAS2Rs were also detected in most tissue samples and showed less variability than the downstream signaling effectors. Even in control uncinate samples in which we could detect no downstream effectors, most expressed the bitter taste receptors.

To validate the integrity of the extracted RNA, we performed at least two of the following on all samples; RT-PCR for β actin, RNA sample analysis on the GE NanoVue

spectrophotometer (Pittsburg, PA), and/or analyzed the concentration and integrity of the RNA samples in the Agilent 2100 Bioanalyzer (Santa Clara, CA). All RNA samples were intact and thus the lack of expression in some samples did not reflect poor quality of the mRNA.

Immunohistochemistry

Taste signaling effectors, particularly the bitter taste receptors, have been observed in airway tissues other than SCCs, including cultured tracheal epithelial cells ^(30,31).

Immunohistochemistry was used to determine if cells with morphological characteristics of SCCs could be found in the tissue biopsies. We used antibodies against PLC β 2 and TRPM5 to identify putative SCCs, because we were able to validate these antisera using sections containing taste buds from a human circumvallate papilla, where PLC β 2 and TRPM5 are expressed in Type II taste cells (Figure 2C). Panels A & B of Figure 2 illustrate PLC β 2-immunoreactive cells in the nasal epithelium that morphologically resemble SCCs in rodents. In the human tissue, immunoreactive cells were 5-10 μ m in width and varied in height according to the thickness of the epithelium (Figure 2A, B). In whole mounts reacted for TRPM5, numerous immunoreactive cells were evident (Figure 2D-F), but the immunoreactive cells were distributed non-uniformly, often occurring in clusters (Figure 2F) with broad swaths of epithelium exhibiting no immunoreactive cells. This corresponded to our observations of sectioned biopsy specimens; some sections lacked immunolabeled cells while others had multiple cells.

Quantitative PCR

To test for a possible relationship between disease severity and the expression level of taste signaling effectors, qPCR was used to quantify the relative expression of α -Gustducin and TRPM5 in the uncinate of control and diseased patients relative to a reference gene (GAPDH). TRPM5 and α -Gustducin were chosen as they are less likely to be present in the ciliated cells but are required for transduction of irritants and bacterial quorum signaling molecules in the SCCs ⁽¹⁰⁾. Further, we failed to detect any immunoreactivity for TRPM5 in ciliated cells in our biopsy material (Figure 2), suggesting that all expression should reflect the abundance of SCCs. The mRNA used for qPCR was obtained from seven control patients and the eight diseased patients with the highest SNOT-22 scores. No significant difference in TRPM5 and α -Gustducin expression was found in the uncinate process between the control and diseased patients (Figure 3). Further studies with larger sample sizes will be needed to confirm that SCC density is unrelated to sinonasal disease. We also analyzed relative expression on an individual patient basis, by correlating the relative expression of α -Gustducin and TRPM5 obtained from each patient with their visual analog pain and SNOT-22 scores. No correlation was observed (Figure 4).

Discussion

Whitear first described scattered chemosensory cells in the epidermis of fish and referred to them as solitary chemosensory cells (SCCs) ⁽³²⁾. Since then, morphologically similar cells have been identified in mammals and studied for their possible role in detection of noxious stimuli within the airway epithelium. In mice, SCCs express the transduction elements utilized for bitter taste transduction, but respond to a variety of irritants and may detect the presence of bacteria before they reach a pathogenic state ⁽⁹⁾. Activation of SCCs through taste transduction pathways results in protective airway reflexes, including changes in respiration and local inflammatory responses, mediated by the trigeminal nerve ⁽⁹⁾. Importantly, in knockout mice lacking either TRPM5 or α -Gustducin, certain irritant or bacterial stimuli fail to evoke either respiratory changes or inflammatory response, indicating that SCCs are necessary for trigeminally mediated responses to particular

irritants⁽⁹⁾. SCCs, therefore, if found in humans may be expected to respond to the inhalation of irritants or to expanding pathogenic bacterial populations, and may alert the innate immune system to the presence of a pathogenic state.

In this paper we provide molecular and morphological evidence for the presence of solitary chemosensory cells (SCCs) in human nasal epithelium. Taste signaling effectors were broadly expressed in several regions of the sinonasal cavity, including the inferior turbinates and uncinate process, regions associated with inflammation and pain in allergic rhinitis, and the septum and middle turbinates, regions of high SCC density in rodents. Our immunohistochemical data, although preliminary, corroborate our molecular expression data and show that both PLC β 2 and TRPM5 are expressed in cells with morphological characteristics of SCCs in rodents: they have apical processes that extend to the surface of the epithelium and have an elongate morphology typical of SCCs. Importantly, no PLC β 2 or TRPM5 immunoreactivity was detected in ciliated epithelial cells. These data extend the findings of Braun et al., who first showed that SCCs could be detected in human nasal tissue, from a specialized region near the vomeronasal pit, a remnant of the vomeronasal organ that forms embryonically but disappears before birth in humans⁽³³⁾. Our data suggest SCCs are much more broadly expressed, including in areas relevant for chronic sinonasal disease.

The number of SCCs detected immunohistochemically appeared lower than expected based on previous experiments in rodent airways. However, the irregularity of detection reflects a patchy distribution in humans as in rodents, so more samples will be required before the prevalence and distribution of SCCs within the human airways is clarified. In the current study, the gross prevalence of signaling effectors was similar in healthy and diseased states; however a number of other mechanisms of dysregulation are feasible.

Although we observed expression of taste signaling effectors in all biopsied regions of both control and diseased patients, our quantitative PCR did not show an upregulation of signaling effectors in diseased patients, and there was no correlation of either SNOT-22 score or VAS pain score with expression of mRNA for either α -gustducin or TRPM5. These data suggest that expression level of taste signaling effectors, and presumably SCC density, is independent of disease state, at least in the uncinate process, where qPCR was performed. An important caveat in this study is that all diseased patients are on topical steroids and this could, in principle, affect the expression of taste signaling effectors or number of SCCs. Lack of changes in SCC density, however, does not imply that SCCs are not involved in mediating inflammation and pain, since they are present in areas affected in chronic sinus disease. In mice, activation of SCCs through taste transduction pathways results in protective airway reflexes, including changes in respiration and local inflammatory responses, mediated by the trigeminal nerve⁽⁸⁻¹⁰⁾. Strictly identifying nonallergic rhinitis (NAR) and the subgroup idiopathic rhinitis (IR) patients can be very complicated as these diagnoses contain heterogeneous groups. If SCCs play a similar role in humans, they may be at least partly responsible for producing the inflammation that is associated with inflammatory diseases of the nasal cavity -- including rhinosinusitis and allergic rhinitis. With the relatively small sample size in our study, we decided to include AR patients in the disease group to preliminarily examine those patients with chronic rhinitis. Nasal hyperreactivity to nonspecific stimuli such as smoke, intense odorants, and environmental irritants is a classic feature of patients with chronic rhinitis, regardless of an infectious, allergic or nonallergic origin⁽³⁴⁾. Additionally, mucosal hyperinnervation has been demonstrated with increased expression of the neuropeptides calcitonin gene related peptide (CGRP) and Substance P in periglandular nerve fibers in patients with AR, with a comparable level in IR, indicating a similar degree of neurogenic inflammation in both diseases⁽³⁵⁾.

These diseases are often associated with pain, pressure, and poor quality of life. A number of inflammatory pathways have been implicated, including local pro-inflammatory cytokine accumulation, increased tissue recruitment of inflammatory cell populations, and T-cell or nerve-mediated increases in vascular permeability to name a few ^(3,6,30). An important goal for future research will be to determine whether the SCCs in human nasal tissue are innervated by the trigeminal nerve, as is the case in rodents. Innervation by the trigeminal nerve would provide a mechanism for linking activation of the SCCs to neurogenic inflammation.

Conclusion

We have demonstrated the presence of SCCs and bitter taste transduction in functionally relevant areas of the human sinonasal tract. These cells may be implicated in the pathogenesis of chronic inflammatory diseases such as chronic rhinosinusitis and chronic rhinitis. Further study is clearly necessary to further classify mechanisms of cell stimulation, signaling pathways, and potential therapeutic targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Mattos JL, Woodard CR, Payne SC. Trends in common rhinologic illnesses: analysis of U.S. healthcare surveys 1995-2007. *Int Forum Allergy Rhinol.* 2011; 1(1):3–12. [PubMed: 22287302]
2. Bhattacharyya N. The economic burden and symptom manifestations of chronic rhinosinusitis. *Am J Rhinol.* 2003; 17:27–32. [PubMed: 12693653]
3. Benninger MS, Ferguson BJ, Hadley JA, et al. Adult chronic rhinosinusitis: definitions, diagnosis, epidemiology, and pathophysiology. *Otolaryngol Head Neck Surg.* 2003; 129:S1–32. [PubMed: 12958561]
4. Jones NS. Sinogenic facial pain: diagnosis and management. *Otolaryngol Clin North Am.* 2005; 38(6):1311–25. [PubMed: 16326187]
5. Gliklich RE, Metson R. The health impact of chronic sinusitis in patients seeking otolaryngologic care. *Otolaryngol Head Neck Surg.* 1995; 113:104–109. [PubMed: 7603703]
6. Fokkens W, Lund V, Mullol J, European Position Paper on Rhinosinusitis and Nasal Polyps group. European position paper on rhinosinusitis and nasal polyps. *Rhinol Suppl.* 2007; (20):1–136. [PubMed: 17844873]
7. Finger TE, Bottger B, Hansen A, et al. Solitary chemoreceptor cells in the nasal cavity serve as sentinels of respiration. *Proc Natl Acad Sci USA.* 2003; 100:8981–8986. [PubMed: 12857948]
8. Tizzano M, Finger TE. Nasal epithelium inflammation: involvement of solitary chemosensory cells after short term presentation of the irritating compound denatonium benzoate. *Chem Senses.* 2012 (in press):Abst #p49 in press.
9. Tizzano M, Cristoforetti M, Sbarbati A, Finger TE. Expression of taste receptors in solitary chemosensory cells of rodent airways. *BMC Pulm Med.* 2011; 11:3. [PubMed: 21232137]

10. Tizzano M, Gulbransen BD, Vandenbeuch A, et al. Nasal chemo- sensory cells use bitter taste signaling to detect irritants and bacterial signals. *Proc Natl Acad Sci USA*. 2010; 107:3210–3215. [PubMed: 20133764]
11. Gulbransen BD, Clapp TR, Finger TE, Kinnamon SC. Nasal solitary chemoreceptor cell responses to bitter and trigeminal stimulants in vitro. *J Neurophysiol*. 2008; 99:2929–2937. [PubMed: 18417634]
12. Finger TE, Kinnamon SC. Taste isn't just for taste buds anymore. *F1000 Biol Rep*. 2011; 3:20. [PubMed: 21941599]
13. Kinnamon SC. Taste receptor signaling-from tongues to lungs. *Acta Physiol (Oxf)*. 2012; 204(2): 158–68. [PubMed: 21481196]
14. Ogura T, Krosnowski K, Zhang L, et al. Chemoreception regulates chemical access to mouse vomeronasal organ: role of solitary chemosensory cells. *PLoS One*. 2010; 5:e11924. [PubMed: 20689832]
15. Krasteva G, Canning BJ, Hartmann P, et al. Cholinergic chemosensory cells in the trachea regulate breathing. *Proc Natl Acad Sci USA*. 2011; 108:9478–83. [PubMed: 21606356]
16. Tizzano M, Merigo F, Sbarbati A. Evidence of solitary chemosensory cells in a large mammal: the diffuse chemosensory system in *Bos Taurus* airways. *J Anat*. 2006; 209(3):333–7. [PubMed: 16928202]
17. Meltzer EO, Hamilos DL, Hadley JA, et al. Rhinosinusitis: establishing definitions for clinical research and patient care. *J Allergy Clin Immunol*. 2004; 114:155–212. [PubMed: 15577865]
18. Lund VJ, Kennedy DW. Quantification for staging sinusitis. The Staging and Therapy Group. *Ann Otol Rhinol Laryngol Suppl*. 1995; 167:17–21. [PubMed: 7574265]
19. Lund VJ, Mackay IS. Staging in rhinosinusitis. *Rhinology*. 1993; 31:183–184. [PubMed: 8140385]
20. Paul A, Harris Robert Taylor, Robert Thielke, et al. Research electronic data capture (REDCap) - A metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009; 42(2):377–81. [PubMed: 18929686]
21. Lee BJ, Naclerio RM, Bochner BS, et al. Nasal challenge with allergen upregulates the local expression of vascular endothelial adhesion molecules. *J Allergy Clin Immunol*. 1994; 94:1006–16. [PubMed: 7528231]
22. Liu F, Zhang J, Liu Y. Inflammatory profiles in nasal mucosa of patients with persistent vs intermittent allergic rhinitis. *Allergy*. 2010; 65(9):1149–57. et al. [PubMed: 20192943]
23. Kennedy, DW. *Diseases of the Sinuses: Diagnosis and Management*. 1st ed. B.C. Decker Inc; Ontario: 2001. *Functional Endoscopic Sinus Surgery: Concepts, Surgical Indications, and Instrumentation*; p. 197-210.
24. Keyhani K, Scherer PW, Mozell MM. Numerical simulation of airflow in the human nasal cavity. *J Biomech Eng*. 1995; 117(4):429–41. [PubMed: 8748525]
25. Schroeter JD, Kimbell JS, Asgharian B. Analysis of particle deposition in the turbinate and olfactory regions using a human nasal computational fluid dynamics model. *J Aerosol Med*. 2006; 19(3):301–13. [PubMed: 17034306]
26. Zhao K, Scherer PW, Hajiloo SA, Dalton P. Effect of anatomy on human nasal air flow and odorant transport patterns: implications for olfaction. *Chem Senses*. 2004; 29(5):365–79. [PubMed: 15201204]
27. Vandenbeuch A, Tizzano M, Anderson CB, et al. Evidence for a role of glutamate as an efferent transmitter in taste buds. *BMC Neurosci*. 2010; 11:77. [PubMed: 20565975]
28. Ozdener MH, Brand JG, Spielman AI, et al. Characterization of Human Fungiform Papillae Cells in Culture. *Chem Senses*. 2011; 36(7):601–612. [PubMed: 21471186]
29. Zhang Z, Zhao Z, Margolskee R, Liman E. The transduction channel TRPM5 is gated by intracellular calcium in taste cells. *J Neurosci*. 2007; 27(21):5777–86. [PubMed: 17522321]
30. Lee RJ, Xiong G, Kofonow JM, et al. T2R38 taste receptor polymorphisms underlie susceptibility to upper respiratory infection. *J Clin Invest*. Oct 8.2012 2012. pii: 64240. doi: 10.1172/JCI64240.
31. Shah AS, Ben-Shahar Y, Moninger TO, et al. Motile cilia of human airway epithelia are chemosensory. *Science*. 2009; 325(5944):1131–4. [PubMed: 19628819]

32. Whitear, M. Solitary Chemoreceptor Cells. In: Hara, TJ., editor. Chemoreception in Fishes. 2nd ed. Elsevier Press; London: 1992. p. 103-125.
33. Braun T, Mack B, Kramer MF. Solitary chemosensory cells in the respiratory and vomeronasal epithelium of the human nose: a pilot study. *Rhinology*. 2011; 49(5):507–12. [PubMed: 22125779]
34. Gerth van Wijk RG, de Graaf-in 't Veld C, Garrelds IM. Nasal hyperreactivity. *Rhinology*. 1999; 37:50–55. [PubMed: 10416248]
35. Knipping S, Holzhausen HJ, Riederer A, Schrom T. Allergic and idiopathic rhinitis: an ultrastructural study. *Eur Arch Otorhinolaryngol*. 2009; 266:1249–1256. [PubMed: 19125268]

Figure 1.

GelRed stained RT-PCR products from the mRNA of human nasal tissue taken from a patient with CRS amplified with primers designed for (A) PLC β 2, (B) α -Gustducin, (C) TRPM5, (D) TAS2R4, (E) TAS2R14, and (F) TAS2R46. Expected sizes for PCR products are as followed: PLC β 2: 333bp, α -Gustducin: 386bp, TRPM5: 301bp, TAS2R4: 474bp, TAS2R14: 250bp, and TAS2R46: 250bp. Lanes represent (in order): negative control (no template); positive control, human tongue (HT, Clontech); inferior turbinate (IT); middle turbinate (MT); septum (S); and uncinate (U). In general, all regions biopsied showed expression of taste signaling effectors in both diseased patients and control patients (refer to Table 1).

Figure 2. Immunohistochemistry for PLC β 2 (A-C) and TRPM5 (D-F) from human biopsy material. **A.** A presumed PLC β 2-immunoreactive solitary chemosensory cell from the septum of a CRS patient. **B.** PLC β 2-immunoreactive cells in the inferior turbinate of a control subject. **C.** PLC β 2-immunoreactive taste cells in a circumvallate papilla for validation of antibody. **D.** TRPM5-immunoreactive cell in a whole mount of the posterior part of the middle turbinate. **E.** Cluster of TRPM5-immunoreactive cells in the middle turbinate in sections taken from the whole mount specimen shown in panel D. **F.** Low magnification view of the whole mount middle turbinate showing the density of labeled cells in a region containing many presumed SCCs. Scale bar: 25 μ M (Panels A-E); 50 μ M for panel F.

Figure 3.

Quantitative PCR for α -Gustducin and TRPM5 mRNA in the uncinate from control and diseased (CRS/AR) patients. Expression is shown relative to an internal calibrator. There was no significant difference in expression of either gene between the two groups of patients. Data are represented as means $\pm$ SEM from control (n=7) and diseased (n=8) patients, each run in triplicates.

Figure 4.

Relationship between SNOT-22 score and relative expression of α -Gustducin and TRPM5 in control (open circles) and diseased (closed circles) patients (upper panels) and VAS pain score and α -Gustducin and TRPM5 (lower panels). Linear regression analysis showed no correlation between SNOT-22 score and relative expression level of either gene ($r^2 = 0.01$ for α -Gustducin and 0.003 for TRPM5) and VAS pain score and either gene ($r^2 = 0.004$ for α -Gustducin and 0.017 for TRPM5). In order to control for confounding effects, multiple regression of SNOT-22 and VAS scores were run as independent variables. No significant correlations were observed.

Table 1

RT-PCR and qPCR primers for TAS2R bitter taste receptors and downstream signaling effectors: α -Gustducin, PLC β 2, and TRPM5.

	Gene	Accession #	Forward Primer (5'→3')	Reverse Primer (5'→3')	bp	Anneal °C
RT-PCR	TRPM5	NM_014555	TGGTAGAGCGCATGATGAAG	ACCAACAGGAAGGTGACCAG	301	63°
	α -Gustducin	NM_001102386	TCTGGGTATGTGCCAAATGA	GGCCCAGTGATTCTGGAAA	386	51°
	PLC β 2	NM_004573	GTCACCTGAAGGCATGGTCT	TTAAAGGCGCTTTCTGCAAT	333	53°
	TAS2R4	NM_016944	ACTCGAGCAGTGTCTGGTTTGTGA	ACTGGACCAGGGTAGCAACTGAAT	474	60°
	TAS2R14	NM_023922	TGCTGCTTCTTGTAAGTTCGGTCT	GTGTGCTGCATCTTCTTGCGATGT	250	58°
	TAS2R46	NM_176887	AGATCCCAGCATGAAGGTCCACAT	TTTACCCAGTACCTCACATGCCA	250	60°
qPCR	TRPM5	NM_014555	ACAGATCAACTACTGCTCGGTGCT	TGTTCCCAGCCATCTAAACCACCT	144	
	α -Gustducin	NM_001102386	ATACCCTGGAAGATGGTGGCATGA	TTTACAGATGCCCTTTCAAAGCAGGC	101	
	Gapdh	NM_002046	CATGTTTCGTCATGGGTGTGAACCA	AGTGATGGCATGGACTGTGGTCAT	137	

Table 2

RT-PCR results showed consistent expression of the three signaling molecules and TAS2Rs in the inferior turbinate (IT), middle turbinate (MT), and septum (S), whereas the uncinate process in three control patients did not express any of the three signaling effectors.

<i>I7+MT+S</i>	α -Gust	PLC β 2	TrpM5	TAS2R4	TAS2R14	TAS2R46
Diseased	18/45 (40%)	45/45 (100%)	39/45 (86%)	28/30 (93%)	29/30 (97%)	22/30 (73%)
Control	12/21 (57%)	19/21 (90%)	17/21 (81%)	23/24 (96%)	23/24 (96%)	16/24 (67%)
<i>Uncinate</i>						
Diseased	13/15 (86%)	15/15 (100%)	13/15 (86%)	10/10 (100%)	10/10 (100%)	7/10 (70%)
Control	4/7 (57%)	4/7 (57%)	4/7 (57%)	7/8 (88%)	8/8 (100%)	6/8 (75%)