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A role for airway taste receptor modulation in the treatment of upper respiratory infections

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Summary

Taste receptors, initially identified in the oral epithelium, have since been shown to be widely distributed, being found in the upper and lower respiratory tracts, gastrointestinal epithelium, thyroid, and brain. The presence of taste receptors in the nasal epithelium has led to the discovery of their role in innate immunity, defending the paranasal sinuses against pathogens. This article addresses the current paradigm for understanding the role of extraoral taste receptors, specifically the T2R38 bitter taste receptor and the T1R2+3 sweet taste receptor, in respiratory innate defenses and presents evidence for the use of these and other taste receptors as therapeutic targets in the management of chronic rhinosinusitis. Future studies should focus on understanding the polymorphisms of taste receptors beyond T2R38 to fully elucidate their potential therapeutic use and lay the groundwork for their modulation in a clinical setting to decrease the health impact and economic burden of upper respiratory disease.

Keywords

Airway physiology; taste receptors; chronic rhinosinusitis; TAS2R; epithelial biology; hostpathogen interactions; upper respiratory disease; innate immunity

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Introduction

The pathogenesis of upper respiratory disease requires that bacteria evade a number of airway defenses, among them mucociliary clearance and other components of the respiratory innate immune system. Despite such protective barriers, the incidence of chronic rhinosinusitis (CRS) invokes a significant disease and economic burden and is responsible for a worrisome trend toward microbial antibiotic resistance [1–3]. A thorough understanding of this process of invasion and infection therefore presents a valuable opportunity for intervention in the interest of antimicrobial stewardship, cost containment, and most important, novel therapeutics.

A diagnosis of CRS is made after signs and symptoms of an upper respiratory infection, including rhinorrhea, nasal obstruction, decreased sense of smell, and sinus pressure, persist for 12 or more weeks despite optimal medical management. Initial management strategies include local irrigation, nasal steroids, and multiple rounds of culture-directed antibiotics. In the setting of long-term treatment failure, patients are referred for functional endoscopic sinus surgery to reestablish patency and normalize drainage of the paranasal sinuses. The impact of rhinosinusitis on patients is fairly dramatic, with sufferers reporting lower quality of life scores than do patients with chronic obstructive pulmonary disease, congestive heart failure, or angina [4]. Of note, disease burden in CRS is typically measured using the SNOT-22 questionnaire, which investigates aspects of sinonasal symptomatology, sleep, and emotional and psychosocial functioning, with higher scores representing a worse quality of life [5].

Many recent studies have demonstrated the role of extraoral taste receptors present on the nasal epithelium in the innate immune response to invading pathogens, with the T2R38 bitter taste receptor and T1R2+3 sweet taste receptor being best characterized [6]. In the interest of enhancing treatment responsiveness, we argue that modulation of these receptors offers a unique frontier for the development of targeted therapeutics in the treatment of upper respiratory disease.

Historical perspective

From the earliest studies, it has been clear that taste represents a sense in which two individuals can fundamentally differ. The first documented instance of so-called taste blindness was detailed by Roger Williams in 1931 and involved the ability to taste creatine, a nitrogenous organic acid [7]. The following year, Arthur Fox reported a similar finding in the ability to taste the bitter compound phenylthiocarbamide (PTC) after accidentally dispersing the compound in his laboratory [8]:

“Another inhabitant of the laboratory...complained of the bitter taste of the dust, but [I] observed no taste.”

At the time, the difference in taste was hypothesized to stem from variable solubility of PTC in saliva, with some individuals producing saliva containing a protein rendering PTC insoluble and therefore tasteless, and others lacking this protein, rendering PTC soluble and strongly bitter tasting. While this hypothesis was ultimately disproved, Fox’s observations

spurred decades of research on the genetics of taste perception that continues to this day. The ability to taste PTC became one of the most studied traits in human genetics, second only to the study of ABO blood groups [9]; however, the specific gene responsible remained unknown. Researchers proposed a variety of inheritance mechanisms, including multiple genes, incomplete dominance, and multiple alleles of a single gene [10,11]. Numerous linkage studies were performed, with the strongest associations demonstrated with chromosome 5p15 and the KELL region on chromosome 7 [9,12,13]. Interesting in the context of current knowledge, early studies showed a correlation between PTC taste blindness and susceptibility to infectious diseases, among them the primarily respiratory illness of tuberculosis [14].

It was not until the completion of the Human Genome Project in 2003 that a family of G protein-coupled receptors (GPCRs), the so-called T2Rs, was identified as candidate bitter taste receptors due their linkage to loci known to influence bitter taste [15,16]. These receptors were found on chromosomes 5, 7, and 12 but differed from GPCRs previously implicated in taste, the T1Rs: the T2Rs featured a short extracellular N-terminal domain, while T1R2 exhibited a long N-terminus [17]. Heterologous expression experiments provided further evidence for the singularity of T2Rs as bitter taste receptors, showing that both a human receptor and a mouse receptor (hT2R4 and mT2R8) respond to compounds similar to PTC: denatonium benzoate and 6-n-propyl-2-thiouracil (PROP) [16]. Subsequently, through functional expression studies, the *in vitro* function of one member of this family, T2R38, encoded by the *TAS2R38* gene on chromosome 7, was formally identified as responsible for sensing PTC [18]. This discovery marked a milestone in the study of taste receptors, and the T2R38 bitter taste receptor continues to rank as one of the best-studied taste receptors in the current literature.

Basic biology of taste

The human sensory system detects five unique tastes: salty, sweet, sour, bitter, and umami (also known as savory). While moderately salty, sweet, and umami are typically viewed as pleasant tastes and alert the body to the presence of sodium chloride, sugar, and amino acids, respectively, the opposite is true for sour and bitter, which alert the body to the presence of toxic substances such as acidic products of bacterial fermentation and toxic plant alkaloids such as strychnine [19]. The tastes of salty and sour are transduced through the activation of ion channels, facilitating the transport of sodium chloride in type I and hydrogen ions in type III taste cells, respectively.

The remaining tastes, sweet, umami, and bitter, are transduced through the activation of GPCRs existing as one of a number of dimers and are found in type II taste cells [20,21]. In humans, umami and sweet are each transduced through their own unique GPCR (Figure 1, left and center); however, bitter perception is transduced through approximately 25 different T2R isoforms (Figure 1, right), the genetic sum of which is referred to as the “bitterome” (Figure 2) [22,23]. Some bitter agonists have been shown to stimulate multiple T2R isoforms, and some receptor isoforms also respond to multiple agonists, creating a redundant pattern of encoding [24]. Further, of the 25 T2Rs, the collective receptive range of 19

receptors accounts for approximately 80% of the established bitter library; the handful of remaining “orphan” receptors have ligands that are yet to be identified [24].

The initial point of activation for each GPCR-linked taste differs, with cells expressing T1R2+3 responding to sweet, those expressing T1R1+3 responding to savory, and those expressing T2Rs responding to bitter ligands (Figure 1). However, intracellular signal transduction is highly conserved within these type II taste receptor cells, as illustrated in Figure 3. The cascade begins with G $\beta\gamma$ activation of phospholipase C isoform $\beta 2$ (PLC $\beta 2$), which results in production of inositol 1,4,5-trisphosphate (IP $_3$), activating the intracellular type 3 IP $_3$ receptor (IP $_3$ R) and stimulating the release of intracellular calcium (Ca $^{2+}$) stores from the endoplasmic reticulum. (The type 3 IP $_3$ R is the major isoform found in type II taste cells). A separate pathway involving G α -gustducin is stimulated in parallel, which activates phosphodiesterases, reducing levels of cyclic adenosine monophosphate (cAMP) and decreasing protein kinase A (PKA) activity. Because PKA phosphorylates and thereby inhibits the activity of IP $_3$ R, this reduction in PKA by the G α -gustducin pathway enhances IP $_3$ R-mediated calcium signaling [25–27]. Subsequently, Ca $^{2+}$ stimulates the cation channel TRPM5 (transient receptor potential cation channel subfamily M member 5), located on the plasma membrane, depolarizing the membrane potential and activating voltage-gated sodium (Na $^+$) channels to produce an action potential. This results in the release of adenosine triphosphate, which activates purinergic receptors on presynaptic cells and afferent sensory fibers carrying sensory information to the brain for further processing [19,28].

The persistence of taste receptors is highly dependent on their active use in feeding behavior. Specifically, carnivorous animals such as the sea lion and bottlenose dolphin exhibit dysfunctional (pseudogenized) *TAS1R1* and *TAS1R3* receptor genes and may also have lost bitter receptor genes. This is hypothesized to occur because these animals consume their food whole and thus are not consistently activating the receptors [29].

Despite what the name “taste receptor” may imply, a growing literature exists surrounding the expression of these receptors beyond the epithelium of the tongue, as well as their role in the innate immune system. This phenomenon is discussed in detail below. In this review, we focus on the T1R2+3 (sweet) and T2R (bitter) taste receptors, which have been shown to exhibit the strongest association with the innate immunity of the upper respiratory tract.

TAS2R38: A model system for human knockout studies

Following the discovery of *TAS2R38*, the previously puzzling variation in PTC sensitivity was found to be due to haplotype variation. A genetic haplotype is a set of single nucleotide polymorphisms (SNPs), or variations in the DNA sequence, that tend to be inherited together. The most common *TAS2R38* polymorphisms are observed at amino acid position 49, where either proline or alanine is encoded; position 262, where either alanine or valine is encoded; and position 296, where either valine or isoleucine is encoded. This yields two frequent haplotypes, PAV and AVI, and multiple other less common haplotypes.

While exposure of the PAV variant to PTC results in increased intracellular Ca $^{2+}$ in a concentration-dependent manner, the AVI variant shows no response. Outcomes are comparable when the structurally similar compound PROP is applied. *In vivo* testing further

shows a positive correlation with psychophysical bitter perception, with individuals homozygous for the active (PAV/PAV) form exhibiting sensitivity to bitterness in compounds containing a thiourea moiety ($-N-C=S$; e.g., PTC, PROP, and the plant compound goitrin, common in certain foods, such as some green vegetables) [30]. Individuals homozygous for the inactive (AVI/AVI) form require high concentrations to taste PTC, if they are able to taste it at all. These results provided the first direct molecular link between variability in PTC perception and variants of the bitter taste receptor T2R38.

Numerous studies of population genetics have been performed to better characterize the frequency of each genotype. Results indicate that the frequency of the non-taster (AVI/AVI) genotype is much higher than would be predicted by traditional Mendelian genetics, and ranges anywhere from zero to 66.7% non-taster individuals in a given population [9]. Apart from one small group of Brazilian Indians [31], the non-taster form is present in all populations and has also been identified in other mammalian species [9]. Such widespread persistence of a non-functional receptor allele makes the study of *TAS2R38* genotype particularly compelling.

The phenomenon of linkage between a particular taste receptor and its agonist has since been extended to pairings beyond T2R38 and PTC. For example, nearly 50% of individual ability to perceive PROP is related to the T2R38 receptor, and approximately 6% of the variability in perception of quinine has been linked with the T2R19 receptor [32]. Given these examples, it is clear that not every bitter receptor and its agonist(s) exhibit a correlation as strong as that of T2R38 and PTC. In fact, recent studies have shown that bitter sensitivity may be inherited in a manner that spans the bitterome. Specifically, of the 93 coding SNPs identified within the 25 T2Rs, 67 were considered more common (i.e., frequency > 0.05); however, while polymorphisms were distinct between individuals, the variation exists within only six regions, with one to five haplotypes for each region [23]. This complexity in the genetics of bitter perception highlights that the T2R38 and PTC pairing is more unique than originally thought and that an individual's sensitivity to a bitter compound has less to do with an individual T2R genotype and more to do with the gestalt of the individual's bitterome (Figure 2) [23]. While the genotype-phenotype data regarding T2R38 have been reliably localized to three SNPs, this new knowledge complicates our interpretation of other genotype-phenotype relationships [33].

Extraoral taste receptors

Taste receptors and the associated signaling cascade were initially discovered within the taste epithelium. Interestingly, later studies also noted their presence in bipolar cells in scattered nasal respiratory epithelium of mice by identifying the presence of α -gustducin, a component of the taste signaling pathway (Figure 3). This concept has since become known as "extraoral" expression, and studies have been extended to humans, identifying extraoral taste receptors in such locations as the sinonasal cavity, brain, thyroid, pancreas, gastrointestinal tract, bladder, and testes [19,34–39]. Given the fact that these sites, apart from the gastrointestinal tract, do not come into direct contact with ingested food, the potential roles of extraoral taste receptors have generated much speculation. Apart from the role in upper airway innate immunity described below, T1Rs have also been shown to play a

role in pancreatic β cells and gut endocrine cells by regulating glucose metabolism [40,41]. Many oral pharmaceuticals and even compounds secreted by bacteria have a bitter taste, leading to the hypotheses that extraoral expression may produce unintended drug side effects or play a role in the detection of invading pathogens [36,42–47].

Historical studies have also characterized an association between genetic variability and susceptibility to various infections, such as sinonasal disease [48,49], and the risk for developing CRS requiring surgical intervention [50]. A critical observation was made by Tizzano et al demonstrating that T2Rs on murine nasal solitary chemosensory cells (SCCs) are stimulated by compounds secreted by bacteria and stimulate an apneic response [45]. From this observation grew the concept that other T2Rs may contribute to respiratory innate defense in response to bacterial factors. Below we discuss the state of knowledge on the role of extraoral T2R bitter and T1R2+3 sweet taste receptors in the innate immune system of the sinonasal cavity.

Upper airway defenses and the role of taste receptors

As a conducting pathway through which respiration occurs, the sinonasal cavity represents one of the body's first points of contact with invading respiratory pathogens [51]. It therefore features multiple components aimed at combating the continual onslaught of pathogens with each respiratory cycle. The backbone of these protective barriers is mucociliary clearance, which involves the secretion of mucus by airway goblet cells. Mucus, made up of cross-linked glycosylated mucin macromolecules, bathes the nasal epithelium, sticking to pathogens such as virus particles, bacteria, fungal spores, and particulates [52–54]. Through the coordinated beating of ciliated epithelial cells, the mucus-pathogen combination is propelled from the paranasal sinuses and nasal cavity toward the throat, where it is either swallowed or expectorated. This pattern of ciliary beating and mucus propulsion has been heavily studied and is well summarized [55].

Additionally, reactive oxygen species and reactive nitrogen species are produced to limit pathogen invasion. These compounds, the most important of which is nitric oxide (NO), possess intrinsic antibacterial, antifungal, and antiviral effects through the induction of direct DNA and membrane damage [56]. NO is further thought to be particularly important in upper airway defenses due to the high local expression of NO synthase, the enzyme that produces NO, in nasal epithelial cells [57]. These compounds combine with a variety of other antimicrobial compounds, including lysozyme, defensins, lactoferrin, and cathelicidins, to limit the invasion of microbes [58].

Microbes use quorum-sensing molecules, such as acyl-homoserine lactones (AHLs) used by gram-negative organisms, for bacterial cross-talk to determine population density and coordinate virulence through toxin secretion, biofilm formation, and antibiotic resistance [59–62]. Additionally, microbes secrete factors to counteract the innate defenses discussed above. For example, *Pseudomonas aeruginosa*, a known respiratory pathogen, produces pyocyanin and pyoverdine, which are cilia toxins that act to paralyze beating cilia and thus dismantle the mucociliary component of host defenses, affording the invading bacteria a less hostile “landing surface”. Because many lactones are known to be bitter [63], AHLs were

hypothesized to also be bitter, and it has since been shown that AHLs are in fact agonists of T2Rs [44].

Specifically, the gram-negative quorum-sensing molecule N-butyryl-L-homoserine lactone (C4HSL) has been shown to activate T2R38 in cultures derived from PAV/PAV but not AVI/AVI individuals, inducing a genotype-dependent intracellular calcium response that requires transduction of taste signaling components [64]. Further, heterologous expression experiments prove that this process occurs through the direct binding of C4HSL to T2R38 [60,65]. Other T2Rs, notably T2R46 and -14, have also been shown to be activated by bitter sesquiterpene lactones [66]. Murine SCCs, the heavily innervated sensory cells of the nasal epithelium, were the first cells identified to express extraoral taste receptors and are now known to feature both T2Rs and T1Rs [67]. In response to AHLs, SCCs produce the Ca^{2+} response of intracellular taste receptor transduction illustrated in Figure 3 [44].

T2R38: The antimicrobial receptor

The intracellular calcium response provided evidence of the presence of taste receptors in SCCs of the upper respiratory tract [44], and a 2009 publication [68] furthered the idea that taste receptors may additionally be present in ciliated upper airway epithelial cells (Figure 4). This publication identified the presence of T2R bitter taste receptors in human bronchial epithelial cells and found that agonist stimulation induced calcium responses that increase ciliary beat frequency (CBF), a process that was hypothesized to serve the purpose of clearing noxious chemicals [68]. This finding is not surprising in the context that ciliated airway epithelial cells also express toll-like receptors, which play a vital role in the early innate immune response [69].

Our lab utilizes a technique known as the air-liquid interface (ALI) culture that enables analysis of the airway epithelial cells' responses by mimicking the natural polarization of the epithelium [70,71]. This setup mirrors the *in vivo* environment and enables the direct stimulation and evaluation in real time of downstream effects through live cell imaging techniques [47,60].

Both SCCs and ciliated epithelial cells feature taste-receptor-dependent pathways (Figure 4), which regulate innate immune function. First, SCCs have been shown to contain crucial components of the canonical taste receptor signaling pathway (Figures 3, 4) and, when stimulated by bitter compounds including denatonium benzoate, cause an intracellular calcium response that in the mouse triggers acetylcholine (ACh) release and activation of local trigeminal nerve fibers yielding “neurogenic inflammation” [72]. In human SCCs, T2R stimulation leads to the secretion of antimicrobial peptides including β -defensin 1 and 2, which have potent activity against both gram-negative (e.g., *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and gram-positive (e.g., *Staphylococcus epidermidis* and methicillin-resistant *Staphylococcus aureus*) bacteria (Figure 5, left) [47,73]. This paracrine signaling requires propagation of the SCC calcium response to the surrounding ciliated epithelial cells via gap junctions. Notably, SCCs have only recently been identified in humans [73,74] but are not activated by AHLs in contrast to the murine SCCs [46,47,73,74]. This leads us to caution against the direct application of mouse studies to human disease, as

the humans and mice inhabit unique ecological niches with obvious differences in physiology.

Second, stimulation of T2R38 receptors in human ciliated epithelial cells by bitter agonists such as PTC and AHLs induces the production of NO [60]. Nitric oxide is directly bactericidal and additionally, increases CBF to facilitate mechanical expulsion of the pathogen (Figure 5). The above processes occur in a genotype-dependent manner, with those individuals homozygous for the active (PAV/PAV) form of *TAS2R38* exhibiting robust anti-microbial NO responses, while those individuals homozygous for the inactive (AVI/AVI) form of *TAS2R38* lack the NO response [60]. These responses have important clinical ramifications, with AVI/AVI (PTC non-taster) individuals having increased susceptibility to gram-negative upper respiratory infections when compared with PAV/PAV (taster) individuals [60]. The AVI/AVI genotype also serves as an independent risk factor for chronic rhinosinusitis requiring surgical intervention after failed medical therapy [50]. Interestingly, individuals who are heterozygous (AVI/PAV) exhibit significant variability in their sensitivity to bitter compounds, with some acutely sensitive and others needing high concentrations to taste them at all [75]. While one component of this difference is taste papillae density, we have recently shown the concurrent role of allele-specific expression, or variable proportions of mRNA expression of the PAV (active) and AVI (inactive) alleles, in this variability of bitter perception [76].

T1R2+3: Not so sweet

SCCs additionally express the T1R2+3 sweet taste receptor [46,47]. The presence of apical sugars such as glucose and sucrose block the denatonium-induced calcium response in human SCCs described above in a dose-dependent manner [47]. Thus, in the human respiratory epithelium, the T1R2+3 receptor functions as a “rheostat” by adjusting the magnitude of the SCC mediated antimicrobial activity (Figure 5). The glucose and sucrose inhibition is additionally blocked by antagonists of T1R2+3 such as lactisole [47,77] and amiloride [47,78], but not by glucose transport inhibitors such as phloretin and phlorizin [47].

Glucose is present in the airway surface liquid (ASL) due to paracellular leakage and is optimally maintained at 0.5 mM [79]. Given this, there exists a tonic level of T1R-induced inhibition of the T2R SCC antimicrobial response. In the setting of bacterial infection, ASL glucose is decreased due to bacterial consumption, leading to the hypothesis that the T1R pathway exists to attenuate the SCC function in the presence of physiologic ASL glucose, but signal local infection when ASL glucose levels drop to augment the T2R-induced immune response. This is further supported by knowledge that individuals with diabetes mellitus, a disease of chronically impaired glucose homeostasis, exhibit increased ASL glucose [80] and are more likely to experience respiratory infections than are non-diabetic patients [81]. These patients also have significantly less improvement in CRS disease burden after surgical management as measured by the SNOT-22 questionnaire [82].

Individual variability in T1R phenotypes has not been as extensively studied as that of T2R38. However, a recent Canadian study focusing on difficult to treat CRS found allele

frequency differences exceeding 10% for 16 different SNPs of the *TAS1R1* and *TAS1R2* genes [83].

Taste as therapy

Knowledge of the antimicrobial pathways associated with extraoral taste receptors has created significant discussion regarding their potential therapeutic use in managing CRS. As described above, this would involve one of three methods: (1) enhancing the T2R38-induced stimulation of NO production by ciliated epithelial cells, (2) enhancing the T2R-induced secretion of antimicrobial peptides by SCCs, and (3) inhibiting T1R inhibition of the SCCs' T2R response. In the interest of the first two pathways, it has been hypothesized that intranasal application of a T2R agonists such as PTC could mimic the natural sinonasal response to infection and facilitate clearing of locally colonized bacteria in the setting of CRS. This could be delivered in either spray or lavage formulation, similar to the current options for delivery of nasal steroids. Given that the T2R38 antimicrobial pathway is most active in individuals homozygous for the active (PAV) form of T2R38, less active in heterozygous individuals, and inactive in individuals homozygous for the inactive (AVI) form, this method would be preferentially beneficial to patients of the PAV/PAV genotype although patients with this genotype are less likely to suffer from CRS [84].

Because heterozygous individuals have variable responses to bitter agonists, the question then becomes how to identify those with a strong response, as well as how to drive this pathway in individuals with a smaller response. Based on preliminary data indicating that individual bitter sensitivity of heterozygous patients occurs in an allele-specific manner, we hypothesize that these same differences in upper airway immunity may be due to this same mechanism. Should this be the case, a simple taste test assessing for oral T2R38 agonist sensitivity by comparison with normative data could identify individuals who may benefit from local (topical) T2R38 therapy.

In individuals homozygous for the inactive (AVI) allele, a simple taste test as proxy for T2R38 genotype could be used to identify them as non-responders, saving economic resources by avoiding multiple antibiotic courses or even topical T2R38 therapy (should that come to fruition) in a setting where it is unlikely to be effective. These individuals may benefit from early aggressive surgical intervention or alternative taste receptor mediated therapies (see below).

T1R represents an enticing target for therapeutic use. Topical application of sweet receptor antagonists such as lactisole and amiloride have been shown to block the T1R inhibition of T2R mediated SCC antimicrobial defenses [46]. Since the SCC innate pathway is independent of T2R38, PTC non-tasters may benefit from augmenting this pathway in combating upper airway infection. Importantly, this approach directly attacks the immunosuppression resulting from a leaky epithelial barrier, i.e. elevated ASL glucose, and thus by eliminating or reducing the microbial load in the airways with antimicrobial peptide release, the epithelial barrier may be able to recover.

The possibility also exists for the concurrent use of T2R38 agonists and T1R antagonists, though studies have yet to analyze the effects of their simultaneous stimulation. Concomitant

use of both pathways may even induce a synergistic response and represent an effective treatment modality independent of T2R38 phenotype. However, it must be mentioned that excessive NO production or antimicrobial peptide release can have local detrimental effects on the respiratory epithelium [85,86]. Additionally, stimulation of murine SCCs generates localized neurogenic inflammation (see below), which if replicated in humans could limit the utility of SCC stimulation as a therapeutic modality.

It is also interesting to note that in the lower airway, stimulation of T2R receptors by various bitter agonists (quinine, chloroquine, and a number of others) leads to bronchodilation in a calcium-dependent manner. This phenomenon does not exhibit tachyphylaxis, which is a frequent problem encountered with traditional treatment methods for asthma, such as β -agonist therapy [87]. Tachyphylaxis is also a complicating factor of use of upper airway α -agonists (e.g., phenylephrine, oxymetazoline). When administered for more than five days, a condition known as rhinitis medicamentosa can occur, where individuals suffer from rebound nasal congestion and obstruction in the setting of otherwise resolved sinus symptoms (e.g., rhinorrhea, postnasal drip). This is thought to be due to the unmasking of local β -receptor-induced vasodilation, which at baseline is balanced by endogenous α -receptor-dependent vasoconstriction. However, in the setting of prolonged pharmacotherapy, the alpha response wanes and vascular congestion occurs. The preferential use of extraoral taste therapy could circumvent this problem in the upper airway as well.

Neurogenic inflammation

While SCCs represent an important new therapeutic target for the treatment CRS, overstimulation of these cells could possibly activate a pro-inflammatory cascade, which could trigger idiopathic nonallergic rhinitis. Idiopathic nonallergic or vasomotor rhinitis is typified by rhinorrhea, postnasal drip, irritation, and inflammation of the nose in the absence of allergic reaction or infection [88]. While the exact cause of idiopathic rhinitis is unclear, the current animal model of SCC-induced nasal irritation triggers symptoms that are typical of nonallergic rhinitis [72].

Currently, the mouse is the main animal model for studying SCC-induced irritation [67]. As in the human nasal epithelium, in the murine nasal epithelium SCCs are immunoreactive for canonical taste signaling proteins and co-express several T1R and T2R family members [43,46]. Genetic knockouts or pharmacological blockade of these signaling elements—such as α -gustducin, TRPM5, or PLC β 2—prevents SCCs from responding to classically bitter substances and AHLs [45]. In addition to taste signaling proteins, murine SCCs are also immunoreactive for choline acetyltransferase, an enzyme required for the synthesis of the neurotransmitter ACh [72,89,90]. Murine cholinergic SCCs make intimate contact with class C peptidergic polymodal nociceptive nerve fibers of the nose, which are commonly referred to as trigeminal pain fibers [91]. Ultrastructural studies have revealed cytoplasmic vesicles in SCCs positioned for release onto nerve fibers [43], and other studies indicate that these nerve fibers express functional nicotinic ACh receptors (nAChRs) [92,93]. Taken together, these studies provide support for the idea that SCCs are sensory end-organs that release ACh onto trigeminal nerve fibers.

In the mouse, stimulation of nasal SCCs triggers reflexive behaviors associated with trigeminal irritation and several classic symptoms of neurogenic inflammation. SCC activation by traditional bitter receptor agonists or AHLs results in a patterned decrease in respiration rate (i.e., apnea) [45,89], which has long been considered indicative of trigeminal irritation [94,95]. Likewise, SCC stimulation results in local inflammation, including edema and mast cell activation [72]. This local inflammation appears primarily neurogenic, because chemical lesioning of epithelial pain fibers with the capsaicin analogue resiniferatoxin prevents inflammatory symptoms [72]. Additionally, blocking receptors for the pro-inflammatory peptide substance P, which is released from stimulated nerve terminals, also prevents SCC-induced inflammation [72]. Likewise, nAChR blockers prevent SCC stimulation from triggering respiratory rate depression and neurogenic inflammation [72,89]. These data support a model of nasal inflammation where, on stimulation with bacterial metabolites, SCCs release ACh to activate nAChRs on trigeminal pain fibers [72]. The stimulation of pain fibers results in changes to respiratory rate and triggers a pro-inflammatory cascade that recruits the adaptive immune system.

SCC-mediated inflammation stands in direct contrast to the SCC-mediated release of antimicrobial peptides in that the former process requires intact neural innervation [72], whereas the latter is intrinsic to nasal epithelium [47,96]. This observation suggests that SCC stimulation may trigger at least two parallel mechanisms which combat infection. The segregation of these mechanisms is further supported by experiments in which the long-term stimulation of ALI cultures with AHLs did not trigger the secretion of 18 common inflammatory cytokines [60]. As mentioned above, these data warrant caution when we consider the possible use of bitter receptor agonists to treat upper respiratory tract infections. Specifically, even though T2R agonists would trigger the SCC-mediated release of antimicrobial peptides to fight off nascent infection [60], SCC stimulation could also trigger a pro-inflammatory cascade, which can sensitize trigeminal pain fibers to future stimuli, causing symptoms consistent with idiopathic rhinitis [72].

It is also worth considering whether pathological SCC sensitization could cause nonallergic rhinitis. As mentioned before, the symptoms of nonallergic rhinitis are consistent with the result of SCC overstimulation in animal models [72]. In addition to this similarity, many of the chemicals that activate murine SCCs *in vitro* are found in commercial products (e.g., soaps and perfumes) that individuals with nonallergic rhinitis report to be exceedingly noxious [97]. This sensitivity to normally benign odorants could possibly be due to overactive SCCs that release large amounts of ACh onto pain fibers. Conversely, pain fibers that are sensitized would be hyper-responsive to the normal amount of neurotransmitter released by SCCs. This hypothesis is further supported by the observation that anticholinergic agents alleviate some of the symptoms of idiopathic rhinitis [98]. Currently, our ability to determine the exact role that human SCCs play in neurogenic inflammation is limited, as trigeminal pain fibers are missing in traditional ALI cultures of nasal epithelium. Thus, the role that SCCs play in idiopathic rhinitis will remain speculative until new techniques are developed that allow the study of human SCCs in the presence of trigeminal nerve fibers.

Future directions

The role of extraoral taste receptors in upper airway immunity has been well established, with bitter taste receptors enhancing antimicrobial activity and sweet receptors tempering specific components of this response. While T2R receptor polymorphisms have been well characterized, T1R polymorphisms have not been as thoroughly studied. Prior studies began characterizing these allele-frequency differences in the *TAS1R1* and *TAS1R2* genes; however, it would be beneficial to precisely characterize this diversity [83].

Further, given the state of knowledge on the role of T2R38 in upper airway innate immunity, it would be valuable to perform *in vivo* randomized controlled trials of the application of T2R38 agonists in patients with a known diagnosis of CRS. This could also be extended to T1R2+3 antagonists as the knowledge of this receptor expands. Once the independent role of each receptor has been well characterized both *in vitro* and *in vivo*, it would be beneficial to study the effect of T2R38 agonists and T1R2+3 antagonists simultaneously to evaluate their possible synergy.

While we know SCCs play a role in upper respiratory innate immunity, a more thorough understanding of the implications in vasomotor rhinitis would be powerful, and would also influence a second patient population: those with idiopathic nonallergic rhinitis. Thus, the development of ALI cultures with SCCs in the presence of trigeminal nerve fibers or organoid explant models with viable nerve fibers, as well as the identification of the precise T2R SCC ligand(s) would allow for greater therapeutic use.

As previously discussed, gram-negative bacteria have been most strongly implicated in the T2R38 mediated antimicrobial pathway discussed in this review. However, gram-positive bacteria are responsible for a significant portion of CRS and respiratory infections, making it imperative to seek greater understanding of the endogenous pathways involved in defending against gram-positive bacteria [99].

Beyond the presence of extraoral taste receptors in the upper airway, the receptors have also been identified in lower airway, brain, pancreas, gastrointestinal tract, bladder, thyroid, and testes [19,34–39]. The inferred question, therefore, is whether the extraoral expression in these tissues could have a role in local infectious processes. Research on an immune role in the lower airway has shown significant promise, with implications for the treatment of asthma [87]. Additionally, the potential for involvement of other extraoral taste receptors in the upper airway and auxiliary immune pathways should not be overlooked.

Expert commentary

The T2R38 bitter taste receptor represents a prototypical taste receptor that has served as the model for decades of research into the genetics of psychophysical taste perception and dietary preferences, the results of which have led to a more recent understanding of the generalized, extraoral expression of such receptors. Specifically, the expression of the T2R38 as well as other bitter T2Rs and T1R2+3 sweet taste receptors has been shown to play an important and dynamic role in innate immunity of the upper airway and paranasal sinuses. This process occurs through pathways that are uniquely dependent on the genetic

makeup of taste receptors, the bitterome, enabling the potential use of a simple taste test to evaluate genotype and extraoral receptor expression. Thus, the modulation of airway taste receptors presents a putative opportunity for therapeutic intervention in airway diseases.

Five-year view

As health care expenditures in the United States continue to rank highest among international peers, and with the expansion of access to medical care, containment of expenses related to chronic diseases such as CRS will be prioritized to an even greater degree [1]. The data presented here show that specific patient populations preferentially benefit from differential management: T2R38 homozygous tasters respond to medical management, homozygous nontasters often necessitate surgical intervention, and diabetics respond poorly to both modalities. Thus, providers will become obligated to better characterize these patients preoperatively. We anticipate that rapid, point-of-care taste tests will be incorporated into the health care providers' armamentarium, aiding in optimizing patient selection for various therapeutic modalities. A better understanding of individual variance in taste-receptor-dependent upper airway immunity and the implementation of local modulation will thus lead to improvements in patient quality of life, decreased health care expenditures, and greater antimicrobial stewardship.

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Reference annotations

* Of interest

** Of considerable interest

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Key issues

- Chronic rhinosinusitis (CRS) represents a spectrum of chronic disease with significant morbidity and causes a greater decrease in quality of life than does chronic obstructive pulmonary disease, congestive heart failure, or angina.
- The T2R38 bitter taste receptor exists primarily in one of two polymorphisms: the active (PAV) form senses bitter compounds and exhibits antimicrobial response in the nasal epithelium, and the inactive (AVI) form does not. The response of individuals heterozygous for the receptor (AVI/PAV) is variable.
- The majority of bitter taste receptors contain common polymorphisms with possible innate immune functional consequences
- The role of extraoral taste receptors has been suggested in the pathogenesis of CRS, with studies linking T2R38 genotype and susceptibility to sinonasal infections, and CRS requiring surgical management.
- Two primary cell types contribute to extraoral taste-receptor-mediated antimicrobial responses: (1) Nasal solitary chemosensory cells (SCCs) express taste receptors. Stimulation of the SCC bitter receptor(s) induces secretion of antimicrobial peptides, while stimulation of sweet receptor T1R2+3 blocks the T2R antimicrobial peptide release. (2) Ciliated epithelial cells express T2R38, whose stimulation induces NO production, with direct bactericidal activity, and simultaneous increase in ciliary beat frequency and mucociliary clearance.
- Stimulation of the T2R38 and antagonism of the T1R2+3 sweet taste receptors represents a putative therapeutic target in the management of CRS.
- Taste receptor pharmacotherapy represents an alternative to local alpha agonist therapy with a mechanism of action that bypasses the tachyphylaxis and rhinitis medicamentosa plaguing current therapeutics.
- SCCs may contribute to the pathophysiology of nonallergic, vasomotor rhinitis. This may secondarily create a limitation to their therapeutic use in the T2R-dependent modulation of upper airway disease.
- Stratification of patients by T2R38 genotype could become the standard of care in managing patients with refractory CRS.

Figure 1. Umami, sweet, and bitter receptors

The receptors for umami (savory), sweet, and bitter are dimers of two G protein-coupled receptors. The umami and sweet receptors are heterodimers formed by T1R3 plus T1R1 or T1R2, respectively. In contrast to those receptors, there are 25 unique human bitter taste receptor genes or T2Rs, which function as monomers, or homodimers and heterodimers although the function of dimerization is uncertain.

Figure 2. Genomic organization and diversity of the TAS2R gene family

TAS2R genes, known collectively as the “bitterome,” are found on chromosomes 5, 7, and 12. Single nucleotide polymorphisms (SNPs) within the TAS2R loci exhibit significant individual variability, and are present as homozygous for the more common allele (black), homozygous for the less common allele (light grey), or heterozygous (dark grey). SNPs unidentifiable for a subject are indicated in white. Each column represents a unique SNP; data from individual patients are organized by row.

Figure 3. Canonical type II taste receptor cell signaling

On stimulation with taste receptor agonists, the G-protein subunits Gα-gustducin and Gβγ dissociate from the G-protein coupled receptor. Subsequently, Gα-gustducin activates phosphodiesterase (PDE), which depletes cyclic adenosine monophosphate (cAMP) by converting it into adenosine monophosphate (AMP). Under normal conditions, the presence of cAMP activates protein kinase A (PKA), which inhibits several elements of the parallel Gβγ-mediated pathway. When released, Gβγ triggers phospholipase Cβ2 (PLCβ2), which produces inositol 1,4,5-trisphosphate (IP₃) from the phospholipid phosphatidylinositol 4,5-bisphosphate (PIP₂). Once produced, IP₃ activates the type 3 IP₃ receptor (IP₃R3) on the endoplasmic reticulum, releasing calcium stores. Increased intracellular calcium opens the nonspecific cation channel TRPM5 (transient receptor potential cation channel subfamily M member 5), further depolarizing the cell and resulting in transmitter release.

Figure 4. Ciliated cells and solitary chemosensory cells (SCCs) express taste-related proteins
A: Explant of human nasal epithelium immunoreactive for bitter taste receptor T2R38 and the cilium marker β -tubulin IV. **B:** Air-liquid interface culture of nasal epithelium from a transgenic mouse co-expressing green fluorescent protein (GFP) with TRPM5 allows for the identification of SCCs. SCCs are also immunoreactive for the taste transduction protein α -gustducin.

Figure 5. Model of taste receptor signaling in the respiratory epithelium

Under healthy conditions, glucose present in the airway activates the sweet receptor dimer T1R2+3, silencing solitary chemosensory cells (SCCs). In the case of bacterial infection, this glucose is metabolized by the growing bacteria, which also release bitter compounds including acylhomoserine lactones (AHLs). Bitter bacterial metabolites activate T2Rs on SCCs and trigger an intracellular signaling cascade similar to canonical taste receptor cell signaling, including phospholipase C β 2 (PLC β 2). **In the mouse** (dashed circle), this signaling cascade results in the release of acetylcholine (ACh) and activation of free trigeminal nerve fibers triggering neurogenic inflammation, mast cell degranulation, and an apneic reflex. **In human primary cell cultures**, activation of SCCs triggers a calcium wave via gap junctions to the surrounding epithelial cells stimulating the release of antimicrobial peptides from these cells, which is presumed to halt the growing bacterial infection. Furthermore, in humans, AHLs stimulate T2R38 on ciliated cells, which results in the

activation of nitric oxide synthase (NOS) and the production of nitric oxide (NO). The production of NO increases ciliary beat frequency and thus accelerates mucociliary clearance. Additionally, NO diffuses into the mucus and airway where it is directly bactericidal.

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