

# Taste receptors in respiratory innate immunity

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## Abstract

Over the past several years, taste receptors have emerged as key players in the regulation of innate defenses in the mammalian respiratory tract. Several cell types in the airway, including ciliated epithelial cells, solitary chemosensory cells, and bronchial smooth muscle cells all display chemoresponsive properties that utilize taste receptors. A variety of bitter products secreted by microbes are detected with resultant downstream inflammation, increased mucous clearance, antimicrobial peptide secretion, and direct bacterial killing. Genetic variation of bitter taste receptors also appears to play a role in susceptibility to infection in respiratory disease states, including chronic rhinosinusitis. Ongoing taste receptor research may yield new therapeutics that harness innate defenses in the respiratory tract and offer alternatives to antibiotic treatment.

## Introduction

Canonically, taste has been thought of as an adaptive sense for organisms that feed on matter in the environment: food that nourishes is considered to have a pleasant taste, while poisons and inedible material tend to be far less palatable. Specifically, bitter taste receptors are often tuned to respond to toxic chemicals or products that compromise digestive health. Over the past several years, a growing body of literature supports a broader role for taste receptors throughout the body, with functions extending far beyond the sensory capacity of the tongue [1-6]. Both bitter and sweet taste receptors are expressed in the airway, where they appear to play several important roles in innate defenses [7, 8].

## Taste receptor mechanisms

Bitter and sweet taste receptors are G-Protein Coupled Receptors (GPCR's) that were first identified in taste bud type II cells [9, 10]. Those from Taste Receptor Family 1 subtype 2 and 3 (TAS1R2/TAS1R3) respond to sugars [5, 11] such as glucose, fructose, and sucrose [12]. Bitter taste receptors, from Taste Receptor Family 2 (TAS2R's), have a much wider diversity of subtypes, with each tuned to specific bitter compounds [13]. These compounds include the plant sesquiterpene lactones, strychnine, and denatonium [14]. Humans are known to have at least 25 TAS2R subtypes [11, 15], and there are many others that have been discovered in mammalian species [16]. The type II taste cells of the tongue most often express only one taste modality, but some cells do express multiple unique receptors [17].

The pre-synaptic mechanisms for taste receptor stimulation and signal transduction are relatively conserved in the tongue and the airway. Briefly, a bitter or sweet ligand binds its respective GPCR, triggering activation of phospholipase C isoform  $\beta 2$  (PLCB2). PLC $\beta 2$  then causes inositol 1,4,5-trisphosphate (IP3) production, activating the IP3 receptor on the endoplasmic reticulum (ER) with release of calcium (Ca<sup>2+</sup>) [18]. While this process occurs, the GPCR stimulation also activates phosphodiesterases (PDE's) that cause the reduction of cAMP levels and corresponding protein kinase A (PKA) activity.

PKA acts as an inhibitor of the type III IP3R through phosphorylation, so removal of this inhibitory pathway further enhances calcium release from the ER [19]. The released calcium activates the TRPM5 channel [20], which depolarizes the cell membrane, activates voltage-gated sodium (Na<sup>+</sup>) channels generating an action potential that causes ATP release through the CALHM1 ion channel [5, 19, 21, 22]. In the tongue, this ATP release activates purinergic receptors on presynaptic taste cells and sensory fibers, transmitting the sensation of taste to the central nervous system [5, 22, 23].

### **Taste receptors and airway immunity**

GPCR taste receptors are expressed in a number of organ systems, including the brain, pancreas, testicles, bladder, respiratory and GI tracts [1-4, 24] The present review will focus on taste receptors expressed in the airways.

#### *Overview of innate airway immunity*

Several respiratory immune mechanisms work in concert to achieve a relatively low microbial biomass in the lower airway, in spite of the vast number of bacteria, fungi, and viruses that are inhaled into the upper respiratory tract with each breath. During infection or debris inhalation, ciliary beat frequency (CBF) increases to speed up mucociliary clearance (MCC) [25]. In addition to transporting the mucus to the pharynx where it is cleared by swallowing, innate immune products are disseminated on the airway surface [26]. These immune products include direct anti-microbial compounds such as defensins, lactoferrin, cathelicidins, and lysozyme, in addition to reactive oxygen species (ROS) and nitric oxide (NO) that also display potent antimicrobial activity [27].

In order to activate all of these defense mechanisms, recognition of foreign organisms or toxins both immediately and throughout bacterial colonization is paramount. Toll-like receptors (TLR's) are expressed by airway epithelial cells and recognize pathogen-associated molecular patterns (PAMP's), which are bacterial cell wall components or bacterial products. TLR signaling and downstream immune effect takes up to 12 hours and works through gene expression, creating a sustained immune response [28]. However, a portion of antimicrobial peptide secretion and changes in MCC in response to pathogens occurs almost immediately [29], suggesting the existence of a molecular pathway that rapidly detects foreign compounds and effects timely responses. Bitter taste receptors may provide a missing link in this pathway as initiators of these rapid defenses.

#### *Airway bitter taste receptors*

A wide variety of bitter taste receptors are expressed in various parts of both the human and rodent airway [8, 29-32]. While some bitter taste receptors in the airway are upstream of a nervous system signaling cascade [33], others act in a cell-autonomous fashion without any nervous innervation with the bitter products detected an entirely local phenomenon. In 2009, bronchial epithelial cells were shown to have Ca<sup>2+</sup> increases following bitter compound stimulation, thus increasing CBF thereby accelerating clearance of the noxious compound [32]. These TAS2R receptors are located on the motile cilia themselves. In response to phenylthiocarbamide (PTC) stimulation of sinonasal epithelial cell TAS2R's, an increase in NO production is also observed, with potent bactericidal consequences [8]. NO diffuses rapidly into bacteria such as *P. aeruginosa*, where it causes cellular destruction and death [34]. However, recent *in vitro* experiments demonstrated differential bactericidal activity of NO depending on the specific organism in question [35]. In addition to this direct antimicrobial activity, NO

acts as a second messenger to activate protein kinase G (PKG) and guanylyl cyclase to phosphorylate proteins within the cilia and speed up CBF [36]. Other experiments have further investigated this NO pathway and found that both the TRPM5 channel and PLCB2, two of the components in canonical taste transduction, are necessary for NO production but not the canonical taste G-protein gustducin [8].

Lactones are bitter chemicals that can stimulate TAS2R's in the airway [8, 37], and acyl-homoserine lactones (AHL's) are a subclass of lactones that are produced by many gram-negative bacteria [38, 39]. AHL's serve as biofilm "quorum-sensing molecules" for the bacteria. Once a sufficient concentration of AHL's are produced in a localized environment, bacteria will form a biofilm, which confers increased protection for the bacteria from host immune defenses [40]. It is proposed that detection of these AHL's before bacteria reach a density adequate for biofilm formation is an adaptive mechanism, allowing for an increased immune response before microbial protection occurs in the biofilm formation [7].

### *Solitary chemosensory cells*

Ciliated epithelial cells are not the only cells to express bitter taste receptors in the airway. Over a decade ago, a class of cells that is sparsely scattered in rodent respiratory epithelium was shown to be immunoreactive with alpha-gustducin (a component of taste signaling) [41]. These cells were named "solitary chemosensory cells" (SCC's), and they share many similarities with cells found in the taste buds of the tongue [30]. Approximately one out of every hundred cells in the sinonasal cavity is a SCC [33]. The function of these airway taste-like cells were explored further, and it was discovered that they express sweet and bitter taste receptors [29, 42], and in the mouse capable of responding to AHL's and other bitter agonists [7, 43, 44]. These murine SCC's show intracellular calcium responses in the presence of AHL's [33], but they do not appear to activate downstream NO production. Instead, when mouse sinonasal SCC's are stimulated with AHL's or denatonium, the calcium response results in acetylcholine (ACh) release that stimulates trigeminal nerve peptidergic nociceptors, with downstream effects of breath holding and inflammatory mediator release [7, 33, 43]. The inflammatory response is intuitively antimicrobial, while the breath holding response may also represent an adaptive reflex to limit toxin or organism aspiration in the host.

SCC's have been identified in human upper airway tissue as well [29, 45], along with additional physiological function beyond what has been elucidated in the rodent system. TAS1R1 and 2, and TAS2R4, 10, and 47 are all expressed on SCC's in the human nasal cavity [31, 45]. Denatonium, a bitter compound that shows activity in mouse SCC signaling [30], also stimulates a Ca<sup>2+</sup> response in human SCC's that spreads to neighboring cells via gap junctions [31]. Just as in the NO response seen in ciliated cells, the calcium signaling requires canonical taste signaling pathways, including gustducin, PLCβ2, the IP3 receptor, and TRPM5 [31]. Gap junction spread of the signal causes immediate release of antimicrobial peptides (AMP's) from the adjacent ciliated cells [29]. These AMP's include beta defensin 1 (DEFB1) and beta defensin 2 (DEFB2), and the secreted products have potent activity in killing of gram-positive and gram-negative organisms [46], including methicillin-resistant *S. aureus* and *P. aeruginosa*. This rapid secretion of antimicrobial products contrasts directly with the TLR mechanism of AMP messenger RNA upregulation, causing a sustained response that does not appear until several hours after bacterial stimulation [28]. Pre-formed stores of AMP's are released in the TAS2R response, rather than *de-novo* synthesis [46].

## T2R38

TAS2R's are very genetically diverse, a phenomenon that helps to explain the wide variety of taste preference both within and between cultures [47, 48]. Many individuals find bitter foods such coffee or herbs to be detestable, while others do not have an aversive response. This genetic variation of TAS2R's is not exclusively found in the tongue; TAS2R receptor variation in the airway appears to also play a key role in respiratory defense. TAS2R38, a receptor that is localized to motile cilia in humans, responds to at least three AHL's produced by *P. aeruginosa*, N-butyl-L-homoserine lactone, N-hexanoyl-L-homoserine lactone and N-3-oxo-dodecanoyl-L-homoserine lactone [8]. Additionally, PTC and propylthiouricil (PROP) are bitter compounds that also agonize TAS2R38 in a similar fashion [49]. When TAS2R38 in nasal cells is stimulated by AHL's, PTC, or PROP, NO is produced to speed up MCC and directly kill pathogens in the human upper airway [31]. However, the genetic locus for TAS2R38, has three common polymorphisms that tend to segregate together, yielding a functional receptor (PAV) and a non-functional receptor (AVI) [48]. Individuals who have an AVI/AVI genotype do not taste the bitter compounds PTC or PROP [50], and epithelial cells from these patients grown at an air-liquid interface (ALI) show significantly lower NO production in response to AHL's when compared to epithelial cells from a PAV/PAV individual. The consequent reductions in MCC and bacterial killing are also significant in the AVI/AVI group [51].

The implications of these differences are broad. Patients with chronic rhinosinusitis (CRS) have pathological mucociliary stasis, which harbors bacteria and allows infection to perpetuate [52]. This creates a very stagnant and favorable environment for bacteria to proliferate, and for bacterial toxins to continually cause destruction of both cells and cilia [53]. It was previously shown that sinonasal epithelial explants from patients with CRS show an attenuated response to a variety of compounds that stimulate CBF in normal controls [54]. Additionally, further studies demonstrated that there were differences in NO levels in patients with CRS or other airway diseases [55]. However, a review of the nasal NO literature was unable to demonstrate any trends in rhinopathologies with regard to nasal NO measurements [56]. The pathophysiology behind this disparity is not entirely clear, but the TAS2R38 genotype (or not controlling for TAS2R38 genotype) may help to explain the conflicted literature. Individuals who have the PAV/PAV genotype are less likely to need surgical intervention for their CRS symptoms than those with the AVI/AVI genotype [50, 57]. PAV/PAV patients are additionally less prone to developing gram-negative infection, such as that of *P. aeruginosa* [50, 57, 58]. In light of this data, it appears that variation in bitter taste receptor function in humans has a phenotypic effect on upper respiratory disease. In the near future, bitter taste testing with PTC or PROP could potentially help to stratify CRS patients who are more likely to benefit from standard sinus procedures as well as those who should receive alternative or more aggressive management [8]. Further, the bitter compounds themselves could even serve as therapeutic agents, in speeding up MCC and strengthening host responses to counter bacterial proliferation in CRS [59].

**Table 1:** Overview of bitter and sweet receptors and their functions in airway immune defense.

Cell Type	Receptor(s) Expressed	Animal	Function
Solitary Chemosensory Cells (sinuses)	TAS2R bitter receptors	Mouse	Breath holding, inflammation
		Human	Antimicrobial peptide release
Ciliated cells (sinuses)	TAS1R sweet receptors	Mouse	Silence TAS2R stimulation
		Human	Unknown
Ciliated cells (bronchi)	TAS2R38	Human	NO production (MCC stimulation and direct killing)
Brush cells (trachea)	TAS2R bitter receptors	Human	MCC stimulation
Smooth muscle cells (bronchi)	TAS2R bitter receptors	Mouse	Breath holding
		Human	Bronchodilation

Sources: [7, 8, 30-32, 43, 45, 51, 60-64]

### Sweet taste receptors

The TAS1R receptors (dimer of isoform 2 and 3) detect sweet compounds and are also found in the respiratory mucosa [30]. They have been isolated in the human vomeronasal duct [30] as well as in SCC's [29]. In the sinuses, the sweet receptors respond to concentrations of glucose and other sugars that are far lower than those detected on the tongue [65]. Normally, individuals have a glucose concentration of approximately 0.5 mM in the airway surface liquid (ASL), and there is a constant leak and reuptake of glucose from the serum that maintains this constant concentration [31]. The T1R2/3 sweet receptors are tonically activated by this low level of glucose, and appear to function in an antagonistic role to that of the bitter taste receptors. Depletion of ASL glucose is a harbinger of bacterial infection, as the bacteria consume the sugar rapidly. It is hypothesized that this reduction in glucose deactivates the sweet receptors, which then release their inhibition on the action of the TAS2R receptors to bitter compounds [31]. While low-level colonization by bacteria is expected in the sinonasal tract, any perturbation in this homeostasis towards glucose depletion (i.e., more than colonization) causes a balance in favor of TAS2R activation with subsequent mobilization of local defenses against the pathogen, resulting in decreased microbial numbers and restoration of physiologic airway surface glucose concentrations. Paradoxically, a recent study correlated in vitro SCC hyper-activation to disease recurrence for patients with chronic rhinosinustitis [66].

This hypothesis has been supported by several experiments. The addition of glucose and sucrose (both TAS1R2/3 agonists) to the ASL of an ALI culture blocked the Ca<sup>2+</sup> response of bitter taste receptors to denatonium, while mice that did not express these sweet receptors [67] showed a normal response to the compound [31]. Antagonists of the TAS1R2/3 receptors, such as lactisole [68] and amiloride [31], also could release the inhibition of the denatonium response. D-amino acids produced by bacteria in the airway also could activate TAS1R2/3 sinonasal taste receptors [69]. Work by Lee and colleagues demonstrated that *S. aureus* produced at least two TAS1R2/3-activating D-amino acids, and these D-amino acids could suppress sinonasal SCC innate immune responses with resultant decreased secretion of antimicrobial peptides. These D-amino acids may be produced by the bacteria for protection from host innate defenses and may allow for increased colonization and potential opportunistic infection. Just as is the case with bitter receptors, there is genetic variation in TAS1R genes that manifests as individual preference in sweet taste [70]. While no single locus has yet been identified, there are allele variations among the TAS1R genes that show frequency differences of >10% in 16 loci between patients with CRS and controls [58]. TAS1R2/3 antagonists such as lactisole

may prove useful in the future in augmentation of host airway bitter taste receptor responses.

#### *Additional functions of taste receptors in the airway*

The previous experiments discussed focused on SCC's and ciliated cells that populate the upper airway, and SCC cells are unique to that location of the respiratory tract. Bronchial tissue, which contains an abundance of smooth muscle cells, do not demonstrate SCC responses or secretion of AMP's following stimulation [31]. However, the smooth muscle cells do express several TAS2R's, and activation of these receptors causes bronchodilation [30, 51]. This phenomenon potentially occurs due to an increase in  $Ca^{2+}$  that modifies potassium currents within the muscle cells that causes them to become hyperpolarized and relax [63]. These cells lack innervation, so this response is similar to that of the NO production within ciliated cells, in that it is a local defense. Interestingly, asthmatics have an upregulation in TAS2R gene expression [71].

Allele expression studies in patients with CRS showed that TAS2R38 is not the only genetic determinant of disease severity. Several other loci, such as that of TAS2R14 and TAS2R49 show an allele frequency difference of >10% between CRS patients and controls [58]. It will be important for future research to determine the full expression pattern of taste receptors throughout the length of the respiratory tract, as well as explore the full complement of bitter products that are secreted by organisms.

#### **Conclusions**

Airway taste receptors play an important role in innate respiratory defense, and they function in regulating inflammation and antimicrobial activity within the respiratory tract. These responses are quick in onset and are complementary to traditional antimicrobial pathways, such as those involving TLR's. Dysfunction or genetic variation of bitter or sweet taste receptors appears to play a key role in respiratory disease, including CRS and increased susceptibility to infection in diabetes. Conventional management of respiratory diseases often involves antibiotics, but strengthening endogenous defense mechanisms may be possible by using TAS1R and TAS2R receptors as novel therapeutic targets.

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