Bitter taste receptors in the treatment of asthma: Opportunities and challenges

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Asthma is a chronic respiratory disease of high prevalence worldwide. Exaggerated airway smooth muscle (ASM) contraction, inflammation and remodeling of the airways, and airway hypersensitivity are key features that result in lumen narrowing and airflow obstruction, which cause wheezing, coughing, and shortness of breath. Most subjects with asthma respond adequately to bronchodilators and anti-inflammatory medicines. However, for some, in particular those with severe asthma, unmet therapeutic need persists.

Members of the G protein–coupled receptor (GPCR) family regulate both ASM contraction and relaxation and constitute targets for asthma therapy. Bitter taste receptors (TAS2R), the third largest GPCR subfamily, are potential new candidates to treat obstructive lung diseases.

EXTRAORAL EXPRESSION AND FUNCTION OF TAS2R

TAS2R are evolutionary conserved proteins in animals that presumably form part of a survival mechanism of chemoperception to sense and avoid potentially harmful food sources. Functional expression studies show these receptors are activated by synthetic as well as natural bitter substances.[1] The human genome contains 25 functional, different, relatively small and intronless *TAS2R* genes expressed on the taste buds of the tongue and palate, as well as 8 pseudogenes. It is thought that the large number of bitter taste receptor genes and numerous allelic variants identified might equip humans and other species to better improve their adaptation to the environment by enabling the detection of estimated thousands of structurally diverse bitter tastants.

The relationship of bitter compounds with bitter receptors is complex. Although there is some specificity, a marked dual promiscuity exists where a single receptor subtype can recognize multiple unrelated bitter substances, and one bitter substance can stimulate more than 1 receptor.[1] Moreover, some compounds including cyclamate, the artificial sweetener with bitter aftertaste, can act as both agonists and antagonists of TAS2R.

TAS2R also are found in the gut, brain, heart, vasculature, skin, breast, kidney, immune and genitourinary systems, thyroid, bone marrow, and the skeletal and smooth muscles where they mediate additional physiological functions apart from bitter taste perception. For example, stimulation of TAS2R in the gut regulates gastrointestinal motility, releases anorexigenic peptides, and can invoke vomiting, all of which theoretically limit further ingestion of bitter toxicants when detection at the first barrier, the tongue, fails. Activation of TAS2R in the nasal and tracheal epithelium can promote an innate immune response against inhaled irritants and bacterial products with subsequent secretion of antimicrobial substances, increased cilia beat frequency, and production of nitric oxide. Genetic polymorphism within the *TAS2R38* gene is implicated in the susceptibility, severity, and outcomes following sinus surgery of subjects with chronic rhinosinusitis.[2] Stimulation of TAS2R in ASM with chloroquine or quinine induces relaxation that is slightly more efficacious than that elicited with isoproterenol, a β-agonist. This makes TAS2R activators an attractive new class of potential direct bronchodilators.

TAS2R FUNCTION IN ASM

β2 agonists, muscarinic acetylcholine receptor antagonists, corticosteroids, and xanthines are used to treat various obstructive airway diseases. However, they are not always effective, especially in severe cases, and their prolonged use raises both efficacy and safety concerns due to adverse effects, for example, tachyphylaxis. Although TAS2R are expressed at low levels, significant physiological effects are achieved because GPCRs use a powerful intracellular amplification cascade for signaling. The inhibitory effect of TAS2R on smooth muscle contraction is rapid and reversible and, importantly, is mostly independent of disease and inflammatory state,[3] although *in vitro* pretreatment of ASM cells with the TAS2R agonist quinine causes some degree of receptor desensitization and a reduction in quinine-promoted relaxation. Moreover, because the relaxing effect of TAS2R agonists is not impaired by β2-adrenergic receptor (AR) desensitization, bitter tastants could be useful as an add-on medication or as a replacement for subjects unresponsive to β-agonists.

The TAS2R subtypes 10, 14, and 31 are the most abundant among those expressed in human ASM (Table I).[3-8] The β2-AR signaling cascade leading to bronchodilation is known, but some controversy remains about the signaling downstream of TAS2R in ASM. Various mechanisms of ASM relaxation distinct from the mechanism that operates in the taste buds of the tongue are proposed. Deshpande et al[4] describe relaxation of cultured human ASM cells that occurs, paradoxically, with an increase in Ca^{2+} signaling. The α and βγ subunits of the G protein (gustucin

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TABLE I. Bitter taste receptor research pathways and their opportunities and difficulties

Area of research	Opportunities and difficulties
Role of TAS2R subtypes in human ASM*	Elucidation of the mode of regulation and relative contribution in driving bronchodilation and antiproliferative action Determine the subtype(s) most relevant to asthma pathogenesis and potential antiasthma therapy
TAS2R desensitization in ASM	Determination of occurrence, extent, and molecular mechanism
Molecular mechanisms to inhibit ASM contraction Role of calcium	Promotion of bronchodilation within the context of airway inflammation and obstructive lung disease
Improved preclinical model systems Development of antagonists/inhibitors	Link between biochemical events in cells, tissues, and animals to clinically relevant physiological responses in humans
Drug discovery	High-throughput screening of small-molecule libraries and computer modeling to identify and develop more potent agonists Development of palatable compounds Bitter compounds can be agonistic to one TAS2R but antagonistic to another TAS2R
Clinical studies	Trial design considering genetic variability, disease heterogeneity, concurrent use of other medications
Repurposing approved medications	Understanding of the receptor biology and signaling pathways Establish optimal dose and interpatient differences

*TAS2R10 is activated by chloroquine,[3-7] denatonium,[4,6] and quinine[3,4,7]; TAS2R14 is activated by diphenhydramine and flufenamic acid[3,7] and by quinine and saccharine[3,4,7]; TAS2R31 is activated by quinine[3,4,7]; TAS2R46 is activated by absinthin.[8]

or G_i) dissociate upon receptor activation after ligand binding. The βγ dimer activates phospholipase C (PLC) β, causing a release of Ca^{2+} from inositol trisphosphate (IP3)-sensitive Ca^{2+} stores. Localized elevation of calcium and opening of large-conductance Ca^{2+}-activated potassium (BK-Ca^{2+}) channels results in hyperpolarization of the cell membrane and relaxation.[4] Thus, this mechanism is Gβγ-, PLCβ-, and IP3 receptor (IP3R)-dependent. On the contrary, studies by Zhang et al[5] indicate that TAS2R agonists oppose contraction of freshly isolated mouse ASM in a Gβγ-dependent but PLCβ- and IP3R-independent manner. Relaxation in this system occurs through inhibition of L-type voltage-dependent Ca^{2+} channels that block extracellular calcium influx into the cell. Pulkkinen et al[9] showed that BK-Ca^{2+} channels are required for denatonium- but not chloroquine-induced relaxation of guinea pig tracheas. Tan and Sanderson[6] used mouse lung slices, a less reductionist and more integrative system, to report a mechanism of TAS2R10-mediated bronchodilation of small airways that does not stimulate Ca^{2+} signaling, is independent of Gβγ, and inhibits IP3R, Ca^{2+} oscillations, and Ca^{2+} sensitivity induced by bronchoconstrictors. Finally, Talmon et al[8] showed that absinthin activates TAS2R46, a minor bitter taste receptor present in ASM, and reduces histamine-induced cytosolic Ca^{2+} elevation through a rapid and positive modulation of mitochondrial Ca^{2+} uptake. This effect is sensitive to mitochondria uncoupling, actin cytoskeletal disruption, inhibition of Epac (exchange factor directly activated by cAMP), and not evident when using carbachol or bradykinin-exposed smooth muscle cells. The bronchodilator salbutamol, which activates the β2-AR, also increases histamine-induced Ca^{2+} uptake into the mitochondria, a proposed novel pathway that mediates the effect of β2-agonists.

Regardless of the mechanism of action, whether it is agonist-driven or species- or system-specific, activation of TAS2R by quinine, denatonium, and chloroquine efficaciously relaxes precontracted airways with a relatively low potency (high μM to mM range) in the studies cited above (Table I). The effect is observed with ASM prestimulated by exposure to agonists of various procontractile GPCRs (pcGPCR) including the muscarinic, serotonin, histamine, and leukotriene receptors.

We discovered a new physiologically relevant TAS2R function that opposes human ASM contraction evoked by simultaneous, rather than prior, stimulation of bronchoconstrictors with bitterants.[7] This novel inhibiting effect occurs at low concentrations of TAS2R agonists that are incapable of eliciting Ca^{2+} mobilization (IC_{50} in the low μM range), is associated with a decrease in cell membrane depolarization, and is highly selective because it is contingent on the pcGPCR being activated. This selectivity is not apparent in the canonical TAS2R pathways and might rely on the formation of specific associations between certain TAS2R and pcGPCRs. Activation of the canonical[3] and the novel pathways seems to be intact in ASM derived from patients with asthma.

TAS2R AS TARGETS FOR ASTHMA TREATMENT

The ASM-relaxing effect of activated TAS2R is thought to be protective. Other beneficial outcomes of activated bitter taste receptors are their reported anti-inflammatory, antiproliferative, and antifibrotic properties. These effects could have an impact on airway remodeling, hyperplastic growth, and inflammation associated with asthma (Fig 1). Furthermore, an elevation of TAS2R mRNA expression occurs in leukocytes of children with severe asthma versus healthy controls as well as inhibition of proinflammatory cytokines secretion from leukocytes of adult patients with asthma and inhibition of IgE-mediated release of histamine from mast cells (Fig 1). The fact that some TAS2R subtypes are functional in organs and cells not in contact with the external environment raises intriguing questions about the identity of their physiological ligands. Although certain substances from resident bacteria can stimulate few TAS2R, to our knowledge, no endogenous ligands of these receptors are described, which may be related to what is anticipated of a "taste" receptor.

Important progress has been made in understanding bitter taste receptor signaling and function in the airways during the last decade. Yet, several factors, including poor expression of recombinant receptors at the cell membrane using heterologous systems, the availability of a handful specific TAS2R antagonists,

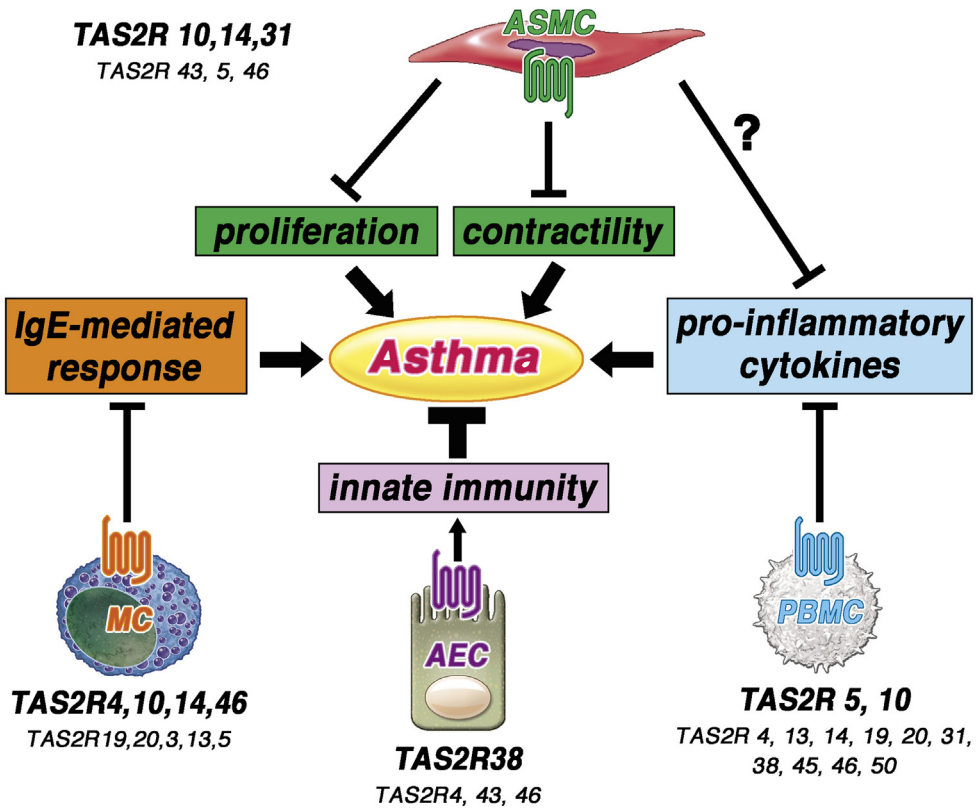

FIG 1. Bitter taste receptors and asthma. Stimulation of TAS2R in ASM cell inhibits cell proliferation and cell contractility, which lead to remodeling and hyperresponsiveness. TAS2R agonists inhibit IgE-dependent mast cell release of inflammatory mediators. Activated TAS2R38 in the airway epithelial ciliated and solitary chemosensory cells promotes innate immunity to protect against inhaled insults such as allergens and microbes. Activated TAS2R in PBMCs prevent proinflammatory mediator release, an effect not determined yet for stimulated receptors in ASM. Major and minor TAS2R subtypes expressed in each cell type are in big and small font, respectively.

the lack of a crystal structure for any bitter taste receptor, and the difficulty in assigning mouse orthologs to the human genes that could enlighten functional discoveries are serious limitations to progress. Furthermore, the vast majority of the bitter taste agonists used in preclinical studies are not ideal candidates for human use because of the high concentration needed to fully relax precontracted ASM. Another hindrance for the clinical application of bitterants, as in the case for chloroquine, the antimalaria drug that relaxes human ASM by stimulating TAS2R10, is the degree to which these bitter tastants can be unpleasant, which makes it difficult for such medications to be inhaled or given orally. Nevertheless, it is hoped that drug discovery efforts could develop new activators of TAS2R suitable to treat human disease because some bitter compounds can exhibit high pharmacological potency, at least *in vitro*.

DRUG DISCOVERY OF TAS2R LIGANDS AND FUTURE INVESTIGATIONS

Computationally and molecular modeling approaches developed in the last 5 years can predict the bitterness and toxicity of chemicals, difficult tasks to perform in humans and animals. The database BitterDB lists more than 600 molecules that taste bitter to humans. Among them, almost 10% are medications and about 70% are predicted to be drug-like, a concept that considers oral bioavailability, metabolism, clearance, toxicity, and *in vitro* pharmacology of candidate medications. Compounds in BitterDB include ions, peptides, glycosides, quinolones, phenols, alkaloids, flavonoids, and terpenoids. This chemical and physicochemical richness creates a recognition puzzle toward a clear understanding of structure- and mechanism-based studies. Bitterness-toxicity relationship studies show that about 60% of bitter compounds are toxic. Moreover, their acute oral LD_{50} value distribution (LD_{50} is the median lethal dose, the amount sufficient to kill 50% of the animals tested during a specified duration) suggests that most bitterants are not fatal or even very toxic to rats and suggests that when applied to humans, unrealistically high oral consumption (above 21 g for a 70-kg person during 24 h) is needed to induce harmful effects.

Many modern medications, including some prescribed antibiotics, are bitter and could potentially exhibit other beneficial effects through the activation of TAS2R expressed in various tissues of the body. This prospect may have important implications for medical practice and drug development as it opens the possibility of both repurposing these pharmaceuticals for other indications and recommending characterization of new drugs for their potential efficacy as TAS2R ligands. For example, azithromycin, a macrolide with anti-inflammatory properties, is gaining more interest for asthma treatment. Azithromycin mobilizes intracellular Ca^{2+} through activation of TAS2R4. Flufenamic

acid, an anti-inflammatory and analgesic, and diphenhydramine, an antihistamine, both activate TAS2R14 and thereby induce human ASM relaxation and bronchodilation.[7]

Preclinical investigations of bitter taste receptors remain an area of great interest (Table I) and in need of appropriate animal models to carry out cross-species comparative analyses. Deletion of individual *Tas2r* genes in mice might have limited value because of the undesirable overlapping ligand specificities and the broad tuning of this receptor family. Generation of "humanized" mice carrying human knock-in receptor(s) in a null host background is feasible by taking advantage of deleting the tight clustering of most *Tas2rs* genes.

Ultimately, human clinical trials that consider the significant genetic variation in *TAS2R* are required to establish the effectiveness of TAS2R agonists in asthma and other chronic obstructive pulmonary diseases.

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