



Taste receptors in asthma

Stanislas Grassin-Delyle^{a,b}, Emmanuel Naline^{a,b}, and Philippe Devillier^{a,b}

Purpose of review

Although today's cornerstone therapies for asthma (inhaled bronchodilators and corticosteroids) target airway narrowing and lung inflammation, about half of treated asthmatic patients do not achieve good disease control. There is a clear need for new therapeutic approaches and novel drug targets. Recent research has unexpectedly revealed that certain taste receptors (particularly those involved in bitter taste transduction) are expressed in lung tissue.

Recent findings

Bitter taste receptors are expressed in several cell types in the lungs (such as chemosensory cells, epithelial cells, smooth muscle cells, lymphocytes, and macrophages) and variously involved in ciliary beating, muscle relaxation, and/or inhibition of the production of inflammatory mediators. Here, we review recent research on the role of bitter taste receptors in experimental models of asthma and in asthmatics.

Summary

The currently available data suggest that bitter taste receptor agonists have therapeutic potential in chronic obstructive airway diseases such as asthma.

Keywords

asthma, bitter taste receptors, lung

INTRODUCTION

Asthma is a widespread, chronic airway disease in which episodic airway obstruction is caused by a combination of airway inflammation and airway hyperresponsiveness [1]. Although cluster analysis has identified several clinical phenotypes, the cornerstones of pharmacological asthma therapy are still inhaled β_2 -adrenoreceptor agonists, inhaled corticosteroids, leukotriene modifiers, and (in severe allergic asthma) anti-IgE agents [2]. Corticosteroids are effective in controlling asthma, improving lung function, and reducing the frequency of exacerbations, whereas β_2 -adrenoreceptor agonists are mainly used as bronchodilators. A spectrum of different molecules is available in each of these classes. The drugs differ in terms of their efficacy/potency, onset of action, duration of action, and side-effects. However, all the molecules in a given drug class share common drawbacks such as relative refractoriness to β_2 -adrenoreceptor agonists in some patients or resistance to corticosteroids in others. Moreover, about half of the treated asthmatic patients do not achieve good disease control [3], mainly as a result of poor treatment adherence and/or poor inhaler technique [4,5]. There is a clear need for new, easily administered drug classes that efficiently and safely control asthma and improve outcomes.

In recent years, research in experimental models of lung disease has revealed that bitter taste receptors (TAS2Rs) are potential drug targets. Here, we review recent data obtained in this field (from in-vitro models to clinical studies). The main relevant studies are summarized in Table 1 [6,7,8^{*},9,10^{*},11–26].

BITTER TASTE RECEPTORS

In humans, taste receptors detect the five basic tastes: sweet, bitter, sour, salty, and umami. The TAS2Rs are a large family of about 25 G-protein-coupled receptors [27,28]. Although the TAS2R's role in taste perception in the oral cavity has been well characterized, it has recently been found that these receptors are also expressed in the nose, lung [6,7,29], gut [30], and heart [31]. A large number of natural or synthetic molecules stimulate TAS2Rs,

^aLaboratoire de Pharmacologie Respiratoire UPRES EA220, Hôpital Foch, Suresnes and ^bUniversité Versailles Saint-Quentin, UFR Sciences de la Santé Simone Veil, Montigny-Le-Bretonneux, France

Correspondence to Stanislas Grassin-Delyle, Laboratoire de Pharmacologie Respiratoire, UPRES EA220, Hôpital Foch, 11 rue Guillaume Lenoir, F-92150 Suresnes, France. Tel: +33 146 252 791; fax: +33 140 999 657; e-mail: s.grassin-delyle@hopital-foch.org

Curr Opin Allergy Clin Immunol 2015, 15:63–69

DOI:10.1097/ACI.0000000000000137

KEY POINTS

- Bitter taste receptors are expressed outside the oral region (notably by several cell types in the lung).
- Activation of bitter taste receptors induces airway relaxation, an increase in cilia beat frequency, and a decrease in the production of proinflammatory mediators.
- Although the available clinical trial data are coherent with observations in in-vitro and in-vivo models, higher levels of evidence must be obtained before bitter taste receptors can become true drug targets in the treatment of obstructive lung diseases.

but an endogenous ligand has not been identified [32]. However, some Gram-negative bacteria are able to produce acylhomoserine lactones that bind to and activate TAS2Rs [33]. Similarly, several drugs currently marketed for a wide range of indications activate TAS2Rs; these include erythromycin, carisoprodol, flufenamic acid, dapson, quinine, chloroquine, caffeine, azelastine, and colchicine [32]. Most of these compounds have poor selectivity and weak potency for TAS2Rs, meaning that high (millimolar) concentrations are required to activate the receptors. When combined with a lack of selectivity, these concentrations may cause off-target effects [32]. Conversely, some subtypes (such as TAS2Rs 19, 41, 42, 45, and 60) have no known agonists and are referred to as orphan receptors.

DATA ACQUIRED IN IN-VITRO MODELS

The first report of the expression of bitter taste receptors in airway tissues dates from 2009, when a research group from the University of Iowa identified four different TAS2Rs subtypes located in the cilia of human airway epithelial cells [7]. The application of bitter compounds (such as denatonium, an agonist for TAS2Rs 4, 10, and 46) to the airway epithelium induced an increase in the cilia beating frequency. This finding suggests that TAS2Rs in the airway epithelium have a defensive role by speeding up the elimination of noxious substances. Furthermore, acylhomoserine lactones produced by bacteria in spoiled food can stimulate epithelial TAS2R38, resulting in the generation of bactericidal amounts of nitric oxide [34]. As patients with asthma display poor mucociliary clearance (involving an abnormally low ciliary beat frequency [35]), the stimulation of TAS2Rs might also help to restore normal mucociliary clearance.

In 2010, another research group from the University of Maryland described the expression

of TAS2Rs in human airway smooth muscle cells [6]. The transcripts of TAS2Rs 10, 14, and 31 were found to be about three-fold more abundant than those of β_2 -adrenoreceptors. The expression levels of other receptor subtypes (such as TAS2Rs 4, 5, or 19) were also significant [6]. The TAS2R agonists chloroquine, denatonium, and quinine caused a reduction in the basal tone of isolated mouse airways and a dose-dependent relaxation in airways precontracted with acetylcholine or serotonin. The maximal relaxation response corresponded to as much as 90% of the contraction [6]. Similarly, TAS2Rs 3, 4, and 10 were found to be expressed in guinea pig trachea, and agonists to these three receptors (thiamine, chloroquine, denatonium, and noscapine) evoked concentration-dependent relaxations of carbachol-precontracted guinea pig trachea [36].

The expression of TAS2Rs and the relaxant effects of their agonists have also been reported in human tissues [6,8[•],37,38]. Although most of the TAS2R subtypes are expressed, the responses to a large panel of agonists (chosen according to their relative selectivity for various receptor subtypes) suggested that TAS2Rs 5, 10, and 14 have a preponderant role in the relaxation of human bronchi [8[•]]. The relaxation to TAS2R agonists was intense, with chloroquine, quinine, phenanthroline, erythromycin, flufenamic acid, and dapson producing more than 90% of the maximal effect. However, the concentrations required to relax the human bronchi were 4 to 5 log units higher than the concentrations of the β_2 -adrenoreceptor agonists isoproterenol or formoterol required to provoke similar degrees of relaxation [8[•]]. The efficacy and potency of TAS2R agonists were truly comparable to those obtained with theophylline in human bronchi. In addition to the well known inhibition of phosphodiesterases at high concentrations, theophylline may also relax the airways through activation of TAS2Rs as the structurally similar methylxanthine caffeine is known to be a TAS2R agonist [8[•],32].

Data on tissues from asthmatic patients are scarce. The mRNA expression of TAS2Rs 10, 14, and 31 does not appear to be abnormal in airway smooth muscle cells from patients with fatal asthma, and the same is true of the calcium flux in response to chloroquine, diphenhydramine, flufenamic acid, saccharin, and quinine [9]. Furthermore, the relaxant response to quinine shown by carbachol-precontracted, precision-cut lung slices of normal human airways was unaffected by preincubation with IL-13 (a major T_H2 cytokine), whereas the response to formoterol was altered [9]. These data suggest that the relaxant response to TAS2R agonists may be maintained in an asthmatic or

Table 1. Summary of the main studies reviewed which support an interest for bitter taste receptors in asthma

Reference	Main findings
Deshpande <i>et al.</i> [6]	First description of the expression of TAS2R in human airway smooth muscle cells. The expression of some TAS2Rs was more abundant than the expression of the β_2 -adrenergic receptor. TAS2R agonists induced relaxation of isolated smooth muscle cells and decreased airway obstruction in a mouse model of asthma
Shah <i>et al.</i> [7]	Description of the expression of TAS2Rs in motile cilia of the airway epithelium. TAS2R agonists induced an increase in ciliary beat frequency
Grassin-Delyle <i>et al.</i> [8 [■]]	Characterization of the relaxant response of human bronchi to several TAS2R agonists, suggesting a predominant role for TAS2R5 10 and 14 and the involvement of phosphatidylinositol-3-kinases
Robinett <i>et al.</i> [9]	The expression of TAS2R10, 14 and 31 is observed in the airway smooth muscle from patients with asthma. TAS2R agonist-induced relaxant response in human precision-cut lung slices was unaltered by treatment with IL-13, whereas the response to formoterol was reduced
Ekoff <i>et al.</i> [10 [■]]	The expression of TAS2Rs is reported in cord blood-derived mast cells, and TAS2R agonists inhibited the IgE-dependent release of histamine and PGD ₂
Orsmark-Pietras <i>et al.</i> [11]	Description of an upregulation of the expression of some TAS2R transcripts in blood leukocytes from asthmatic children. TAS2R agonists also inhibited the LPS-induced cytokine production from blood leukocytes
Bukowskyj and Nakatsu [12] Gong <i>et al.</i> [13] Becker <i>et al.</i> [14] Kivity <i>et al.</i> [15] Colacone <i>et al.</i> [16] Henderson <i>et al.</i> [17]	Characterization of the bronchodilator effects of caffeine in asthma or in exercise-induced or histamine-induced bronchoconstriction
Miyatake <i>et al.</i> [18]	Report of the reduction of bronchial responsiveness by a 10 week treatment with erythromycin in patients with atopic or nonatopic bronchial asthma
Popa [19]	Description of a bronchodilator effect of the H ₁ antihistamine chlorpheniramine given at high IV doses in asthmatic patients
Ollier <i>et al.</i> [20] Albazzaz and Patel [21] Rafferty and Holgate [22]	Description of the inhibition of histamine-induced or allergen-induced bronchoconstriction by azelastine in patients with asthma
Kelly <i>et al.</i> [23]	Studies on the effects of colchicine on airway responsiveness in allergic asthmatic patients and of the use of colchicine as a corticosteroid-sparing agent
Fish <i>et al.</i> [24] Charous [25] Charous <i>et al.</i> [26]	Studies on the effects of the chloroquine derivative hydroxychloroquine on lung function in asthmatic patients

LPS, lipopolysaccharide.

asthma-like inflammatory environment – in contrast to the clear alteration in the response to β_2 -adrenoreceptor agonists. In addition to their direct effects on muscle contraction, the TAS2R agonists' ability to inhibit smooth muscle cell growth may also be of value. Indeed, chloroquine and quinine appear to inhibit the growth of smooth muscle cells (whether isolated from healthy controls or patients with asthma) in a concentration-dependent manner and without affecting cell viability; this may be of value in limiting the airway remodeling observed in asthma [39].

The TAS2R agonists' effects are not restricted to bronchial reactivity. Inflammation is a major feature of asthma, and the role of specific immune cells (such as mast cells, eosinophils, lymphocytes, and

macrophages) in inflammation has been clearly demonstrated. These cells are involved in the production of mediators such as cytokines, histamine, arachidonic acid metabolites, and growth factors. TAS2R expression has been observed in cord blood-derived mast cells. Incubation of these cells with chloroquine (a TAS2R 3 and 10 agonist) or denatonium (a TAS2R 4, 10, and 46 agonist) inhibited the IgE-dependent release of both histamine and prostaglandin D₂, whereas dextromethorphan (a TAS2R10 agonist) inhibited the production of histamine only and noscapine (a TAS2R14 agonist) inhibited the production of prostaglandin D₂ only [10[■]]. These observations suggest that TAS2Rs can regulate mast cell activation. Similar data have been reported for lymphocytes; TAS2Rs are expressed

in peripheral blood mononuclear cells in general and in lymphocytes in particular. The expression of transcripts of TAS2Rs 13, 14, and 19 (of the 11 subtypes studied) was higher in blood leukocytes from children with severe asthma than in cells from healthy controls. Furthermore, expression levels of TAS2R5 in cells from asthmatic children were negatively correlated with lung function [forced expiratory volume in the first second of expiration (FEV₁) and forced vital capacity (FVC)] and positively correlated with bronchial hyperresponsiveness to methacholine [11]. The lipopolysaccharide (LPS)-induced production of certain cytokines (including TNF- α , CCL2, IL-4, IL-13, and IL-10) by blood leukocytes is reduced by chloroquine (and, to a lesser extent, denatonium) in a concentration-dependent manner [11]. The LPS-induced release of other mediators involved in inflammation was also impacted. For example, chloroquine and denatonium inhibited the production of growth factors granulocyte-colony stimulating factor and granulocyte macrophage-colony stimulating factor (G-CSF and GM-CSF) and prostaglandin E₂ [11]. Furthermore, recent research has shown that human primary lung macrophages also express TAS2R mRNA, and TAS2R agonists inhibit the LPS-induced release of cytokines by these cells or by human lung parenchyma explants [40]. Hence, the data obtained with isolated cells and whole lung tissue suggest that TAS2Rs downregulate the production of inflammatory mediators. The *in-vitro* concentrations of most TAS2R agonists causing the relaxation of isolated human bronchi, the inhibition of IgE-mediated histamine release from mast cells, and the inhibition of the LPS-induced release of cytokine from blood leukocytes and lung macrophages are often at least 10-fold to 100-fold higher than the plasma concentrations in patients treated with these drugs, although chloroquine concentrations in leukocytes from patients are in the same range as those found to be effective *in vitro* [41]. Although the lung concentrations of these drugs are not often reported, the clinically relevant use of such medicines to treat lung diseases would probably require high lung concentrations, making administration by the inhaled route almost mandatory. Indeed, inhalation of denatonium may enable active concentrations to be achieved in the lung in the absence of local toxicity (at least in animals) [42].

DATA ACQUIRED IN IN-VIVO MODELS

Few data on TAS2Rs have been collected in *in-vivo* animal models. In the mouse, brush cells in the tracheal epithelium express the receptors TAS2R4 and TAS2R5 (formerly known as TAS2R108 and

TAS2R105, respectively) and enzymes of the cholinergic transmission pathway (such as the choline acetyltransferase and the vesicular acetylcholine transporter). Moreover, these brush cells are in contact with cholinergic nerve fibers [43]. Although the brush cells' function in the respiratory epithelium remains unclear, experiments with cycloheximide (a TAS2R5 agonist) in the mouse have shown that cholinergic tracheal brush cells detect bitter compounds. This induces the release of acetylcholine, which in turn activates cholinergic nicotinic receptors on adjacent sensory nerve fibers and prompts a decrease in the respiratory rate [43]. Hence, TAS2Rs in the airways may also be involved in the vagal regulation of breathing.

With respect to airway responsiveness, the TAS2R agonists quinine and denatonium reduced airway resistance by 50 and 57%, respectively, in the ovalbumin-sensitized mouse model of bronchial hyperresponsiveness [6]. In the same model, albuterol was used as a positive control but exhibited a smaller reduction in airway resistance than quinine in sensitized animals, suggesting that TAS2R agonists may be more effective than β_2 -adrenoreceptor agonists under inflammatory conditions [6]. Although there are several reports of the *in-vivo* effects of several other known TAS2R agonists on lung inflammation in conditions other than asthma, the involvement of TAS2R activation has not been established. For example, caffeine inhibits hyperoxia-induced pulmonary cytokine production and leukocyte influx in the rat [44], dapsone decreases LPS-induced intraepithelial neutrophil accumulation in the ferret [45], colchicine decreases neutrophil influx, protein accumulation, and changes in both expiratory resistance and dynamic compliance after phosgene exposure in the rat [46], and erythromycin reduces the LPS-induced recruitment of neutrophils in the rat lung [47]. Further studies are needed to assess the involvement of TAS2R in these effects, such as by using TAS2R knockout model or (as-yet unavailable) specific TAS2R agonists and/or antagonists.

EFFECT OF TAS2R AGONISTS ON LUNG FUNCTION IN ASTHMATIC PATIENTS

No clinical trials have been designed to address the pulmonary effects of TAS2R agonists in either healthy subjects or patients with asthma. This is because the pathway has only recently been described as a potential drug target, and the currently known TAS2R agonists are not sufficiently selective and may induce harmful off-target effects. Although effects on lung function have already been reported for several TAS2R activators, the level of evidence remains low.

Caffeine (a TAS2R 7, 10, 14, 43, and 46 agonist) has been studied in most detail in patients with asthma. However, its effects are not intense and its clinical relevance is uncertain. In a study [12] of eight adults with asthma, 5 mg/kg caffeine increased FEV₁ by 12%. In nine other patients with asthma, caffeine induced concentration-dependent increases in FEV₁ and airway conductance of up to 15 and 40%, respectively [13]. In 13 young patients with asthma, 10 mg/kg caffeine was associated with significant improvements in FVC, FEV₁, and forced expiratory flow rates [14]. In 10 patients with bronchial asthma, 7 mg/kg oral caffeine increased FEV₁ at rest (by about 0.35 l/s) and prevented exercise-induced bronchoconstriction [15]. Accordingly, oral administration of 5 mg/kg caffeine to 10 subjects with mild asthma was associated with only a small increase in the provocation concentration of histamine causing a 20% fall in FEV₁ (PC₂₀) [16]. In another set of eight patients with mild asthma, the same dose of caffeine also induced an increase in PC₂₀ [17]. In the two latter studies, the resting FEV₁ was unaffected. The authors of a recent *Cochrane Database Systematic Review* concluded that caffeine induced a moderate improvement in airway function in people with asthma [48]. Caffeine's mechanism of action remains to be determined. A number of hypotheses have been suggested, such as an increase in cyclic adenosine monophosphate levels, a change in intracellular calcium levels, the blockade of adenosine receptors, and indirect effects (such as a change in actin polymerization). The putative involvement of TAS2Rs in caffeine's effects may reconcile many of these assumptions. *In vivo*, a small proportion of caffeine (4%) is metabolized into theophylline, which may account for the parent compounds' moderate effects on lung function. Ingestion of the caffeine equivalent of 1 to 3 cups of coffee produces plasma caffeine concentrations of between 20 and 60 µmol/l [13], whereas the plasma concentration peaks at 28 µmol/l after the ingestion of 5 mg/kg caffeine [16]. Although these values are much lower than the concentrations required to relax bronchi *in vitro*, the lung distribution of caffeine has barely been investigated.

Similarly, erythromycin has beneficial effects that are unrelated to its antimicrobial properties. In 23 patients with asthma receiving 200 mg oral erythromycin three times daily for 10 weeks, the mean PC₂₀ to histamine (but not the FEV₁) increased significantly (relative to the value before treatment) [18]. After a course of 250 mg erythromycin four times a day in patients with mild COPD, the plasma concentration is about 1–3 µmol/l [49]. The concentration in lung tissue at a standard dosage is usually between 1 and 15 µmol/g [50,51]. In isolated

human bronchi precontracted with histamine, erythromycin's relaxant effect starts at about 50 µmol/l [8[¶]]. However, it is not at all clear that this concentration can be achieved at the site of action site *in vivo* – even after repeated doses.

A study [19] of lung function in 10 patients with asthma reported that high-dose, intravenous administration of chlorpheniramine (an H₁ antihistamine that also acts as an agonist for TAS2Rs 10 and 14) has bronchodilatory properties, with an increase in FEV₁. Azelastine (another H₁ antihistamine, with a bitter taste) was shown to protect patients with asthma from an allergen-induced fall in specific airway conductance [20] and allergen-induced or histamine-induced bronchoconstriction [21,22]. However, direct effects (related at least in part to H₁ receptor antagonism) are probably involved in this context [21]. Despite the lack of relevant data in humans, azelastine is known to preferentially distribute into the guinea pig lung, with a lung/blood concentration ratio of 22 [52].

Colchicine (an agonist for TAS2Rs 4, 39, and 46) has also been investigated as an antiinflammatory drug in asthma. In a two-period crossover trial, nine patients with mild asthma received either placebo or 0.6 mg colchicine twice daily for 13 doses. Relative to the placebo, colchicine was associated with inhibition of the early and late allergen-induced fall in FEV₁ but did not affect methacholine responsiveness [23]. In a subsequent trial, 71 patients with moderate asthma received inhaled corticosteroids prior to initiation of colchicine. A 4-week run-in period (2 weeks of triamcinolone alone and then 2 weeks of triamcinolone + 0.6 mg colchicine twice a day) was followed by a 6-week treatment period in which triamcinolone was discontinued (i.e. patients continued receiving colchicine or placebo alone). When comparing baseline and study end values, there were no differences in FEV₁ or peak expiratory flow (among other criteria) [24]. However, the 6-week treatment period after the discontinuation of triamcinolone may have been too short to change the FEV₁. From a pharmacokinetic standpoint, blood colchicine concentrations are rather low (<20 ng/ml). However, the compound is known to diffuse into the lungs [53] and concentrate 60-fold to 600-fold inside leukocytes [54].

Lastly, chloroquine has been trialed in respiratory diseases other than asthma. In 19 patients with sarcoidosis, a 6-month course of chloroquine led to statistically significant increases in FEV₁ and FVC (relative to values at inclusion). In the 10 patients who continued to receive chloroquine for 6 to 48 months after this open-label period, only a significant decrease in FEV₁ (relative to patients receiving placebo) was observed [55]. The concentrations

of chloroquine achieved in treated patients are in the same range as those found to be effective in in-vitro models [26]. Interestingly, hydroxychloroquine (a chloroquine derivative that also tastes bitter) was also shown to improve lung function in two trials on patients with asthma [25,26].

CONCLUSION

In summary, TAS2Rs are expressed in most cell types in the proximal to distal airways. Stimulation of those receptors evokes responses that may be of value in the treatment of respiratory diseases (such as an increase in epithelial ciliary beat frequency, smooth muscle relaxation, and inhibition of cytokine production in several in-vitro and in-vivo models). However, consistent data have yet to be obtained in clinical trials. Moreover, the pharmacology of these receptors remains to be explored; there is a need to identify the receptor subtypes involved in the observed effects and determine the intracellular signaling pathways activated by TAS2Rs. Recent reports have suggested the absence of tachyphylaxis with TAS2Rs for smooth muscle relaxation (in contrast with β_2 -adrenoreceptors). Although confirmation in the clinic is required, the currently available data suggest that TAS2R agonists may become a new class of drugs that can be combined with or used instead of conventional anti-inflammatory or bronchodilatory drugs for the management of inflammatory and obstructive lung diseases.

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Martinez FD, Vercelli D. Asthma. *Lancet* 2013; 382:1360–1372.
2. Halder P, Pavord ID, Shaw DE, *et al.* Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med* 2008; 178:218–224.
3. Demoly P, Annunziata K, Gubba E, Adamek L. Repeated cross-sectional survey of patient-reported asthma control in Europe in the past 5 years. *Eur Respir J* 2012; 21:66–74.
4. Chapman KR, Boulet LP, Rea RM, Franssen E. Suboptimal asthma control: prevalence, detection and consequences in general practice. *Eur Respir J* 2008; 31:320–325.
5. Global Initiative for Asthma. Global strategy for asthma management and prevention. Update 2014. http://www.ginasthma.org/local/uploads/files/GINA_Report_2014_Aug12.pdf. [Accessed 24 September 2014]
6. Deshpande DA, Wang WC, McIlmoyle EL, *et al.* Bitter taste receptors on airway smooth muscle bronchodilate by localized calcium signaling and reverse obstruction. *Nat Med* 2010; 16:1299–1304.
7. Shah AS, Ben-Shahar Y, Moninger TO, *et al.* Motile cilia of human airway epithelia are chemosensory. *Science* 2009; 325:1131–1134.
8. Grassin-Delyle S, Abrial C, Fayad-Kobeissi S, *et al.* The expression and relaxant effect of bitter taste receptors in human bronchi. *Respir Res* 2013; 14:134.
- Pharmacological characterization of the relaxation of human bronchi to bitter taste receptor agonists.
9. Robinett KS, Koziol-White CJ, Akoluk A, *et al.* Bitter taste receptor function in asthmatic and nonasthmatic human airway smooth muscle cells. *Am J Respir Cell Mol Biol* 2014; 50:678–683.
10. Ekoff M, Choi JH, James A, *et al.* Bitter taste receptor (TAS2R) agonists inhibit IgE-dependent mast cell activation. *J Allergy Clin Immunol* 2014; 134:475–478.
- Reports the TAS2R-mediated inhibition of the production of proinflammatory mediators in mast cells.
11. Orsmark-Pietras C, James A, Konradsen JR, *et al.* Transcriptome analysis reveals upregulation of bitter taste receptors in severe asthmatics. *Eur Respir J* 2013; 42:65–78.
12. Bukowskyj M, Nakatsu K. The bronchodilator effect of caffeine in adult asthmatics. *Am Rev Respir Dis* 1987; 135:173–175.
13. Gong H Jr, Simmons MS, Tashkin DP, *et al.* Bronchodilator effects of caffeine in coffee. A dose-response study of asthmatic subjects. *Chest* 1986; 89:335–342.
14. Becker AB, Simons KJ, Gillespie CA, Simons FE. The bronchodilator effects and pharmacokinetics of caffeine in asthma. *N Engl J Med* 1984; 310:743–746.
15. Kivity S, Ben Aharon Y, Man A, Topilsky M. The effect of caffeine on exercise-induced bronchoconstriction. *Chest* 1990; 97:1083–1085.
16. Colaccone A, Bertolo L, Wolkove N, *et al.* Effect of caffeine on histamine bronchoprovocation in asthma. *Thorax* 1990; 45:630–632.
17. Henderson JC, O'Connell F, Fuller RW. Decrease of histamine induced bronchoconstriction by caffeine in mild asthma. *Thorax* 1993; 48:824–826.
18. Miyatake H, Taki F, Taniguchi H, *et al.* Erythromycin reduces the severity of bronchial hyperresponsiveness in asthma. *Chest* 1991; 99:670–673.
19. Popa VT. Bronchodilating activity of an H1 blocker, chlorpheniramine. *J Allergy Clin Immunol* 1977; 59:54–63.
20. Ollier S, Gould CA, Davies RJ. The effect of single and multiple dose therapy with azelastine on the immediate asthmatic response to allergen provocation testing. *J Allergy Clin Immunol* 1986; 78:358–364.
21. Albazzaz MK, Patel KR. Effect of azelastine on bronchoconstriction induced by histamine and leukotriene C4 in patients with extrinsic asthma. *Thorax* 1988; 43:306–311.
22. Rafferty P, Holgate ST. The inhibitory effect of azelastine hydrochloride on histamine- and allergen-induced bronchoconstriction in atopic asthma. *Clin Exp Allergy* 1989; 19:315–320.
23. Kelly SJ, Uri AJ, Freeland HS, *et al.* Effects of colchicine on IgE-mediated early and late airway reactions. *Chest* 1995; 107:985–991.
24. Fish JE, Peters SP, Chambers CV, *et al.* An evaluation of colchicine as an alternative to inhaled corticosteroids in moderate asthma. National Heart, Lung, and Blood Institute's Asthma Clinical Research Network. *Am J Respir Crit Care Med* 1997; 156:1165–1171.
25. Charous BL. Open study of hydroxychloroquine in the treatment of severe symptomatic or corticosteroid-dependent asthma. *Ann Allergy* 1990; 65:53–58.
26. Charous BL, Halpern EF, Steven GC. Hydroxychloroquine improves airflow and lowers circulating IgE levels in subjects with moderate symptomatic asthma. *J Allergy Clin Immunol* 1998; 102:198–203.
27. Andres-Barquin PJ, Conte C. Molecular basis of bitter taste: the T2R family of G protein-coupled receptors. *Cell Biochem Biophys* 2004; 41:99–112.
28. Chandrashekar J, Hoon MA, Ryba NJ, Zuker CS. The receptors and cells for mammalian taste. *Nature* 2006; 444:288–294.
29. Manson ML, Safholm J, Al-Ameri M, *et al.* Bitter taste receptor agonists mediate relaxation of human and rodent vascular smooth muscle. *Eur J Pharmacol* 2014; 740C:302–311.
30. Kim KS, Egan JM, Jang HJ. Denatonium induces secretion of glucagon-like peptide-1 through activation of bitter taste receptor pathways. *Diabetologia* 2014; 57:2117–2125.
31. Foster SR, Blank K, See Hoe LE, *et al.* Bitter taste receptor agonists elicit G-protein-dependent negative inotropy in the murine heart. *FASEB J* 2014; 28:4497–4508.
32. Meyerhof W, Batram C, Kuhn C, *et al.* The molecular receptive ranges of human TAS2R bitter taste receptors. *Chem Senses* 2010; 35:157–170.
33. Tizzano M, Gulbransen BD, Vandenbeuch A, *et al.* Nasal chemosensory cells use bitter taste signaling to detect irritants and bacterial signals. *Proc Natl Acad Sci U S A* 2010; 107:3210–3215.
34. Lee RJ, Xiong G, Kofonow JM, *et al.* T2R38 taste receptor polymorphisms underlie susceptibility to upper respiratory infection. *J Clin Invest* 2012; 122:4145–4159.
35. Thomas B, Rutman A, Hirst RA, *et al.* Ciliary dysfunction and ultrastructural abnormalities are features of severe asthma. *J Allergy Clin Immunol* 2010; 126:722–729; e722.

36. Pulkkinen V, Manson ML, Safholm J, *et al.* The bitter taste receptor (TAS2R) agonists denatonium and chloroquine display distinct patterns of relaxation of the guinea pig trachea. *Am J Physiol Lung Cell Mol Physiol* 2012; 303:L956–L966.
37. Belvisi MG, Dale N, Birrell MA, Canning BJ. Bronchodilator activity of bitter tastants in human tissue. *Nat Med* 2011; 17:776; Author reply 776–778.
38. Morice AH, Bennett RT, Chaudhry MA, *et al.* Effect of bitter tastants on human bronchi. *Nat Med* 2011; 17:775.
39. Panebra A, Hershfild A, Deshpande DA. Bitter taste receptor (BTR) agonists inhibit mitogenic signaling in human airway smooth muscle (ASM) cells. *Am J Respir Crit Care Med* 2014; 189 Abstracts Issue: A1047–A1047.
40. Grassin-Delyle S, Abrial C, Brollo M, *et al.* Characterization of the expression and the role of bitter taste receptors in human lung parenchyma and macrophages. *Am J Respir Crit Care Med* 2014; 189 Abstracts Issue: A5749–A5749.
41. Jang CH, Choi JH, Byun MS, Jue DM. Chloroquine inhibits production of TNF-alpha, IL-1beta and IL-6 from lipopolysaccharide-stimulated human monocytes/macrophages by different modes. *Rheumatology (Oxford)* 2006; 45:703–710.
42. Cosmetic Ingredient Review Expert P. Final report of the safety assessment of Alcohol Denat., including SD Alcohol 3-A, SD Alcohol 30, SD Alcohol 39, SD Alcohol 39-B, SD Alcohol 39-C, SD Alcohol 40, SD Alcohol 40-B, and SD Alcohol 40-C, and the denaturants, Quassin, Brucine Sulfate/Brucine, and Denatonium Benzoate. *Int J Toxicol* 2008; 27 (Suppl 1):1–43.
43. Krasteva G, Canning BJ, Hartmann P, *et al.* Cholinergic chemosensory cells in the trachea regulate breathing. *Proc Natl Acad Sci U S A* 2011; 108:9478–9483.
44. Weichelt U, Cay R, Schmitz T, *et al.* Prevention of hyperoxia-mediated pulmonary inflammation in neonatal rats by caffeine. *Eur Respir J* 2013; 41:966–973.
45. Kanoh S, Tanabe T, Rubin BK. Dapsone inhibits IL-8 secretion from human bronchial epithelial cells stimulated with lipopolysaccharide and resolves airway inflammation in the ferret. *Chest* 2011; 140:980–990.
46. Ghio A, Lehmann J, Winsett D, *et al.* Colchicine decreases airway hyper-reactivity after phosgene exposure. *Inhal Toxicol* 2005; 17:277–285.
47. Sanz MJ, Nabah YN, Cerda-Nicolas M, *et al.* Erythromycin exerts in vivo anti-inflammatory activity downregulating cell adhesion molecule expression. *Br J Pharmacol* 2005; 144:190–201.
48. Welsh EJ, Bara A, Barley E, Cates CJ. Caffeine for asthma. *Cochrane Database Syst Rev* 2010; 1:CD001112.
49. Matera MG, Tufano MA, Polverino M, *et al.* Pulmonary concentrations of dirithromycin and erythromycin during acute exacerbation of mild chronic obstructive pulmonary disease. *Eur Respir J* 1997; 10:98–103.
50. Brun Y, Forey F, Gamondes JP, *et al.* Levels of erythromycin in pulmonary tissue and bronchial mucus compared to those of amoxycillin. *J Antimicrob Chemother* 1981; 8:459–466.
51. Wollmer P, Pride NB, Rhodes CG, *et al.* Measurement of pulmonary erythromycin concentration in patients with lobar pneumonia by means of positron tomography. *Lancet* 1982; 2:1361–1364.
52. Chand N, Adusumalli VE, Nolan K, *et al.* Pharmacodynamic and pharmacokinetic studies with azelastine in the guinea pig: evidence for preferential distribution into the lung. *Allergy* 1993; 48:19–24.
53. Rochdi M, Sabouraud A, Baud FJ, *et al.* Toxicokinetics of colchicine in humans: analysis of tissue, plasma and urine data in ten cases. *Hum Exp Toxicol* 1992; 11:510–516.
54. Ben-Chetrit E, Bergmann S, Sood R. Mechanism of the anti-inflammatory effect of colchicine in rheumatic diseases: a possible new outlook through microarray analysis. *Rheumatology (Oxford)* 2006; 45:274–282.
55. Baltzan M, Mehta S, Kirkham TH, Cosio MG. Randomized trial of prolonged chloroquine therapy in advanced pulmonary sarcoidosis. *Am J Respir Crit Care Med* 1999; 160:192–197.