

How does rhinovirus cause the common cold cough?

Samantha K Atkinson, Laura R Sadofsky, Alyn H Morice

To cite: Atkinson SK, Sadofsky LR, Morice AH. How does rhinovirus cause the common cold cough? *BMJ Open Resp Res* 2016;**3**:e000118. doi:10.1136/bmjresp-2015-000118

Received 20 October 2015
Accepted 18 December 2015

ABSTRACT

Cough is a protective reflex to prevent aspiration and can be triggered by a multitude of stimuli. The commonest form of cough is caused by upper respiratory tract infection and has no benefit to the host. The virus hijacks this natural defence mechanism in order to propagate itself through the population. Despite the resolution of the majority of cold symptoms within 2 weeks, cough can persist for some time thereafter. Unfortunately, the mechanism of infectious cough brought on by pathogenic viruses, such as human rhinovirus, during colds, remains elusive despite the extensive work that has been undertaken. For socioeconomic reasons, it is imperative we identify the mechanism of cough. There are several theories which have been proposed as the causative mechanism of cough in rhinovirus infection, encompassing a range of different processes. Those of which hold most promise are physical disruption of the epithelial lining, excess mucus production and an inflammatory response to rhinovirus infection which may be excessive. And finally, neuronal modulation, the most convincing hypothesis, is thought to potentiate cough long after the original stimulus has been cleared. All these hypotheses will be briefly covered in the following sections.

INTRODUCTION

Cough is a common symptom associated with upper respiratory tract infections (URTIs).¹⁻⁴ In some patients, coughing can persist leading to a syndrome known as postviral or postinfectious cough which is arbitrarily defined as lasting from 3 to 8 weeks, with normal chest radiograph findings.^{5 6} In some individuals, cough persists even longer, when it is termed a chronic cough.⁷ While a normal cough is a vital protective reflex preventing aspiration, cough hypersensitivity is the mechanism thought to underlie almost all types of pathological cough.⁸ This has been demonstrated in URTI.⁹⁻¹¹

Cough causes a plethora of complications affecting the cardiovascular, gastrointestinal and respiratory systems, with far-reaching psychological, neurological and musculoskeletal effects.^{6 7 12} While there are many agents on the market to reduce the frequency of cough

and aid in the clearance of mucus, a systematic review of over-the-counter preparations failed to recommend any available treatment.¹³ For example, the use of codeine in respiratory tract infection-associated cough was found to be no more effective than its vehicle,¹⁴ and prescription-only medications are often unsuitable for certain groups of individuals.¹⁵

There are three metrics which are used to study cough: cough challenge, cough counting, and subjective end points such as visual analogue scale or quality of life. Cough challenge studies include the use of pro-tussive agents, such as capsaicin and citric acid, which stimulate transient receptor potential (TRP) ion channels to induce cough.¹⁶⁻¹⁸ TRP channels have been popularised as pro-tussive irritant receptors.^{19 20} However, on account of repeated clinical trial failures in patients with chronic cough using both cough counting and subjective measures, TRPV1 and A1 antagonists as anti-tussives have failed to reach the clinic,²¹ and an unpublished RCT of inhaled TRPA1 antagonist GRC 17536 (personal communication AHM, 2015). A recent shift of focus now proposes that other channels and receptors, such as P2X receptors, different TRP channels including TRPV4 and TRPM8²²⁻²⁴ may be responsible for the observed hypersensitivity. It seems unlikely that one single channel, or receptor, is responsible for causing cough hypersensitivity in all participants in cases of postviral and chronic cough.

Research into URTI and cough faces many problems. Research which relies on natural infection of human volunteers is open to a range of uncontrollable variability including incubation time and causative agent (virus genus and serotype). There is also a lack of suitable animal models for studying HRV due to high host specificity of attachment receptors. Major group HRV requires human ICAM-1 receptor²⁵ which is not present in guinea pigs, an animal classically used for studying the cough reflex. However, pathogens such as parainfluenza virus, a rarer



CrossMark

Centre for Cardiovascular and Metabolic Research (CCMR), The Hull York Medical School (HYMS), The University of Hull, Hull, UK

Correspondence to

Samantha K Atkinson;
Samantha.atkinson@hyms.ac.uk

cause of the common cold, can infect guinea pigs and produce a postviral cough with a hypersensitive airway response to capsaicin.²⁶ As a result, studying the effects of HRV infection is often carried out in vitro using cell systems. There is a plethora of research into viral induced effects characterised from various respiratory cell lines, leading to a variety of proposed mechanisms for the induction of cough.

MECHANISMS

Inflammatory mediators

HRV infection results in the production a broad profile of inflammatory mediators in the host. The primary inflammatory cytokines reported in HRV infection are interferon (IFN), interleukin (IL) 1, IL-6, IL-8, tumour necrosis factor (TNF) α , granulocyte-macrophage colony-stimulating factor and RANTES. The infection leads to massive upregulation,²⁷ and, consequently, it is often described as a 'cytokine disease'.²⁸ Many symptoms are thought to occur as a result of the effects of inflammatory cytokines releasing of mediators. For example, sore throat may occur as a result of the release of bradykinin.²⁹ The role of these endogeneous mediators is discussed below.

Bradykinin

The proinflammatory mediator bradykinin has been suggested as a potent tussive modulator of TRPA1 and TRPV1.^{30–32} It is thought to work through phospholipase C (PLC) causing channel phosphorylation and subsequent sensitisation.³³ Elevated levels of bradykinin are found in the BAL fluid of patients with inflammatory airway conditions.³⁴ Bradykinin has also been suggested to mediate ACE inhibitor cough³² which affects 15% of patients.³⁵ Bradykinin and PGE2 possess the ability to sensitise the airways to cough stimulus in animal studies which can be effectively abolished on simultaneous application of antagonists to both TRPV1 and TRPA1.³⁶

Tachykinins

Tachykinin peptides, neurokinin A and B, and substance P, are inflammatory neuropeptides, which collectively induce airway hyper-responsiveness, bronchial constriction,³⁷ and increased vascular permeability.³⁸ They also generate substantial mucus secretion³⁹ and the secretion of inflammatory mediators from immune cells.^{40–42} It has been suggested that inhibition of tachykinin metabolism by ACE inhibitors is an alternate mechanism for ACE inhibitor cough.⁴³ In HRV infection, tachykinins are released from neurons on TRPV1 activation.⁴⁰ Unfortunately, the mechanism underlying this activity is currently unknown, and understanding such channel interaction may hold the key to modulating the development of viral and postviral cough. Reduction of degradation of tachykinins by neutral endopeptidases in respiratory viral infection⁴⁴ are likely to enhance the noxious effects of tachykinins.

Despite its scarcity, substance P in humans has been found to be upregulated, both in nasal epithelium and plasma in chronic cough sufferers.^{45–47} The efficacy of substance P, mediated by tachykinin NK-1 receptors,⁴⁸ is greatly enhanced by prior inflammation. Furthermore, when in excess, it is suggested to lower the threshold of pain perception to noxious stimuli, as demonstrated in several pain-associated disease states.^{49 50} In guinea pigs, the role of substance P in cough has been extensively investigated. Substance P results in bronchoconstriction but highly variable cough.⁵¹ Likewise, in healthy individuals, inhalation of substance P does not cause cough. However, at the same concentration, substance P has the ability to elicit cough in patients with common colds,⁵² suggesting a hypersensitive state induced by the virus. Additionally, the microvascular leakage of substance P is thought to activate rapidly adapting receptors (RARs)⁵³ which may add to the irritant effect in common cold.

Calcitonin gene-related peptide

Evidence for the role of another neuropeptide calcitonin gene-related peptide (CGRP) is mixed. TRP channel (TRPV1) activation induces and controls the release^{40 54} from C-fibre terminals.⁵⁵ CGRP has an inhibitory effect in substance P-induced bronchoconstriction,⁵⁶ and when deficient, causes airway hyper-responsiveness.⁵⁷ However, an increase in CGRP has been shown in many pain-associated conditions, including migraine and various forms of inflammation.^{58 59} Chronic cough sufferers have been shown to have increased neuronal levels of CGRP^{60 61} associated with the enhanced sensitivity to capsaicin.⁶¹ These effects appear to be mediated through the cytokines, IL-1 β and TNF- α .⁵⁹ In respiratory syncytial virus (RSV) infection, a rarer cause of URTI in the adult, the development of airway hyper-responsiveness appears to arise through a disruption of CGRP balance.^{57 62} However, this fails to explain the increased levels found in sufferers of chronic cough. Unlike substance P, CGRP does not directly induce mucus secretion,⁶³ but may indirectly enhance through vasodilation.

The likelihood that substance P, CGRP or neurokinins have some role in cough hypersensitivity is high. Opiates have been shown to be highly effective in a subgroup of patients with cough.¹⁵ A possible mode of action is through prejunctional inhibition of peptide release-preventing neurotransmission either centrally, or from afferent nerves adjacent to inflammatory mediator receptors.⁴⁰

Leukotrienes

Leukotrienes are potent inflammatory mediators of chemotaxis, bronchoconstriction and vascular permeation,⁶⁴ which are predominately produced by leucocytes, but also by other inflammatory immune cells. Data is scarce on the extent leukotrienes play in HRV infection. However, Seymour *et al*⁶⁵ identified a significant increase of precursor enzymes within the airways during

HRV infection in healthy individuals, which can potentially increase the capacity of leukotriene B₄ and C₄ synthesis. Muscarinic receptor involvement has been implicated in the production of leukotriene B₄.⁶⁶ In cough-associated eosinophilic inflammation, blockade of leukotriene receptors has been shown to be efficacious.⁶⁷ However, in a recent study, montelukast, a potent inhibitor of the receptor of leukotriene C₄ and D₄, has no effect on cough in the common cold.⁶⁸

Eosinophils

Eosinophils are important mediators of cough in allergic disease but their role in viral infection is less clear. Eosinophils, if activated, during viral infection release a multitude of molecules including leukotrienes, growth factors, cytokines and major basic protein (MBP).⁶⁹ MBP binds to as well as alters prejunctional M2 receptor function,^{70 71} and increases tachykinin release.⁴⁴ MBP is cytotoxic⁷² and has been implicated in peripheral nerve remodelling.⁷³ It is found in higher levels in nasal aspirates from children during HRV infection.⁷⁴ Not only are eosinophils implicated in tachykinin modulation, but during degranulation they also release a potent peroxidase which generates reactive oxygen species (ROS) and reactive nitrogen species. These harmful oxidants are known potent TRPA1 agonists,^{75 76} which are receptors ascribed to cause cough in humans and guinea pigs.²⁰

Muscarinic receptors

Muscarinic receptors are highly characterised in airway diseases such as asthma and chronic obstructive pulmonary disease with limited evidence of their involvement in respiratory infections. Of the five muscarinic receptors (M1-5), only M1-3 can be found in the respiratory system. M1 is expressed on epithelial cells and submucosal glands of pulmonary veins.⁷⁷ M3 receptors are heavily expressed on smooth muscle, inflammatory and submucosal cells⁷⁸ where they mediate bronchoconstriction, mucosal secretion and inflammatory responses.⁷⁹ M2 receptors predominately regulate cardiac contraction, but can also be localised to respiratory smooth muscle.⁷⁷ However, the most important role of M2 receptors in the airway is the prejunctional inhibition of acetylcholine release to limit the degree of bronchoconstriction.⁸⁰ Respiratory viral agents, parainfluenza and RSV, have been shown to cause depletion and dysfunction of M2 receptors,⁸¹ thereby exaggerating cholinergic activity. This has been further shown in double-stranded (ds) RNA animal models, independent of inflammation,⁸¹ and mediated by IFN release.⁸² Therefore, viral infection-induced bronchoconstriction, airway hyperresponsiveness and mucus secretion causing cough may be indirectly mediated through M2 receptors.⁸² M2 dysregulation can be reversed with dexamethasone⁸³ or pilocarpine.⁸⁰ However, Lowry *et al*⁸⁴ found anticholinergic bronchodilators to be ineffective against cough in natural URTI. The potent topical corticosteroid, fluticasone, was also ineffective in significantly reducing

symptoms of viral URTI and, indeed, significantly increased the bacterial colonisation of the upper airway.⁸⁵

Many of the inflammatory mediators discussed above do not directly evoke cough but work synergistically through other pulmonary fibres where the threshold for cough is lowered to provoke the urge to cough. Thus, this change to sensory nerve functionality secondary to these mediators may potentiate and prolong a cough response during and after respiratory viral infection.

Physical damage

By comparison with other respiratory viruses such as influenza, HRV is renowned for its minimal cytopathic effects.⁸⁶ It has been suggested that influenza has a higher incidence of cough than that seen with HRV infections.⁸⁷ Thus, physical disruption of airway integrity may be a factor in a heightened cough response. HRV, or synthetic dsRNA stimuli polyinosinic:polycytidylic acid (poly(I:C)), is able to disrupt airway epithelial cells via disruption of tight junction complexes at apicolateral membranes through dissociation of zona occludin (ZO) 1, occludin, claudin-1, E-cadherin and β -catenin. This leads to a significant reduction in transepithelial resistance⁸⁸⁻⁹⁰ indicating a loss of epithelial integrity (figure 1A). Transepithelial resistance can also be decreased through respiratory-localised TRPV4 activation,⁹¹ and causes disruption of tight junction complexes leading to increased permeability. But whether these are related or not, are unknown. Whether this viral induced loss of integrity is dependent or independent of an inflammatory response is open to debate.

Inflammatory independent

ROS and other oxidants cause barrier disruption and affect permeability in tissues throughout the body⁹² through cytoskeletal and tight junction interruption. This effect is mirrored in polarised epithelial cells, such as those within the airways.^{88 93} During infection, HRV causes oxidative stress independent of viral replication or ICAM-1-mediated viral attachment.⁹⁴ The mechanism is thought to be via a NOD-like receptor X-1 (NLRX-1) interaction with dsRNA causing a translocation of NADPH oxidase-1 (NOX-1) leading to the generation of ROS and oxidants.^{93 95} By contrast, ROS production is necessary for clearance of viral infections, but requires stringent regulation. Interaction with dsRNA receptor NLRX-1 induces mitochondrial ROS generation.^{96 97} This is likely a result of mitochondrial antiviral signalling protein interaction with dsRNA,⁹⁸⁻¹⁰⁰ an important component of HRV lifecycle.¹⁰¹ ROS are also potent agonists of TRPA1 and TRPV1,^{75 76} which poses a potential interaction route between HRV and TRP channels.

Inflammatory dependent

Other investigators propose that physical disruption is an inflammatory-dependent process caused by TNF- α , IFN- γ , IL-4 and IL-8 which mediate the tight junction

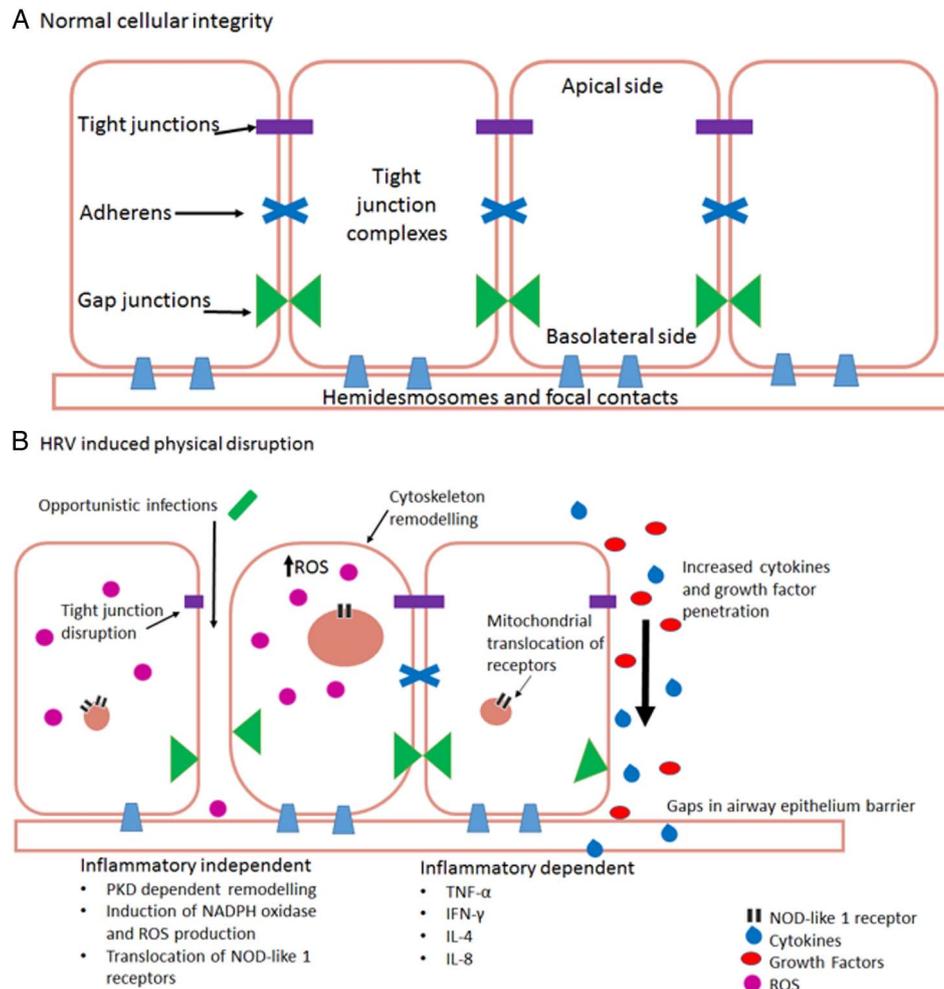


Figure 1 (A) Normal healthy airway barrier. In a healthy airway, cells are connected together by tight junction complexes including tight junctions, adherens and gap junctions. Cells are attached to basement membranes by hemidesmosomes and focal contacts. Barrier permeability is minimal and tightly regulated to prevent the excessive release of essential molecules, ions and proteins. The barrier is protective against infection. (B) Human rhinovirus infection in airway epithelial cells. There are two main ways that HRV causes physical disruption of airway barriers, inflammatory-dependent and independent. Both replicating and non-replicating viruses can interfere with airway membrane integrity by disrupting tight junction complexes. This causes a reduction of transepithelial resistance with the potential consequence of contracting a secondary infection. Cytoskeletal remodelling mediated by protein kinase D (PKD) causes an actin reorganisation within infected cells, altering their structure and integrity, further allowing cells to lose their adjoining contacts. Replicating HRV produces a dsRNA intermediate structure which can interact and activate NOD-like receptor X-1 ultimately producing reactive oxygen species. These alone are capable of reducing transepithelial resistance and barrier disruption. Loss of gap junctions and cells leaves gaps within epithelial layers. These allow cytokines, growth factors, immune cells and further viral particles to penetrate deeper layers within the airways, causing dysregulation of cellular signalling. This dysregulation causes further upregulation of various molecules including growth factors, which, in turn, can lead to an increase of receptor expression, such as transient receptor potential channels which have a prolific effect to cause cough (TNF, tumour necrosis factor; IFN, interferon; IL, interleukin).

dysregulation.^{102–104} A cytokine-induced effect may, however, be secondary to signalling pathways aforementioned, and which mechanism predominates may be dependent on specific cell type.

Consequently, varying degrees of barrier disruption and physical damage have the potential to cause a multitude of effects. A loss of integrity to airway barriers enables the transmigration of opportunistic bacteria causing secondary respiratory infection.⁹⁰ It can also lead to dysregulation of intracellular signalling facilitating the upregulation of growth factors.^{105–107} Finally, in

exposed animals, an enhanced activation of sensory nerve fibres leads to airway hyper-responsiveness¹⁰⁸ and epithelial repair is delayed.¹⁰⁹ Thus, there is a cycle of cytokine-induced barrier damage (figure 1B) leading to epithelial shedding. Airway epithelial lining begins to become permeable to larger molecules⁸⁸ leading to a cycle of hypersensitivity and further damage.

Physical disruption to airways described above is a well-characterised part of the pathophysiology of lung diseases including asthma and cystic fibrosis, but the role it plays in URTI, such as HRV, has only recently begun to

become clear, but may be crucially important in patients with pre-existing respiratory disease.

Mucus

Excessive mucus production and secretion is common in URTI^{1 110} initiating symptoms such as a cough and sneezing, and thus facilitating transmission of infection.^{3 111} HRV, in particular, upregulates the transcription of various mucin genes including MUC5AC.^{112–114} This pathway is particularly involved in mucus production and release, but this complex process (figure 2) has yet to be completely characterised. Using nuclear factor (NF) κ B and mitogen-activated protein kinase inhibitors, the pathway induced during HRV infection was originally identified. The mechanism is independent of serotype and genotype and is inducible by artificial genomic stimulus using poly(I:C).¹¹² NF κ B is upregulated as part of HRV lifecycle,^{115 116} so it is unsurprising that it plays a pivotal role in the production of symptoms during infection, and is essential for MUC5AC production. HRV is able to induce mucosal cell metaplasia through a novel TLR3-epidermal growth factor receptor (EGFR) coupling and the induction of EGFR ligands,¹¹³ including transforming growth factor α . This results in the

production and secretion of mucins via MUC5AC promoter regions.¹¹² The process of mucus secretion and tight junction disruption go hand in hand. A loss of epithelial integrity where a dissociation of E-cadherens from adherens tight junction complexes causes the uncoupling of EGFR where it becomes readily activated. However, an excess of EGFR activation promotes goblet metaplasia and, thus, excessive mucus secretion.¹¹⁷ Muscarinic receptors are also involved in mucus secretion, mediated predominately through M3 in cooperation with M1¹¹⁸ and are regulated by M2.¹¹⁹ Since stimulation of muscarinic receptors transactivate EGFR to stimulate goblet cell mucus secretion,^{120 121} it is possible that HRV possesses the ability to interact with muscarinic receptors to cause this process. The implications of this are far-reaching as not only may it begin to explain the aetiology of a viral mucosal cough but also chronic mucus secretion such as occurs in chronic bronchitis.

A counter-regulatory mechanism to HRV-induced TLR-EGFR coupling is by induction of the transcription factor sterile- α -motif-pointed domain ETS-factor (SPDEF). SPDEF inhibits TLR signalling and type I IFN release to dampen the proinflammatory response

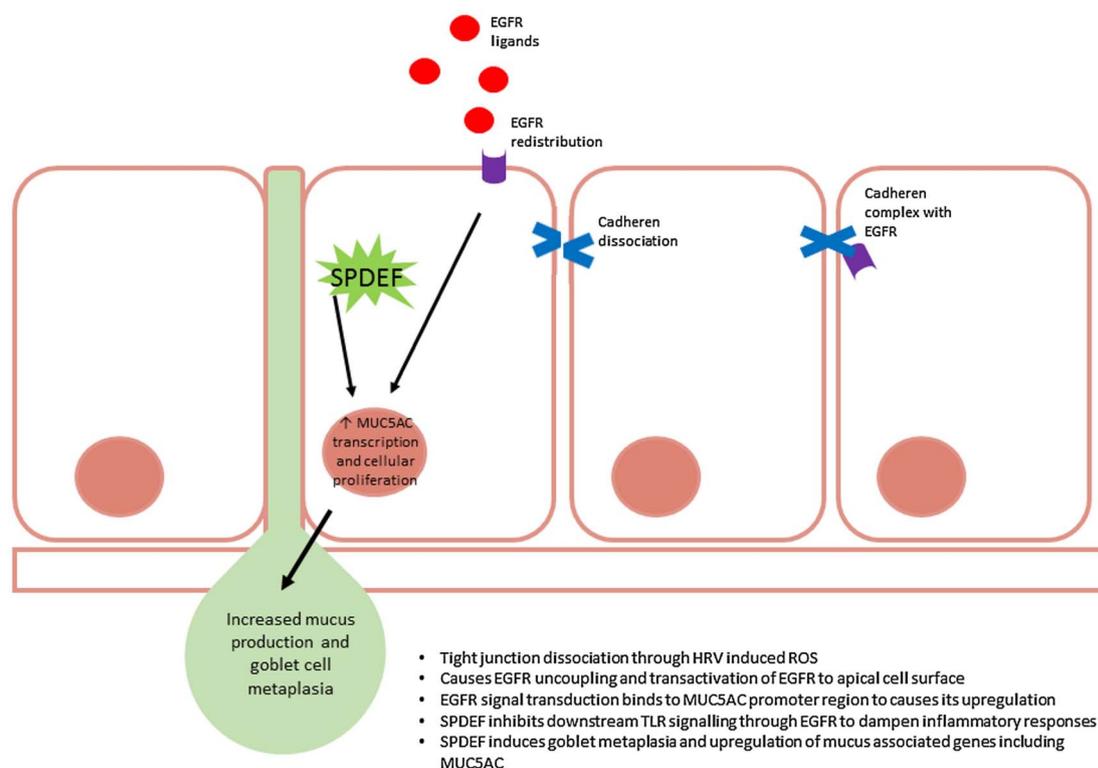


Figure 2 Sterile- α -motif-pointed domain ETS-factor (SPDEF) and epidermal growth factor receptor (EGFR) regulation of mucus production and goblet cell metaplasia within the airways during upper respiratory tract infection (URTI). Reactive oxygen species (ROS)-induced uncoupling of EGFR permits its translocation to the apical membrane of cells which readily allows its activation. Excessive activation of EGFR promotes various cellular processes including goblet cell metaplasia and upregulation of mucus-associated genes including MUC5AC. Simultaneously, SPDEF is activated to dampen the inflammatory response mounted against the rhinovirus infection through blockade of TLR signal transduction. SPDEF also functions as a transcription modulator and causes upregulation of MUC5AC. Ultimately, increased MUC5AC and goblet metaplasia results in the hyperproduction of mucus, characteristic of rhinovirus infection.

induced by HRV.¹²² SPDEF is a transcriptional modulator with a wide variety of roles including endocrine and androgen interaction,^{123 124} however, recently this transcription modulator was found to regulate goblet cell metaplasia^{123 125} and upregulate genes associated with mucus production,¹²⁶ including MUC5AC.¹²⁵ It has been suggested that SPDEF initiates recovery and protects against excessive inflammatory damage during metaplasia. Dysregulation of this pathway may permit the cycle of cough and damage to persist, exposing basal membranes and nerve fibres, allowing HRV-induced physical damage and mucus overproduction to act synergistically.

Many patients have a dry or non-productive cough, and thus, an important cofactor mucus production alone is insufficient to explain coughing in URTI. Excessive mucus may exacerbate cough by several mechanisms including a stretch response and altered tonicity. Present within mucus are nucleosides and nucleotides, namely ATP and its breakdown products.¹²⁷ ATP release occurs through cellular swelling mediated via pannexin-1^{128–130} and subsequent ATP-induced ATP release.¹³¹ Purinergic receptors are increasingly thought to be important mediators of cough hypersensitivity, and are discussed further in the section on neuronal mechanisms. In terms of mucus secretion, P2Y receptors enhance intracellular calcium concentrations and subsequent ciliary beat frequency.^{132 133} P2X7 is known to colocalise and interact with Pannexin-1.^{134–136} As part of the pore-forming complex of P2X7 receptor, pannexin-1 forms a death complex through extended pore dilation and increased permeability.^{135 137 138}

In recent years, an increasing number of studies have begun to implicate TRPV4 in mucociliary clearance and airway defence, as it is essential for epithelial barrier function,⁹¹ and is highly expressed in ciliated tracheal cells.^{139–141} As well as arachidonic acid metabolites,¹⁴² TRPV4 can be activated through mechanical and osmotic stimulus,¹⁴³ such as viscous and hypotonic mucus, to induce and regulate calcium release.¹⁴⁴ Not only does this activate the channel but it also regulates ciliary beat frequency¹⁴¹ and mucus secretion, mediated by aquaporin 5.¹⁴⁵

Neuronal modulation

Cough is clearly a neuronal reflex, so the hypothesis that neuronal modulation underlies the pathogenesis of viral cough is the most convincing. However, at present, there is no single comprehensive mechanism which explains cough induced by HRV or indeed any other respiratory pathogen. Theories include a cooperative role of pulmonary oxidative stress in vagal sensory nerves between TRPV1, TRPA1 and P2X receptors.¹⁴⁶ Direct viral damage to mitochondria leading to ROS production may modulate or influence cough.

During URTI sensitivity to capsaicin, citric acid and histamine are transiently increased with a reduction in cough threshold,^{10 11 147 148} without concurrent

hyper-responsiveness to methacholine.¹¹ This suggests that HRV-induced cough is independent of bronchial smooth-muscle tone. This was originally proposed to occur through the sensitisation of RARs,¹⁴⁹ but despite convincing evidence, RARs do not express TRPV1 receptors and are insensitive to chemical stimuli.¹⁵⁰ As such, they are no longer proposed to be the primary fibre involved in the cough reflex,²³ but may have a synergistic interaction with C-fibres.¹⁵¹ As a result of these findings, capsaicin-sensitive nerves are not the same nerves known to initiate pathological cough, despite the clear observation that inhaled aerosolised capsaicin produces a cough.¹⁸ These observed differences may be explained by phenotypic changes to nerve fibres induced during inflammation.¹⁵²

Phenotypic changes imply altered gene expression and differentiation. In the guinea pig, low threshold mechanosensitive sensory nerves express TrkA,¹⁵³ and application of other growth factors induce the functional expression of TRPV1 and TRPA1 de novo.¹⁵² In vivo research in the rat and guinea pig models have found inflammatory states through increased nerve growth factor (NGF) levels causing a phenotypic change of A-delta fibres. They now resemble C-fibres as shown by the coexpression of substance P and NGF.^{154 155} NGF is transported to sensory neurons via DRG and is able to alter transcription of various proteins and peptides.¹⁵⁶ Further research into the effect of NGF on TRPV1 by Chuang *et al*⁸³ and Ganju *et al*¹⁵⁷ found that NGF activates the PLC pathway. TRPV1 associates with phosphatidylinositol 4,5-bisphosphate (PIP₂) in its resting state, but PLC activation causes the hydrolysis of PIP₂ to release TRPV1 from constitutive inhibition, thus increasing the opening probability of TRPV1.

Similarly, HRV infection in the human has been shown to cause the upregulation of a number of growth factors.^{105–107} Specifically, HRV can induce the upregulation of NGF¹⁵⁸ and inhalation of aerosolised NGF can enhance cough in the guinea pig citric acid-induced cough model through TRPV1 and TrkA (NGF receptor) activation.^{159 160} Despite the differences between human and guinea pig airway innervation, it is these modifications in expression that may lead to a phenotypic change during URTI.

Interest into the role of TRPV4 and P2X receptors in cough has been growing exponentially. A recent abstract publication showed that the application of a TRPV4 agonist facilitated the subsequent activation of P2X3 receptor through sensitisation of airway sensory nerves.¹⁶¹ This is not the first time TRPV4 and P2X3 receptors have been hypothesised to play a cooperative role in pathophysiology.¹⁶² It has become apparent that there is significant overlap between TRP channel and purinergic receptor functionality, which have given rise to the persuasive theory that TRPV4 and purinergic receptors play a cooperative role in pathological cough. ATP, which has been shown to enhance cough reflex sensitivity²⁴ in response to citric acid and histamine

challenge,¹⁶³ potentiates through P2X2/X3-mediated bronchoconstriction.^{164 165} A successful clinical trial using AF-219, a P2X3 antagonist, reduced the incidence of coughing in patients with chronic cough by 75%,¹⁶⁶ provides more convincing evidence that cough sensitivity may be strongly upregulated by the P2X pathway.

Neurogenic inflammation from afferent sensory neurons¹⁶⁷ is mediated mostly, but not exclusively, by neuropeptides CGRP, neurokinins and substance P. When present, it is thought to be a protective reflex, facilitating healing and modulation of local immunity. Unfortunately, there is wide interspecies and, in man, intersubject variability in afferent sensory innervation making interpretation of experimental findings difficult to translate into clinical relevance. However, neurogenic inflammation is well characterised in several diseases, including migraine,¹⁶⁸ and more controversially in asthma¹⁶⁹ and rhinitis, the latter of which is common in URTI,¹⁷⁰ where it likely amplifies maladaptive responses. Neurogenic inflammation is essential for sensory neurons to prime and respond to noxious stimuli quickly. Consequently, afferent neurons are abundant in TRP channels, P2X, PAMPs and DAMP receptors. Only a limited set of TLRs, 3, 4, 7 and 9, are present within nociceptive neurons,^{171–173} therefore, not all pathogens are capable of directly causing neurogenic inflammation. Stimulation of these TLRs induce an inward depolarisation to elicit neuronal sensitisation to pain stimuli.^{171–173} A recent interesting finding identified TLR 7 stimulation leading to an itch-specific sensory pathway,¹⁷³ through intracellular microRNA let-7b. This, in turn, induces a rapid inward current in neurons, coexpressing TLR7 and TRPA1 to generate pain.^{174 175} Extracellular ATP is a crucial damage-associated molecular pattern molecule ligand which is released during damage and injury. In nociceptive neurons, P2X3 receptors are key to ATP recognition and pain production.¹⁷⁶ Purinergic receptor P2Y2 is also responsive to ATP, and is capable of TRPV1 sensitisation and activation in the absence of TRPV1 stimuli,¹⁷⁷ a similar sensation previously ascribed to TRPV4 through HRV-induced mucus overproduction. Cytokines, namely IL-1 β and TNF- α , produced as a result of infection cause TRP channel sensitisation and activation through membrane phosphorylation.^{178 179} The resultant effect means that TRP channels respond to innocuous stimuli as noxious stimuli causing allodynia and, perhaps, allotussia.

There is a surprising degree of overlap in the physiological location and functionality of thermo-TRP channels and P2X receptors. TRPA1 is reported to possess mechanotransductive properties likely attributable to their distinct characteristic ankyrin repeats¹⁸⁰ in the inner ear to permit hearing,¹⁸¹ although this is disputed by other groups.¹⁸² However, despite this, an interesting notable finding was the identification of P2X receptors, also within the inner ear, and that their initial action potential firing is necessary for the maturation of hearing.¹⁸³ Likewise in the bladder, TRPV1, TRPV2,

TRPV4, TRPA1 and TRPM8 all have mechanosensory roles¹⁸⁴ to interpret stretch and pain perception,¹⁸⁵ whilst P2X2 and P2X3 play a major role in distension sensation to excite a micturition reflex.¹⁸⁶ Most relevant to cough is their influence on pain. TRPA1 and TRPV1 are well characterised to elicit pain signals in response to noxious stimuli^{187 188} P2X2, P2X3, P2X4 and P2X7 having all been identified to play some form of role in various types of pain.^{189–192} Most importantly in neuropathic pain where some pharmacological agents which have shown promising results in clinical trials.¹⁹³ Relevant to these observations is that P2X2 and P2X3 receptors expressed on afferent neurons are home to the classic pain sensation channel TRPV1.¹⁹⁴

The role of TRP channels in HRV-induced cough has recently been explained in a first-of-its-kind study by Abdullah *et al.*¹⁹⁵ A novel infection site of HRV was identified in neuronal cell lines with concomitant upregulation of expressed TRP channels TRPA1, TRPV1 and TRPM8 found on airway sensory nerves. HRV infection accounts for up to 50% of exacerbations in asthmatics,¹⁹⁶ and both asthmatic¹⁹⁷ and chronic cough sufferers^{198 199} have higher levels of TRP channels expressed in their airways. This finding adds to the mounting evidence that TRP channels play a major role in cough during HRV infections, but requires further investigation to definitively confirm this.

SUMMARY

A postviral cough is generally unresponsive to conventional pharmacological intervention, but has shown limited responsiveness to the anticholinergic drug, tiotropium,²⁰⁰ which leaves a major hole in our therapeutic armamentarium. Currently, treatment merely consists of dampening the inflammatory response with the use of antiinflammatories (such as naproxen),²⁰¹ and cough suppressants (codeine-containing products and dextromethorphan),¹⁵ in the hope of reducing the frequency, severity and transmission of cough. Opiates, although commonly prescribed, are not generally recommended for viral cough due to their poor efficacy and significant adverse effect profile.¹⁵

The multiple mechanisms described above provide a confusing and inter-related ‘soup’ of potential therapeutic targets, dissecting which, are the key players involved in this common affliction that will be challenging. We suggest that modulation of the afferent neuronal hypersensitivity will provide the most fruitful target in what is essentially a benign and self-limiting disease. Other strategies, such as systemic immune modulation, run the risk of generating unforeseen off-target effects. The rewards for understanding the mechanism of viral-induced cough will have enormous impact on human morbidity.

Competing interests None declared.

Provenance and peer review Commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

Open Access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

REFERENCES

- Witek TJ, Ramsey DL, Carr AN, *et al.* The natural history of community-acquired common colds symptoms assessed over 4-years. *Rhinology* 2015;53:81–8.
- Eccles R, Loose I, Jawad M, *et al.* Effects of acetylsalicylic acid on sore throat pain and other pain symptoms associated with acute upper respiratory tract infection. *Pain Med* 2003;4:118–24.
- Curley FJ, Irwin RS, Pratter MR, *et al.* Cough and the common cold. *Am Rev Respir Dis* 1988;138:305–11.
- Reid DD, Williams RE, Hirsch A. Colds among office workers an epidemiological study. *Lancet* 1953;262:1303–6.
- Braman SS. Postinfectious cough: ACCP evidence-based clinical practice guidelines. *Chest* 2006;129:138S–46S.
- Irwin RS, Baumann MH, Bolser DC, *et al.* Diagnosis and management of cough executive summary: ACCP evidence-based clinical practice guidelines. *Chest* 2006;129:1S–23S.
- Morice AH, Fontana GA, Belvisi MG, *et al.* ERS guidelines on the assessment of cough. *Eur Respir J* 2007;29:1256–76.
- Morice AH. The cough hypersensitivity syndrome: a novel paradigm for understanding cough. *Lung* 2010;188(Suppl):S87–90.
- Dicpinigaitis PV, Tibb AS, Ramsey DL, *et al.* Stability of cough reflex sensitivity during viral upper respiratory tract infection (common cold). *Pulm Pharmacol Ther* 2014;28:154–7.
- Dicpinigaitis PV, Bhat R, Rhoton WA, *et al.* Effect of viral upper respiratory tract infection on the urge-to-cough sensation. *Respir Med* 2011;105:615–18.
- O'Connell F, Thomas VE, Studham JM, *et al.* Capsaicin cough sensitivity increases during upper respiratory infection. *Respir Med* 1996;90:279–86.
- McGarvey LPA, Morice AH. Clinical cough and its mechanisms. *Respir Physiol Neurobiol* 2006;152:363–71.
- Schroeder K, Fahey T. Systematic review of randomised controlled trials of over the counter cough medicines for acute cough in adults. *BMJ* 2002;324:329–31.
- Eccles R, Morris S, Jawad M. Lack of effect of codeine in the treatment of cough associated with acute upper respiratory tract infection. *J Clin Pharm Ther* 1992;17:175–80.
- Morice AH, McGarvey L, Pavord I, *et al.* Recommendations for the management of cough in adults. *Thorax* 2006;61(Suppl 1):i1–24.
- Morice AH, Kastelik JA, Thompson R. Cough challenge in the assessment of cough reflex. *Br J Clin Pharmacol* 2001;52:365–75.
- Laude EA, Higgins KS, Morice AH. A comparative study of the effects of citric acid, capsaicin and resiniferatoxin on the cough challenge in guinea-pig and man. *Pulm Pharmacol* 1993;6:171–5.
- Collier JG, Fuller RW. Capsaicin inhalation in man and the effects of sodium cromoglycate. *Br J Pharmacol* 1984;81:113–17.
- Benemei S, Patacchini R, Trevisani M, *et al.* TRP channels. *Curr Opin Pharmacol* 2015;22:18–23.
- Birrell MA, Belvisi MG, Grace M, *et al.* TRPA1 agonists evoke coughing in guinea pig and human volunteers. *Am J Respir Crit Care Med* 2009;180:1042–7.
- Khalid S, Murdoch R, Newlands A, *et al.* Transient receptor potential vanilloid 1 (TRPV1) antagonism in patients with refractory chronic cough: a double-blind randomized controlled trial. *J Allergy Clin Immunol* 2014;134:56–62.
- Morice AH. Developing antitussives the clinician's pipeline—what do we need? *J Thorac Dis* 2014;6:S735–8.
- Grace MS, Dubuis E, Birrell MA, *et al.* Pre-clinical studies in cough research: role of Transient Receptor Potential (TRP) channels. *Pulm Pharmacol Ther* 2013;26:498–507.
- Kamei J, Takahashi Y, Yoshikawa Y, *et al.* Involvement of P2X receptor subtypes in ATP-induced enhancement of the cough reflex sensitivity. *Eur J Pharmacol* 2005;528:158–61.
- Tomassini JE, Graham D, DeWitt CM, *et al.* cDNA cloning reveals that the major group rhinovirus receptor on HeLa cells is intercellular adhesion molecule 1. *Proc Natl Acad Sci USA* 1989;86:4907–11.
- Ye XM, Zhong NS, Liu CL, *et al.* Cough reflex sensitivity is increased in guinea pigs with parainfluenza virus infection. *Exp Lung Res* 2011;37:186–94.
- Papadopoulos NG, Johnston SL. Rhinoviruses as pathogens of the lower respiratory tract. *Can Respir J* 2000;7:409–14.
- Stöckl J, Vetr H, Majdic O, *et al.* Human major group rhinoviruses downmodulate the accessory function of monocytes by inducing IL-10. *J Clin Invest* 1999;104:957–65.
- Proud D, Reynolds CJ, Lacapra S, *et al.* Nasal provocation with Bradykinin induces symptoms of rhinitis and a sore throat. *Am Rev Respir Dis* 1988;137:613–16.
- Bandell M, Story GM, Hwang SW, *et al.* Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron* 2004;41:849–57.
- Carr MJ, Kollarik M, Meeker SN, *et al.* A role for TRPV1 in bradykinin-induced excitation of vagal airway afferent nerve terminals. *J Pharmacol Exp Ther* 2003;304:1275–9.
- Fox AJ, Laloo UG, Belvisi MG, *et al.* Bradykinin-evoked sensitization of airway sensory nerves: a mechanism for ACE-inhibitor cough. *Nat Med* 1996;2:814–17.
- Chuang HH, Prescott ED, Kong H, *et al.* Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5) P2-mediated inhibition. *Nature* 2001;411:957–62.
- Baumgarten CR, Lehmkuhl B, Henning R, *et al.* Bradykinin and other inflammatory mediators in BAL-fluid from patients with active pulmonary inflammation. *Agents Actions Suppl* 1992;38(Pt 3):475–81.
- Yeo WW, Foster G, Ramsay LE. Prevalence of persistent cough during long-term enalapril treatment: controlled study versus nifedipine. *Q J Med* 1991;80:763–70.
- Grace M, Birrell MA, Dubuis E, *et al.* Transient receptor potential channels mediate the tussive response to prostaglandin E2 and bradykinin *Thorax* 2012;67:891–900.
- Joos GF, Germonpre PR, Kips JC, *et al.* Sensory neuropeptides and the human lower airways: present state and future directions. *Eur Respir J* 1994;7:1161–71.
- Lundberg JM, Brodin E, Hua X, *et al.* Vascular permeability changes and smooth muscle contraction in relation to capsaicin-sensitive substance P afferents in the guinea-pig. *Acta Physiol Scand* 1984;120:217–27.
- Rogers DF, Aursudkij B, Barnes PJ. Effects of tachykinins on mucus secretion in human bronchi in vitro. *Eur J Pharmacol* 1989;174:283–6.
- Holzer P. Neurogenic vasodilatation and plasma leakage in the skin. *Gen Pharmacol* 1998;30:5–11.
- Numao T, Agrawal DK. Neuropeptides modulate human eosinophil chemotaxis. *J Immunol* 1992;149:3309–15.
- Lotz M, Vaughan JH, Carson DA. Effect of neuropeptides on production of inflammatory cytokines by human monocytes. *Science* 1988;241:1218–21.
- Morice AH, Lowry R, Brown MJ, *et al.* Angiotensin-converting enzyme and the cough reflex. *Lancet* 1987;2:1116–18.
- Jacoby DB. Pathophysiology of airway viral infections. *Pulm Pharmacol Ther* 2004;17:333–6.
- Bae Y-J, Moon KA, Kim T-B, *et al.* The role of nitrosative stress in the pathogenesis of unexplained chronic cough with cough hypersensitivity. *Am J Rhinol Allergy* 2012;26:e10–14.
- Otsuka K, Niimi A, Matsumoto H, *et al.* Plasma substance P levels in patients with persistent cough. *Respiration* 2011;82:431–8.
- Cho Y, Park S, Lee CK, *et al.* Elevated substance P levels in nasal lavage fluids from patients with chronic nonproductive cough and increased cough sensitivity to inhaled capsaicin. *J Allergy Clin Immunol* 2003;112:695–701.
- Moore KA, Udem BJ, Weinreich D. Antigen inhalation unmasks NK-2 tachykinin receptor-mediated responses in vagal afferents. *Am J Respir Crit Care Med* 2000;161:232–6.
- Appelgren A, Appelgren B, Kopp S, *et al.* Substance P-associated increase of intra-articular temperature and pain threshold in the arthritic TMJ. *J Orofac Pain* 1998;12:101–7.
- Evengard B, Nilsson CG, Lindh G, *et al.* Chronic fatigue syndrome differs from fibromyalgia. No evidence for elevated substance P levels in cerebrospinal fluid of patients with chronic fatigue syndrome. *Pain* 1998;78:153–5.
- El-Hashim AZ, Amine SA. The role of substance P and bradykinin in the cough reflex and bronchoconstriction in guinea-pigs. *Eur J Pharmacol* 2005;513:125–33.
- Katsumata U, Sekizawa K, Inoue H, *et al.* Inhibitory actions of procaterol, a beta-2 stimulant, on substance P-induced cough in normal subjects during upper respiratory tract infection. *Tohoku J Exp Med* 1989;158:105–6.

53. Bonham AC, Kott KS, Ravi K, *et al.* Substance P contributes to rapidly adapting receptor responses to pulmonary venous congestion in rabbits. *J Physiol* 1996;493(Pt 1):229–38.
54. Kichko TI, Reeh PW. TRPV1 controls acid- and heat-induced calcitonin gene-related peptide release and sensitization by bradykinin in the isolated mouse trachea. *Eur J Neurosci* 2009;29:1896–904.
55. Mak JCW, Barnes PJ. Autoradiographic localization of calcitonin gene-related peptide (CGRP) binding sites in human and guinea pig lung. *Peptides* 1988;9:957–63.
56. Cadieux A, Monast NP, Pomerleau F, *et al.* Bronchoprotector properties of calcitonin gene-related peptide in guinea pig and human airways. Effect of pulmonary inflammation. *Am J Respir Crit Care Med* 1999;159:235–43.
57. Dakhama A, Kanehiro A, Mäkelä MJ, *et al.* Regulation of airway hyperresponsiveness by calcitonin gene-related peptide in allergen sensitized and challenged mice. *Am J Respir Crit Care Med* 2002;165:1137–44.
58. Lassen L, Haderslev P, Jacobsen V, *et al.* CGRP may play a causative role in migraine. *Cephalalgia* 2002;22:54–61.
59. Hua X-Y, Chen P, Fox A, *et al.* Involvement of cytokines in lipopolysaccharide-induced facilitation of CGRP release from capsaicin-sensitive nerves in the trachea: studies with interleukin-1beta and tumor necrosis factor-alpha. *J Neurosci* 1996;16:4742–8.
60. Chang AB, Gibson PG, Ardill J, *et al.* Calcitonin gene-related peptide relates to cough sensitivity in children with chronic cough. *Eur Respir J* 2007;30:66–72.
61. O'Connell F, Springall DR, Moradoghli-Haftvani A, *et al.* Abnormal intraepithelial airway nerves in persistent unexplained cough? *Am J Respir Crit Care Med* 1995;152:2068–75.
62. Dakhama A, Park J-W, Taube C, *et al.* Alteration of airway neuropeptide expression and development of airway hyperresponsiveness following respiratory syncytial virus infection. *Am J Physiol Lung Cell Mol Physiol* 2005;288:L761–70.
63. Webber SE, Lim JC, Widdicombe JG. The effects of calcitonin gene-related peptide on submucosal gland secretion and epithelial albumin transport in the ferret trachea in vitro. *Br J Pharmacol* 1991;102:79–84.
64. Hammarström S. Leukotrienes. *Annu Rev Biochem* 1983;52:355–77.
65. Seymour ML, Gilby N, Bardin PG, *et al.* Rhinovirus infection increases 5-lipoxygenase and cyclooxygenase-2 in bronchial biopsy specimens from nonatopic subjects. *J Infect Dis* 2002;185:540–4.
66. Profita M, Di Giorgi R, Sala A, *et al.* Muscarinic receptors, leukotriene B4 production and neutrophilic inflammation in COPD patients. *Allergy* 2005;60:1361–9.
67. Takemura M, Niimi A, Matsumoto H, *et al.* Clinical, physiological and anti-inflammatory effect of montelukast in patients with cough variant asthma. *Respiration* 2012;83:308–15.
68. Wang K, Birring SS, Taylor K, *et al.* Montelukast for postinfectious cough in adults: a double-blind randomised placebo-controlled trial. *Lancet Respir Med* 2014;2:35–43.
69. Hogan SP, Rosenberg HF, Mochel R, *et al.* Eosinophils: biological properties and role in health and disease. *Clin Exp Allergy* 2008;38:709–50.
70. Evans CM, Fryer AD, Jacoby DB, *et al.* Pretreatment with antibody to eosinophil major basic protein prevents hyperresponsiveness by protecting neuronal M2 muscarinic receptors in antigen-challenged guinea pigs. *J Clin Invest* 1997;100:2254–62.
71. Larsen GL, Fame TM, Renz H, *et al.* Increased acetylcholine release in tracheas from allergen-exposed IgE-immune mice. *Am J Physiol* 1994;266:L263–70.
72. Hisamatsu K, Ganbo T, Nakazawa T, *et al.* Cytotoxicity of human eosinophil granule major basic protein to human nasal sinus mucosa in vitro. *J Allergy Clin Immunol* 1990;86:52–63.
73. Pégorier S, Wagner LA, Gleich GJ, *et al.* Eosinophil-derived cationic proteins activate the synthesis of remodeling factors by airway epithelial cells. *J Immunol* 2006;177:4861–9.
74. Teran LM, Seminario MC, Shute JK, *et al.* RANTES, macrophage-inhibitory protein 1alpha, and the eosinophil product major basic protein are released into upper respiratory secretions during virus-induced asthma exacerbations in children. *J Infect Dis* 1999;179:677–81.
75. Nishio N, Taniguchi W, Sugimura YK, *et al.* Reactive oxygen species enhance excitatory synaptic transmission in rat spinal dorsal horn neurons by activating TRPA1 and TRPV1 channels. *Neuroscience* 2013;247:201–12.
76. Andersson DA, Gentry C, Moss S, *et al.* Transient receptor potential A1 is a sensory receptor for multiple products of oxidative stress. *J Neurosci* 2008;28:2485–94.
77. Mak JC, Barnes PJ. Autoradiographic visualization of muscarinic receptor subtypes in human and guinea pig lung. *Am Rev Respir Dis* 1990;141:1559–68.
78. Gosens R, Zaagsma J, Meurs H, *et al.* Muscarinic receptor signaling in the pathophysiology of asthma and COPD. *Respir Res* 2006;7:73.
79. Rogers DF. Motor control of airway goblet cells and glands. *Respir Physiol* 2001;125:129–44.
80. Fryer AD, Maclagan J. Muscarinic inhibitory receptors in pulmonary parasympathetic nerves in the guinea-pig. *Br J Pharmacol* 1984;83:973–8.
81. Bowerfind WML, Fryer AD, Jacoby DB. Double-stranded RNA causes airway hyperreactivity and neuronal M2 muscarinic receptor dysfunction. *J Appl Physiol* 2002;92:1417–22.
82. Jacoby DB, Xiao HQ, Lee NH, *et al.* Virus- and interferon-induced loss of inhibitory M2 muscarinic receptor function and gene expression in cultured airway parasympathetic neurons. *J Clin Invest* 1998;102:242–8.
83. Jacoby DB, Yost BL, Kumaravel B, *et al.* Glucocorticoid treatment increases inhibitory m(2) muscarinic receptor expression and function in the airways. *Am J Respir Cell Mol Biol* 2001;24:485–91.
84. Lowry R, Wood A, Higenbottam T. The effect of anticholinergic bronchodilator therapy on cough during upper respiratory tract infections. *Br J Clin Pharmacol* 1994;37:187–91.
85. Puhakka T, Mäkelä MJ, Malmström K, *et al.* The common cold: effects of intranasal fluticasone propionate treatment. *J Allergy Clin Immunol* 1998;101:726–31.
86. Winther B, Gwaltney JM, Hendley JO. Respiratory virus infection of monolayer cultures of human nasal epithelial cells. *Am Rev Respir Dis* 1990;141:839–45.
87. Monto AS, Gravenstein S, Elliott M, *et al.* Clinical signs and symptoms predicting influenza infection. *Arch Intern Med* 2000;160:3243–7.
88. Rezaee F, Meednu N, Emo JA, *et al.* Polyinosinic:polycytidylic acid induces protein kinase D-dependent disassembly of apical junctions and barrier dysfunction in airway epithelial cells. *J Allergy Clin Immunol* 2011;128:1216–24.e11.
89. Yeo N-K, Jang YJ. Rhinovirus infection-induced alteration of tight junction and adherens junction components in human nasal epithelial cells. *Laryngoscope* 2010;120:346–52.
90. Sajjan U, Wang Q, Zhao Y, *et al.* Rhinovirus disrupts the barrier function of polarized airway epithelial cells. *Am J Respir Crit Care Med* 2008;178:1271–81.
91. Reiter B, Kraft R, Günzel D, *et al.* TRPV4-mediated regulation of epithelial permeability. *FASEB J* 2006;20:1802–12.
92. Yamaya M, Sekizawa K, Masuda T, *et al.* Oxidants affect permeability and repair of the cultured human tracheal epithelium. *Am J Physiol* 1995;268:L284–93.
93. Comstock AT, Ganesan S, Chatteraj A, *et al.* Rhinovirus-induced barrier dysfunction in polarized airway epithelial cells is mediated by NADPH oxidase 1. *J Virol* 2011;85:6795–808.
94. Kaul P, Biagioli MC, Singh I, *et al.* Rhinovirus-induced oxidative stress and interleukin-8 elaboration involves p47-phox but is independent of attachment to intercellular adhesion molecule-1 and viral replication. *J Infect Dis* 2000;181:1885–90.
95. Unger BL, Ganesan S, Comstock AT, *et al.* Nod-like receptor X-1 is required for rhinovirus-induced barrier dysfunction in airway epithelial cells. *J Virol* 2014;88:3705–18.
96. Arnout D, Soares F, Tattoli I, *et al.* An N-terminal addressing sequence targets NLRX1 to the mitochondrial matrix. *J Cell Sci* 2009;122:3161–8.
97. Tattoli I, Carneiro LA, Jéhanno M, *et al.* NLRX1 is a mitochondrial NOD-like receptor that amplifies NF- κ B and JNK pathways by inducing reactive oxygen species production. *EMBO Rep* 2008;9:293–300.
98. Kawai T, Takahashi K, Sato S, *et al.* IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. *Nat Immunol* 2005;6:981–8.
99. Meylan E, Curran J, Hofmann K, *et al.* Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. *Nature* 2005;437:1167–72.
100. Seth RB, Sun L, Ea C-K, *et al.* Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF- κ B and IRF 3. *Cell* 2005;122:669–82.
101. Hewson CA, Jardine A, Edwards MR, *et al.* Toll-like receptor 3 is induced by and mediates antiviral activity against rhinovirus infection of human bronchial epithelial cells. *J Virol* 2005;79:12273–9.
102. Soyka MB, Wawrzyniak P, Eiwegger T, *et al.* Defective epithelial barrier in chronic rhinosinusitis: the regulation of tight junctions by IFN- γ and IL-4. *J Allergy Clin Immunol* 2012;130:1087–96.e10.

103. Bruewer M, Luegering A, Kucharzik T, et al. Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. *J Immunol* 2003;171:6164–72.
104. Biagioli MC, Kaul P, Singh I, et al. The role of oxidative stress in rhinovirus induced elaboration of IL-8 by respiratory epithelial cells. *Free Radic Biol Med* 1999;26:454–62.
105. Wang JH, Kwon HJ, Jang YJ. Rhinovirus upregulates matrix metalloproteinase-2, matrix metalloproteinase-9, and vascular endothelial growth factor expression in nasal polyp fibroblasts. *Laryngoscope* 2009;119:1834–8.
106. Leigh R, Oyelusi W, Wiehler S, et al. Human rhinovirus infection enhances airway epithelial cell production of growth factors involved in airway remodeling. *J Allergy Clin Immunol* 2008;121:1238–45.e4.
107. Psarras S, Volonaki E, Skevaki CL, et al. Vascular endothelial growth factor-mediated induction of angiogenesis by human rhinoviruses. *J Allergy Clin Immunol* 2006;117:291–7.
108. Newcomb DC, Sajjan US, Nagarkar DR, et al. Human rhinovirus 1B exposure induces phosphatidylinositol 3-kinase-dependent airway inflammation in mice. *Am J Respir Crit Care Med* 2008;177:1111–21.
109. Bossios A, Psarras S, Gourgiotis D, et al. Rhinovirus infection induces cytotoxicity and delays wound healing in bronchial epithelial cells. *Respir Res* 2005;6:114.
110. Yuta A, Doyle WJ, Gaumont E, et al. Rhinovirus infection induces mucus hypersecretion. *Am J Physiol Lung Cell Mol Physiol* 1998;274:L1017–23.
111. Phipps RJ, Richardson PS. The effects of irritation at various levels of the airway upon tracheal mucus secretion in the cat. *J Physiol* 1976;261:563–81.
112. Hewson CA, Haas JJ, Bartlett NW, et al. Rhinovirus induces MUC5AC in a human infection model and in vitro via NF- κ B and EGFR pathways. *Eur Respir J* 2010;36:1425–35.
113. Zhu L, Lee P-K, Lee W-M, et al. Rhinovirus-induced major airway mucin production involves a novel TLR3-EGFR-dependent pathway. *Am J Respir Cell Mol Biol* 2009;40:610–19.
114. Inoue D, Yamaya M, Kubo H, et al. Mechanisms of mucin production by rhinovirus infection in cultured human airway epithelial cells. *Respir Physiol Neurobiol* 2006;154:484–99.
115. Zhu Z, Tang W, Gwaltney JM, et al. Rhinovirus stimulation of interleukin-8 in vivo and in vitro: role of NF-kappaB. *Am J Physiol* 1997;273:L814–24.
116. Zhu Z, Tang W, Ray A, et al. Rhinovirus stimulation of interleukin-6 in vivo and in vitro. Evidence for nuclear factor kappa B-dependent transcriptional activation. *J Clin Invest* 1996;97:421–30.
117. Casalino-Matsuda SM, Monzón ME, Forteza RM. Epidermal growth factor receptor activation by epidermal growth factor mediates oxidant-induced goblet cell metaplasia in human airway epithelium. *Am J Respir Cell Mol Biol* 2006;34:581–91.
118. Ishihara H, Shimura S, Satoh M, et al. Muscarinic receptor subtypes in feline tracheal submucosal gland secretion. *Am J Physiol* 1992;262:L223–8.
119. Ramnarine SI, Haddad EB, Khawaja AM, et al. On muscarinic control of neurogenic mucus secretion in ferret trachea. *J Physiol* 1996;494(Pt 2):577–86.
120. Iwase N, Sasaki T, Oshiro T, et al. Differential effect of epidermal growth factor on serous and mucous cells in porcine airway submucosal gland. *Respir Physiol Neurobiol* 2002;132:307–19.
121. Kanno H, Horikawa Y, Hodges RR, et al. Cholinergic agonists transactivate EGFR and stimulate MAPK to induce goblet cell secretion. *AJP Cell Physiol* 2003;284:C988–98.
122. Korfhagen TR, Kitzmiller J, Chen G, et al. SAM-pointed domain ETS factor mediates epithelial cell-intrinsic innate immune signaling during airway mucous metaplasia. *Proc Natl Acad Sci USA* 2012;109:16630–5.
123. Park K-S, Korfhagen TR, Bruno MD, et al. SPDEF regulates goblet cell hyperplasia in the airway epithelium. *J Clin Invest* 2007;117:978–88.
124. Oettgen P, Finger E, Sun Z, et al. PDEF, a novel prostate epithelium-specific ets transcription factor, interacts with the androgen receptor and activates prostate-specific antigen gene expression. *J Biol Chem* 2000;275:1216–25.
125. Chen G, Korfhagen TR, Xu Y, et al. SPDEF is required for mouse pulmonary goblet cell differentiation and regulates a network of genes associated with mucus production. *J Clin Invest* 2009;119:2914–24.
126. Bai J, Miao B, Wu X, et al. Enhanced expression of SAM-pointed domain-containing Ets-like factor in chronic rhinosinusitis with nasal polyps. *Laryngoscope* 2015;125:E97–103.
127. Kreda SM, Seminario-Vidal L, van Heusden CA, et al. Receptor-promoted exocytosis of airway epithelial mucin granules containing a spectrum of adenine nucleotides. *J Physiol* 2010;588:2255–67.
128. Seminario-Vidal L, Okada SF, Sesma JI, et al. Rho signaling regulates pannexin 1-mediated ATP release from airway epithelia. *J Biol Chem* 2011;286:26277–86.
129. Ransford GA, Fregien N, Qiu F, et al. Pannexin 1 contributes to ATP release in airway epithelia. *Am J Respir Cell Mol Biol* 2009;41:525–34.
130. Bao L, Locovei S, Dahl G. Pannexin membrane channels are mechanosensitive conduits for ATP. *FEBS Lett* 2004;572:65–8.
131. Locovei S, Wang J, Dahl G. Activation of pannexin 1 channels by ATP through P2Y receptors and by cytoplasmic calcium. *FEBS Lett* 2006;580:239–44.
132. Lieb T, Frei CW, Frohock JI, et al. Prolonged increase in ciliary beat frequency after short-term purinergic stimulation in human airway epithelial cells. *J Physiol* 2002;538:633–46.
133. Korngreen A, Priel Z. Purinergic stimulation of rabbit ciliated airway epithelia: control by multiple calcium sources. *J Physiol* 1996;497(Pt 1):53–66.
134. Iglesias R, Locovei S, Roque A, et al. P2X7 receptor-Pannexin1 complex: pharmacology and signaling. *Am J Physiol Cell Physiol* 2008;295:C752–60.
135. Locovei S, Scemes E, Qiu F, et al. Pannexin1 is part of the pore forming unit of the P2X(7) receptor death complex. *FEBS Lett* 2007;581:483–8.
136. Pelegrin P, Surprenant A. Pannexin-1 mediates large pore formation and interleukin-1beta release by the ATP-gated P2X7 receptor. *EMBO J* 2006;25:5071–82.
137. Yan Z, Khadra A, Li S, et al. Experimental characterization and mathematical modeling of P2X7 receptor channel gating. *J Neurosci* 2010;30:14213–24.
138. Jiang L-H, Rassendren F, Mackenzie A, et al. N-methyl-D-glucamine and propidium dyes utilize different permeation pathways at rat P2X(7) receptors. *Am J Physiol Cell Physiol* 2005;289:C1295–302.
139. Alenmyr L, Uller L, Greiff L, et al. TRPV4-mediated calcium influx and ciliary activity in human native airway epithelial cells. *Basic Clin Pharmacol Toxicol* 2014;114:210–16.
140. Lorenzo IM, Liedtke W, Sanderson MJ, et al. TRPV4 channel participates in receptor-operated calcium entry and ciliary beat frequency regulation in mouse airway epithelial cells. *Proc Natl Acad Sci USA* 2008;105:12611–16.
141. Andrade YN, Fernandes J, Vázquez E, et al. TRPV4 channel is involved in the coupling of fluid viscosity changes to epithelial ciliary activity. *J Cell Biol* 2005;168:869–74.
142. Watanabe H, Vriens J, Prenen J, et al. Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature* 2003;424:434–8.
143. Fernandes J, Lorenzo IM, Andrade YN, et al. IP3 sensitizes TRPV4 channel to the mechano- and osmotransducing messenger 5'-6'-epoxyeicosatrienoic acid. *J Gen Physiol* 2008;131:i2.
144. Fernández-Fernández JM, Andrade YN, Arniges M, et al. Functional coupling of TRPV4 cationic channel and large conductance, calcium-dependent potassium channel in human bronchial epithelial cell lines. *Pflügers Arch* 2008;457:149–59.
145. Liu X, Bandyopadhyay BC, Bandyopadhyay B, et al. A role for AQP5 in activation of TRPV4 by hypotonicity: concerted involvement of AQP5 and TRPV4 in regulation of cell volume recovery. *J Biol Chem* 2006;281:15485–95.
146. Taylor-Clark TE, Udem BJ. Sensing pulmonary oxidative stress by lung vagal afferents. *Respir Physiol Neurobiol* 2011;178:406–13.
147. Grünberg K, Timmers MC, Smits HH, et al. Effect of experimental rhinovirus 16 colds on airway hyperresponsiveness to histamine and interleukin-8 in nasal lavage in asthmatic subjects in vivo. *Clin Exp Allergy* 1997;27:36–45.
148. Empey DW, Laitinen LA, Jacobs L, et al. Mechanisms of bronchial hyperreactivity in normal subjects after upper respiratory tract infection. *Am Rev Respir Dis* 1976;113:131–9.
149. Madison JM, Irwin RS. Pharmacotherapy of chronic cough in adults. *Expert Opin Pharmacother* 2003;4:1039–48.
150. Myers AC, Kajekar R, Udem BJ. Allergic inflammation-induced neuropeptide production in rapidly adapting afferent nerves in guinea pig airways. *Am J Physiol Lung Cell Mol Physiol* 2002;282:L775–81.
151. Mazzone SB, Mori N, Canning BJ. Synergistic interactions between airway afferent nerve subtypes regulating the cough reflex in guinea-pigs. *J Physiol* 2005;569:559–73.
152. Lieu TM, Myers AC, Meeker S, et al. TRPV1 induction in airway vagal low-threshold mechanosensory neurons by allergen challenge and neurotrophic factors. *Am J Physiol Lung Cell Mol Physiol* 2012;302:L941–8.

153. Lieu T, Kollarik M, Myers AC, *et al.* Neurotrophin and GDNF family ligand receptor expression in vagal sensory nerve subtypes innervating the adult guinea pig respiratory tract. *Am J Physiol Lung Cell Mol Physiol* 2011;300:L790–8.
154. Hunter DD, Myers AC, Udem BJ. Nerve growth factor-induced phenotypic switch in guinea pig airway sensory neurons. *Am J Respir Crit Care Med* 2000;161:1985–90.
155. Neumann S, Doubell TP, Leslie T, *et al.* Inflammatory pain hypersensitivity mediated by phenotypic switch in myelinated primary sensory neurons. *Nature* 1996;384:360–4.
156. Woolf CJ. Phenotypic modification of primary sensory neurons: the role of nerve growth factor in the production of persistent pain. *Philos Trans R Soc B Biol Sci* 1996;351:441–8.
157. Ganju P, O'Bryan JP, Der C, *et al.* Differential regulation of SHC proteins by nerve growth factor in sensory neurons and PC12 cells. *Eur J Neurosci* 1998;10:1995–2008.
158. Othumpangat S, Regier M, Piedimonte G. Nerve growth factor modulates human rhinovirus infection in airway epithelial cells by controlling ICAM-1 expression. *Am J Physiol Lung Cell Mol Physiol* 2012;302:L1057–66.
159. El-Hashim AZ, Jaffal SM, Al-Rashidi FT, *et al.* Nerve growth factor enhances cough via a central mechanism of action. *Pharmacol Res* 2013;74:68–77.
160. El-Hashim AZ, Jaffal SM. Nerve growth factor enhances cough and airway obstruction via TrkA receptor- and TRPV1-dependent mechanisms. *Thorax* 2009;64:791–7.
161. Bonvini SJ, Birrell MA, Grace MS, *et al.* TRPV4 and activation of airway sensory nerves: the role of ATP. *Presented at Pharmacology 2014 Basic pharmacology oral communications (VII)*. 2014.
162. Aizawa N, Wyndaele J-J, Homma Y, *et al.* Effects of TRPV4 cation channel activation on the primary bladder afferent activities of the rat. *NeuroUrol Urodyn* 2012;31:148–55.
163. Kamei J, Takahashi Y. Involvement of ionotropic purinergic receptors in the histamine-induced enhancement of the cough reflex sensitivity in guinea pigs. *Eur J Pharmacol* 2006;547:160–4.
164. Basoglu OK, Barnes PJ, Kharitonov SA, *et al.* Effects of aerosolized adenosine 5'-triphosphate in smokers and patients with Chronic Obstructive Pulmonary Disease. *Chest* 2015;148:430–5.
165. Basoglu OK, Pelleg A, Essilfie-Quaye S, *et al.* Effects of aerosolized adenosine 5'-triphosphate vs adenosine 5'-monophosphate on dyspnea and airway caliber in healthy nonsmokers and patients with asthma. *Chest* 2005;128:1905–9.
166. Abdulqawi R, Dockry R, Holt K, *et al.* P2X3 receptor antagonist (AF-219) in refractory chronic cough: a randomised, double-blind, placebo-controlled phase 2 study. *Lancet* 2015;385:1198–205.
167. Chiu IM, von Hehn CA, Woolf CJ. Neurogenic inflammation and the peripheral nervous system in host defense and immunopathology. *Nat Neurosci* 2012;15:1063–7.
168. Durham PL. Calcitonin gene-related peptide (CGRP) and migraine. *Headache* 2006;46(Suppl 1):S3–8.
169. Barnes PJ. Neurogenic inflammation in the airways. *Respir Physiol* 2001;125:145–54.
170. Gentile DA, Skoner DP. Viral rhinitis. *Curr Allergy Asthma Rep* 2001;1:227–34.
171. Diogenes A, Ferraz CCR, Akopian AN, *et al.* LPS sensitizes TRPV1 via activation of TLR4 in trigeminal sensory neurons. *J Dent Res* 2011;90:759–64.
172. Qi J, Buzas K, Fan H, *et al.* Painful pathways induced by TLR stimulation of dorsal root ganglion neurons. *J Immunol* 2011;186:6417–26.
173. Liu T, Xu Z-Z, Park C-K, *et al.* Toll-like receptor 7 mediates pruritus. *Nat Neurosci* 2010;13:1460–2.
174. Park C-K, Xu Z-Z, Berta T, *et al.* Extracellular microRNAs activate nociceptor neurons to elicit pain via TLR7 and TRPA1. *Neuron* 2014;82:47–54.
175. Winkler CW, Taylor KG, Peterson KE. Location is everything: let-7b microRNA and TLR7 signaling results in a painful TRP. *Sci Signal* 2014;7:pe14.
176. Souslova V, Cesare P, Ding Y, *et al.* Warm-coding deficits and aberrant inflammatory pain in mice lacking P2X3 receptors. *Nature* 2000;407:1015–17.
177. Lakshmi S, Joshi PG. Co-activation of P2Y2 receptor and TRPV channel by ATP: implications for ATP induced pain. *Cell Mol Neurobiol* 2005;25:819–32.
178. Binshtok AM, Wang H, Zimmermann K, *et al.* Nociceptors are interleukin-1beta sensors. *J Neurosci* 2008;28:14062–73.
179. Nicol GD, Lopshire JC, Pafford CM. Tumor necrosis factor enhances the capsaicin sensitivity of rat sensory neurons. *J Neurosci* 1997;17:975–82.
180. Nagata K, Duggan A, Kumar G, *et al.* Nociceptor and hair cell transducer properties of TRPA1, a channel for pain and hearing. *J Neurosci* 2005;25:4052–61.
181. Corey DP, García-Añoveros J, Holt JR, *et al.* TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. *Nature* 2004;432:723–30.
182. Kwan KY, Allchorne AJ, Vollrath MA, *et al.* TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. *Neuron* 2006;50:277–89.
183. Tritsch NX, Yi E, Gale JE, *et al.* The origin of spontaneous activity in the developing auditory system. *Nature* 2007;450:50–5.
184. Araki I. TRP channels in urinary bladder mechanosensation. In: Islam S, ed. *Transient receptor potential channels: advances in experimental medicine and biology*, 2011:861–79.
185. Andersson K-E, Gratzke C, Hedlund P. The role of the transient receptor potential (TRP) superfamily of cation-selective channels in the management of the overactive bladder. *BJU Int* 2010;106:1114–27.
186. Kaan TKY, Yip PK, Grist J, *et al.* Endogenous purinergic control of bladder activity via presynaptic P2X3 and P2X2/3 receptors in the spinal cord. *J Neurosci* 2010;30:4503–7.
187. Laing RJ, Dhaka A. ThermoTRPs and Pain. *Neuroscientist* 2015. Published Online First: 21 January 2015.
188. Julius D. TRP channels and pain. *Annu Rev Cell Dev Biol* 2013;29:355–84.
189. Ulmann L, Hatcher JP, Hughes JP, *et al.* Up-regulation of P2X4 receptors in spinal microglia after peripheral nerve injury mediates BDNF release and neuropathic pain. *J Neurosci* 2008;28:11263–8.
190. Chessell IP, Hatcher JP, Bountra C, *et al.* Disruption of the P2X7 purinoceptor gene abolishes chronic inflammatory and neuropathic pain. *Pain* 2005;114:386–96.
191. McGaraughty S, Wismer CT, Zhu CZ, *et al.* Effects of A-317491, a novel and selective P2X3/P2X2/3 receptor antagonist, on neuropathic, inflammatory and chemogenic nociception following intrathecal and intraplantar administration. *Br J Pharmacol* 2003;140:1381–8.
192. Cockayne DA, Hamilton SG, Zhu QM, *et al.* Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. *Nature* 2000;407:1011–15.
193. Gum RJ, Wakefield B, Jarvis MF. P2X receptor antagonists for pain management: examination of binding and physicochemical properties. *Purinergic Signal* 2012;8:41–56.
194. Guo A, Vulchanova L, Wang J, *et al.* Immunocytochemical localization of the vanilloid receptor 1 (VR1): relationship to neuropeptides, the P2X3 purinoceptor and IB4 binding sites. *Eur J Neurosci* 1999;11:946–58.
195. Abdullah H, Heaney LG, Cosby SL, *et al.* Rhinovirus upregulates transient receptor potential channels in a human neuronal cell line: implications for respiratory virus-induced cough reflex sensitivity. *Thorax* 2014;69:46–54.
196. Johnston SL. Overview of virus-induced airway disease. *Proc Am Thorac Soc* 2005;2:150–6.
197. McGarvey LP, Butler CA, Stokesberry S, *et al.* Increased expression of bronchial epithelial transient receptor potential vanilloid 1 channels in patients with severe asthma. *J Allergy Clin Immunol* 2014;133:704–12.e4.
198. Mitchell JE, Campbell AP, New NE, *et al.* Expression and characterization of the intracellular vanilloid receptor (TRPV1) in bronchi from patients with chronic cough. *Exp Lung Res* 2005;31:295–306.
199. Groneberg DA, Niimi A, Dinh QT, *et al.* Increased expression of transient receptor potential vanilloid-1 in airway nerves of chronic cough. *Am J Respir Crit Care Med* 2004;170:1276–80.
200. Dicipinigaitis PV, Spinner L, Santhyadka G, *et al.* Effect of tiotropium on cough reflex sensitivity in acute viral cough. *Lung* 2008;186:369–74.
201. Pratter MR. Cough and the common cold: ACCP evidence-based clinical practice guidelines. *Chest* 2006;129:72S–4S.