BJP British Journal of Pharmacology

DOI:10.1111/bph.12538
www.brjpharmacol.org

REVIEW

Transient receptor potential (TRP) channels in the airway: role in airway disease

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Keywords
asthma; COPD; TRP channels; airway

Received
7 August 2013
Accepted
18 November 2013

Over the last few decades, there has been an explosion of scientific publications reporting the many and varied roles of transient receptor potential (TRP) ion channels in physiological and pathological systems throughout the body. The aim of this review is to summarize the existing literature on the role of TRP channels in the lungs and discuss what is known about their function under normal and diseased conditions. The review will focus mainly on the pathogenesis and symptoms of asthma and chronic obstructive pulmonary disease and the role of four members of the TRP family: TRPA1, TRPV1, TRPV4 and TRPM8. We hope that the article will help the reader understand the role of TRP channels in the normal airway and how their function may be changed in the context of respiratory disease.

Abbreviation

4αPDD, 4α-phorbol-12,13-didecanoate; AHR, airway hyper-responsiveness; CGRP, calcitonin gene-related peptide; COPD, chronic obstructive pulmonary disease; DRG, dorsal root ganglia; HASM, human airway smooth muscle; LAR, late asthmatic response: OTC, over-the-counter; PAR, protease-activated receptors; SNP, single nucleotide polymorphism; TRP, transient receptor potential

Introduction to transient receptor potential (TRP) ion channels

TRP channels were discovered in the eye of the *Drosophila melanogaster* fly, and named for their transient response to bright light (Montell and Rubin, 1989). Several homologues have since been identified that have a well conserved 'TRP domain' consisting of 23–25 amino acids. There are 28 mammalian TRP subunits, categorized in to six related protein subfamilies, based on sequence homology (Clapham, 2003). TRP ion channels are widely expressed throughout the body, and can respond to a remarkable diversity of intracellular and extracellular stimuli. This capacity to be activated by seemingly disparate mechanisms has led to the perception of TRP channels as multiple signal integrators. The TRP channel superfamily comprises a group of cation-selective proteins, which display a general preference for calcium ions. TRPs exhibit six transmembrane-spanning domains with the channel pore located between transmembrane domains 5 and 6, intracellular C and N termini, and varying degrees of ankyrin repeats (Caterina *et al.*, 1997; Ramsey *et al.*, 2006). Current evidence suggests that active TRP channels are formed by four subunits, and could assemble as homo- or hetero-tetramers (Latorre *et al.*, 2009). For more in-depth information on TRP channels, we suggest consulting some of the excellent reviews including Clapham, 2003; Nilius *et al.* 2005; Bessac & Jordt, 2008; Preti *et al.* 2012.

Aim of the review

The aim of this review is to describe the TRP channels, which have been explored in more depth regarding their role in physiological and pathological mechanisms in the airways

(TRPA1, TRPV1, TRPV4 and TRPM8; receptor nomenclature follows Alexander *et al.*, 2013). In particular, we will focus on the pathogenesis and symptoms associated with asthma and chronic obstructive pulmonary disease (COPD). Both of these diseases are the subject of many excellent reviews and we suggest the reader consults these for a more complete understanding (Barnes, 2010; 2013; Murphy and O'Byrne, 2010; Pawankar *et al.*, 2012; Vestbo *et al.*, 2013). Below, we have provided an overview of asthma and COPD designed to briefly highlight key features of the diseases and emphasize aspects that will be referred to later when we discuss the role of TRP channels.

Asthma

Asthma is a chronic inflammatory disease of the airways, which is defined based on descriptive phenotypes, including variable airflow obstruction, airway hyper-responsiveness (AHR) and the presence of symptoms such as cough, dyspnoea, wheezing and chest tightness (Lemanske and Busse, 2003; Bateman *et al.*, 2008). Asthma is now one of the most prevalent diseases in the world, and the global prevalence is increasing, particularly in developing countries (Bateman *et al.*, 2008; Barnes, 2010).

Asthma is often associated with allergy and patients typically present with increased levels of IgE (atopy). It is thought that it is the repeated exposure to these allergen(s) that leads to airway inflammation and the activation of resident cells such as epithelial cells and alveolar macrophages. The inflammatory profile typically presents as an increase in cells such as Th-2 cells, eosinophils, and mast cells and of mediators such as IL-4, IL-5, IL-13, eotaxin (CCL11) and eicosanoids. The dogma is that this increase in inflammatory status subsequently drives the pathophysiological changes in the asthmatic airway and the associated symptoms. Furthermore, exposure to the allergen often leads to an early asthmatic response, which manifests as brief, acute bronchoconstriction thought to be driven by the release of preformed mediators. This can be followed by a late asthmatic response (LAR), which lasts longer and has been reported by the asthmatic sufferers as the defining feature of the disease (O'Byrne *et al.*, 2009). This LAR type phenotype can be induced by other triggers such as isocyanate in patients suffering from occupational asthma (Kenyon *et al.*, 2012), exercise and cold air. In addition, work has indicated that mediators are released from sensory nerves, such as tachykinins and calcitonin gene-related peptide (CGRP), that induce a local inflammatory response, termed 'neurogenic inflammation' (see Barnes *et al.*, 1991a,b), which can comprise extravasation of plasma and leukocytes, mucus hypersecretion and airway constriction (Holzer, 1988; Fozard *et al.*, 2001; Reilly *et al.*, 2003; Karmouty-Quintana *et al.*, 2007). The relevance of neurogenic inflammation in the normal and diseased human airway is still unclear.

Asthma patients can also suffer from disease exacerbations. These are often associated with respiratory infections and are characterized by episodes of progressive increases in dyspnoea, cough, wheezing and/or chest tightness (Barnes, 2010). During these episodes, the effectiveness of the normal medication is often compromised.

Typically, treatment is aimed at either alleviating the bronchospasm with inhaled bronchodilators such as short- or long-acting β_2 adrenoceptor agonists. These are thought to act by relaxing the smooth muscle, which leads to opening of the airways and increased air flow (Barnes, 2011a). The second treatment approach is to target the inflammation thought to be responsible for the compromised lung function; these include inhaled or systemic (in more severe patients) glucocorticoids (Barnes, 2011b). Often, these two approaches are prescribed as a combined treatment (Barnes, 2011b). While the current treatments are effective in many asthmatics, their safety profile is not ideal and there are a proportion of asthma sufferers, often the ones with the more severe disease phenotype, for which current therapies are ineffective (Barnes, 2013). Furthermore, the current treatments are largely aimed at the control of the manifestations of disease rather than prevention or cure (Bateman *et al.*, 2008; Barnes, 2011b).

COPD

COPD is a term encompassing a heterogeneous group of airway pathologies, including chronic bronchitis, bronchiolitis and emphysema, and is associated with chronic airway inflammation (Bateman *et al.*, 2008). In contrast to asthma, COPD is characterized by airflow limitation that is not fully reversible and is usually progressive, leading to increasing levels of disability and ultimately death. COPD is a global health problem, currently ranked as the fourth most common cause of death worldwide (WHO, 2004). Key symptoms in COPD include shortness of breath, wheezing, lack of mobility and excessive coughing. The vast majority of COPD is linked to smoking status (Sethi and Rochester, 2000), although exposure to smoke from biomass fires (i.e. wood smoke) is also an important factor (Salvi and Barnes, 2009). The increasing prevalence of COPD is probably driven by the growing popularity of smoking, particularly in developing countries, as well as higher global life expectancy. It is believed that smoke-induced chronic inflammation drives pathogenesis of the disease. This, typically, involves activation of lung resident cells, an increase in inflammatory cells (such as neutrophils) and mediators (such as IL-1β, IL-8). Unlike in the asthmatic lung, treatment with glucocorticoids does not appear to affect the inflammation present in COPD. Indeed, currently, there are no treatments that reduce COPD-associated mortality (Calverley *et al.*, 2007; Barnes, 2013). As with asthmatics, COPD patients can have episodes of exacerbations, and it is these that are often responsible for the majority of the social and economic burden.

It is clear that asthma and COPD represent diseases that affect a large proportion of the world's population and, furthermore, the numbers of patients are actually increasing. They are a huge burden on health care and society, and thus, there is an urgent need for new, safe, effective medication. Understanding the normal physiological processes in the airways and how they change in disease conditions is likely to help develop new treatments. In this review, we will discuss the role of four TRP channels (TRPA1, TRPV1, TRPV4 and TRPM8) in both physiological and pathological mechanisms in the airway. Many activators of these TRP channels are

known to be increased in the diseased airway, including eicosanoids and their breakdown products, factors associated with reactive oxidant stress, low pH, osmolarity changes and altered temperature. Moreover, the disease state is known to increase sensitivity to endogenous and exogenous TRP ligands. Thus, it appears that the normal protective role of these channels can change to drive the disease status and be responsible for the associated symptoms. The focus of the review will be based on discussing the published literature on: expression of the TRP channels (under normal and disease settings), role in sensory nerve activation (predominantly cough), role in the control of airway tone (directly via actions on airway smooth muscle or through a central parasympathetic reflex) and involvement in the inflammation in the airway. The results discussed will focus on data measuring the target at the mRNA level, or implied in the functional studies because currently, the tools for measuring the expression of these TRP channels at the protein level are of limited use. The IUPHAR database is an excellent source of information on TRP expression patterns in various tissues. Furthermore, we recommend that anyone interested in this area also read some of the excellent reviews available, for example, Li *et al*. 2003; Nassini *et al*. 2010; Banner *et al*. 2011; Guibert *et al*. 2011; Grace *et al*. 2012b; Preti *et al*. 2012; Abbott-Banner *et al*. 2013.

TRPA1 (ankyrin 1) channels

Human TRPA1 channels were first isolated from cultured fibroblasts (Jaquemar *et al*., 1999), and originally named ANKTM1 due to the large number of ankyrin repeat domains. Only one homologue of the ankyrin subfamily has been identified in mammals. The human trpa1 gene is composed of 27 exons, spanning 55 701 base pairs of the human chromosome 8q13 (Nilius *et al*., 2012).

The TRPA1 channel has emerged as an important sensor of noxious stimuli and tissue damage (Rech *et al*., 2010). TRPA1 agonists include extracts from spicy foods, such as mustard oil, garlic, cinnamon and wasabi (Bandell *et al*., 2004; Jordt *et al*., 2004; Macpherson *et al*., 2005), environmental irritants present in air pollution, vehicle exhaust and cigarette smoke (isothiocyanates and acrolein; Bautista *et al*., 2006; Facchinetti *et al*., 2007; Andrè *et al*., 2008; Brone *et al*., 2008) and products of oxidation (4-oxynonenal and 4-hydroxynonenal; Trevisani *et al*., 2007; Taylor-Clark *et al*., 2008). There is also evidence that TRPA1 channels are activated by cold temperatures below 17°C (Story *et al*., 2003; Zurborg *et al*., 2007; see Caspani and Heppenstall, 2009), although this finding is controversial (Zhou *et al*., 2011). TRPA1 channels are also reported to play a role in GPCR signalling in sensory nerves. There is evidence for both sensitization and activation of TRPA1 channels by bradykinin (via the B_2 receptor; Bandell *et al*., 2004; Grace *et al*., 2012a) and PGE_2 (via the EP_3 receptor; Bang *et al*., 2007; Maher *et al*., 2009).

TRPA1 channels in the airways

TRPA1 ion channels are thought to be mainly expressed in neurons, including the spinal dorsal root ganglia (DRG), nasal trigeminal and vagal airway neurons (Story *et al*., 2003; Bandell *et al*., 2004; Bautista *et al*., 2005; Nassenstein *et al*., 2008; Jang *et al*., 2012). Although there are some reports to suggest TRPA1 is present in non-neuronal tissues including human and mouse lung (Kunert-Keil *et al*., 2006; Stokes *et al*., 2006). In addition, there are reports of expression of TRPA1 channels in asthma/COPD-relevant cell types, that is, CD4+ and CD8+ T cells, B cells and mast cells (Prasad *et al*., 2008; Banner *et al*., 2011). It is not clear whether these expression levels change under disease conditions; furthermore, we could find very little information on genetic changes in TRPA1 channels. Indeed, Smit *et al*. (2012) presented data to suggest there are no single nucleotide polymorphisms (SNPs) of TRPA1 channels that associate smoking or occupational exposures with cough.

Triggering TRPA1 channels causes the activation of vagal bronchopulmonary C-fibres in rodent lungs (Bessac and Jordt, 2008; Nassenstein *et al*., 2008; Taylor-Clark *et al*., 2008; 2009; Andrè *et al*., 2009; Birrell *et al*., 2009; Grace *et al*., 2012b). Activation of TRPA1 channels has been shown to stimulate isolated human vagal tissue and evoke cough in guinea pigs and in healthy human subjects (Birrell *et al*., 2009). Thus, it seems that the TRPA1 channel is part of the lung's defence system. Indeed, TRPA1 activation has become a key mechanism for evoking cough associated with environmental and occupational exposure to respiratory irritants (Grace *et al*., 2011). Harmful environmental irritants and industrial pollutants have been shown to activate TRPA1 in the airways (Deering-Rice *et al*., 2011; Shapiro *et al*., 2013). Similar irritants have also been reported to cause asthma-like symptoms such as cough, wheezing, dyspnoea and subsequent hypersensitivity to chemical and physical stimuli (Preti *et al*., 2012). Furthermore, the TRPA1 channel may be involved in hypersensitivity to noxious stimuli in disease states (Petrus *et al*., 2007). The possibility exists that chronic cough associated with disease states is caused by the inflammation in the airways, that is, the release of endogenous TRPA1 agonists such as reactive oxygen species and products of lipid peroxidation, and indirect channel openers such as PGs and bradykinin (Grace and Belvisi, 2011).

TRPA1 activation has been linked to increased bronchoconstriction and AHR. Recently, a role for TRPA1 channels in the LAR was established in rodent models of allergic asthma (Raemdonck *et al*., 2012). This study demonstrated that antigen challenge leads to the activation of airway sensory nerves which in turn triggers a central neuronal reflex and a subsequent parasympathetic cholinergic constrictor response. The data showed that blockade of the TRPA1 , but not TRPV1, channels inhibited the LAR. This suggests that TRPA1 ligands are induced in the allergic lung and trigger activation of sensory nerves or that this channel is responsible for a key aspect in the response (e.g. affecting mast cells). Another possibility is that the TRPA1 channel is involved in the control of airway tone. Indeed, Andrè *et al*. (2008) reports that activation of TRPA1 channels also causes constriction of isolated guinea pig bronchus secondary to the release of neuropeptides via a local axon reflex event. So it would seem, as with the cough reflex, that TRPA1 channels are present in the airway to trigger bronchoconstriction as a protective measure but under disease conditions they could be involved in the development of a disease phenotype.

Recently, it has been reported that TRPA1 channels are involved in non-allergic AHR (Hox *et al.*, 2013). The authors modelled the increase in incidents of AHR reported to occur in swimmers (via increased exposure to hypochlorite) in the mouse and showed an involvement of TRPA1-dependent stimulation of sensory neurons and mast cell activation. Similarly, Caceres *et al.* (2009) reported that the TRPA1 channel was central to the AHR observed in murine models of allergic asthma. The authors used genetically altered mice and a TRPA1 antagonist, and showed a reduction in allergen-induced mediator production, leukocyte infiltration and mucus levels. It was postulated that the reduced inflammation could account for the lack of AHR development in these mice, and that inhibition of neuropeptide release could explain the impaired inflammatory response (Caceres *et al.*, 2009).

TRPA1 channels have also been implicated in the pathogenesis of COPD. It has been suggested that TRPA1-induced neurogenic inflammation could contribute to the progression of COPD (Andrè *et al.*, 2008). Animal models show that TRPA1 channels play a major role in the early phase of bronchial inflammation to cigarette smoke (Andrè *et al.*, 2008). Cigarette smoke is known to contain several irritants that activate TRPA1, including acrolein and crotonaldehyde (Bautista *et al.*, 2006; Facchinetti *et al.*, 2007; Andrè *et al.*, 2008; 2009; Lin *et al.*, 2010), and nicotine has been reported to directly activate TRPA1 channels (Talavera *et al.*, 2009). Moreover, Shapiro *et al.* (2013) have recently shown that exposure to wood smoke activates ganglia cells and epithelial cells through a TRPA1-dependent mechanism. They suggest this is evidence to implicate TRPA1 channels in symptoms and disease associated with exposure to wood smoke. Activation of human lung fibroblasts and other structural cells by cigarette smoke also promotes the release of IL-8 (Keatings *et al.*, 1996; Crooks *et al.*, 2000; Gompertz *et al.*, 2001; Mukhopadhyay *et al.*, 2011). Nassini *et al.* (2012) reported that cultured human airway fibroblasts, epithelial and smooth muscle cells released IL-8 after stimulation of TRPA1 channels with cigarette smoke extract. Furthermore, they showed that activation of TRPA1 in the airways caused the release of the murine orthologue of IL-8, KC, through a non-sensory nerve driven mechanism. Thus, it would seem that TRPA1 channels in non-neuronal tissues/cells could be involved in triggering airway inflammation, possibly as a lung-defensive mechanism, but overstimulation or oversensitivity could be associated with disease states.

TRPV1 (vanilloid 1) channels

The TRPV1 (originally vanilloid receptor 1 or VR1) channel was the founding member for this subclass of TRP channels, cloned from rodent DRG neurons, and named for the essential vanilloid moiety on the classical agonists capsaicin and resiniferatoxin (Caterina *et al.*, 1997). Subsequent members of the vanilloid family of channels, TRPV2-6, were categorized according to sequence homology. The human trpv1 gene is located on human chromosome 17p13 and composed of 17 exons (Birnbaumer *et al.*, 2003). Early studies identified TRPV1 as a neuronally expressed ion channel that senses noxious heat and pain (Caterina *et al.*, 1997). TRPV1 is now known to be a polymodal channel that is activated by diverse stimuli such as irritant chemicals, acidic pH, hot temperatures (>43°C) and endogenous mediators, and is thought to be important in integrating these disparate signals (Caterina *et al.*, 1997; Zygmunt *et al.*, 1999; Hwang *et al.*, 2000; Jordt *et al.*, 2000; Jia *et al.*, 2002; Kagaya *et al.*, 2002; Carr *et al.*, 2003; Kollarik and Undem, 2004; Moriyama *et al.*, 2005; Tominaga and Tominaga, 2005; Zhou *et al.*, 2011). Noxious exogenous irritants such as capsaicin and resiniferatoxin, and some endogenous mediators such as acid (Jordt *et al.*, 2000) and anandamide (an endocannabinoid eicosanoid derivative; Zygmunt *et al.*, 1999; Jia *et al.*, 2002; Kagaya *et al.*, 2002), bind directly and open TRPV1 ion channels (Caterina *et al.*, 1997). Some agonists are thought to be able to both activate and sensitize TRPV1 channels, and it is not always possible to distinguish whether a stimulus acts as a direct opener or rather a sensitizer, which lowers the activation threshold for another stimulus. For example, low pH and heat can gate TRPV1, causing channel opening, or lower the activation threshold of TRPV1, leading to channel sensitization (Caterina *et al.*, 1997; Tominaga *et al.*, 1998; Trevisani *et al.*, 2002). TRPV1 is also thought to play a role in some GPCR signalling pathways, for example, bradykinin (Chuang *et al.*, 2001; Carr *et al.*, 2003; Kollarik and Undem, 2004; Bautista *et al.*, 2006; Grace *et al.*, 2012a), prostanoids such as PGE_2 (Moriyama *et al.*, 2005; Grace *et al.*, 2012a), nerve growth factor (Chuang *et al.*, 2001), histamine (Kajihara *et al.*, 2010) and activating peptides of protease activated receptors (PARs; Amadesi *et al.*, 2004; Gatti *et al.*, 2006).

TRPV1 channels in the airways

As with TRPA1, TRPV1 channels were initially thought to be confined to nociceptive neurons and was shown to be highly expressed in dorsal root, trigeminal and vagal ganglia (Caterina *et al.*, 1997; Ahluwalia *et al.*, 2000; Ichikawa and Sugimoto, 2004). TRPV1-positive nerve fibres innervate the entire respiratory tract, including the nose, larynx and trachea of the upper airways, lung parenchyma, alveoli, smooth muscle and blood vessels (Watanabe *et al.*, 2006). However, TRPV1 channel expression has now been identified in a range of tissues and organs. Expression levels in the lung tissue and airway structural cells are reported to be low (Kunert-Keil *et al.*, 2006; Banner *et al.*, 2011; Jang *et al.*, 2012). But preliminary data suggests that TRPV1 mRNA expression in the lung tissue is increased in patients with emphysema compared with healthy non-smokers and non-smokers (Baxter *et al.*, 2012). Furthermore, SNPs in TRPV1 channels were associated with cough among subjects without asthma from two independent studies in eight European countries. TRPV1 SNPs may enhance susceptibility to cough in current smokers and in subjects with a history of workplace exposures (Smit *et al.*, 2012). Cantero-Recasens *et al.* (2010) also report on the association of a functional SNP, TRPV1-I585V, with childhood asthma. This SNP was genotyped in a population of 470 controls without respiratory symptoms and 301 asthmatics. Although this SNP did not modify the risk of suffering from asthma, carriers of the TRPV1-I585V genetic variant showed a lower risk of current wheezing or cough. Functional analysis of TRPV1-I585V, using the Ca^{2+}-sensitive

dye fura-2 to measure intracellular Ca^{2+} concentrations, revealed a decreased channel activity in response to two typical TRPV1 stimuli, heat and capsaicin. These data provide genetic evidence that TRPV1 channels play an important role in intracellular Ca^{2+} dysregulation in asthma pathophysiology.

Due to its ability to be activated and functionally sensitized by pro-inflammatory mediators and noxious stimuli, TRPV1 is considered to be a 'pathological receptor' that plays an important role in the transduction of noxious stimuli, and in the maintenance of inflammatory conditions (Ferrer-Montiel *et al.*, 2012). The vast majority of research to date has focused on the role of TRPV1 channels in sensory nerves, including the mechanisms driving the cough reflex. Agonists such as capsaicin and citric acid stimulate cough in humans and animal models, which is inhibited by TRPV1 antagonists (Collier and Fuller, 1984; Lalloo *et al.*, 1995; Trevisani *et al.*, 2004; McLeod *et al.*, 2006; Bhattacharya *et al.*, 2007; Grace *et al.*, 2012b). Moreover, allergen-induced cough responses in ovalbumin-sensitized guinea pigs (modelling asthma) are also inhibited by TRPV1 antagonism (McLeod *et al.*, 2006). Furthermore, as with TRPA1, the TRPV1 channel is present on airway sensory nerve endings as part of the lung defence mechanism. Indeed, Deering-Rice *et al.* (2012) recently showed that environmental particulate matter pollutants are sensed by TRPV1.

Interestingly, asthma and COPD are associated with increased cough sensitivity to capsaicin inhalation (O'Connell *et al.*, 1996; Doherty *et al.*, 2000; Higenbottam, 2002; Nakajima *et al.*, 2006; Plevkova *et al.*, 2006; Pecova *et al.*, 2008). Theories could be put forward as to how this occurs and which population of afferents and targets are involved. Firstly, inflammation could lead to oedema formation or bronchoconstriction, which in turn activate Aδ-fibre, low-threshold mechanosensors; secondly, inflammatory mediators could 'sensitize' C-fibre afferent nerve endings, which may lead to an increase in the electrical excitability of afferent nerves so that they have a reduced threshold for activation; thirdly, certain pro-tussive inflammatory mediators are increased in airway inflammatory diseases (Baumgarten *et al.*, 1992; Profita *et al.*, 2003), which could lead to the persistent activation and increased action potential discharge in vagal C-fibre afferent nerves. Finally, in some cases, cough still persists after an inflammatory response or viral/bacterial infection subsides. This is probably due to a change in the gene expression profile in the airway afferent nerves leading to a phenotype change. This phenomenon has been reported in the somatosensory system and can lead to phenotype changes in which neurons with low-threshold mechanoreceptors take on a chemosensitive or nociceptor phenotype by expressing the neuropeptide substance P, which is typically limited to C-fibre afferents (Neumanns *et al.*, 1996). This has been mimicked in animal models of disease, whereby cigarette smoke exposure, allergy and virus have been observed to cause hypersensitivity to TRPV1 agonist inhalation and in some cases a phenotypic change in the airway nerves has been proposed as the mechanism responsible (Karlsson *et al.*, 1991; Carr *et al.*, 2002; Myers *et al.*, 2002; Lewis *et al.*, 2007; Zhang *et al.*, 2008; Maher *et al.*, 2010; Grace *et al.*, 2011; Ye *et al.*, 2011; Lieu *et al.*, 2012). Phenotype changes in airway vagal Aδ-fibres in an asthma model in sensitized guinea pigs following allergen challenge (Zhang *et al.*, 2008) have been reported. In these studies, allergic airway inflammation can induce the expression of TRPV1 channels in tracheal Aδ-fibres (that do not express TRPV1 under 'normal' circumstances) secondary to neurotrophic factor production. Furthermore, sensitization or up-regulation of the cough reflex in humans is associated with increased expression of TRPV1 channels in airway nerves in comparison to healthy controls (Groneberg *et al.*, 2004; Mitchell *et al.*, 2005; Butler *et al.*, 2010). This data suggests that the peripheral nervous system is malleable and capable of adapting to its environment by expanding the range of stimuli that activate certain nerve fibres. This could help to explain the augmented expression of TRPV1 channels and excessive tussive response observed during chronic inflammatory pathologies both in animal models and in human respiratory disease.

Other molecular mechanisms (some of which are mentioned above) may also contribute to the enhanced TRPV1-mediated cough response in disease states. For example, patients with inflammatory airway disease exhibit more acidic exhaled breath condensate than healthy controls (Takemura *et al.*, 2008; MacNee *et al.*, 2011) and inhalation of low pH solutions is known to induce coughing partly via the TRPV1 ion channel. Furthermore, it is known that pH levels in the diseased airway are lower than normal, which may either directly activate TRPV1 channels or increase their opening potential thus making them more sensitive to other stimuli (Jack *et al.*, 1994). In addition, enhanced release of pro-tussive inflammatory mediators that activate TRPV1 (e.g. PGE_2, bradykinin, lipoxygenase metabolites), as well as mediators that act to sensitize TRPV1 (e.g. proteases via PARs) are also associated with inflammatory disease (Gatti *et al.*, 2006; Grace *et al.*, 2012a). Therefore, enhanced release of mediators that activate or sensitize TRPV1 channels could contribute to chronic cough pathologies. As a result, TRPV1 channels have been identified as a potential pharmacological target for the development of novel anti-tussive therapies.

TRPV1 channel agonists have been shown to cause bronchoconstriction in humans and animals (Fuller *et al.*, 1985; Lalloo *et al.*, 1995). In animals, the response is thought to involve local 'neurogenic inflammation' and activation of a central reflex. Isolated guinea pig trachea smooth muscle contracts in response to activation of TRPV1 channels (Belvisi *et al.*, 1992). This response is thought to involve the release of sensory neuropeptides, such as substance P and neurokinin A, which subsequently activates neurokinin receptors on the airway smooth muscle. Neurogenic mediators are released from stores in the nerve endings after stimulation of TRPV1 channels; these stores are finite, which may explain why these responses are poorly maintained and not repeatable in the same tissue (Barnes *et al.*, 1991a,b). In the whole animal, responses to inhaled TRPV1 agonists was partly blocked with neurokinin receptor antagonists and partly with muscarinic receptor antagonists (Barnes *et al.*, 1991a,b). This data demonstrates the role of the central reflex. The effect of inhaling TRPV1 agonists is less clear in man; some groups report bronchoconstriction which is inhibited by ipratropium bromide indicating that it was dependent on a cholinergic vagal reflex rather than on local release of substance P from nerves in the airway (Fuller *et al.*, 1985) while others suggest that capsaicin causes bronchodilation (Ichinose *et al.*, 1988; Preti *et al.*,

2012). These last observations are similar to observations reported in isolated mouse trachea. Manzini (1992) reported that capsaicin triggers the release of substance P, activation of a neurokinin receptor leading to the release of PGE_2 and relaxation. TRPV1-mediated actions on isolated human airway are varied (Lundberg and Saria, 1983; Chitano *et al.*, 1994; Preti *et al.*, 2012). Apparent differences between animal and human experiments could be explained by the relatively sparse release of tachykinins from human tissue (Spina *et al.*, 1998; Preti *et al.*, 2012). In summary, it is currently not clear if activation of TRPV1 channels causes bronchospasm in humans and whether these responses are altered under disease conditions or whether blockade of the channel affects airway obstruction in disease. We suggest a study designed at addressing this would be very interesting.

The importance of non-neuronal TRPV1 channels in the airways is being increasingly recognized, particularly in inflammatory processes. As mentioned, epithelial cells are thought to play an important role in both asthma and COPD. Recently, there has been a lot of interest in studying the role of the TRPV1 channels in these cell types. Exposure to inhaled pollutants was reported to increase TRPV1 receptor gene expression in rat bronchus (Costa *et al.*, 2010). A TRPV1 antagonist has been shown to attenuate airborne particulate matter-induced apoptosis in human epithelial cells (Agopyan *et al.*, 2004). Similarly, TRPV1 agonists have been shown to cause cell death in alveolar epithelial cell lines (Reilly *et al.*, 2003; Thomas *et al.*, 2007). There are several reports that activation of TRPV1 channels causes the release of pro-inflammatory cytokines from airway bronchial epithelial cells (Reilly *et al.*, 2003; 2005; Seki *et al.*, 2007; Sadofsky *et al.*, 2012; Yu *et al.*, 2012; Mabalirajan *et al.*, 2013).

Few studies to date have examined the role of either neuronal or non-neuronal TRPV1 channels in airway inflammatory disease processes *in vivo*. Recently, mice genetically deficient for TRPV1 were shown to be protected against airway inflammation and bronchial hyperactivity induced by LPS (Helyes *et al.*, 2007), and another group have reported that pretreatment with a TRPV1 agonist attenuated neutrophil influx and cytokine release (Tsuji *et al.*, 2010). The data from asthma models is less clear, some studies showed that inhibition or genetic ablation of TRPV1 channels did not play a role in animal models of ovalbumin-induced LAR or acetylcholine-induced AHR in ovalbumin-sensitized animals (Caceres *et al.*, 2009; Raemdonck *et al.*, 2012). On the other hand, Rehman *et al.* (2013) reported that, in BALB/c mice, modulation of TRPV1 channels attenuated their IL-13 driven model of asthma, and TRPV1 played an important role in histamine-induced AHR in ovalbumin-sensitized guinea pigs (Delescluse *et al.*, 2012). Finally, Mori *et al.* (2011) reported that TRPV1 channels had a protective role in their intranasal, but not intraperitoneal, driven ovalbumin/house dust mite model of asthma. There is even less work published in models of COPD; recently, data was presented to suggest that TRPV1 KO mice had significantly less airway neutrophilia after exposure to cigarette smoke compared with the relevant wild-type animals (Baxter *et al.*, 2012). It seems that more preclinical and clinical work is required before we can fully understand the role of TRPV1 channels in airway inflammation.

TRPV4 (vanilloid 4) channels

TRPV4 (also VR-OAC) was originally isolated from rat kidney, and identified as a vertebrate homologue of the *Caenorhabditis elegans* gene *Osm-9*, with sequence homology similar to TRPV1 and TRPV2 channels (Liedtke *et al.*, 2000). The human trpv4 gene was subsequently mapped to chromosome 12q24 (Liedtke *et al.*, 2000). TRPV4 is expressed in a wider range of tissues than TRPA1 and TRPV1 channels, including the heart, lung, kidney, DRG neurons, CNS, skin and sweat glands, with the most prominent expression in epithelial and endothelial cells (Liedtke *et al.*, 2000; Strotmann *et al.*, 2000; Grant *et al.*, 2007; Willette *et al.*, 2008). The TRPV4 channel is a thermosensor, activated by innocuous warm temperatures in the range of 27–35°C (Liedtke *et al.*, 2000; Strotmann *et al.*, 2000; Watanabe *et al.*, 2003). Genetic deletion of TRPV4 channels confirmed a role in mechanosensation and osmotic stress, whereby deficient mice showed impaired responses to hypoosmotic stimuli, and reduced sensitivity to pressure and acidic nociception; but retained taste sensation, olfaction and heat avoidance (Liedtke and Friedman, 2003; Suzuki *et al.*, 2003). Chemical stimuli directly gate TRPV4 channels, including 4α-phorbol-12,13-didecanoate (4αPDD), the synthetic agonist GSK1016790A and endogenous metabolites of arachidonic acid (e.g. 5′,6′- epoxyeicosatrienoic acid; Watanabe *et al.*, 2002; 2003; Willette *et al.*, 2008). By contrast, osmotic stress has been suggested to indirectly cause channel gating via the PLA2 pathway (Vriens *et al.*, 2004). In fact, previous reports describing accumulation and release of arachidonic acid upon hypotonic stimulation make it likely that calcium entry upon hypotonic induction is mediated in an arachidonic acid-dependent manner via TRPV4 channel activation by agents such as epoxyeicosatrienoic acid (Mizuno *et al.*, 2003; Watanabe *et al.*, 2003; Vriens *et al.*, 2005). The PAR2 receptor has also been shown to indirectly couple to and sensitize TRPV4 channels, most likely mediated by release of arachidonic acid metabolites and phosphorylation of a key tyrosine residue (Grant *et al.*, 2007; Poole *et al.*, 2013).

TRPV4 channels in the airways

In the airways, TRPV4 channels are expressed on the smooth muscle, alveolar wall, lung tissue and lung vessels (Jia *et al.*, 2004; Alvarez *et al.*, 2006; Dietrich *et al.*, 2006; Yang *et al.*, 2006), with the highest levels of expression found in the epithelial linings of the trachea, bronchi and lower airways, and in the alveolar septal walls (Alvarez *et al.*, 2006; Fernández-Fernández *et al.*, 2008; Willette *et al.*, 2008). Inflammatory cells also express TRPV4, including mononuclear cells (Delany *et al.*, 2001), alveolar macrophages and neutrophils (Liedtke *et al.*, 2000; Banner *et al.*, 2011). Interestingly, TRPV4 channels have been reported to be highly expressed in macrophages but not in differentiated surrogates (Groot-Kormelink *et al.*, 2012).

Interestingly, data is now emerging suggesting that there are genetic variants TRPV4, which may be associated with the pathophysiology and symptomatology of asthma and COPD. Cantero-Recasens *et al.* (2010) has described the association

of a functional SNP, TRPV4-P19S, with childhood asthma. This SNP was identified, together with a functional SNP of TRPV1 described above, in a population of 470 controls without respiratory symptoms and 301 asthmatics. In contrast to TRPV1-I585V functional analysis of TRPV4-P19S, despite its loss-of-channel function, showed no significant association with asthma or the presence of wheezing (Cantero-Recasens *et al.*, 2010). Conversely, a genome-wide association study highlighted seven TRPV4 SNPs with susceptibility to COPD (Zhu *et al.*, 2009). These results suggest that the modulation of TRPV4 protein structure and activity may impact COPD pathogenesis, which may suggest that treating COPD through targeting TRPV4 channels could deliver a successful therapeutic strategy. However, further functional studies are needed to clarify the molecular mechanism of TRPV4 variants in the pathophysiology of COPD.

In contrast to TRPV1, there is very little known about the role of TRPV4 channels in activation of airway sensory nerves. Belvisi *et al*. (2013) presented data recently to suggest that activation of this channel leads to activation of airway ganglia cells and vagal (including human) tissue *ex vivo*. They showed that the same channel agonist caused afferent nerve firing and cough in a conscious guinea pig model. Thus, like TRPV1 and TRPA1, these channels are likely to be present to protect the airway. However, as many of the activators of these channels are believed to be increased in the diseased airway, it seems likely that the TRPV4 channel could be central to disease symptoms associated with sensory nerves. For example, it is postulated that bronchial smooth muscle cells and nerve endings may become exposed to hypotonic bronchial fluid in asthmatics due to airway remodelling (Liedtke and Simon, 2004). It is not clear yet whether TRPV4-mediated sensory nerve activation leads to a reflex bronchospasm but the limited data published so far suggests that TRPV4 channel activation can cause direct constriction of airway smooth muscle. Indeed, functional expression of TRPV4 channels has been demonstrated in human airway smooth muscle (HASM) cells, and a TRPV4 agonist and hypotonic solution elicits calcium movement in HASM cells in culture. Furthermore, hypotonic solution caused contraction in isolated human bronchus and guinea pig trachea (Jia *et al.*, 2004). Recently, Bonvini *et al*. (2013) demonstrated that a selective TRPV4 channel activator caused contraction of human and guinea pig airway smooth muscle, which was blocked by a selective antagonist and bronchoconstriction in anaesthetized guinea pigs that had their vagal nerves cut. These results indicate that the TRPV4 channel may play a crucial role in the bronchoconstriction observed in respiratory diseases such as asthma.

As discussed, epithelial cells play an important defensive role in the lung but are also thought to be central to the pathogenesis and symptoms in inflammatory airways disease. The TRPV4 channel may play a role in the control of epithelial and endothelial barrier function, particularly in response to increased vascular pressure and stretch (Alvarez *et al.*, 2006; Li *et al.*, 2011a). It has also been implicated in the development of neurogenic inflammation in animal models, where hypotonic solutions and 4αPDD induce neuropeptide release from isolated murine airways (Vergnolle *et al.*, 2010). Moreover, stimulation of alveolar macrophages with 4αPDD *ex vivo* causes the release of reactive oxygen and nitrogen species. This demonstrates functional expression of TRPV4 channels in airway macrophages, and suggests a potential role in macrophage activation by mechanical stress (Hamanaka *et al.*, 2010). Given this profile of activity, it is not surprising that the TRPV4 channel is important in the development of ventilator-induced injury (Wu *et al.*, 2009; Hamanaka *et al.*, 2010). The TRPV4 channel is also involved in permeability induced by high vascular pressure, as demonstrated by endothelial permeability and alveolar flooding in a murine isolated perfused lung model (Jian *et al.*, 2008). This response was absent in lungs from TRPV4 channel-deficient mice. TRPV4 channel agonists have also been suggested to play a role in heart failure-induced lung oedema (Thorneloe *et al.*, 2012).

Functional expression of TRPV4 has been demonstrated in murine ciliated tracheal cells and been shown to play a role in the increased ciliary beat frequency associated with mild temperature and ATP stimulation, implicating it in mucociliary clearance of the lungs (Lorenzo *et al.*, 2008). Furthermore, osmotic stress can cause ATP release from human bronchial epithelial cells via a cell swelling/TRPV4 channel – Rho kinase – pannexin axis (Seminario-Vidal *et al.*, 2011).

To our knowledge, few studies have investigated the role of TRPV4 channels in the specific context of asthma and COPD. It would seem that, with the reported role played by the channel in control of sensory nerves, airway smooth muscle and epithelial cells, together with the knowledge that many mediators thought to activate the channel are present in disease, the TRPV4 channel could be important in disease and associated symptoms.

TRPM8 (melastatin 8) channels

The TRPM subfamily was named after its founding member TRPM1 (Duncan *et al.*, 1998). In contrast to TRPA1, the TRPM subfamily is characterized by a lack of ankyrin repeat domains in the N-terminus (Peier *et al.*, 2002). TRPM8 was isolated from mouse DRG neurons via a genomic search of thermo-TRP-like protein sequences (Peier *et al.*, 2002), and the trpm8 gene is located on human chromosome 2q37. TRPM8 is predominantly expressed in a subpopulation of cold-responsive primary afferent sensory neurons within the DRG and the trigeminal ganglia, and are largely distinct from neurons expressing TRPV1 or TRPA1 (Clapham *et al.*, 2001; Peier *et al.*, 2002; Story *et al.*, 2003). TRPM8 is a thermosensor, activated by physiologically cool temperatures in the range of 15–28°C (McKemy *et al.*, 2002; Peier *et al.*, 2002). Aptly, direct activators of TRPM8 include compounds, which elicit a cooling sensation, for example, menthol, icilin and eucalyptol (Peier *et al.*, 2002; Zhou *et al.*, 2011). The mechanism of action of cold temperature in some cases is known to be distinct from that of 'cooling' compounds, as several channel mutations have abolished the effect of one but not the other (Bandell *et al.*, 2006). As yet, no endogenous TRPM8 ligands have been reported.

There are many conflicting data on the role of the TRPM8 channel and this confusion is likely to be confounded by the lack of selective tools. For example, in addition to their effects on TRPM8, both menthol and icilin activate TRPA1 at high concentrations. A number of novel TRPM8 inhibitors have

recently been developed, but these compounds have yet to be thoroughly characterized and validated (Preti *et al.*, 2012).

TRPM8 channels in the airways

The TRPM8 channel has been shown to be expressed in trigeminal ganglia but sparsely expressed in murine airway vagal sensory nerve fibres (Nassenstein *et al.*, 2008). Very little expression is reported in whole lung tissue but the TRPM8 channel is expressed in human bronchial epithelial cells (Tsavaler *et al.*, 2001; Sabnis *et al.*, 2008a,b; Banner *et al.*, 2011; Li *et al.*, 2011b). To our knowledge, there are no SNPs that are reported to be associated with asthma or COPD.

The role of TRPM8 channels in the normal and diseased airway is relatively unexplored. Breathing cold air can induce respiratory autonomic responses, which include cough, airway constriction, plasma protein extravasation and mucosal secretion. Such responses can trigger an asthma attack or exacerbate pre-existing disease although it has not been conclusively demonstrated that this effect is mediated via TRPM8 channels (Yoshihara *et al.*, 1996; Carlsen and Carlsen, 2002; Fisher, 2011). TRPM8 channels have been implicated in the cough and airway constriction associated with inhaling cold air but this has yet to be substantiated (Peier *et al.*, 2002; Xing *et al.*, 2008). Conversely, activation of these channels inhibited the cough reflex, though the current data on this is also contradictory (Laude *et al.*, 1994; Kenia *et al.*, 2009; Buday *et al.*, 2012; Preti *et al.*, 2012; Wise *et al.*, 2012; Millqvist *et al.*, 2013). In some of the papers that demonstrate an inhibitory action of menthol on tussive responses, the authors conclude that menthol suppresses cough evoked in the lower airways primarily through a reflex initiated from the nose. Consistent with this view are molecular analyses confirming the expression of TRPM8 channels in a subset of nasal trigeminal afferent neurons that do not coincidently express TRPA1 or TRPV1 channels and the failure to detect significant expression of TRPM8 channels in vagal sensory ganglia (Buday *et al.*, 2012; Plevkova *et al.*, 2013). Despite this conflicting data, and the fact that

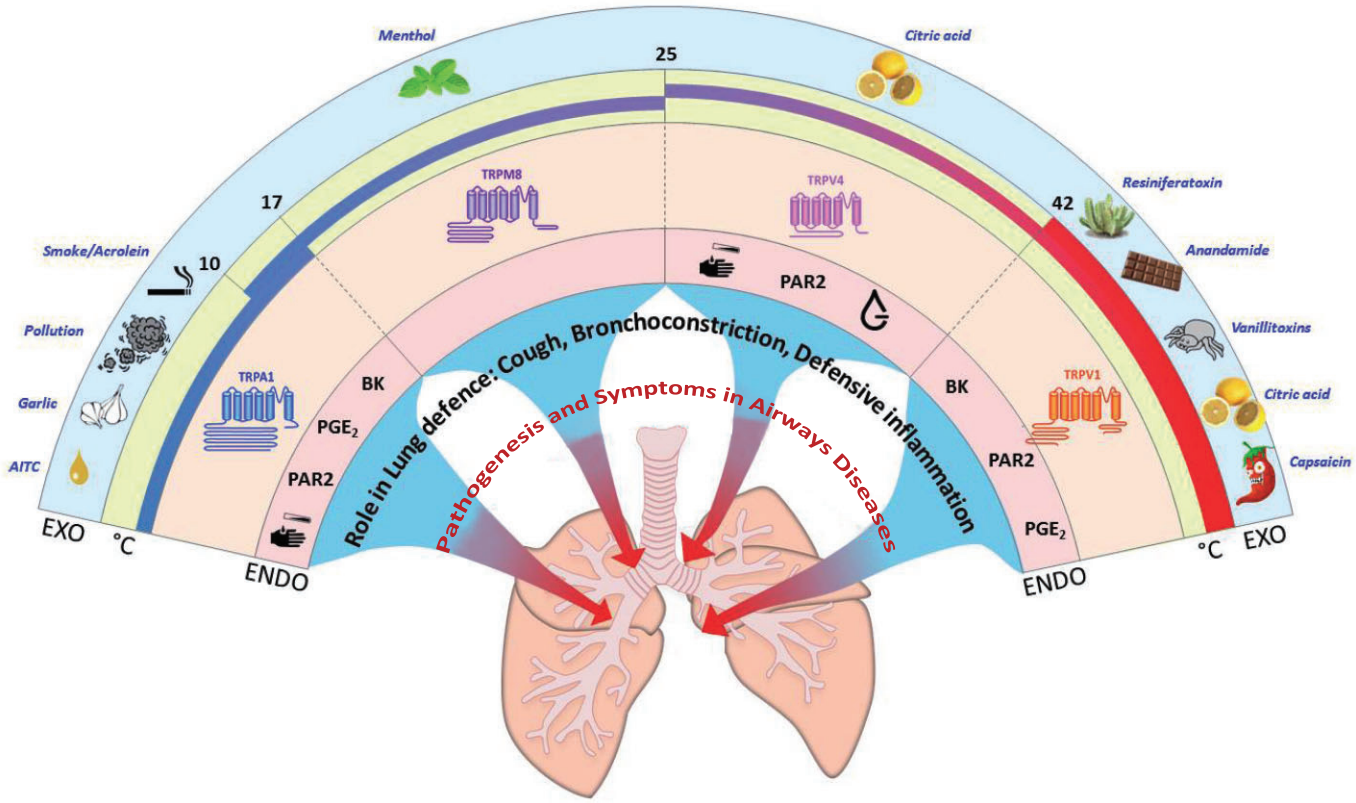

Figure 1

Overview of the role of TRP channels in the airway. The figure represents an overview of how TRP channels are involved in the physiology of the normal airway and how they could be involved in the pathogenesis of asthma and COPD. The middle arch indicates the four TRP channels, with examples of exogenous activators (EXO) in the outer arch, the temperatures (°C) that trigger the channels on the second arch. Examples of endogenous channel modulators (ENDO) including osmolarity-induced changes in cell membrane tension (Osmolar) and acidification of the epithelial surrounding (Acid) are displayed on the inner arch.

AITC = allyl isothiocyanate.

= Osmolar.

= Acid.

menthol does not convincingly outperform placebo in clinical trials, it is widely used in over-the-counter (OTC) antitussive therapies (Laude *et al.*, 1994; Morice *et al.*, 1994; Xing *et al.*, 2008; Kenia *et al.*, 2009; Preti *et al.*, 2012; Wise *et al.*, 2012). The OTC use could also be due to other perceived activities. Menthol has been associated with relief of dyspnoea in diseases such as COPD and is regularly used as a nasal decongestant (Eccles, 2003). A recent study showed no effect of menthol on nasal air flow, although it did increase the perception of nasal patency (Kenia *et al.*, 2009).

There is some published data to suggest that triggering the TRPM8 channel could result in the production of inflammatory mediators. For example, activation of the truncated TRPM8 variant in human bronchial epithelial cells by cold or menthol can elicit enhanced expression of a range of pro-inflammatory cytokines, an effect inhibited by the modestly selective TRPM8 antagonist BCTC, and siRNA knockdown (Sabnis *et al.*, 2008a,b). Furthermore, activated TRPM8 channels were crucial in cold-induced mucus production in lung epithelium (Li *et al.*, 2011b), and Cho *et al.* (2010) found that TRPM8 channels were involved in cold-triggered mast cell activation. Together, these data suggests that the TRPM8 channel could be involved in the innate protection of the airway, and perhaps, the inflammation present in airway disease. Over the next few years, it will be interesting to determine the role of the TRPM8 channel in the control of airway sensory nerves, airway tone and inflammatory status under normal and disease conditions, using truly selective ligands to conclusively demonstrate a role for this channel.

Conclusions

In summary, our understanding of the role of TRP channels in the normal and diseased airway is ever increasing. In Figure 1, we have tried to summarize the current data, indicate endogenous and exogenous stimuli and illustrate the role these various channels may play in the airway. It would seem that TRP channels make up an important part of the endogenous defence system of the lungs but are also likely to be central to the pathogenesis and symptoms associated with respiratory diseases. Over the next decade, as potent and selective TRP ligands are developed, more information will be accumulated regarding their role in host defence and in the protective mechanisms in normal individuals and how these channels may become dysfunctional in disease patients.

Acknowledgements

M. B. was supported by a Medical Research Council, (MRC) COPD studentship; E. D. was funded by a project grant from the (MRC, UK) (MR/K020293/1).

Conflict of interest

The authors have no conflict of interest with respect to the content of this review article.

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