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REVIEW

Targeting TRP channels for chronic cough: from bench to bedside

Sara J. Bonvini · Mark A. Birrell · Jaclyn A. Smith · Maria G. Belvisi

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Abstract Cough is currently the most common reason for patients to visit a primary care physician in the UK, yet it remains an unmet medical need. Current therapies have limited efficacy or have potentially dangerous side effects. Under normal circumstances, cough is a protective reflex to clear the lungs of harmful particles; however, in disease, cough can become excessive, dramatically impacting patients' lives. In many cases, this condition is linked to inflammatory diseases such as asthma and chronic obstructive pulmonary disease (COPD), but can also be refractory to treatment and idiopathic in nature. Therefore, there is an urgent need to develop therapies, and targeting the sensory afferent arm of the reflex which initiates the cough reflex may uncover novel therapeutic targets. The cough reflex is initiated following activation of ion channels present on vagal sensory afferents. These ion channels include the transient receptor potential (TRP) family of cation-selective ion channels which act as cellular sensors and respond to changes in the external environment. Many direct activators of TRP channels, including arachidonic acid derivatives, a lowered airway pH, changes in temperature, and altered airway osmolarity are present in the diseased airway where responses to challenge agents which activate airway sensory nerve activity are known to be enhanced. Furthermore, the expression of some TRP channels is increased in airway disease. Together, this makes them promising targets for the treatment of chronic cough. This review will cover the current understanding of the role of the TRP family of ion channels in the activation of airway sensory nerves and cough, focusing on four members, transient receptor potential vanilloid (TRPV) 1, transient receptor potential ankyrin (TRPA) 1, TRPV4, and transient receptor potential melastatin (TRPM) 8 as these represent the channels where most information has been gathered with relevance to the airways. We will describe recent data and highlight the possible therapeutic utility of specific TRP channel antagonists as antitussives in the clinic.

Keywords Cough · TRP channels · Airway · Sensory nerves

S. J. Bonvini · M. A. Birrell · M. G. Belvisi (✉)
Respiratory Pharmacology Group, Airway Disease Section, National Heart & Lung Institute, Imperial College, Exhibition Road, London SW7 2AZ, UK
e-mail: m.belvisi@imperial.ac.uk

J. A. Smith
Respiratory and Allergy Centre, University of Manchester, University Hospital of South Manchester, Manchester, UK

Introduction

Cough is a troublesome symptom and is currently the most common reason for visiting a doctor in the UK (Schappert and Burt 2006; Schappert and Rechtsteiner 2011). Epidemiological studies have shown that at any one time, up to 40 % of the population reports cough (Janson et al. 2001). Cough is normally an important defensive reflex, which protects the airways from the inhalation of harmful substances and foreign materials and aids in immune defence (Widdicombe 1995; Fontana et al. 1999; Irwin et al. 1998); indeed when cough is ineffective, this can lead to a variety of pathological conditions including atelectasis, bronchiectasis, pneumonia, lung abscesses, and pulmonary scarring (Madison and Irwin 2010). However, in certain diseases, the cough response can become augmented, leading to excessive coughing which can be due to increased activation of the neuronal pathways responsible (Young and Smith 2011).

Clinically, the aetiology and treatment of cough can broadly be classified into either acute (less than 3 weeks) (Irwin et al. 1998), which is largely as a result of viral upper respiratory tract infections (URTIs) and bacterial infections (Curley et al. 1988; Irwin et al. 1998), and chronic cough (more than 8 weeks in duration) which can lead to an increase in both

frequency and intensity of cough (McGarvey et al. 2007). Chronic cough has been estimated to affect up to 40 % of the population at any one time (Cullinan 1992; Janson et al. 2001; Morice et al. 2001). Chronic cough is often associated with inflammatory airway diseases including chronic obstructive pulmonary disease (COPD), asthma, pulmonary fibrosis, lung cancer, or conditions outside the lung such as gastro-oesophageal reflux and rhinosinusitis (Fuller and Choudry 1987; Irwin et al. 1998; Morice et al. 2007a) and is also a side effect of drug treatments such as ACE inhibitors (Sesoko and Kaneko 1985; Lalloo et al. 1996; Fahim et al. 2011; Faruqi et al. 2014). Chronic cough can also be idiopathic in origin, and this can account for 18–42 % of patients at specialist cough clinics (Polley et al. 2008; Haque et al. 2005), and chronic cough severe enough to interfere with the normal activities of daily life is thought to affect approximately 7 % of the population (Ford et al. 2006). Chronic cough patients report cough on exposure to a broad range of normally innocuous stimuli including environmental, mechanical, and temperature changes (Birrell et al. 2009; Khalid et al. 2014). This is a debilitating condition, where long-term excessive cough has side effects including sleep disturbance, nausea, chest pains, social embarrassment, incontinence and depression, alongside affecting social relationships with family and colleagues (Brignall et al. 2008). This condition can affect patients, for months or years at a time, ultimately impacting on the quality of life for sufferers (French 1998).

Antitussive medications represent one of the most widely sold over-the-counter (OTC) medications, and in the USA alone, over $3 billion a year is spent on antitussive therapies (Footitt and Johnston 2009) and £100 million in the UK (Dicpinigaitis et al. 2011). A survey carried out in the USA of over 8000 subjects reported that over half of preschool children had been given OTCs, of which 67 % were composed of cough medications (Kogan et al. 1994). This is despite the fact that a systematic review including 2100 participants, found a lack of evidence to support the use of OTC cough therapies (Schroeder and Fahey 2002). Many OTC medications are thought to have no pharmacological effects and instead act as a lubricant to coat the throat (Eccles 2006). The American College of Chest Physicians (ACCP) now advises against OTC therapy for cough with URTI (Bolser 2006), and furthermore, some children's cough and cold medications in the USA have now been removed from public use due to potentially life-threatening side effects in children under 2 years, in the absence of evidence of efficacy (Centers for Disease Control and Prevention 2007; Vassilev et al. 2009).

Treatments can be broadly classified into those that act centrally, such as opiates, and those which act peripherally, outside the central nervous system targeting airway sensory nerves. Opiates, which exert their actions through the central nervous system, are the only class of drug currently in use that have shown any efficacy and are therefore the current gold standard in cough treatment. A clinical trial demonstrated that morphine sulphate was efficacious in treating chronic cough in patients that did not respond to specific antitussive treatments (Morice et al. 2007b). Clinically, codeine is currently the reference antitussive of choice, but doses used have been shown to have side effects such as dependence, respiratory depression, and GI problems (Belvisi and Geppetti 2004). In addition, a clinical trial has shown that treatment with codeine in patients with COPD and cough was no more effective than placebo (Smith et al. 2006a).

Despite the high prevalence, treatment of chronic cough is a significant unmet medical need, and there is an urgent requirement for new, safe and effective therapies with fewer side effects. Currently, most therapies are targeted at treating the underlying cause of cough (if they can be identified), but in many cases, these are not effective; therefore, treatments specifically targeting cough are needed. Patients with asthma, COPD, post viral cough and interstitial lung diseases exhibit increased cough reflex sensitivity to inhaled tussive agents, suggesting that targeting sensory nerve activation and the peripheral arm of the reflex response may be effective in treating the excessive cough (Key et al. 2010). Members of the transient receptor potential (TRP) family of ion channels are present on vagal sensory nerves, which, when activated, initiate the cough reflex. TRP channels are a family of cation-selective ion channels which act as cellular sensors and respond to changes in the external environment. Many direct activators including arachidonic acid derivatives, a lowered airway pH, changes in temperature, reactive oxygen species (ROS), oxidative stress by-products and altered airway osmolarity are present in the diseased airway of patients that exhibit increased symptoms such as cough. Furthermore, some TRP channels have shown altered or increased expression in airway disease. Together, this makes them promising targets for the treatment of chronic cough.

The aims of this article are to review the current understanding of airway sensory nerves and the cough reflex and to highlight the role of the TRP family of ion channels present on vagal nerve afferents as potential therapeutic targets.

Physiology of the cough reflex

The cough reflex is a forced event, which clears the larynx, trachea and large bronchi of secretions. There are three phases, which begin with a rapid deep inspiration, followed by a compressive phase where the glottis closes and there is a build-up of intrathoracic pressure. The cough ends with an expiratory phase where the glottis opens and there is a rapid expulsion of air and characteristic cough sound, which clears the airways of irritant material. During this final phase, air expulsion speed can reach 500 mph (Bianco et al. 1988). Coughs can occur in bouts where a number of repetitive

coughs occur in succession (Nasra and Belvisi 2009). Although the cough reflex is involuntary, cough can be induced and mimicked voluntarily.

Cough and airway sensory nerves

Regardless of cause, the cough reflex is generally initiated following activation of airway sensory nerves. Studies carried out in animals have determined that the cough reflex is regulated by vagal afferent nerves (Canning et al. 2006), which innervate the airways along with other midline organs and tissues (Standring 2005). Receptors present on the vagal nerve termini situated in and under the airway epithelium can be activated by a wide variety of stimuli. These include mechanical stimuli such as punctate touch and lung inflation (Canning 2004), inflammatory mediators released during disease such as PGE_2 and bradykinin (Kawakami et al. 1973; Choudry et al. 1989; Maher et al. 2009; Grace et al. 2012), environmental irritants such as cigarette smoke and pollutants (Lee et al. 2007; Belvisi et al. 2011), changes in osmolarity of the airways (Lowry et al. 1988; Koskela et al. 2005) and changes in pH or temperature (McGarvey et al. 1998; Wong et al. 1999). Activation of these receptors causes a localized membrane depolarization, and if this reaches a certain threshold, then, an action potential is generated following activation of voltage-gated sodium channels (VGSCs) and carried up by the vagus nerve to the brainstem. The nerve terminals then synapse in the nucleus tract solitarius (nTS), where the reflex is processed, and finally, the information is carried by efferent motor neurons to the respiratory muscles and larynx to cause cough (Fig. 1). Initiation of cough requires a sustained high frequency activation of the afferent nerves that creates an urge to cough in patients preceding the reflex itself (Canning and Mori 2011; Dicpinigaitis et al. 2014)

The cell bodies for airway sensory nerves are mostly housed in the nodose and jugular ganglia although around 1 % originate from the thoracic dorsal root ganglia (DRG) (Mazzone 2004). The nodose and jugular ganglia have different embryonic origins; the nodose is epibranchial placode-derived and the jugular neural crest-derived (Nasra and Belvisi 2009), and as a result, they express different growth peptides and neurotrophins (Ernfors et al. 1992; Begbie et al. 2002) and house different populations of nerve fibres. There are several known sensory nerve subtypes present in the lung, which can be identified based on adaptation indices, physiochemical sensitivity, neurochemistry, origin, myelination conduction velocities and sites of termination (Canning 2006a; Adcock et al. 2003). Of these sensory nerve subtypes, three are more mechanically sensitive; the rapidly adapting receptors (RARs), slowly adapting receptors (SARs) and the subtype known as the 'cough' receptor. There are also two fibre types which are more chemosensitive: C fibres and Aδ nociceptors.

Rapidly adapting receptors

RARs, or irritant receptors, are myelinated, conducting action potentials in the range of 14–23 m/s (Fox et al. 1993; Riccio et al. 1996). Although distributed throughout the airways, they are mostly found in the larger airways where they respond rapidly to mechanical stimuli such as light touch and lung hyperinflation/deflation (Mortola, Sant'Ambrogio, and Clement 1975; Sant'Ambrogio et al. 1978; Canning 2004; Nasra and Belvisi 2009). RARs are thought to be mostly nodose-derived (Riccio et al. 1996; Canning 2006) and were originally thought to be directly activated by a chemical stimulus such as capsaicin and bradykinin, although it is thought that these stimuli can indirectly activate these fibres following bronchoconstriction and mucus production (Widdicombe 2003). However, emerging evidence has suggested that a subset of RARs is responsive to capsaicin regardless of conduction velocity in naive guinea pigs (Adcock et al. 2014). Due to their mechanical sensitivity and their ability to cause cough even under anaesthesia, RARs are thought to provide the defensive cough reflex that clears the lungs from harmful particles (Nasra and Belvisi 2009; Dicpinigaitis et al. 2014).

Slowly adapting receptors

SARs are also fast-conducting, nodose-derived fibres that are sensitive to mechanical stimulation (Nasra and Belvisi 2009). However, they are less sensitive than RARs to sustained lung inflation (Schelegle and Green 2001) and are involved in tidal breathing and are important in the Hering-Breur reflex and sense when the lungs are sufficiently inflated during inspiration and initiate expiration (Schelegle 2003). SARs are not thought to play a direct role in the cough response.

The cough receptor

The more recently described cough receptors are found in the larynx, trachea and bronchi and, similarly to RARs, are nodose-derived, activated by citric acid and changes in pH, and are extremely sensitive to light punctate touch, with a low threshold for activation (Canning 2004; Nasra and Belvisi 2009). However, they differ from RARs as they have slower conduction velocities (5 m/s) and are insensitive to stretch, increased pressure, and bronchoconstriction which all activate RARs (Canning 2004; Mazzone 2004). It should be noted that the relevance of the cough receptor remains unclear in man, as the majority of work has been carried out in the anaesthetized guinea pig (Nasra and Belvisi 2009; Canning 2004; Mazzone 2004).

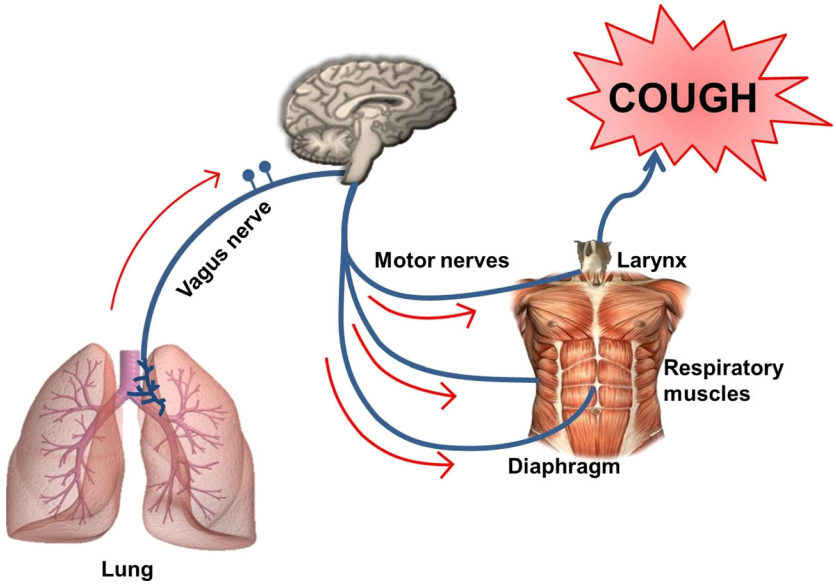

Fig. 1 The cough reflex. The cough reflex can be separated into three components, the peripheral arm which initiates the reflex following sensory nerve activation, a central component where the reflex is processed in the brainstem and, finally, the efferent arm which causes the cough response following activation of motor nerves. The cough reflex initiates when endogenous or exogenous irritants activate receptors present on sensory nerves in the airways. If this activation reaches a certain threshold, then an action potential is produced, which is carried up by the vagus nerve which synapses in the nucleus tractus solitarius (nTS) in the brainstem. A signal is then sent down to motor neurons to the diaphragm, respiratory muscles and larynx to cause the characteristic cough response

Aδ nociceptors

Aδ nociceptors are chemosensitive, fast-conducting myelinated fibres that differ from RARs, as they originate in the jugular ganglia and are activated by transient receptor potential vanilloid (TRPV) 1 agonists such as capsaicin; however, they do remain relatively insensitive to mechanical stimuli (Riccio et al. 1996; Kajekar et al. 1999; Yu 2005). Although they show similar effects pharmacologically to C fibres, their contribution to the cough reflex is not clear, as they are yet to be fully investigated.

C fibres

C fibres make up the majority of nerve fibres that innervate the airways (Takemura et al. 2008), are found predominantly in the airway epithelium and can originate in both the nodose and jugular ganglia. They are unmyelinated, slower conducting fibres which have a conduction velocity of ~1 m/s (Fox et al. 1993; Riccio et al. 1996; Mazzone 2004; Canning 2006). C fibres are blocked by anaesthesia and are therefore responsible for the conscious perception of airway irritants (Mazzone 2004).

C fibres are relatively less sensitive to mechanical stimuli but are activated by agonists of the TRPV1 (capsaicin, resiniferatoxin (RTX)) and transient receptor potential ankyrin (TRPA) 1 (allyl isothiocyanate (AITC), acrolein, cinnamaldehyde) ion channels, which have also been shown to cause cough in animals and in man (Coleridge and Coleridge 1984; Canning 2004; Dicpinigaitis and Alva 2005; Birrell et al. 2009; Grace et al. 2012; Dicpinigaitis et al. 2014; Adcock et al. 2014; Lalloo et al. 1995). Single-cell PCR has demonstrated the expression of TRPA1 and TRPV1 in C fibres in animals (Nassenstein et al. 2008; Brozmanova et al. 2012), and TRPV1 expression has been shown on nerves in man (Groneberg 2004). As the fibres are relatively unresponsive to mechanical stimuli, the chemical stimuli activate the nerves directly (Undem 2004; Chuaychoo et al. 2005).

C fibres are further differentiated from RARs and Aδ nociceptors by their ability to participate in local axon reflex events leading to the release of neuropeptides such as CGRP, substance P and neurokinin A (Lundberg et al. 1985; Hunter and Undem 1999; Myers, Kajekar, and Undem 2002; Baluk et al. 1992) which can evoke responses such as bronchoconstriction and mucus production (Nasra and Belvisi 2009). However, although local axon reflex events, characterized by the release of neuropeptides, have been demonstrated in rodent and guinea pig airways, it is still not clear if this happens in human airways.

There are two distinct populations of C fibres, bronchial and pulmonary C fibres, which are distinguished according to their site of termination, responsiveness to different stimuli and ganglionic origin. Bronchial C fibres are thought to innervate the extrapulmonary airways and are derived mostly from the jugular ganglia and release neuropeptides and pulmonary C fibres which innervate the lower airways (Coleridge and Coleridge 1984; Carr and Undem 2003; Undem 2004). In

some cases, pulmonary C fibres have been found to be inhibitory against cough in animal models (Widdicombe and Undem 2002; Tatar et al. 1994; Chou et al. 2008), so it is thought that it is the bronchial C fibres that mediate the cough response to chemicals such as capsaicin.

Preclinical systems for assessing airway sensory nerves

Guinea pigs, dogs, cats, rabbits and rodents have all been used to investigate the neural pathways, physiology and pharmacological intervention of the cough (Hanácek et al. 1984; Gardiner and Browne 1984; Kamei et al. 1993; Tatar et al. 1994; Patel et al. 2003). However, it has been widely agreed that the guinea pig provides the most suitable animal model (Belvisi and Hele 2003), as there are a great number of similarities in the cough response between the guinea pig and man, including their sensitivity to tussive stimuli such as capsaicin and citric acid (Karlsson and Fuller 1999), they have similar lung anatomy and physiology to humans, and the lungs are also innervated similarly, unlike the mouse (Canning 2006). There are a number of experimental systems that can investigate the cough reflex both in vitro and in vivo in the guinea pig, some of which are outlined below.

Isolation of vagal ganglia

The cell bodies from airway-specific neurons in nodose and jugular ganglia can be identified and isolated for assessment. One technique involves using the retrograde tracer dye DiIC18(3),1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI). The dye is given into the airways at least 12–14 days prior to harvesting the ganglia. The tracer travels back up the neuron by retrograde transport into the cell body (Undem et al. 2002; Lieu et al. 2012; Kwong and Lee 2005). Once isolated, the airway-specific neurons can be identified under a fluorescent microscope and used in a number of ways including the following:

- Assessment of target expression at the messenger RNA (mRNA) level (Nassenstein et al. 2008).
- Measurement and location of the target at the protein level (Kwon et al. 2014).
- Intracellular Ca^{2+} movement (i.e. using fluorescent tags (Grace et al. 2012; Dubuis et al. 2013).
- Biochemical study (i.e. understanding signalling transduction (Hadley et al. 2014)).
- Electrophysiological assessment (i.e. patch clamp systems (Dubuis et al. 2014))

The key advantage of this technique is that it allows both functional phenotyping and target expression in primary airway nodose and jugular ganglia (a schematic is shown in Fig. 2a). Disadvantages are that studies are performed with cell bodies rather than the nerve terminal, and as yet, it is not possible to access human primary ganglia cells for translational conformation studies.

In vitro-isolated vagus nerve recordings

The use of the vagal trunk to assess sensory nerve activity has also been used effectively. One system involves isolation of the two ends of the vagus trunk in a grease-gap recording chamber. One end can then be challenged with stimuli/test solutions, and membrane depolarization recorded. An example of the system and traces generated is as shown in Fig. 2b and reported in various publications including (Grace and Belvisi 2011; Fox et al. 1995; Birrell et al. 2009).

This is a relatively high throughput, pharmacologically amenable method where many stimuli that activate the vagus nerve have been shown to cause cough in both animals and man (Maher et al. 2009; Patel et al. 2003). It also holds the key advantage of being able to record from human vagal tissue to provide translational data (Usmani et al. 2005; Belvisi 2008). There are, however, several limitations to consider, including the use of nerve trunk rather than the nerve terminal; the inability to specifically record from airway nerves and the recordings taken are a summation of the activity from the whole trunk, and action potential generation is not measured. Nonetheless, the recordings are robust and reproducible and have allowed the investigation of a number of tussive and antitussive compounds.

In vivo single fibre

The in vivo single-fibre recording technique allows the investigation of specific tussive or antitussive stimuli on action potential firing of single afferent airway terminating fibres (Adcock et al. 2003; Canning 2004). This technique measures the effects of compounds on the nerve termini in the whole animal and is therefore more physiologically relevant than the isolated vagus or ganglia. Figure 2c shows a representative scheme and trace from one model system. Fibre type can be identified using a range of criteria (Adcock et al. 2003). However, the disadvantage of this technique is that it is relatively low throughput, requires relatively large amounts pharmacological/biological tools with appropriate characteristics for in vivo work and is extremely technically demanding.

In vivo cough recordings

The most common model of cough is the conscious guinea pig cough model. This often involves placing conscious animals into individual Perspex chambers where

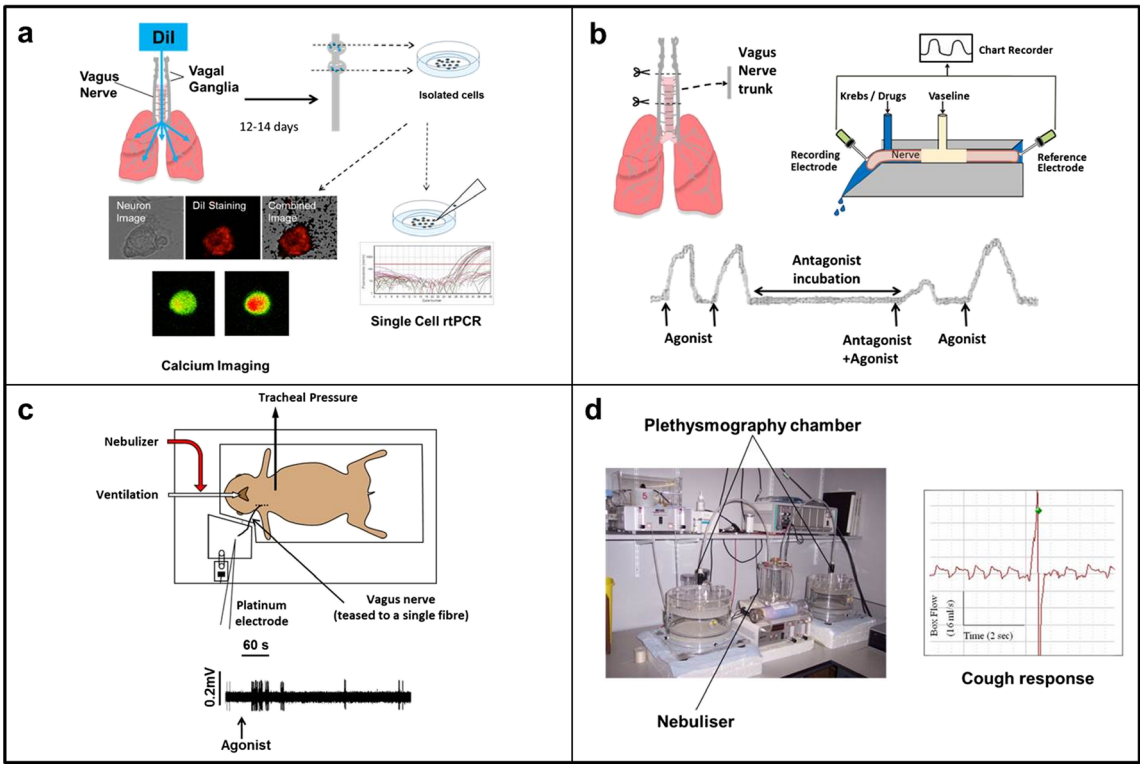

Fig. 2 Preclinical models of cough. **a** Isolation of airway-specific ganglia. Guinea pigs are intranasally dosed with the retrograde tracer dye DiI 12–14 days prior to dissection and enzymatic disassociation of the jugular and nodose ganglia. Airway-specific cells can then be identified using fluorescence imaging indicated by the overlay image, and then, cells can be used for calcium imaging or single cells taken and used for RT-PCR. **b** In vitro-isolated vagal nerve preparation. The vagus nerve trunk is isolated and dissected and placed into a grease gap recording chamber, where each end of the nerve is electrically and chemically isolated using Vaseline™. One end of the nerve is then attached to a recording electrode and perfused with either Krebs-Hensleitt (KH) solution or drug, and the other end of the nerve is attached to a reference electrode but bathed in KH for the duration of the experiment. When drugs are perfused, the difference between the two electrodes can be amplified and recorded as compound depolarization. The *bottom panel* indicates an example trace of a classical agonist/antagonist experiment; the nerve is exposed to two reproducible responses to agonist, following which the nerve is exposed to an antagonist for 10 min. The nerve is then restimulated with the agonist in the presence of the antagonist, and % inhibition of the original response can be assessed. After a brief washout period, the nerve is restimulated with agonist to ensure nerve viability. **c** In vivo single fibre. Guinea pigs are anaesthetized and ventilated and both cervical vagal nerves cut at the central end. The left vagus nerve is then dissected down to a single fibre and attached to a platinum recording electrode. Compounds are then aerosolized into the lungs, and firing from the single fibre is recorded. The *bottom panel* is an example trace of firing from a C fibre to capsaicin. **d** In vivo cough. Guinea pigs are placed into the two plethysmography chambers and compounds aerosolized in via a central nebulizer. The number of coughs can then be counted by a trained observer and also monitored by the Buxco Cough Analyzer software, which would show a characteristic spike (*right*) if a cough occurred

they can be exposed to tussive agents (Fig. 2d). Coughs can be counted by both automated cough counting software which uses an custom-designed algorithm to recognize cough (Lewis et al. 2007), measurement of airflow and assessment by a trained observer who will recognize both the sound and characteristic change of posture adopted by the animal (Nasra and Belvisi 2009; Andrè et al. 2009; Birrell et al. 2009; Lee et al. 2006; Grace et al. 2012; Maher et al. 2009; Trevisani et al. 2004). The key advantage of this technique is that studies have shown that tussive responses induced in guinea pigs closely resemble that seen in man (Laude, Higgins, and Morice 1993; Birrell et al. 2009; Lee et al. 2006); however, this model does not allow the identification of the fibre type responsible and requires large amounts of test compound.

Disease models

Much of the research into the cough response has been carried out in naive animals and healthy cells/tissue, but it is known that the cough reflex and presumably sensory nerve activity are altered in disease. Therefore, investigating sensory nerves under disease conditions is vital. Generally, the approach used is to model the disease in the animal and study how the sensory nerve phenotype has been altered. One example is the modelling of COPD, a disease where the patients are known to cough more and be more sensitive to inhaled tussive agents including the TRPV1 agonist capsaicin (Sumner et al. 2013). To model COPD, researchers expose the guinea pigs to the main causative agent linked to the development of COPD, cigarette smoke

(CS). The exposed guinea pigs are then shown to be more sensitive to tussive stimuli (Karlsson et al. 1991b; Lewis et al. 2007; Wortley et al. 2011; Bergren 2001; Karlsson et al. 1991a). Furthermore, tissue and ganglia harvested from the CS-exposed guinea pigs have an altered phenotype. These data will be discussed in greater detail later.

Measuring cough in the clinic

Although guinea pigs have similar pharmacology and physiology to the human airways, there are several caveats to using animal models (Morice et al. 2007a, b), and therefore, cough models may not always translate to the clinical situation. Alongside animal models, cough can also be measured in the clinic in both healthy and diseased patients. There are three main techniques used in the clinic to monitor cough and to assess the validity of specific antitussive therapies. These are subjective quality of life questionnaires, ambulatory cough counting and cough reflex sensitivity testing.

Cough questionnaires

Specialist qualitative cough questionnaires have been established that measure the impact of chronic cough on daily life. They provide a subjective retrospective analysis of cough severity (Nasra and Belvisi 2009). There are currently three questionnaires in common use that look at cough as a primary end point and are used it to assess the impact of cough on quality of life. These are the Leicester Cough Questionnaire (LCQ) (Birring et al. 2003), where a lower score indicates a poorer quality of life, and the Cough Quality of Life Questionnaire (CQLQ) (French et al. 2002) and Chronic Cough Impact Questionnaire (CCIQ) (Baiardini et al. 2005), where a higher score indicates a lower quality of life. All questionnaires measure the physical, psychological and social impact of cough using a specialized scoring system and are both validated and comparable in establishing the severity of cough.

Ambulatory cough counting

Cough monitors provide a non-invasive method of measuring cough over a 24-h time period (Kelsall et al. 2011). This is a more representative measure of the symptom of cough, as it does not rely upon patient recall and is carried out in the patient's natural environment, rather than under laboratory conditions (Smith 2010). Patients wear a discrete recording device equipped with a microphone which records cough sounds over a set time period (Smith and Woodcock 2008; Smith 2010).

There are currently several systems in use, but none are commercially available at present; the LifeShirt (VivoMetrics Incorporate, Ventura, CA) (Coyle et al. 2005; Smith 2010) and the CoughCOUNT (KarmelSonix, Haifa, Israel) (Vizel et al. 2010) are no longer in production. The other systems in use include the Leicester Cough Monitor, which, although widely published, is not yet fully automated (Birring et al. 2008) (McGuinness et al. 2008). The sensitivity of the system is most recently reported as 83.8 % in patients (n=12) and 82.3 % in healthy controls (n=8) (Yousaf et al. 2013) on pooled data; how the sensitivity varies between individual subjects is not reported. The Hull Automated Cough Counter which uses neural network-based technology to recognize sounds is still in development (Barry et al. 2006). The final cough counting system is the VitaloJAK (Vitalograph Limited, Buckinghamshire, UK), which uses a custom-made digital recording device to record cough through sounds detected by microphones on the chest wall and lapel (Smith and Woodcock 2008). This monitoring system no longer requires calibration (Smith 2010) and consists of a compression algorithm that removes silence and the bulk of non-cough sounds from 24-h acoustic recordings. This provides a sound file an average of 85 min in length containing median 100.0 % (range 97.3–100 %) of coughs in the original 24-h recording when tested on a group of 47 subjects, including chronic cough, asthma, COPD, lung cancer and healthy controls (McGuinness et al. 2012 and unpublished data).

Cough reflex sensitivity

Capsaicin and citric acid are the most common agonists used to induce cough in the clinic, and in the case of capsaicin, this is because it has been shown to have no adverse effects (Dicpinigaitis and Alva 2005) and induces cough in a dose-dependent, reproducible manner (Dicpinigaitis 2007). Other tussive agents include bradykinin, prostaglandins, fog and hypertonic saline (Nasra and Belvisi 2009). This technique involves the inhalation of irritants and counting the number of coughs over a set time period, which allows a comparison between healthy and diseased individuals (Nasra and Belvisi 2009).

The most common method of measuring cough reflex sensitivity is using the C2 and C5 parameters, which is the concentration of tussive stimulus (commonly capsaicin) required to reach two or five coughs (Doherty et al. 2000; Dicpinigaitis 2006; Morice et al. 2007a, b), reached by inhaling single doubling doses of stimulus (Hilton et al. 2013). This technique has been found to be highly reproducible (Dicpinigaitis 2003) and easy to perform (Morice et al. 2007a, b), and clinicians can discern differences between disease groups.

Recently, however, the use of C2 and C5 parameters has come into question, as they have been shown to only weakly correlate with the spontaneous cough frequency measured using ambulatory 24-h cough counts (Hilton et al. 2013; Decalmer et al. 2007; Birring et al. 2006). Instead, a new

capsaicin challenge has been put forward using non-linear mixed effects dose-response modelling, which uses EMax or ED50 rather than C2 and C5 to distinguish between health and disease, which correlates well with cough frequency (Hilton et al. 2013). This has also been shown to be more similar to conscious cough in animals (Smith et al. 2012).

TRP channels in cough

Airway sensory nerves are known to express a variety of receptors and ion channels that are activated by a variety of exogenous and endogenous mediators to cause cough. A greater understanding of the mechanisms of how these receptors and ion channels are involved in cough may lead to the development of new and effective therapies. In this part of the review, the role of several members of the TRP family of ion channels, TRPV1, TRPA1, TRPV4 and transient receptor potential melastatin (TRPM) 8, in the activation of airway sensory nerves and cough is outlined.

The TRP superfamily of ion channels is a set of cation-dependent transmembrane proteins which show a preference for Ca^{2+}, initially discovered in the Drosophila fly and so named because of their transient response to bright light (Montell and Rubin 1989; Ramsey et al. 2006; Caterina et al. 1997). TRP channels are known as cellular sensors (Clapham 2003), as they respond to changes including temperature, stretch, chemicals, oxidation, osmolarity and pH (Picazo-Juárez et al. 2011; Moran et al. 2011) in the cellular environment. They are also sensors for certain herbs, spices, venoms and toxins (Vriens et al. 2008). This family of ion channels has 28 members in six subfamilies (TRPC, TRPV, TRPM, TRPA, TRPP, TRPML) based on sequence homology and amino acid sequence (Clapham 2003), and all have six transmembrane domains with the pore situated between domains 5 and 6, variable degrees of ankyrin repeats and an intracellular N and C terminus (Caterina et al. 1997; Ramsey et al. 2006). Functional channels are mostly formed as homotetramers, but heteroteteramization can also occur (Latorre et al. 2009; Cheng et al. 2010). Activation of TRP channels allows cations to pass through the channel on the membrane, causing depolarization of the cells and causing a wide range of cellular responses (Kaneko and Szallasi 2014).

As cellular sensors, TRP channels are therefore linked to sensory perception, and many are associated with the pathogenesis of several respiratory diseases including COPD, asthma, cancer and cystic fibrosis (Caterina et al. 1997; Arniges et al. 2004; Nilius 2007; Zhu et al. 2009). Members of the TRP family of ion channels have been outlined as strong candidates for sensory receptors that modulate cough in humans (reviewed in Grace et al. 2014b; Grace et al. 2013; Materazzi et al. 2009), and there is a large body of evidence outlining the role of TRPV1 and TRPA1 in cough and evidence indicating that both TRPV4 and TRPM8 could also play a role.

TRPV1

The receptor for the tussive stimulus capsaicin was identified to be the vanilloid receptor TRPV1 (Szallasi and Blumberg 1990; Caterina et al. 1997), and this was the first TRP channel to be identified as a key regulator of tussive reflexes (Lalloo et al. 1995). TRPV1 is a polymodal ion channel which is activated by a diverse range of stimuli including irritant chemicals (i.e. capsaicin), low pH, increases in temperature (around 43 °C) and a number of endogenous mediators (Jordt et al. 2000; Moriyama et al. 2005; Zhou et al. 2011; Jia et al. 2002; Kagaya et al. 2002; Zygmunt et al. 1999; Caterina et al. 1997; Carr and Undem 2003; Kollarik and Undem 2004; Hwang et al. 2000). In addition, activation of certain G protein-coupled receptors (GPCRs) by inflammatory mediators has been shown to result in the opening of TRPV1 ion channels. These include bradykinin (Grace et al. 2012), PGE_2 (Grace et al. 2012; Moriyama et al. 2005) and peptides which activate protease-activated receptors (PARs) (Amadesi et al. 2004; Gatti et al. 2006). Table 1 contains a summary of some of the reported TRPV1 agonists (direct and indirect activators) and antagonists.

TRPV1 expression

TRPV1 mRNA has been shown to be expressed in vagal ganglia (Caterina et al. 1997; Ahluwalia et al. 2000; Ichikawa and Sugimoto 2004), with single-cell reverse transcription (RT)-PCR showing TRPV1 in airway neurons (Nassenstein et al. 2008; Lieu et al. 2012). As with all the TRP receptors, it is currently difficult to determine expression of TRPV1 at the protein level because of the lack of selective antibodies for accurate staining although papers describing protein expression have been published. Instead, specific ligands can be used to trigger a functional response (i.e. Ca^{2+} flux, nerve depolarization and firing) to demonstrate the presence of the ion channels. Using this approach, it has been shown that TRPV1 is functionally expressed on naive guinea pig airway jugular neurons with less activation by TRPV1 ligands on the nodose neurons (Grace et al. 2012; Hu et al. 2014). Interestingly, in a cigarette smoke (CS)-driven model system and also following allergen exposure, it has been shown that the nodose ganglia cells become much more responsive to capsaicin, suggesting a phenotype change in disease (Wortley et al. 2011; Lieu et al. 2012). Indeed, others have reported that the expression of TRPV1 ion channels on sensory nerves is increased in patients with idiopathic cough (Groneberg 2004). Furthermore, two independent studies in eight

Table 1 TRP channel agonists and antagonists

Agonists	Antagonists
TRPV1	
Capsaicin (Caterina et al. 1997)	Capsazepine (Dickenson and Dray 1991)
Resiniferatoxin (Caterina et al. 1997)	IRTX (Seabrook et al. 2002)
Acid (Jordt et al. 2000)	JNJ17203212 (Bhattacharya et al. 2007)
Anandamide (Smart et al. 2000)	JNJ39729209 (Maher et al. 2011)
HPETE (Hwang et al. 2000)	BCTC (McLeod et al. 2006)
LTB_4 (Hwang et al. 2000)	SB705948[a] (Khalid et al. 2014)
Temperature >43 °C (Caterina et al. 1997)	XEN-DO501[a] (Round et al. 2011)
Oleamide (Zygmunt et al. 1999)	
9- and 13-HODE (Patwardhan et al. 2010)	
TRPA1	
Acrolein (Bautista et al. 2006)	HC030031 (McNamara et al. 2007)
Ozone (Taylor-Clark and Undem 2010)	AP-18 (Brozmanova et al. 2008)
AITC (Jordt et al. 2004)	GRC17536[a] (Mukhopadhyay et al. 2014)
Cinnamaldehyde (Bandell et al. 2004)	CB189625 (Radresa et al. 2013)
Allicin (Jordt et al. 2004)	
Wood smoke (Shapiro et al. 2013)	
Formalin (McNamara et al. 2007)	
Cigarette smoke (Andrè et al. 2009)	
Aβ-unsaturated aldehydes (Andrè et al. 2008)	
ROS (Bessac et al. 2008)	
4-Hydroxynonenal (Trevisani et al. 2007)	
Chlorine/hypochlorous acid (Bessac et al. 2008)	
TRPV4	
GSK1016790a (Thorneloe et al. 2008)	HC067047 (Everaerts et al. 2010)
4αPDD (Watanabe et al. 2002)	GSK2193874 (Thorneloe et al. 2012)
Bisandrographalide (Smith et al. 2006b)	RN1737 (Vincent et al. 2009)
Hypo osmolarity (Strotmann et al. 2000; Liedtke et al. 2000; Suzuki et al. 2003).	
RN1747 (Vincent et al. 2009)	
5′6′-Epoxyeicosatrienoic acid (Watanabe et al. 2003)	
TRPM8	
Menthol (McKemy et al. 2002)	AMTB (Lashinger et al. 2008)
Linalool (Peier et al. 2002; Zhou et al. 2011)	Anandamide (De Petrocellis et al. 2008)
Geraniol (Peier et al. 2002; Zhou et al. 2011)	NADA (De Petrocellis et al. 2008)
Icilin (Peier et al. 2002; Zhou et al. 2011)	JNJ41876666 (Valero et al. 2012)
Eucalyptol (McKemy et al. 2002)	
WS-3 (Behrendt et al. 2004)	
WS-12 (Ma et al. 2008)	

[a] Undergoing phase I/II trials in clinic for treatment of chronic cough

European countries have identified six TRPV1 single-nucleotide polymorphisms (SNPs) associated with a higher risk for chronic cough (Smit et al. 2012).

TRPV1 activation

A number of TRPV1 ligands including capsaicin and RTX have been shown to activate isolated animal vagal tissues (Maher et al. 2009; Grace et al. 2012; Birrell et al. 2014; Fox et al. 1995). As discussed above, parallel data is reported in vagal tissue harvested from human lungs, thus providing translational evidence (Birrell et al. 2009; Birrell et al. 2014; Usmani et al. 2005). Furthermore, using this technique, it has been shown that under disease conditions, vagal tissue exhibits enhanced responses to TRPV1 agonists following CS exposure (Wortley et al. 2011).

Initially, it was thought that only C fibres expressed functional TRPV1 ion channels (Geppetti et al. 2006); however, using techniques like in vivo single airway fibre recordings, it is becoming clear that capsaicin can cause action potentials in a subset of RAR Aδ fibres regardless of conduction velocity in naive animals (Adcock et al. 2014). We wait to see if this profile of TRPV1 responses is altered under disease conditions.

It is established that the numbers of coughs to inhaled capsaicin are increased in disease models (Lewis et al. 2007; Maher and Belvisi 2010; Carr et al. 2002; Myers, Kajekar, and Undem 2002; Karlsson et al. 1991a; Wortley et al. 2011). It is well known that capsaicin causes coughing in guinea pigs and man (Lalloo et al. 1995; Fox et al. 1993; Caterina et al. 1997; Nasra and Belvisi 2009; Riccio et al. 1996); indeed, it is often the tussive agent of choice when performing clinical trials/cough sensitivity assessment (discussed in greater detail previously). Other agents that trigger cough such as citric acid/low pH, PGE_2 and bradykinin do so at least partially through via the TRPV1 ion channel (Fuller and Choudry 1987; Morice et al. 2007a, b; Karlsson and Fuller 1999; Doherty et al. 2000; Laude, Higgins, and Morice 1993; Lalloo et al. 1995; Morice, Kastelik, and Thompson 2001; Kollarik and Undem 2002; Maher et al. 2009; Grace et al. 2012). This data indicates that TRPV1 is central to cough responses in guinea pigs and man. Furthermore, as a number of clinical studies have shown a heightened cough response to inhaled capsaicin in patients with disease such as COPD and idiopathic cough (Choudry and Fuller 1992; Groneberg 2004; Nieto et al. 2003; Prudon et al. 2005; Ternesten-Hasséus et al. 2006; Sumner et al. 2013), the fact that many of the TRPV1 ligands are altered in a diseased airway (i.e. pH) implicates TRPV1 in enhanced troublesome cough.

TRPV1 antagonists in the clinic

Several TRPV1 antagonists have been developed and profiled in clinical trials; however, most of those that have entered clinical trials cause hyperthermia as a side effect. In both animal and human studies, the use of TRPV1 antagonists is known to cause hyperthermia and impaired perception of noxious heat which is clearly an issue for potential development compounds (Gavva et al. 2007; Gavva et al. 2008; Eid 2011; Krarup et al. 2011). Antagonizing TRPV1 with certain compounds has been shown to raise the core body temperature of the patient to an unacceptable level and also impairs noxious heat sensation raising the probability of scalding injuries (Preti et al. 2012). Nevertheless, several small-molecule TRPV1 antagonists have been developed that have overcome these problems which are outlined below.

In 2005, Janssen released a novel TRPV1 antagonist JNJ17203212 which was shown to be highly selective and well tolerated and also significantly inhibited capsaicin-induced cough to the same level as codeine (Bhattacharya et al. 2007). However, development into the clinic was inhibited because of hyperthermia (Swanson et al. 2005). More recently, JNJ39729209 has been developed which also inhibited capsaicin-induced cough in guinea pigs and had a low clearance rate and good orally bioavailability (Maher et al. 2011). In addition, this compound seems to have circumvented the problem of hyperthermia, as it only showed a small increase in core temperature (1 °C) at a fully efficacious dose (Maher et al. 2011). This is yet to be taken into the clinic, but following the promising preclinical data, it would be interesting to see how it would fare at inhibiting capsaicin-induced cough reflex sensitivity in proof of mechanism studies and spontaneous coughing in patients with chronic cough.

A study was recently published profiling the impact of a TRPV1 inhibitor, SB 705498 (Gunthorpe and Chizh 2009), on both capsaicin cough sensitivity and spontaneous cough responses in patients with refractory chronic cough (Khalid et al. 2014). SB 705498 also appears to be devoid of hyperthermia issues and is rapidly absorbed with a long elimination half-life of 50–60 h (Chizh and Sang 2009). The study used both capsaicin cough challenge and 24-h ambulatory cough monitoring. However, SB 705498 caused a very small shift in the capsaicin-induced cough response curve and had no effect on spontaneous cough (Khalid et al. 2014). This could suggest that TRPV1 is not involved in idiopathic cough or more likely that this compound did not possess the optimum efficacy profile or pharmacokinetic characteristics to fully engage the target. Whatever the case, this data does not rule out a role for TRPV1 in coughing in other conditions such as increased cough in basal COPD and during exacerbations. Indeed a phase II clinical trial (double-blind, randomized, placebo-controlled) is ongoing in COPD patients with chronic cough with a highly potent TRPV1 inhibitor, XEN-DO501. The primary endpoint is objective cough monitoring in chronic cough associated with COPD together with a proof of target engagement by including a capsaicin challenge in the protocol. This compound has undergone phase I clinical studies where it has been shown to be safe and well tolerated and has no significant hyperthermia issues (Round et al. 2011). Preclinical studies have demonstrated that XEN -DO501 inhibited capsaicin-induced cough in naive animals and also inhibited a heightened capsaicin cough response in guinea pigs exposed to CS (Wortley et al. 2014).

TRPA1

TRPA1 is a voltage-dependent Ca^{2+} permeable cation channel, where the major mode of activation is through covalent modification of N-terminal cysteines or lysines by electrophilic compounds (Bandell et al. 2004; Banner et al. 2011;

Hinman et al. 2006). TRPA1 was initially identified as a noxious cold sensor and a mechanosensor (Story et al. 2003), but it has since been shown to be activated by a wide range of pungent natural and environmental irritants which cause pain and inflammation (Bandell et al. 2004; Dicpinigaitis et al. 2014; Macpherson et al. 2005; Bautista, Pellegrino, and Tsunozaki 2013). The number of activators of TRPA1 far exceeds those for any other TRP channels. Similarly to TRPV1, it can also be activated by the indirect endogenous mediators PGE_2 and bradykinin (Grace et al. 2012; Bautista et al. 2006), and activation of PAR_2 also leads to activation of TRPA1 (Dai et al. 2007; Terada et al. 2013). A number of agonists and antagonists for the TRPA1 channel are summarized in Table 1.

TRPA1 expression

TRPA1 is expressed in a subset of TRPV1 expressing small-diameter nociceptive neurons (Story et al. 2003; Bautista 2005), and single-cell PCR has indicated that TRPA1 mRNA levels are found in airway vagal neurons (Nassenstein et al. 2008; Jang et al. 2012). TRPA1 has also been reported to be expressed in a number of non-neuronal cell types including epithelial cells, airway smooth muscle cells and fibroblasts (Jaquemar et al. 1999; Kunert-Keil et al. 2006; Stokes et al. 2006; Mukhopadhyay et al. 2011). A functional response in neurons has also been demonstrated as TRPA1 ligands induce calcium release in airway-specific jugular ganglia cells (Grace et al. 2012). It is not yet known if expression levels of TRPA1 are altered in disease conditions, despite the fact that many CS components including acrolein and crotonaldehyde activate TRPA1 (Bautista et al. 2006; Andrè et al. 2008; Andrè et al. 2009; Lin et al. 2010; Volpi et al. 2011). However, Smit et al. have reported that there were no TRPA1 SNPs which associate smoking or occupational exposure with cough although this does not rule out changes in protein expression or the activation status of the channel (Smit et al. 2012).

TRPA1 activation

TRPA1 is the molecular target for by-products of oxidative stress including reactive oxygen species (ROS) and other electrophilic compounds, including hypochlorite, hydrogen peroxide and nitric oxide (Bautista 2005; Bessac et al. 2008; Takahashi et al. 2011; Takahashi et al. 2008; Sawada et al. 2008; Andersson et al. 2008). TRPA1 activation leads to activation of vagal bronchopulmonary C fibres in rodent lungs (Bessac et al. 2008; Taylor-Clark et al. 2008; Nassenstein et al. 2008; Birrell et al. 2009; Taylor-Clark et al. 2009; Andrè et al. 2009; Grace et al. 2012) and cough in both animals and human volunteers (Birrell et al. 2009; Andrè et al. 2009)

A number of TRPA1 ligands including acrolein, cinnamaldehyde and AITC have been shown to cause depolarization in an in vitro model of sensory nerve activation (Birrell et al. 2009; Grace et al. 2012), with translational data in human tissue (Birrell et al. 2009; Grace et al. 2012). Unlike the TRPV1 agonist capsaicin, acrolein appears to only activate C fibres and had no effect on Aδ fibres in a guinea pig model of airway single-fibre activation (Adcock et al. 2014).

Acrolein, cinnamaldehyde, crotonaldehyde and AITC have been shown to cause cough in conscious naive guinea pigs (Birrell et al. 2009; Caceres et al. 2009; Andrè et al. 2009; Brozmanova et al. 2012), and in addition, CS itself is known to cause cough through activation of TRPA1 (Andrè et al. 2009). Furthermore, cinnamaldehyde has been shown to induce a concentration-dependent cough response in human volunteers (Birrell et al. 2009). Unlike TRPV1 agonists, responses to TRPA1 agonists have not yet been shown to be enhanced in disease states.

TRPA1 antagonists in the clinic

Selective TRPA1 inhibitors are also potential therapeutic agents for chronic cough, as TRPA1 plays an important role in respiratory symptoms induced by endogenous and exogenous irritants and is also a sensor of oxidative stress. In addition, TRPA1 antagonists have not yet been shown to have the same temperature regulation safety concerns as TRPV1, which would suggest that, perhaps, this channel is a more suitable target. However, despite this, currently, there is only one TRPA1 channel antagonist in the clinic to combat chronic cough, although several are in preclinical development outlined below for potential use in cough and also asthma.

Cubist pharmaceuticals have developed a novel small-molecule TRPA1 antagonist CB-189625, which is currently in phase I clinical trials in the Netherlands used to target acute pain and inflammatory conditions (Preti et al. 2012). Janssen also has a compound in the preclinical stage of testing, and this has been suggested to have antitussive activity (Preti et al. 2012). However, the only antagonist currently in clinical trials for the treatment for respiratory disorders is GRC17356 by Glenmark pharmaceuticals. Inhalation studies are planned to be initiated in both healthy and asthmatic volunteers in an allergen challenge model (Preti et al. 2012) and have recently been entered into a 4-week phase II study to evaluate efficacy, safety and tolerability of inhaled GRC17356 in patients with refractory chronic cough. It has already been shown to inhibit citric acid-induced cough in guinea pigs (Mukhopadhyay et al. 2014) and has also shown positive data in a phase 2a proof of concept study in patients with painful diabetic neuropathy.

TRPA1 also activates non-neuronal cells including fibroblast epithelial cells and airway smooth muscle, which can also add to the cough response. Activation of TRPA1 on these cells has been shown to trigger the release of inflammatory mediators (Keatings et al. 1996; Crooks et al. 2000; Gompertz

et al. 2001; Mukhopadhyay et al. 2011). Many inflammatory mediators are endogenous TRPA1 agonists, including ROS, prostaglandins and bradykinin (Grace and Belvisi 2011). Therefore, blocking TRPA1 would not only have an effect on nerves, but also aid to block inflammation caused by activating TRPA1 on non-neuronal cells which can also lead to a reduction in cough response.

TRPV4

TRPV4 is a Ca^{2+} permeable polymodal receptor with a proline-rich region and six ankyrin repeats within its cytosolic N terminus (Nilius and Szallasi 2014). TRPV4 is activated by moderate temperatures (>24 °C) and was originally characterized as a sensor of osmotic stress (Strotmann et al. 2000; Suzuki et al. 2003; Liedtke and Friedman 2003), as TRPV4 knockout mice show abnormalities with systemic osmotic and external somatosensory stimuli (Liedtke et al. 2000). Similarly to TRPA1, TRPV4 is also linked to the GPCR PAR_2, which has been shown to sensitize TRPV4 in DRG neurons and amplify neurogenic inflammation and responses to painful stimuli (Alessandri-Haber et al. 2006; Grant et al. 2007). In addition, PAR_2 stimulation has been shown to activate TRPV4 channels (Poole et al. 2013), where PAR_2 activation leads to receptor-operated gating of TRPV4 (Poole et al. 2013; Grace et al. 2014a). A number of selective ligands for the TRPV4 channel are shown in Table 1.

Tissue expression

TRPV4 is expressed in neurons of both the central and peripheral nervous systems including expression in DRG neurons (Grant et al. 2007) and at the mRNA level in pulmonary sensory neurons (Ni et al. 2006). TRPV4 has been shown to be functionally present on airway ganglia, and contrary to both TRPV1 and TRPA1 ligands, TRPV4 ligands were shown to induce calcium signal in the nodose ganglia but had no effect in the jugular ganglia (Belvisi et al. 2013).

Activation of TRPV4

The small-molecule TRPV4 activator GSK1016790a has been shown to induce depolarization of the isolated vagus nerve in both guinea pig and human tissue and also caused cough which was blocked by the selective antagonist HC067047 in conscious naive guinea pigs (Belvisi et al. 2013). This was suggested to be through activation of mechanosensitive Aδ fibres (Belvisi et al. 2013; Adcock et al. 2014). There are currently no studies in man with TRPV4 ligands; however, hypotonic solution, which is known to activate TRPV4 (Strotmann et al. 2000; Liedtke et al. 2000; Jia et al. 2004), has been reported to case cough and bronchoconstriction in asthmatic subjects (Schoeffel et al. 1981; Fuller and Collier 1984). Furthermore, distilled water, or fog, another hypotonic solution, is also used as a tussive agent in the clinic (Morice et al. 2007a). The role of TRPV4 in airway afferents requires further investigation; however, this could provide a novel mechanism for activation of sensory nerves and cough.

TRPV4 antagonists in the clinic

As not much is known about the role of TRPV4 in initiating cough, there are currently no antagonists in the clinic for the treatment of cough or respiratory disorders. However, it has been suggested that TRPV4 does cause cough via a different mechanism to that seen with TRPA1 and TRPV1 (Belvisi et al. 2013). In addition, activation of TRPV4 has been shown to cause contraction of both human and animal airway smooth muscle (Jia et al. 2004; McAlexander et al. 2014). Therefore, a TRPV4 antagonist could prove to be an attractive target for lung disease to combat both bronchoconstriction and cough and could be used alongside a TRPA1 or TRPV1 antagonist to target a different population of airway afferent fibres.

TRPM8

The TRPM8 channel is a thermosensor activated by physiological cool temperatures between 15 and 28 °C (McKemy et al. 2002; Peier et al. 2002). Direct activators of the channel are compounds which elicit a cooling sensation such as menthol, icilin and eucalyptol (Peier et al. 2002; Zhou et al. 2011). The first agonist to be identified was the monoterpene menthol, where the molecular mechanism of its pharmacological activity was determined to be through TRPM8 (McKemy et al. 2002). However, menthol has also been shown to show some biological activity at the TRPA1 channel, where low concentrations of menthol were shown to activate the channel and high concentrations were able to block TRPA1 (Karashima et al. 2007). Table 1 contains a number of agonists and antagonists for TRPM8. The channel functions as a homotetramer (Nilius and Szallasi 2014) and is composed of four identical subunits with six transmembrane domains (Latorre et al. 2007).

Tissue expression

The TRPM8 channel is expressed in a subpopulation of cold responsive primary afferent neurons within the dorsal root and trigeminal ganglia which are different to the neurons which express TRPV1 and TRPA1 (Clapham et al. 2001; Peier et al. 2002; Story et al. 2003). TRPM8 is expressed in cold-

sensitive afferents expressed in the upper and lower airways (Abe et al. 2005; Xing et al. 2008; Zhou et al. 2011; Keh et al. 2011), including vagal sensory neurons (Xing et al. 2008; Nassenstein et al. 2008). Single-cell PCR has indicated that TRPM8 is expressed in around 60 % of nasal trigeminal neurons and is also expressed on retrogradely labelled jugular neurons (Hondoh et al. 2010; Plevkova et al. 2013).

Activation of TRPM8

Although TRPM8 has been suggested to be responsible for cough and bronchoconstriction caused by inhalation of cold air (Peier et al. 2002; Xing et al. 2008), there are a number of animal studies which indicate the antitussive properties of the TRPM8 agonist menthol. Inhalation of menthol vapour reduced citric acid-induced cough by 50 % in guinea pigs (Laude et al. 1994), and inhibition of citric acid-induced cough was also indicated in both conscious and anaesthetized guinea pigs (Plevkova et al. 2013). Menthol was shown to inhibit CS-induced airway irritancy in mice, and this inhibition was reversed by the selective TRPM8 antagonist AMTB (Willis et al. 2011). Menthol's antitussive activity was suggested to be through activation of TRPM8 channels present on nasal trigeminal afferent neurons, which do not express TRPA1 or TRPV1 (Plevkova et al. 2013).

In man, citric acid-induced cough was reduced in healthy volunteers after inhalation of menthol compared to air or pine oil controls (Morice et al. 1994), and menthol vapour caused a short-lasting decrease in capsaicin-induced cough in normal subjects (Wise et al. 2012). In addition, nasal application of menthol has been shown to inhibit capsaicin-induced cough in man (Buday et al. 2012).

TRPM8 antagonists in the clinic

Contrary to activation of TRPA1, TRPV1 and TRPV4, activation of TRPM8 by menthol has instead been shown to have an antitussive effect (Laude et al. 1994; Wise et al. 2012). Menthol has been used for years in a number of OTC therapies, for its antitussive properties (Al Aboud 2010). In addition, menthol has been added to cigarettes for a number of years to reduce airway irritancy (reviewed in (Nilius and Szallasi 2014). If menthol is anti-tussive via its sctivity at TRPM8 thenantagonizing TRPM8 may not be a suitable therapeutic target for the treatment of chronic cough.

Conclusions

In summary, the treatment of chronic cough is currently an urgent unmet medical need. Current therapies have proved ineffective or have side effect liabilities, but novel therapeutics are in development, some of which are in clinical studies, which target sensory afferents and the peripheral arm of the reflex, which may show greater efficacy. TRP channels, present on the vagal sensory nerves, have been shown to play a major role in the tussive response in both animals and man, and therefore, targeting these channels could provide novel antitussive medications. TRPV1 remains an attractive target for treatment, and currently, there are a number of compounds undergoing clinical trials for efficacy in patients with chronic cough. There is also emerging evidence that other members of this family could play a significant role in activation of airway sensory nerves and cough which may provide future therapeutic options.

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