REVIEW

The Prospect for Potent Sodium Voltage-Gated Channel Blockers to Relieve an Excessive Cough

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Summary

An excessive, irritable, productive or non-productive coughing associated with airway inflammation belongs to pathological cough. Increased activation of airway vagal nociceptors in pathological conditions results from dysregulation of the neural pathway that controls cough. A variety of mediators associated with airway inflammation overstimulate these vagal airway fibers including C-fibers leading to hypersensitivity and hyperreactivity. Because current antitussives have limited efficacy and unwanted side effects there is a continual demand for the development of a novel more effective antitussives for a new efficacious and safe cough treatment. Therefore, inhibiting the activity of these vagal C-fibers represents a rational approach to the development of effective antitussive drugs. This may be achieved by blocking inflammatory mediator receptors or by blocking the generator potential associated with the specific ion channels. Because voltage-gated sodium channels (NaVs) are absolutely required for action potentials initiation and conduction irrespective of the stimulus, NaVs become a promising neural target. There is evidence that NaV1.7, 1.8 and 1.9 subtypes are predominantly expressed in airway cough-triggering nerves. The advantage of blocking these NaVs is suppressing C-fiber irrespective to stimuli, but the disadvantage is that by suppressing the nerves is may also block beneficial sensations and neuronal reflex behavior. The concept is that new antitussive drugs would have the benefit of targeting peripheral airway nociceptors without inhibiting the protective cough reflex.

Key words

Airway sensory nerves ● A-fibers ● C-fibers ● Cough ● Voltage-gated sodium channel blockers

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Introduction

Cough is a very frequent symptom and a reason to visit the doctor. On one side cough is an effective defensive reflex that protects the airways and lungs from aspirate, inhaled particulate matter, accumulated secretion or other irritants. On the other side, when cough ceases to fulfil its physiological role and when becomes a pathological one and occurs as a common symptom in a variety of acute and chronic respiratory diseases. This cough becomes too excessive, irritative, strongly productive or non-productive according to the origin. It is often painful and finally adversely impacts patients' quality of life (Kollarik *et al.* 2010, Canning *et al.* 2014).

Acute cough that lasts less than 8 weeks and is usually a result of an acute viral or bacterial upper respiratory tract infection. Chronic cough lasts for over 8 weeks and commonly occurs in chronic respiratory diseases such as bronchial asthma, chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), and lung cancer, or can be idiopathic in origin (Canning *et al.* 2014, Bonvini and Belvisi 2017). Pathological cough in disease is persistent and hypersensitive, occurring in response to stimuli which do not normally evoke cough. This enhanced sensitivity to tussive or non-tussive stimuli is referred to as chronic cough hypersensitivity syndrome which is a common

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symptom in chronic respiratory diseases (Mazzone and Undem 2016, Song and Morice 2017, Brozmanova *et al.* 2008).

Despite the fact that a lot of money is spent every year on the cough medication, it seems from clinical practice that the currently used antitussive therapies are largely ineffective. One of the most effective antitussive drug groups is opiates (codeine), which act both centrally on brainstem via opioid receptors and on receptors located peripherally on sensory nerve terminals in the airways. However, at their effective doses, they have dangerous adverse effects such as dependence, respiratory depression, sedation or gastrointestinal complications (Belvisi and Geppetti 2004, Barnes 2007). Surprisingly, one clinical trial has shown that codeine – the current gold standard in cough treatment, was no more effective than placebo in patients with COPD (Smith *et al.* 2006). Because of the high incidence of chronic cough with adverse consequences, there is a continuous demand for the development of a novel, more effective antitussive for a new efficacious and safe cough treatment. Therefore, the identification of new neural target would be a huge benefit for people suffering from chronic respiratory diseases. The ideal therapeutic strategy would represent inhibition of pathological cough by selectively inhibiting mainly jugular C-fibers with preservation of the protective cough reflex (mainly evoked by nodose Aδ-fibers) for maintaining airway patency and preventing lung infections.

Airway sensory nerves and cough

Extensive experimental studies in animals and an increasing number of studies conducted in human subjects have increased our understanding about neural pathway regulating the cough reflex (Canning *et al.* 2014, Mazzone 2005, Mazzone and Undem, 2016, Song and Morice 2017, Pecova *et al.* 2008, Brozmanova *et al.* 2006, Brozmanova *et al.* 2008, Brozmanova *et al.* 2012, Plevkova *et al.* 2004, Plevkova *et al.* 2013).

Cough is initiated when at least two subtypes of airway vagal sensory nerves are stimulated with distinction in their chemical and mechanical responsiveness according to their anatomic, embryological and physiological characteristics. Studies in guinea pigs indicate that cough reflex is independently regulated by these nerve subtypes. They involve nociceptive C-fibers derived from the jugular ganglia mediating mainly chemically-induced cough. They are unmyelinated and slowly conducting C-fibers with terminals found in and around the mucosal surface of the larynx, trachea, bronchi, and alveoli. C-fibers as nociceptors detect a range of noxious chemical irritants but are relatively insensitive to mechanical stimuli. They are sensitive to a variety of inhaled or locally produced chemical mediators, which may either activate or sensitize nociceptor nerve endings. These nociceptors are also responsive to pro-inflammatory molecules, including bradykinin, prostaglandins, leukotrienes and cytokines. C-fibers are also selectively activated by capsaicin, protons, nicotine or activators of TRPA1. Each of these stimuli initiate coughing in patients and awake animals (Andre *et al.* 2009, Canning *et al.* 2006, Canning *et al.* 2014, Mazzone and Undem 2016, West *et al.* 2015, Taylor-Clark *et al.* 2008, Taylor-Clark *et al.* 2009, Bonvini and Belvisi 2017, Brozmanova *et al.* 2012, Plevkova *et al.* 2004, Pecova *et al.* 2008, Ji *et al.* 2018).

Mechanosensitive "cough receptors" derived from the nodose ganglia mediate mainly mechanically-induced cough, occurring during protection and cleaning of airways. They are myelinated and fast conducting Aδ fibers with terminals found underneath the epithelium in the large airways. They are mainly sensitive to touch-like mechanical stimuli, intraluminal irritants delivered to the mucosal surface such particulate matter but they are relatively insensitive to most chemical mediators with exception of low pH (Canning *et al.* 2006, Canning *et al.* 2014, Mazzone and Undem, 2016, West *et al.* 2015).

Activation of the aforementioned airway sensory nerves causes membrane depolarization of the terminal membrane. If the depolarization is of sufficient magnitude and it reaches a certain threshold (around -55 millivolts), then it leads to the formation of the all-or-nothing action potential via activation of voltage-gated sodium channels (NaVs). Action potentials are conducted along the axon to the central terminations in the brainstem. The nodose neurons have their central terminations well-defined in the nucleus of the solitary tract (nTS), whereas jugular neurons have been recently shown to terminate in the paratrigeminal nucleus (Pa5). These sensory nuclei relay signal to the respiratory central pattern generator within the brainstem, which is responsible for reflex coughing, as well as to higher brain structures for the perception of airway irritation, which are needed for behavioral modulation of coughing (Canning *et al.* 2006, Canning *et al.* 2014, Taylor-Clark 2015, Mazzone and Undem 2016).

Ion channels in activation of airway sensory nerves and cough

Several transient receptor potential (TRP) channels are expressed on airway sensory nerve terminals, namely the temperature-sensitive transient receptor potential vanilloid-1 (TRPV1), transient receptor potential vanilloid-4 (TRPV4), transient receptor potential ankyrin-1 (TRPA1) and transient receptor potential melastatin-8 (TRPM8), which are implicated in the afferent arm of the cough reflex (Grace *et al.* 2014). These TRP channels as ligand-gated ion channels have been described in dorsal root ganglia. They are emerging as sensory transducers that may participate in the generation of pain sensation evoked by chemical, thermal and mechanical stimuli (Levine and Alessandri-Haber 2007). Recent studies in mice deficient in TRP channels indicate that TRP channels may play a crucial role in the hypersensitivity to thermal, chemical and mechanical stimuli that are associated with peripheral inflammation and neuropathic pain (Levine and Alessandri-Haber 2007).

TRPV1, originally named vanilloid receptor 1 (VR 1) is commonly referred to as the capsaicin receptor. TRPV1 is a Ca^{2+} permeable non-selective ion channel. TRPV1 was first described as a polymodal receptor activated by three pain-producing stimuli: vanilloid compounds (capsaicin, resiniferatoxin), moderate heat (≥43 ºC) and a low pH (<5.9) and is highly expressed in dorsal root ganglia, trigeminal and vagal ganglia (Caterina *et al.* 1997). TRPV1 can be also indirectly activated through protein-coupled receptors (GPCRs) when diverse agonists bind to GPCRs and initiate signalling pathway with subsequent activation of TRPV1. These agonists include bradykinin, prostaglandin E_2, extracellular ATP and Protein Activated Receptor 2 (PAR2) (Grace *et al.* 2012, Kollarik and Undem 2004, Chen *et al.* 2017). Noxious exogenous irritants such as capsaicin and resiniferatoxin and some endogenous mediators such as acid bind directly to 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 acts as a direct opener or rather as a sensitizer, which lowers the activation threshold for another stimulus. TRPV1 activation by capsaicin as a common tussive agent to evoke cough has been well documented in human and animals (Lallo *et al.* 1995, Mazzone 2005, Brozmanova *et al.* 2012).

TRPA1 (ankyrin 1) is a nonselective cationic TRP channel expressed in nociceptors, including spinal dorsal root ganglia (DRG), nasal trigeminal neurons and it is widely expressed in vagal airway neurons (Nassenstein *et al.* 2008, Bautista *et al.* 2013). TRPA1 has emerged as an important sensor of noxious stimuli and tissue damage. TRPA1 is activated by many natural compounds that cause pain and inflammation such as mustard oil, garlic, cinnamon and wasabi, environmental irritants present in air pollution, vehicle exhaust and cigarette smoke, or products of oxidation (Taylor-Clark *et al.* 2008, Taylor-Clark *et al.* 2009, Nassenstein *et al.* 2008, Bautista *et al.* 2013). The agonists of GPCRs such as prostaglandin E_2 and bradykinin activate nociceptive neurons indirectly via interaction with TRPA1 (Nassenstein *et al.* 2008, Taylor-Clark *et al.* 2008, Grace *et al.* 2012). TRPA1 channels mediate the tussive response in both animals and human. Several studies in guinea pigs have provided direct and compelling evidence that TRPA1 agonists evoke coughing in a manner that can be antagonized by TRPA1 antagonists (Andre *et al.* 2009, Birrell *et al.* 2009, Brozmanova *et al.* 2012). When we compared the efficacy of TRPA1 *vs.* TRPV1 in our laboratory, we found that TRPA1-induced cough was relatively moderate in naïve guinea pigs compared to the cough initiated by TRPV1, likely due to a lower efficacy of TRPA1 stimulation to induce sustained activation of airway C-fibers. It may be speculated, however, that the efficacy of TRPA1 stimulation to trigger cough is increased in inflammatory conditions (Brozmanova *et al.* 2012).

TRPM8 (melastatin 8) channel has been described as a cold and menthol-activated channel with voltage-dependent gating properties (McKemy *et al.* 2002). TRPM8 is predominantly expressed in cold-responsive primary afferent sensory neurons within the DRG, trigeminal and jugular ganglia and are largely distinct from neurons expressing TRPV1 and TRPA1 (Nassenstein *et al.* 2008, Grace *et al.* 2014, Plevkova *et al.* 2013). TRPM8 is activated by cooling sensation compounds such as menthol, icilin and eucalyptol (McKemy *et al.* 2002, Grace *et al.* 2014, Plevkova *et al.* 2013). Several papers support the fact that TRPM8 channel plays a protective role and suppresses citric acid and capsaicin induced cough in animal and man (Morice *et al.* 1994, Buday *et al.* 2012, Plevkova *et al.* 2013). However, there are still controversial data relating to the role of TRPM8 because both menthol and icilin activate TRPA1 at high concentration (Grace *et al.* 2014).

Acid-sensing ion channels (ASICs) contribute to airway nociception and are partialy responsible for airway defensive mechanisms including cough. ASIC channels are members of the DEG/ENaC family of sodium channels that are the cation channels gated by a proton. Some subunits are expressed in the peripheral sensory neurons and they determinate its acid transduction properties. The pH threshold of ASICs is in a wide range (pH: 7-4). The overall acid responsiveness of a nerve terminal is influenced by other channels modulated by acid. Acid activates both TRPV1 and ASIC channels in sensory neurons. However, TRPV1 is not expressed in Aδ fibers and selective TRPV1 inhibitors have no effect on acid-induced Aδ fibers activation (Kollarik and Undem, 2002).

P2X2 and *P2X3 channels* have been found on sensory C-fibers. ATP activates ionotropic P2X receptors and metabotropic P2Y receptors. P2X3 is predominantly expressed in the afferent airways C and Aδ fibers. ATP has been shown to activate nodose and jugular sensory fibers, which was blocked by the selective P2X3 antagonist (Kwong *et al.* 2008). In human, ATP induced cough and bronchoconstriction in patients with asthma and COPD (Basoglu *et al.* 2015).

Sensory hyperresponsiveness and cough

There are many pieces of evidence that an excessive and troublesome cough may be a consequence of airway inflammation or chronic stimulation of airways by irritants leading to cough hypersensitivity and hyperactivity (Karlsson 1993, Carr 2004, Undem *et al.* 2015, Zaccone *et al.* 2016). Both peripheral and central sensitization of airway sensory nerves has been recognised as potential mechanisms of abnormal acute or chronic cough resulting from airway inflammation. Cough hypersensitivity is most likely associated with dysregulation of sensory neural pathways and cough reflex central regulation (Karlsson 1993, Carr 2004, Undem *et al.* 2015, Zaccone *et al.* 2016).

There are several experimental and clinical aspects for evaluation of cough reflex sensitivity, intensity, frequency and severity. Inhalation airway challenge using tussive agents such as citric acid and capsaicin has been used for both experimental research and clinical trials using regulation according to ERS guideline on the assessment of cough (Morice *et al.* 2007, Birrell *et al.* 2009, Laude *et al.* 1993, Karlsson 1993, Lalloo *et al.* 1995, Pecova *et al.* 2007). Cough reflex sensitivity is measured in individual subjects before and after the intervention and is recorded as the concentration of tussive substances inducing two (C2) or five (C5) coughs (Morice *et al.* 2007).

Previous experimental studies in animals have already shown that airway inflammation or chronic irritation with cigarette smoke may lead to hyperactivity in capsaicin-sensitive afferent nerves. Prolonged exposure of guinea pigs to cigarette smoke caused increased sensitivity to tussive citric acid and reactivity of capsaicin-sensitive nerves mediating cough (Karlsson 1993). Respiratory tract viral infection also induced cough hypersensitivity in response to capsaicin, citric acid and bradykinin in the guinea pig model (Zaccone *et al.* 2016). Many inflammatory mediators, such as histamine, bradykinin and prostaglandins, have been shown to increase nociceptor excitability and lead to development of cough hypersensitivity, as well (Choudry *et al.* 1989, Lee and Morton 1993, Kwong and Lee 2005, Fox *at al.* 1996, Kamei and Takahashi 2006, Taylor-Clark 2015). Increased cough reflex sensitivity and reactivity was observed in animals with allergic rhinitis (Brozmanova *et al.* 2006, Brozmanova *et al.* 2008). Cough reflex was correspondingly modulated by stimulation of nasal mucosa in cats and guinea pigs (Plevkova *et al.* 2004a). An alternative explanation for cough associated with rhinosinusitis would be a sensitizing effect of upper airway afferent nerves, resulting in enhanced cough responsiveness.

The clinical observation supports the existence of cough sensory hyperresponsiveness to tussive agents. Likely as in experimental animals, cough sensitivity was increased in humans with allergic rhinitis (Pecova *et al.* 2008). Coughing in response to experimental airway challenge with a range of physical and chemical irritants, including cold air and aerosols of capsaicin, citric acid, histamine, and charcoal dust, have been reported in both healthy subjects and patients with the disease (Choudry *et al.* 1989, Doherty *et al.* 2000). There is evidence that chronic inflammation of the lower airways with damaged bronchial epithelium and goblet cells hyperplasia may be related to neuropathic modulation. Some of these findings have been associated with capsaicin cough hypersensitivity (Choudry *et al.* 1989, Doherty *et al.* 2000, Niimi and Chung 2015). Increased level of inflammatory biomarkers including histamine, prostaglandin D_2 and E_2, TNFα and IL-8 were found in the sputum of patients with chronic cough (Niimi and Chung 2015). Both bradykinin and PGE_2 increase

capsaicin cough response and indirectly sensitize airway neuronal response to capsaicin via activation of TRPV1 (Niimi and Chung 2015). Cough hypersensitivity correlates with increased expression of TRPV1 receptor on epithelial nerves of chronic cough patients (Groneberg *et al.* 2004). Moreover, the inflammation processes can also change electrical excitability and gene expression. If these changes are long-lasting, they can alter the phenotype of the C- and Aδ-fibers in the airways which is often referred to as neuroplasticity. Subsequently these nerves can be activated by stimuli that normally do not evoke cough. For instance, inflammation of airways leads to expression of TRPV1 channels in nodose Aδ-fibers in the trachea and thus becoming sensitive to TRPV1 activators in guinea pig model (Mazzone and Undem 2016). Interestingly, cough may be sensitized not only from the nose or upper airways respectively but also from outside the respiratory system, such as from oesophagus (Kollarik and Brozmanova 2009, Hennel *et al.* 2015).

Voltage-gated sodium channels as potential targets in the regulation of cough

Coughing associated with acute and chronic inflammation is ascribed to the activation of vagal airway sensory C-fibers. Numerous inflammatory mediators can act on multiple receptors expressed in airway C-fibers. They involve histamine, bradykinin, prostaglandins and others, those have been shown to increase nociceptor excitability and increase the sensitivity of the cough reflex as well (Choudry *et al.* 1989, Lee and Morton 1993, Kwong and Lee 2005, Fox *at al.* 1996, Kamei and Takahashi 2006, Taylor-Clark 2015). Regardless of which receptors are stimulated, afferent activation depends on the gating of membrane ion channels at the airway afferent terminals. This leads to nerve depolarization, which triggers the activation of voltage-gated sodium channels (NaVs), which in turn are responsible for the induction and conduction of action potential (Carr and Undem 2003).

The therapeutic perspectives are still developed with a strategy of looking for effective antitussives mainly targeting C-fibers for peripherally acting anti-tussive drugs. Previously, several studies were conducted when TRPV1 was considered as a strong candidate target for the development of novel antitussive agents (Morice and Geppetti 2004). However, contrary to expectations, a TRPV1 antagonist failed to show any significant benefit in reducing cough (Song and Morice 2017). Similarly, another candidate therapeutic target TRPA1, acting on vagal sensory neurons, failed in the convincing reduction of cough in studies using TRPA1 antagonists (Song and Morice 2017). ATP receptors P2X2/3 antagonists were particularly effective on cough intensity, however, side effects such a loss of taste occurred (Ford and Undem 2013). Many other currently used antitussive therapies are ineffective and act largely through the central nervous system, therefore have numbers of dangerous adverse effects (Dicpinigaitis *et al.* 2014). The most common over-the-counter (OTC) antitussive medications sold are drugs containing dextromethorphan hydrobromide. Dextromethorphan is as effective as codeine and has neurological adverse effects in overdose. There are many other OTC antitussive drugs without available evidence for their effectiveness, but with higher numbers of adverse effects (Dicpinigaitis *et al.* 2014). Nevertheless, advances in the understanding of the mechanism of cough hypersensitivity offer the promise for new therapies targeting voltage-gated sodium channels predominantly peripherally acting antitussive drugs with minimal adverse effects. Among the nine subtypes of NaV1s channels, NaV1.7, NaV1.8 and NaV1.9 channels are almost exclusively expressed by sensory neuron C-fibers and Aδ-fibers that innervate the airways and initiate cough (Kwong *et al.* 2008a, Carr 2013, Muroi *et al.* 2013, Keller *et al.* 2017, Sun *et al.* 2017, Kollarik *et al.* 2018).

Electrophysiological analysis suggests sub-stantial differences in NaVs regulation of different types of cough-triggering nerve afferents. The action potential initiation in nerve terminals of nodose Aδ-fibers strongly depends on tetrodotoxin TTX-sensitive NaV1.7 channels, whereas the action potential initiation in nerve terminals of jugular C-fibers is dependent on TTX-resistant NaV1.8 channels (Sun *et al.* 2017, Kollarik *et al.* 2018). These channels come to be upregulated in response to inflammatory mediators that are known to increase cough sensitivity (Laedermann *et al.* 2015).

It appears that blockers of NaV1.7, NaV1.8 and NaV1.9 channels have a potential for suppressing sensory nerve activity. In addition, NaVs channels are important in sensory nerve excitability and their inhibition may anaesthetize the airways (Keller *et al.* 2017). For instance, non-selective blocking of NaVs channels with local anaesthetics, such as lidocaine reduces but does not abolish cough evoked by mechanical and chemical stimuli (Muroi *et al.* 2013). A similar effect has been observed when using the knockdown of NaV1.7gene-silencing approaches (Muroi *et al.* 2013). Clinical studies

have shown that lidocaine is minimally effective at blocking cough, likely due to its low affinities for NaVs channels and it is likely that at the doses that can be administered safely, they only weakly and briefly inhibit NaVs in the afferent C-fibers and Aδ-fibers terminals involved in cough (Muroi and Undem 2014, Lavorini *et al.* 2016). The lidocaine derivative carcainium chloride has shown some antitussive potential without major local anaesthetic activity (Lavorini *et al.* 2016). Contrary, the novel voltage-gated sodium channel inhibitor GSK2339345 surprisingly increased cough (Smith *et al.* 2017). Among many controversial data in clinical trials using nebulized lidocaine about 50 % of patients reported successful cough suppression and more than 40 % of patients reported some side effects including dysphonia, oropharyngeal numbness, and bitter taste, so larger randomized control trials comparing nebulized lidocaine to placebo need to be conducted in the future (Sunders and Kirkpatrick 1984, Trochtenberg 1994, Lim *et al.* 2013, Truesdale and Jurdi 2013).

We recently reported the effect of lidocaine on citric acid- and capsaicin-induced cough in a guinea pig model. Our data have shown that nebulized lidocaine in a lower concentration of 1 mM was not sufficient to reduce the tussive response evoked by citric acid, but inhalation of nebulized lidocaine in higher concentration (10 mM) was successful in suppressing cough response during citric acid and capsaicin challenge (Svajdova *et al.* 2019). It has been previously established that nebulized lidocaine inhibited chemically induced cough evoked by capsaicin and citric acid in a dose-dependent manner and was ineffective against bronchoconstriction in conscious guinea pig model (Forsberg *et al.* 1992). In anaesthetized rabbits, both chemically (ammonia vapour) and mechanically evoked cough were inhibited by nebulized lidocaine and tetracaine (Karlsson 1987). Adcock and colleagues compared antitussive effects of two distinct local anaesthetics including lidocaine and carcainium chloride (RSD931). In awake guinea pigs, lidocaine aerosol in the concentration of 10 and 30 mg/ml did not significantly reduce the total number of coughs induced by citric acid and capsaicin. On the contrary, RSD931 significantly suppressed citric acid- and capsaicin-evoked cough (Adcock *et al.* 2003). In addition to that, no significant reduction in cough response was observed in guinea pigs pre-treated with nebulized lidocaine hydrochloride in concentrations of 100 mg/ml during capsaicin challenge, but RSD931 was again significantly effective in suppressing capsaicin-induced cough (Venkatasamy *et al.* 2010).

The single-cell RT-PCR data shows that extrapulmonary jugular C-fibers and nodose Aδ-fibers in guinea pig trachea express mainly TTX-sensitive NaV1.7 channels and TTX-resistant NaV1.8 and NaV1.9 channels (Kollarik *et al.* 2018, Sun *et al.* 2017). The significant progress in the understanding of voltage-gated sodium channels (NaVs) proposed a hypothesis that blocking of certain NaV1 subtypes (namely NaV1.7 and NaV1.8) in airway sensory nerves may lead to suppression of pathological coughing (Muroi and Undem 2014).

NaV1.8 blocker and cough

NaV1.8 as one subtype of NaVs channels has a fundamental role on the neuron excitability and capacity for action potential discharge in vagal sensory neurons (Muroi and Undem 2014). NaV1.8 together with NaV1.7 and NaV1.9 appear to be upregulated in the presence of inflammation and specific inflammatory mediators (Strickland *et al.* 2008).

We recently demonstrated that the NaV1.8 inhibitor A-803467 suppressed capsaicin-induced cough in the dose that did not affect the respiratory rate (Brozmanova *et al.* 2019). The inhibitor was administered both in systemic and localized manner – by inhalation. Compared to vehicle, intraperitoneal or inhalation administration of A-803467 did not abolish cough completely but caused 30-50 % inhibition of capsaicin-induced cough in naïve guinea pigs. This is consistent with electrophysiological studies, which demonstrated that bradykinin-induced action potential discharge in guinea pig jugular C-fibers was inhibited by NaV1.8 blocker by 50 % (Kollarik *et al.* 2018). Likewise, NaV1.8 inhibitor A-803467 in a relatively large concentration did not entirely inhibit the TTX-resistant current in guinea pig neurons in the patch-clamp study (Brozmanova *et al.* 2019).

There is evidence that airway inflammatory processes lead to overexpression of NaVs channels such as NaV1.8 together with NaV1.7 and NaV1.9 in afferent nerves mediating cough (Strickland *et al.* 2008). Changes in the expression of NaVs contribute to the sensitization of sensory neurons in chronic pain states as well (Bennett *et al.* 2019). It seems that NaV1.8 blockade with NaV1.7 may normalize C-fibers that are in a hyperexcitable state as a reaction to inflammation. In somatosensory system, blocking NaV1.8 has a limited effect on the response to

painful stimuli in healthy animals, but considerably inhibits the hyperalgesia associated with inflammatory lesions (Lai *et al.* 2004, Jarvis *et al.* 2007). This may most likely explain the fact that Nav1.8 blockade is more effective in hypertussive state accompanied by respiratory diseases than in inhibiting cough in healthy individuals. Moreover, the systemic NaV1.8 blocker A-803467 can affect different parts of C-fibers such as terminals, axons or cell bodies, however, the effect of inhaled A-803467 is most probably limited to C-fiber terminals in the airways.

Joshi with co-workers demonstrated additive antinociceptive effects of the selective NaV1.8 blocker A-803467 and selective TRPV1 antagonists in rat inflammatory and neuropathic pain models. Using such a combination to produce analgesia may potentially provide an improved therapeutic strategy (Joshi *et al.* 2009).

NaV1.7 blocker and cough

NaV1.7 is a tetrodotoxin-sensitive voltage-gated sodium channel and plays a critical role in the generation and conduction of action potentials in excitable tissues. Together with NaV1.8 and NaV1.9, NaV1.7 is abundantly expressed in airway vagal sensory neurons mediated initiating of cough (Kwong *et al.* 2008). NaV1.7 strongly regulates vagal afferent nerve excitability and is essential for conduction of action potential in vagal afferent nerves (Kwong *et al.* 2008, Kollarik *et al.* 2018, Muroi *et al.* 2011, Muroi *et al.* 2013). Single-cell RT-PCR performed on vagal afferent neurons from guinea-pig trachea has shown that almost all of the jugular TRPV1-positive neurons expressed NaV1.7. Tracheal nodose TRPV1-negative neurons also expressed NaV1.7 (Kollarik *et al.* 2018). The expression of NaV1.7 was in accordance with electrophysiology study from isolated vagal nodose and jugular nerve fibers innervating trachea or lung revealing that action potential conduction in the majority of jugular C-fibers was abolished by TTX. Contrary, both action potential initiation and conduction in nodose nociceptors were abolished by TTX or selective NaV1.7 blocker (Kollarik *et al.* 2018). The specific role of NaV1.7 in vagal afferent neurons has been shown when NaV1.7 gene expression was silenced in guinea pig nodose and jugular neurons. NaV1.7 gene expression in vagal ganglia was effectively and selectively reduced without changes in expression of other voltage-gated sodium channels subtypes. Reduced gene expression of NaV1.7 corresponded with a reduction of TTX-sensitive voltage-gated sodium current reflecting in the reduction of action potential discharge (Muroi *et al.* 2011, Muroi *et al.* 2013).

When used silencing NaV1.7 gene expression *in vivo* on citric acid-induced cough in awake guinea pigs, the cough response was almost abolished in animals bilaterally treated with NaV1.7 shRNA although their behavior was normal. In contrast to cough reflex, silencing NaV1.7 expression bilaterally in nodose neurons had no effect on respiratory rate (Muroi *et al.* 2011). It appears that blocking of the NaV1.7 channels prevent action potential conduction in most jugular C-fibers but did not block the action potential initiation (Kollarik *et al.* 2018). Inhibition of action potential activation of jugular C-fibers terminals appeared after the combination of inhaled NaV1.7 and NaV1.8 blockers (Kollarik *et al.* 2018, Patil *et al.* 2019). As seen, the role and function of NaVs channels at the terminals in the airways are more complex. Therefore, other experiments with NaV1.7 blockers focused on cough reflex are required using animal cough challenge model focused on topical - inhaled drug administration.

Conclusion

It is no doubt that increased activation of airway vagal nociceptors in pathological conditions is accompanied by the pathological type of coughing resulting from dysregulation of the neural pathway that controls cough. A large spectrum of mediators associated with airway inflammation overstimulates these vagal airway fibers including C-fibers leading to hypersensitivity and hyperreactivity. Therefore, inhibiting the activity of these vagal C-fibers represents a rational approach to the development of effective antitussive drugs. This may be achieved by blocking inflammatory mediator receptors or by blocking the generator potential associated with the specific ion channels. Despite the strong efforts of pharmacological companies, many antitussive drugs are not effective and mainly centrally acting antitussive drugs have unwanted side effects. Because voltage-gated sodium channels are absolutely required for action potentials initiation and conduction irrespective of the stimulus, NaVs become a promising and attractive neural target. There is evidence that NaV1.7, 1.8 and 1.9 subtypes are predominantly expressed in airway cough-triggering nerves. The advantage of blocking these NaVs is silencing C-fiber in

action irrespective the stimulus, but the disadvantage is that by silencing the nerves it may also block beneficial sensations and neuronal reflex behavior. Therefore, the new strategy of pharmaceutical companies involves blocking the generator potential of nerves and an effort to find novel non-opioid analgesics. One suggests that new antitussive drugs would have the advantage of targeting airway nociceptors topically with an inhaled drug delivery approach without inhibiting the protective cough reflex.

Larger studies are needed for new therapies such as symptom-suppressive antitussives for chronic hypersensitivity syndrome. There are many parallels between chronic neuropathic pain and chronic cough and so this makes voltage-gated sodium channels blockers an attractive therapeutic approach.

Conflict of Interest

There is no conflict of interest.

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