Regulation of cough by voltage-gated sodium channels in airway sensory nerves

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Abstract

Chronic cough is a significant clinical problem in many patients. Current cough suppressant therapies are largely ineffective and have many dangerous adverse effects. Therefore, the identification of novel therapeutic targets and strategies for chronic cough treatment may lead to development of novel effective antitussive therapies with fewer adverse effects. The experimental research in the area of airway sensory nerves suggests that there are two main vagal afferent nerve subtypes that can directly activate cough – extrapulmonary airway C-fibres and Aδ-fibres (described as *cough receptors*) innervating the trachea. There are different receptors on the vagal nerve terminals that can trigger coughing, such as TRP channels and P2X2/3 receptors. However, in many patients with chronic respiratory diseases multiple activation of these receptors could be involved and it is also difficult to target these receptors. For that reason, a strategy that would inhibit cough-triggering nerve afferents regardless of activated receptors would be of great benefit. In recent years huge progress in understanding of voltage-gated sodium channels (NaVs) leads to a hypothesis that selective targeting of NaVs in airways may represent an effective treatment of pathological cough. The NaVs (NaV1.1 – NaV1.9) are essential for initiation and conduction of action potentials in these nerve fibres. Effective blocking of NaVs will prevent communication between airways and central nervous system and that would inhibit provoked cough irrespective to stimuli. This review provides an overview of airway afferent nerve subtypes that have been described in respiratory tract of human and in animal models. Moreover, the review highlights the current knowledge about cough, the sensory nerves involved in cough, and the voltage-gated sodium channels as a novel neural target in regulation of cough.

Key words: airway sensory nerves, A-fibres, C-fibres, cough, voltage-gated sodium channels

INTRODUCTION

The cough reflex represents a physiological response to airway irritation. The problem occurs when cough is dysregulated and becomes excessive and non-productive. This so-called pathological cough is a common symptom in variety of 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 [1].

In recent years the neural pathways that are involved in cough have been investigated. The cough reflex is directly initiated by activation of the two vagal afferent nerve subtypes. Extrapulmonary airway C-fibres with nociceptive properties (derived from jugular ganglia) mediate mainly chemically-induced cough and mechanosensitive Aδ-fibres innervating the trachea (*cough receptors*) mediate primarily mechanically-induced cough. The *cough receptors* originate from nodose ganglia [1]. Cough reflex is initiated only if the activation of these cough-triggering nerves is transmitted to the brainstem in a form of action potentials. Absolute requirement for action potential initiation and conduction are voltage-gated sodium channels (NaVs) that mediate fast sodium current leading to membrane depolarization [2, 3].

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Nine different pore-forming α subunits of NaVs referred to as NaV1.1 – NaV1.9 have been identified. Preliminary studies using single cell RT-PCR expression analysis indicate that the vast majority of jugular C-fibres and nodose Aδ-fibres express mainly NaV1.7, 1.8, and 1.9 channels. Electrophysiological analysis suggests substantial differences in NaVs regulation of different types of cough-triggering nerve afferents. The action potential initiation in nerve terminals of nodose Aδ-fibres strongly depends on NaV1.7 channels, whereas the action potential initiation in nerve terminals of jugular C-fibres is dependent on NaV1.8 channels [3, 4].

Selective silencing of NaVs will prevent generation of action potentials and its conduction to the brainstem and that in turn would block provoked cough reflex irrespective to the triggering stimulus. Therefore, we hypothetize that drugs capable to selectively block of NaVs may represent an effective and safe treatment of pathological cough.

AIRWAY SENSORY NERVES

Sensory (or afferent) nerve fibres carry information about external and internal (visceral) environment towards the central nervous system (CNS). The majority of airway afferent nerve fibres are derived from vagal sensory neurons. Vagal afferent nerve fibres innervate all visceral organs and 20 % of them terminate in respiratory tract [5]. Cell bodies of vagal sensory neurons are located in vagal sensory ganglia – nodose ganglion (inferior vagal ganglion) and jugular ganglion (supranodose or superior vagal ganglion). These neurons have distinct embryological origin. Neurons in the nodose ganglion are embryologically derived from epibranchial placodes, whereas neurons in the jugular ganglion are derived from neural crest. The distinct embryological source of vagal afferent nerves results in different protein expression, different function, and localization of their terminals within respiratory tract [6, 7]. A small population (around 1 %) of airway fibres may originate in dorsal root ganglia (DRG), but little is known about function of spinal afferents in airways [5].

Vagal afferent nerve subtypes innervating respiratory tract (Tab. 1) are classified based on conduction velocity of action potentials as **unmyelinited C-fibres** and **myelinited A-fibres** (Aδ, Aβ). The velocity of action potential conduction depends on axon diameter and degree of myelinization. Thus, Aβ-fibres conduct action potentials with the fastest rate, Aδ-fibres with intermediate rate, and C-fibres are the slowest in conduction [8, 9].

Airway sensory nerves can be functionally classified as low-threshold mechanosensors and chemosensors (nociceptors). **Low-threshold mechanosensors** are activated by one or more mechanical stimuli, such as lung inflation, bronchoconstriction, light touch, but they do not respond directly to chemical stimuli. An exception of this rule is ATP and acid. ATP directly activates intrapulmonary low-threshold mechanosensitive Aβ-fibres (both RAR and SAR fibres) via purinergic P2X2/3 receptors (expressed in nodose neurons) [10, 11, 12]. Nodose Aδ-fibres (*cough receptors*) are sensitive to acid, but only in rapid pH decrease [13]. **Chemosensors** are activated directly by a wide range of chemicals, but they are relatively insensitive to mechanical stimuli. Chemically-sensitive vagal afferents are often defined by their sensitivity to capsaicin (pungent component of chilli peppers) due to the expression of capsaicin-sensitive transient receptor potential vanilloid 1 (TRPV1) channel [10, 14]. However, this definition is not correct because some spieces such as mice have capsaicin-insensitive (TRPV1-negative) chemosensitive population of vagal afferent nerves in airways [15]. It may also be assumed that all airway chemosensors are C-fibres. This assumption is incorrect as well because airway chemosensors that conduct action potentials in Aδ-fibre range have been identified (jugular Aδ-fibres) [16, 17, 18].

C-fibre subtypes

The vast majority of vagal afferents in respiratory tract are unmyelinated C-fibres with low conduction velocity (average 1 m/s). These C-fibres are polymodal sensors of noxious sti-

Table 1 Characteristics of vagal afferent nerve fibres in respiratory tract (modified by [5]).

VAGAL AFFERENT NERVE FIBRES IN RESPIRATORY TRACT

Ganglionic origin	JUGULAR GANGLIA		NODOSE GANGLIA			
Fibre type	C-fibres	Aδ-fibres	C-fibres	Aδ-fibres	Aβ-fibres	
Functional characteristic	Chemosensors (nociceptors)		Chemosensors (nociceptors)	Low-threshold mechanosensors		
		Aδ-nociceptors		*cough receptors*	RARs	SARs
Conduction velocity (m/s)	∽1	∽ 6	∽1	∽ 5	∽ 15	∽ 18
Mechanical threshold [a]	High	High	High	Low	Low	Low
Termination						
– extrapulmonary	Many	Some	Few	Many	Few	Few
– intrapulmonary	Some	Some	Many	Some	Many	Many
Expression						
– substance P (%) [b]	Yes (90-100)	No	Yes (50)	No	No	No
– TRPV1	Yes	Yes	Yes	No	No	No
Sensitivity						
– punctate mechanical stimulation	No	No	No	Yes	Yes	Yes
– tissue stretch	No	No	No	No	Yes	Yes
– bronchoconstriction	No	No	No	No	Yes	Yes
– capsaicin	Yes	Yes	Yes	No	Yes [c]	Yes [c]
– bradykinin	Yes	Yes	Yes	No	Yes [c]	Yes [c]
– acid	Yes	Yes	Yes	Yes	Unknown	Unknown
– ATP	No	No	Yes	No	Yes	Yes
Physiological responses	Apnea, Cough	Unknown	Tachypnea, bronchoconstriction	Cough	Tachypnea, bronchoconstriction	Hering-Breuer, bronchodilation

[a]The threshold for activation of chemosensors is approximately 100-times higher than mechanosensors. [b]The data is expressed as a percentage of cells expressing substance P [11]. [c]RARs and SARs are activated indirectly by bradykinin and capsaicin that act upon airway smooth muscles, glands, and vasculature.

muli and can respond to both chemical and mechanical stimulations. Although they have a high activation threshold (~100-times higher than mechanosensors) for mechanical stimuli which means that C-fibres do not generate action potentials throughout the respiratory cycle but can be activated by inflammation mediators, hyperinflation, and one or more chemical stimuli. Majority of chemicals and inflammatory mediators can sensitize C-fibres and lower their threshold for mechanical activation in the diseased airways [5, 19].

The main hallmark of nociceptive C-fibres is expression of specific receptors for noxious stimuli - transient receptor potential vanilloid 1 (TRPV1) and, thus, sensitivity to agonist of TRPV1 – capsaicin [11, 15, 16, 20]. TRPV1 receptor is polymodal and can be activated by heat and extracellular acidity [21].

Transient receptor potential ankyrin 1 (TRPA1) is commonly co-expressed with TRPV1 and can be activated by a wide range of natural products such as allyl isothiocyanate (AITC – pungent component of wasabi), cinnamaldehyde, and by environmental irritants (e.g. acrolein – present in cigarette smoke and air pollution) [22, 23, 24, 25]. Airway C-fibres can be also stimulated by acid via TRPV1 or other acid-induced mechanism probably mediated by acid sensing ion channels (ASICs) [13, 26, 27].

Nociceptive C-fibres are often subclassified into those localized deep in the lung (pulmonary C-fibres) and those in conducting airways (bronchial C-fibres). This classification arose from studies of the Coleridges and their colleagues. They noted that pulmonary C-fibres are most accessible to stimulants injected into the pulmonary circulation and bronchial C-fibres are accessible to stimulants injected into the bronchial (systemic) circulation [20, 28].

Recent studies in mice and guinea pigs have revealed that different phenotype of pulmonary and bronchial C-fibres can be associated with distinct embryological origin of vagal afferent C-fibres. Based on these findings C-fibres are classificated as **jugular C-fibres** (derived from neural crest) and **nodose C-fibres** (derived from placodes). Jugular C-fibres express more neuropeptides such as substance P, calcitonin gene related peptide (CGRP), whereas the nodose C-fibres express rarely these neuropeptides. Nodose C-fibres are activated by a wide range of chemical stimuli including capsaicin, AITC, bradykinin, citric acid, α,β-methylene ATP (P2X2/3), adenosine (A1 and A2A), and 2-methyl-5-HT (5-HT3). Jugular C-fibres do not express specific receptors – purinergic P2X2 receptors, neither of adenosine A1 and A2A receptors nor ionotropic 5-HT3 receptors, and that s why they are not stimulated by α,β-methylene ATP, adenosine, and 2-methyl-5-HT [7, 11, 29, 30, 31]. However, in anaesthetized animals stimulants such as bradykinin and capsaicin were ineffective at evoking cough. On the contrary, activation of nodose C-fibres by bradykinin, capsaicin, or phenylbiguanide (agonist of 5-HT3 receptor) does not evoke cough but inhibits cough in anaesthetized animals (cats and dogs) [10, 32, 33, 34].

Jugular and nodose vagal C-fibres differ in distinct distribution of nerve terminals in respiratory tract. Most vagal C-fibres terminating in the extrapulmonary airways of guinea pigs are of jugular origin and only 10 – 20 % C-fibres in the trachea originate from nodose ganglion. Regardles of embryological origin, similar numbers of vagal C-fibres terminate in intrapulmonary tissues. The endings of vagal C-fibres are localized in mucosa and submucosa of the airways [11, 16, 35].

A-fibre subtypes

The majority of vagal A-fibres (Aβ, Aδ) innervating respiratory system are derived from nodose neurons. **Nodose Aδ-fibres (*cough receptors*)** have been described in the large airways of guinea pig [10] and they primarily terminate in extrapulmonary bronchi, trachea, and larynx. These myelinated Aδ-fibres conduct action potentials at approximately 5 m/s, which is five times faster than C-fibres and three times slower than Aβ-fibres. This subtype of vagal Aδ-fibres represent low-threshold mechanosensors and they are sensitive to punctate mechanical stimuli (such as touch) and are also sensitive to acidic, hypotonic, and hypertonic solutions. These fibres do not respond to tissue stretch, contraction of airway smooth muscle, and inflammatory mediators such as bradykinin. The Aδ-fibre terminals are insen-

sitive to capsaicin due to the lack of TRPV1 receptor [1, 10, 13, 16]. Stimulation of these fibres causes a strong cough response, even in anaesthetized animals. Comparison of their physiological properties to another intrapulmonary low-threshold mechanoreceptors (RARs and SARs) indicates that nodose Aδ-fibres are a distinct subtype and play a primary role in cough reflex regulation [10, 36].

Cough receptors terminate in subepithelial extracellular matrix and have a characteristic structure [37]. Recently, nerve terminals with the same structure have been described in human bronchi [38]. For comparison, there is an absence of these nerve fibres in species that have not developed cough reflex, such as mice and rats [5].

A subset of low-threshold mechanoreceptors originating from nodose ganglia have been described in the intrapulmonary airways and lung parenchyma. Two types of **mechanosensitive Aβ-fibres (*stretch receptors*)** are subcategorized based on action potential adaptation as rapidly adapting receptors (RARs) and slowly adapting receptors (SARs). These vagal afferent fibres conduct action potentials in the Aβ-range (10-20 m/s) and are sensitive to mechanical stimuli including changes in lung volume, constriction of airway smooth muscle, and airway wall oedema. Accordingly, these fibres display an activity when the lungs are inflated and SARs are more sensitive to lung inflation than RARs. However, RARs are also activated during deflation of the lungs (including lung collapse) [10, 39, 40, 41, 42]. RARs and SARs are generally insensitive to chemical stimuli with exception of cases when the stimulus evokes changes in volume of airway wall, airway smooth muscle tone, or mucus secretion. Substances like capsaicin, bradykinin, histamine, acetylcholine, and substance P that act upon airway smooth muscles, glands, and vasculature can indirectly activate SARs and RARs. Because of indirect chemosensitivity of RARs some researchers used a term *irritant receptors* to define this afferent nerve subtype [43, 44, 45]. This misnamed term is not used anymore. These vagal afferent fibres play a role in physiological control of breathing and only a few chemicals can directly activate these intrapulmonary mechanosensitive nerve fibres. For instance, ATP activates both RARs and SARs via purinergic P2X2/3 receptors (expressed in nodose neurons) [10, 31, 46].

SARs are involved in tidal breathing and have an important role in the Hering-Breuer reflex [47]. It is unlikely that SARs directly evoke coughing. Similarly, RARs do not play a direct role in regulating cough reflex because stimuli that activate intrapulmonary RARs (tromboxane, LTC4 – leukotriene C4, LTD4 – leukotriene D4, histamine, neurokinins, methacholine, inspiratory and exspiratory efforts against closed glottis) do not evoke cough. However, SARs and RARs can modulate cough evoked by the principal afferent pathways [10, 48, 49].

Some functional studies suggest that RARs terminate within or beneath the epithelium and are localized in both intra- and extrapulmonary airways, while SARs may be associated with the airway smooth muscle [50, 51]. RARs terminate in the intrapulmonary airways of all studied animal species and in the extrapulmonary airways of cats and dogs [40, 50]. SARs may be differentially distributed in the airways. In cats, guinea pigs, and rats few SARs and more RARs can be found in the extrapulmonary airways. In dogs SARs may also be localized in extrapulmonary airways [36, 39].

Studies have revealed that half of the extrapulmonary chemosensitive vagal afferents in guinea pigs are fast conducting myelinated Aδ-fibres. These fibres are derived exclusively from the jugular ganglia and are functionally different from the nodose derived Aδ-fibres (*cough receptors*). **Jugular Aδ-fibres (Aδ-nociceptors)** are 15-fold less sensitive to punctate mechanical stimuli than nodose Aδ-fibres and, thus, have greater threshold for mechanical activation. These jugular Aδ-fibres are activated by capsaicin and bradykinin. Chemically sensitive Aδ-fibres express the capsaicin TRPV1 receptor but do not express neuropeptides such as substance P [16, 17, 35]. Although they show similarities to C-fibres their role in cough reflex is not yet fully investigated. Similarly, the terminal structure of these afferents in airways has not been defined [5].

COUGH

Cough is an important defensive reflex, which protects the airways from the inhalation of harmful substances and foreign materials [52, 53]. However, in certain diseases, cough can become excessive and inappropriate as a consequence of increased activation of the neural pathways controlling coughing. Cough can be classified as acute (duration of less than 8 weeks), which is a result of viral or bacterial upper respiratory tract infection, or chronic (more than 8 weeks). This chronic cough is often associated with inflammatory airway diseases such as bronchial asthma, chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), lung cancer, or conditions outside the lungs such as gastroesophageal reflux disease (GERD). Chronic cough can be also idiopathic in origin [1, 54]. Pathological cough in disease is persistent and hypersensitive occuring in response to stimuli which do not normally evoke cough. This enhanced sensitivity to tussive or non-tussive stimuli is referred to as cough hypersensitivity syndrome which is a common symptom in chronic respiratory diseases [5, 55, 56].

Cough reflex is initiated following activation of vagal afferent nerve fibres. Afferent nerve fibres are activated when physical or chemical stimuli interact with specific receptors (TRP channels, P2X2/3 receptors, and others) expressed on vagal nerve termini situated in and under airway epithelium. Activation of these receptors causes membrane depolarization of the terminal membrane. If the depolarization is of sufficient magnitude and it reaches a certain threshold (around -55 millivolts), it leads to 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 in the nucleus of the solitary tract (nTS) well-defined, 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 brainstem which is responsible for reflex coughing, as well as to higher brain structures for the perception of airway irritation which are needed for behavioural modulation of coughing. Finally, the information is carried by motor (efferent) neurons to the respiratory muscles, diaphragm, and larynx to initiate the motor response of cough [5, 57, 58].

Studies in guinea pigs indicate that cough reflex is independently regulated by two vagal afferent nerve subtypes which innervate the airways. Mechanosensitive Aδ-fibres from the nodose ganglia (*cough receptors*) mediate predominantly mechanically-induced cough and nociceptive C-fibres derived from the jugular ganglia mediate mainly chemically-induced cough. Nodose Aδ-fibres are activated by punctate mechanical stimuli and rapid reductions in mucosal pH but are insensitive to activators of TRPV1 and TRPA1 and inflammatory mediators in guinea pig model. These mechanosensitive fibres have an important and irreplaceable role in the defense mechanism of cough because they protect airways against aspiration (e.g. foreign bodies, food). On the contrary, the nociceptive C-fibres from jugular ganglia are stimulated by a wide range of inhaled and locally produced chemicals such as agonists of TRPV1 and TRPA1 receptors and inflammatory mediators. Inflammation in diseased airways leads to production of mediators that activate nociceptive C-fibres and, consequently, these fibres contribute to the symptoms of airway inflammation including pathological cough [1, 5, 10, 13]. Moreover, the inflammation processes can also change electrical excitability and gene expression. If these changes are long-lasting, they can alter phenotype of the C- and A-fibres in the airways which are often referred to as neuroplasticity. In such case 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-fibres in the trachea and, thus, becoming sensitive to TRPV1 activators in guinea pig model. Otherwise, C-fibres are not activated by mechanical stimuli under physiological conditions but after treatment of the lungs with inflammatory mediators such as histamine

the excitability of these nociceptive fibres has been increased to the point that inspiration led to their activation [5].

Generally, there are three types of cough. Firstly, **involuntary subconscious cough type** represents protective cough reflex which depends purely on the activation of peripheral sensory nerves. This type of cough occurs independently of any conscious control from the higher brain structures and can be triggered by stimulation of nodose Aδ-fibres in most mammals. Activation of these fibres leads to coughing in anaesthetized animals. The second type, **non-provoked voluntary cough,** is purely voluntary, which means it is generated willingly and does not require any peripheral sensory input to the brainstem. Most cases of chronic cough in airway diseases are of **provoked conscious cough type**. This type of cough is dependent on peripheral sensory input and can be simultaneously suppressed or enhanced by behavioural modulation from higher brain structures [3, 58].

The currently used antitussive therapies are largely ineffective. One of the most effective antitussive drug groups are opiates (codeine) which act both centrally on brainstem via opioid receptors and on receptors located peripherally on sensory nerve terminals in the airways. Hovewer, at their effective doses they have dangerous adverse effects such as dependence, respiratory depression, sedation, and gastrointestinal problems [59, 60]. Clinical trial has shown that codeine – current gold standard in cough treatment – was no more effective than placebo in patients with COPD [61].

Because of high incidence of chronic cough there is an urgent requirement 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 perfect therapeutic strategy would represent inhibition of pathological cough (mainly evoked by jugular C-fibres) without affecting the protective cough reflex (mainly evoked by nodose Aδ-fibres) maintaining airway patency and preventing lung infections.

VOLTAGE-GATED SODIUM CHANNELS AS A NOVEL NEURAL TARGET IN REGULATION OF COUGH

The communication between peripheral and central nervous system depends on activation of voltage-gated sodium channels (NaVs) which are essential for initiation and conduction of action potentials along nerves. This makes NaVs an attractive neural target. The selective inhibition of certain NaVs in the relevant nerves with specific channel blockers will prevent communication between the airways and brainstem and that would in turn block cough provoked by any stimulus regardless its nature. New peripherally acting antitussive drug would prevent C-fibres activation in the airways and may represent an effective therapy for pathological cough with the minimal adverse effects [2, 3].

NaVs are heteromeric transmembrane protein complexes comprising of the large main α subunit (channel pore) and one or two small auxiliary β subunits. The expression of pore-forming α subunit is sufficient for functional sodium channels [62]. There are nine distinct α subunits of NaVs referred to as NaV1.1 – NaV1.9. With the exception of NaV1.4 (expressed in skeletal muscle) and NaV1.5 (expressed in myocardium) all NaVs are expressed in the nervous system [63]. These NaVs channels can be blocked non-selectively with lidocaine and other local anaesthetics. Tetrodotoxin (TTX) is a potent neurotoxin that selectively blocks the influx of sodium cations through NaV1.1, 1.2, 1.3, 1.4, 1.6 and 1.7 (TTX-sensitive) and has a low affinity for NaV1.5, 1.8, and 1.9 (TTX-resistant). Tetrodotoxin has approximately 1,000-times greater potency than lidocaine and systemic administration of this neurotoxin leads to rapid death due to paralysis of respiratory muscles [64, 65]. The list of mammalian α subunits of NaVs are summarized in Tab. 2.

Table 2 Mammalian α subunits of voltage-gated sodium channels (NaVs) (modified by [63]).

Protein	Gene	Human chromosome	TTX sensitive	Distribution
NaV1.1	*SCN1A*	2q24.3	+	CNS, PNS, myocardium
NaV1.2	*SCN2A*	2q24.3	+	CNS, PNS
NaV1.3	*SCN3A*	2q24.3	+	CNS, PNS, myocardium
NaV1.4	*SCN4A*	17q23.3	+	Skeletal muscle
NaV1.5	*SCN5A*	3p22.2	-	Myocardium
NaV1.6	*SCN8A*	12q13.13	+	CNS, PNS
NaV1.7	*SCN9A*	2q24.3	+	PNS
NaV1.8	*SCN10A*	3p22.2	-	PNS
NaV1.9	*SCN11A*	3p22.2	-	PNS

Abbreviations: TTX – tetrodotoxin, CNS – central nervous system, PNS – peripheral nervous system.

Sodium channels NaV1.7, 1.8, and 1.9 are preferentially expressed in the peripheral nervous system [12, 66]. Equally, the vast majority of nodose Aδ-fibres and jugular C-fibres innervating guinea pig trachea almost exclusivelly expressed NaV1.7, 1.8, and 1.9 channels [3]. These channels are also up-regulated in response to inflammatory mediators that increase cough sensitivity [67]. Electrophysiological analysis suggests substantial differences in NaVs regulation of different type of cough-triggering nerves. The initiation of action potentials in nerve terminals of nodose Aδ-fibres is entirely dependent on NaV1.7 channels. In contrast, the action potential initiation in nerve terminals of jugular C-fibres largely relies on NaV1.8 channels with a minor role of NaV.1.7 channels. In adition, the conduction of action potentials along nodose Aδ- and jugular C-fibres depends on the activity of NaV1.7 channels [4]. This is consistent with the previous findings of Muroi and colleagues that TTX or selective knockdown of NaV1.7 gene expression using AAV-shRNA technology effectively blocked action potential conduction of nodose A- and C-fibres. In electrophysiological study both the nodose A- and C-fibres were inhibited by TTX showing that TTX-sensitive channels are required for action potential conduction in the nodose axons. In the next study the selective silencing of NaV1.7 in the nodose ganglia (with NaV1.7 shRNA) abolished mechanically-induced cough in anaesthetized guinea pigs but capsaicin-induced cough in conscious guinea pigs remained unaffected [66, 68]. Interestingly, individuals with a loss-of-function mutation in NaV1.7 (*SCN9A*) have been described. This is a rare autosomal mutation which leads to congenital insensitivity to pain, yet with otherwise normal neuronal function and normal sensation of non-painful stimuli [69]. Weiss and co-workers examined human patients with this mutation and found out that they have diminished sense of smell [70]. There is no evidence whether cough is altered in individuals with loss-of-function mutation in NaV1.7.

NaV1.8 has a role in the propagation of cold stimuli-induced action potentials and contributes to experimental inflammatory and neuropathic pain [71]. Blocking of NaV1.8 (with A-803467) results in significant reduction in nociception sensitivity in animal models of neuropathic and inflammatory pain [72]. In our preliminary study preinhalation of nebulized potent and highly selective channel blocker of NaV1.8 (A-803467) significantly

decreased capsaicin-induced cough in awake naïve guinea pigs. These data suggest that selective inhibition of NaV1.8 in jugular C-fibres can lead to block of chemically-induced cough (unpublished data). In contrast, TTX selectively administrated to the trachea only marginally inhibits citric acid-induced action potential discharge in jugular C-fibres [4].

Research shows that voltage-gated sodium channels have potential to be a novel neural target in cough treatment. However, the NaVs blocking strategy may also lead to some unwanted changes in respiratory tract such as impaired respiratory sensation or breathing regulation. This may happen due to similar expression profile of NaVs subtypes in nodose Aβ-fibres in the lungs (RARs and SARs) and cough-triggering fibres [12]. Further research needs to be done in order to elucidate this question in greater detail.

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Received: September, 25, 2018
Accepted: November, 22, 2018