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Targeting Peripheral Afferent Nerve Terminals for Cough and Dyspnea

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Abstract

Chronic unproductive coughing and dyspnea are symptoms that severely diminish the quality of life in a substantial proportion of the population. There are presently few if any drugs that effectively treat these symptoms. Rational drug targets for cough and dyspnea have emerged over the recent years based on developments in our understanding of the innervation of the respiratory tract. These drug targets can be subcategorized into those that target the vagal afferent nerve endings, and those that target neural activity within the CNS. This review focuses on targets presumed to be in the peripheral terminals of afferent nerves within the airways. Conceptually, the activity of peripheral afferent nerves involved with unwanted urge-to-cough or dyspnea sensations can be inhibited by limiting the intensity of the stimulus, inhibiting the amplitude of the stimulus-induced generator potential, or inhibiting the transduction between the generator potential and action potential discharge and conduction. These mechanisms reveal many therapeutic strategies for anti-tussive and anti-dyspnea drug development with peripheral sites of action.

I. Introduction

Other than voluntary and so-called psychogenic cough, cough reflexes are initiated by activation of sensory (afferent) nerves carried by the vagi. Activation of certain subsets of vagal afferent nerves can lead to immediate explosive cough reflexes, or to itchy urge-to-cough sensations that are then relieved by a voluntary cough. The dry nonproductive urge-to-cough is often associated with acute infections, but in other cases a chronic cough can develop that is not obviously associated with any specific ongoing pathology. These chronic cough conditions are frequently associated temporally with the resolution of a respiratory virus infection (post-viral cough), but other times can only be described as idiopathic cough.

Unlike the cough reflex, vagal afferent nerves are not necessarily required for the evocation of dyspnea. Nevertheless, several lines of evidence support the hypothesis that activation of vagal afferent nerves likely contributes to the dyspnea associated with airway disease [1].

There are few if any effective drugs on the market that effectively reduce unwanted coughing or dyspnea. In both cases rational and novel targets have emerged based on developments in our understanding of the innervation of the respiratory tract. These drug

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targets can be subcategorized into those that target the vagal afferent nerve endings, and those that target neural activity within the CNS. This review focuses on the former. The discussion below is by necessity more selective than encyclopedic in nature.

II. Vagal Afferent Nerve in Cough and Dyspnea

An accumulation of evidence from studies on laboratory animals has made it abundantly clear that the so-called vagal “cough receptor” cannot easily be constrained to a single nerve phenotype. The specific characteristics of the vagal afferent nerve subtypes involved with cough has been recently reviewed [2] and will not be further discussed here.

There is relatively little that can be definitively stated regarding the afferent nerves subtypes involved in vagally-induced dyspnea. This is due to the regrettable but understandable fact that dyspnea cannot be quantified (or even qualified) in laboratory animals. Any insight into vagal nerve subtypes and dyspnea must be gained by assumption-laden correlations. For example, it is clearly known that autacoids directly activate vagal C-fibers in laboratory animals such as ATP and adenosine lead to dyspnea in humans when inhaled [3]. Likewise, nicotine receptor agonists stimulate vagal C-fibers and cause strong sensations of respiratory irritation and dyspnea when administered intravenously [4] or by inhalation [5], and these sensations are largely absent in lung transplanted subjects [4]. These studies implicate vagal C-fibers as dyspneagenic. These same agents, however, may also lead indirectly (via edema, bronchoconstriction etc) to activation of A β fibers in the lungs. In fact, ATP can directly stimulate A β stretch sensors with an efficacy similar to its action on C-fibers [6]. At the present time it is difficult to rule-in or rule-out a given vagal afferent nerve subtype as being the most important fiber subtype in evoking dyspneic sensations.

It should be kept in mind that the mammalian respiratory tract is also innervated by spinal sensory afferent nerves. These nerves typically get brief mention in discussions about respiratory afferent nerves. This absence of attention is not due to studies showing their lack of involvement in respiratory physiology and sensations, rather it is due to the embarrassing lack of information about these nerves in general. The majority of spinal sensory neurons innervating the airways in lungs are qualitatively similar to jugular vagal neurons in that they are relatively small in diameter and express neuropeptides [7] [8]. Based on these features it is likely that many have a nociceptive phenotype. This observation, along with the fact that they project axons centrally via the spinal thalamic tract, suggests that they may make important contributions to unpleasant respiratory sensations associated with airway disease.

III. Generator Potentials and Action Potentials

For a stimulus in the respiratory tract to be sensed, depolarization of a sensory nerve terminal membrane must occur. The local graded membrane depolarization response to a nonelectrical stimulus is referred to as the “generator potential” [9]. The generator potential will electrotonically fade back to the resting potential unless it is of sufficient magnitude to trigger an action potential. It is the action potential that travels in all-or-none fashion along the nerve fiber to its central terminal where it initiates synaptic transmission with second order neurons in the brain stem (nucleus tractus solitarius).

The generator potential is typically due to a stimulus coupled to the opening of non-selective cation channels leading to membrane depolarization, although inhibition of certain potassium or chloride channels can lead to generator potentials in primary afferent nerves. The threshold and initiation of action potentials is governed by voltage gated ion channels, most notably voltage-gated sodium channels. Local anesthetics have no effect on generator potentials, but block voltage-gated sodium channels and thereby block the formation and conduction of action potentials.

In specialized sensory structures, the generator potential has been quantified and as a general rule its amplitude is approximately proportional to the logarithm of the stimulus. The frequency of action potential discharge, on the other hand, is directly proportional to the amplitude of the generator potential (see Figure 1). Regardless of the specific quantitative relationships, there are basically three strategies available to peripherally inhibit cough and dyspnea: first, decrease the stimulus intensity (e.g. anti-inflammatory strategies); second, decrease the amplitude of the stimulus-induced generator potential; third, decrease the transduction of the generator potential into action potential discharge. This provides a plethora of potential targets for drug development (Figure 2).

IV. Drugs Targeting the Generator Potential

The polymodal nociceptive C-fibers are, by definition, activated by many different stimuli. That is to say that many stimuli lead to suprathreshold generator potentials in these nerves. In many cases the stimulus-induced generator potential of nociceptors is understood sufficiently for consideration of drug discovery. The main activation of respiratory A-fibers is typically mechanical energy either in the form of stretch (RAR, SAR) or in the case of the extrapulmonary cough causing A δ fibers [6], punctuate mechanical forces caused by something impinging on the mucosa. Our knowledge about how these mechanical forces are transmitted into a generator potential is at present lacking. It is therefore premature to discuss drug targets aimed specifically at limiting mechanically-induced generator potentials in vagal A-fibers.

A) Autacoid Receptors

In inflammatory airway diseases, afferent nerves terminating in the mucosa are subjected to a host of inflammatory mediators. Many of these mediators can stimulate specific receptors in the afferent nerve membrane that lead to generator potentials. These membrane receptors can be subclassified as G-protein coupled receptors (GPCRs, metabotropic receptors) or ligand-gated ion channels (ionotropic receptors). Vagal afferent nerves relevant to cough and dyspnea express both GPCRs and ionotropic receptors that are capable of directly evoking generator potentials and action potential discharge. Most of the studies on GPCR and ionotropic receptor mediated activation of vagal afferent nerves have focused on vagal C-fibers. Vagal respiratory C-fibers can be subclassified into at least two distinct phenotypes [10] and the expression of GPCRs is dependent on whether the C-fiber is placodal or neural crest in origin [11].

The advantage of targeting various autacoid receptors for anti-tussive or anti-dyspneic therapy is that these receptors are typically “druggable” targets in which very selective antagonists can readily be developed. The specificity of these drugs often leads to limited side-effect profiles, and useful therapeutic windows. The disadvantage of this strategy is that by blocking a specific autacoid receptor, it is likely that the drug will alleviate the symptoms in only a slice of the overall population of people suffering with abnormal vagal afferent nerve activity-- the efficacy will be limited to those problems that are driven specifically by a single receptor agonist.

1. GPCRs—Among the myriad GPCRs, relatively few appear to directly cause generator potentials and action potential discharge in vagal afferent nerves. When an agonist of a GPCR interacts with the cell surface receptors it leads to a conformational change causing, by definition, activation of a G-protein. The activation of the G-protein in turn leads to production of various second messengers culminating in cascades of protein phosphorylations, changes in cytosolic calcium concentration, and cell function. The precise signal transduction mechanism depends on the nature of G-protein (e.g. Gs, Gi, Gq). Over the past three decades a wealth of knowledge has built up detailing mechanisms of GPCR

signal transduction in a variety of cell types. Lagging far behind, however, is knowledge on the signal transduction of GPCRs at the level of a sensory nerve terminal. Unless the GPCR is capable of influencing the ionic conductance of the terminal membrane such that a generator potential is formed (or inhibited), receptor activation will be of little direct consequence on sensation.

It is more than passing interest to understand how the activation of a GPCR leads to formation of a generator potential. If, for example, all family members of the Gq- or Gs-coupled GPCRs employ the same signaling mechanism to depolarize the membrane, then that signaling mechanism would make for a rational drug target. Such a target would have a broader impact than one that targeted the binding of one specific agonist. By contrast to cell bodies that are replete with a dense endoplasmic reticulum and nucleus, both of which are known to play important roles in GPCR signaling, the nerve terminals are largely devoid of these organelles, and instead are densely packed with mitochondria. Therefore, one must be cautious when inferring information about the afferent nerve terminals from studies carried out at the neuronal cell bodies. In sensory cell bodies of the dorsal root ganglia, stimulation of Gq coupled receptor like the bradykinin B2 receptors and the histamine H1 receptor depolarize the membrane potential largely if not exclusively via the gating mainly of TRPV1, and possibly also TRPA1 [12] [13] [14] (see below). There is also evidence that Gq-coupled receptor activation uses TRPV1 in the evocation of action potential discharge in vagal C-fiber terminals innervating the airways, but only partially [15] [16]. In fact genetically depleting TRPV1 has only a very modest effect on the peak frequency of action potential discharge in the mouse [16]. In guinea pigs, there is evidence that the non-TRPV1 component of the generator potential is due to stimulation of calcium-activated chloride channels [17] [15]. Opening of chloride channels causes chloride to leave the cell resulting in membrane depolarization. Non-selective chloride channel blockers inhibit bradykinin-induced membrane depolarizing current at the cell bodies and action potential discharge at the terminals [15]. Membrane depolarization of vagal sensory cell bodies in response to bradykinin B2 receptors and histamine H1 receptors have also been associated with an increase in electrical input resistance. This is likely due to the inhibition of a resting leak potassium current; an event that would also contribute to membrane depolarization [17] [18].

The specific GPCRs that lead to generator potentials and overt activation of airway afferent nerves are reviewed in this volume. Some of the GPCRs that are expressed by vagal afferent neurons and overtly lead to action potential discharge in vagal afferent terminals include bradykinin via the B2 receptor, adenosine via A1 and A2a receptors, histamine via the H1 receptor and proteases via PAR1 or PAR2 receptors, depending on the species [11].

Stimulation of GPCRs is often associated with increases in C-fiber *excitability* rather than overt generator potential and action potential generation. Stimulation GPCRs linked to elevations of cAMP or phospholipase activation can result in phosphorylation of key channels that fail to activate the nerve, but render it hypersensitive to other stimuli. Such an increase in excitability *in vivo* is often associated with an increase in baseline activity of the nerve [11]. Prostaglandin E2 is an excellent example of an inflammatory mediator that increases C-fiber excitability, generally in the absence of overt activation [19]. GPCRs may also serve to inhibit excitability of vagal afferent nerves to decrease generator potential amplitude in response to an activating stimulus. An example of this may be found in the nociceptive receptor NOP1, as well as certain opioid and cannabinoid receptors [20] [21].

2. Ligand-gated ion channels (ionotropic receptors)—Conceptually, ligand-gated ion channels are more straightforward when it comes to producing generator potentials in vagal afferent nerves since the receptor is an ion channel. An agonist binds to the receptor

with high affinity and selectivity leading to a conformational change in the receptor (ion-channel) and ion permeation. In the vast majority of cases, the ligand gated ion channel responsible for generator potentials is a non-selective cation channel. One exception to this would be the GABA-A receptor; a ligand-gated ion channel that is selective for chloride. Since the equilibrium potential for chloride is at a more depolarized state than the resting membrane potential in primary afferent nerves, opening chloride channels will lead to a membrane depolarization (generator potential). The majority of ligand gated ion channels are receptors that are very selective for a given agonist; however, one family of ligand-gated ion channels relevant to generator potentials in airway vagal afferent nerves are less selective receptors and are able to integrate disparate stimuli. These channels are called members of the transient membrane potential (TRP) family of ion channels. Below is a brief description of the ionotropic receptors particularly relevant to airway afferent nerves.

a. P2X Receptors: ATP can evoke generator potentials and high frequency action potential discharge in vagal C-fibers and in A β stretch-sensors [22] [23]. Purinergic receptors are subclassified into P2Y and P2X subtypes. The P2Y receptors are GPCRs, whereas the P2X receptors are ligand-gated ion channels. ATP-induced action potential discharge in vagal afferent nerves is due to interactions with P2X receptors [22] [23]. There are 7 P2X receptor subtypes and the ligand-gated channel typically consists of three homomeric or heteromeric subunits. The channel most likely responsible for activation of vagal C-fibers in the respiratory tract are heteromeric P2X_{2,3} receptors [24] [25]. In guinea pigs and mice, ATP evokes action potential discharge selectively in placodal afferent nerves, and not in neural crest C-fibers [24,25].

b. 5-Hydroxytryptaine (5-HT): There are a large number of 5-HT receptor subtypes mostly comprising GPCRs. Among 5-HT receptor subtypes, only 5-HT₃ is an ionotropic receptor. Three 5-HT₃ subunits have been described (5-HT_{3A}, 5-HT_{3B} and 5HT-3C) [26]. In the 1970s and 1980s several laboratories were using phenyldiguanide to stimulate pulmonary C fibers (or J-receptors) in classical studies of the pulmonary chemoreflex [10]. These studies predated the discovery that phenyldiguanide is a very selective 5-HT₃ receptor agonist; nevertheless, they provide the first evidence that 5-HT₃ receptor activation can lead to stimulation of pulmonary C-fibers. Incidentally, phenyldiguanide is still often used as a 5-HT₃ selective agonist, but due to slight sequence differences among species, it fails to stimulate 5-HT₃ receptors in some mammals including guinea pigs [27]. One must therefore be cautious when interpreting negative data when phenyldiguanide was used as the putative 5-HT₃ receptor agonist.

In guinea pigs, 5-HT was not effective at stimulating all C-fiber subtypes. As is the case with ATP, the nodose (placodal) C-fibers in the lungs were effectively stimulated by 5-HT (and 5-HT₃ selective agonists), whereas jugular (neural crest)-derived C-fibers were relatively unaffected by 5-HT [28]. In dogs, 5-HT was found to evoke larger responses in bronchial compared to pulmonary C-fibers [29].

c. Nicotinic receptors: Inhalation of cigarette smoke by naïve smokers causes intense airway irritation, chest tightness, and cough. A series of studies revealed that this is largely accounted for by nicotinic receptor activation [5]. Nicotinic receptors are formed by a pentamer of subunits and there are a total of 17 subunits comprising α 1–10, β 1–4, δ , ϵ and γ subunits [30]. This leads to a dizzying array of possible receptor subtypes. The subunit composition of the nicotinic receptor expressed by vagal afferent nerves has not been worked out.

The most parsimonious explanation of how inhaled nicotine leads to sensations of irritation and urge to cough is that nicotine directly activates C-fibers near the airway epithelium.

Nicotine and cigarette smoke both lead to action potential discharge in a population of airway C-fibers by a hexamethonium-sensitive mechanism. Nicotine also activates a population of RAR fibers. Nodose neurons express nicotinic receptors [31], and stimulating these receptors in airway specific nodose neurons in vitro leads to increases in cytosolic calcium [32,33]. The observations that the airways may contain both parasympathetic and non-neural (e.g. epithelial) sources of acetylcholine make the nicotine receptor a potentially interesting vagal afferent nerve drug target.

d. Acid Sensing Channels: Acidic solutions, when inhaled, evoke strong urge to cough sensations, and they are effective stimulators of action potential discharge in vagal bronchopulmonary C-fibers [34] [35]. Increasing alveolar CO₂ concentration activates pulmonary C-fibers, particularly when inflammation is present, likely due to local decreases in pH [36]. As discussed below, TRPV1 is an acid-sensing ion channel in C-fibers. A decrease in extracellular pH, however, also activates capsaicin-insensitive afferent nerves [16]. Available evidence suggests that certain members of the acid sensing ion channel (ASIC) family may be involved in this response, in particular ASIC3 [35].

e. Transient Receptor Potential Vanilloid 1 (TRPV1): The receptors discussed above are each very selective for a given agonist. TRP channels are ligand-gated ion channels expressed largely on vagal afferent C-fibers that can serve to integrate multiple stimuli.

Capsaicin is a vanilloid that binds selectively and with high affinity to TRPV1 [37], and is an effective stimulant of cough in humans and experimental animals. TRPV1 is expressed in virtually all C-fibers (and some A δ fibers) that innervate the respiratory tract. Activation of TRPV1 cause generator potentials that lead to high-frequency action potential discharge. Capsaicin also causes the release of neuropeptides in the airways that, depending on species, can constrict bronchial smooth muscle and cause plasma extravasation which is particularly noticeable in rats and guinea pigs[38]. These neuropeptide-induced effects can in certain circumstances lead indirectly to changes in activity of low threshold RAR or SAR nerve activity in an indirect fashion.

There are two rationales for targeting TRPV1 for anti-tussive and anti-dyspnea drugs. First, TRPV1 is not only stimulated by capsaicin, but can also be activated by many stimuli that may be relevant to airway diseases. TRPV1 is opened by heat, but the temperatures required for this effect is quite high (>42°C). There is a positive interaction between protons and temperature, however, such that at acidic pH (perhaps that associated with local inflammatory reactions) the channel opens at body temperature [37]. Indeed, cough in conscious animals caused by inhalation of citric acid is largely blocked by TRPV1 antagonists [39] [40]. Likewise, reducing the pH leads to strong activation of C-fibers largely via the opening of TRPV1 channels in *ex vivo* preparations [35] [16]. Certain lipid mediators known to be present in inflamed airways may also gate TRPV1. Electrophysiological studies on sensory cell bodies support the hypothesis that anandamide and 5-, 12- and 15-lipoxygenase products of arachidonic acid stimulate TRPV1 [41]. In addition, as discussed above, stimulation of Gq-Coupled GPCRs can lead to signaling events that result in the opening of TRPV1. Bradykinin-induced action potential discharge of C-fibers in guinea pig and murine airways is due to, at least in part, the gating of TRPV1 [42] [15] [16]. The signaling pathway between GPCR stimulation and TRPV1 gating is thought to involve the PLA2 dependent production of endogenous lipoxygenase products of arachidonic acid [12] [42], or to the PLC dependent release of constitutive inhibition of the channel by phosphatidyl inositol bisphosphate (PIP₂) [43].

The second rationale for targeting TRPV1 is based on the observation that prolonged activation of TRPV1 can lead to a functional desensitization of sensory nerves. This

desensitization not only blocks the response to subsequent activation by TRPV1 agonists, but also by other stimuli as well (heterologous desensitization). Since TRPV1 is expressed on virtually all C-fibers, this opens up the possibility of nonselectively inhibiting respiratory C-fiber activity in general. Of course this would require that TRPV1 agonists are used during the desensitizing phase, which comes with the problem that stimulating TRPV1 will lead to coughing and other unwanted effects. This strategy has been used in upper airway disease where the symptoms of rhinitis were inhibited following capsaicin desensitization [44]. Whether the therapeutic effects on cough or dyspnea is sufficient to overcome the negative effects of capsaicin treatment would depend on the efficacy and duration of the desensitization.

TRPV1 antagonists have been developed that are in clinical trials for pain [45]. Unfortunately, in early clinical pain studies, TRPV1 antagonism has been associated with marked hyperthermia [46]. Interestingly, TRPV1 antagonists that do not cause hyperthermia in rats have been identified [47]. Another strategy that may avoid the hyperthermic effects of TRPV1 antagonism is to consider aerosol delivery of the antagonists directly to C-fibers in the airways. This would not, however, inhibit non-airway C-fibers that may sensitize cough, e.g. esophageal C-fibers [48].

f. Transient potential ankyrin 1 (TRPA1): TRPA1 is an ion channel that is co-expressed with TRPV1 on a vast majority of vagal C-fibers in the airways [49]. TRPA1 is a nonselective cation channel like TRPV1 and leads to robust action potential discharge in airway C-fibers when stimulated [49]. Thus, agents known to activate TRPA1 lead to coughing in humans and experimental animals [50] [51].

The prototypical activators of TRPA1 are cinnamaldehyde and mustard oil (allyl isothiocyanate AITC), but what makes targeting TRPA1 particularly rational for anti-tussive development is the observation that this ligand-gated ion channel is also stimulated by numerous respiratory irritants such as acrolein, ozone, toluene diisocyanate, formaldehyde and other reactive electrophilic chemicals [52] [53] [54] [55]. Highly reactive electrophilic chemicals are irritants that are potentially damaging to tissue. TRPA1 may serve to alert the nervous system to the presence of electrophiles by a novel mechanism thought to involve the formation of covalent adducts with three cysteines (and, in human TRPA1, one lysine) within the channel's cytosolic N terminus. This mechanism may underlie the ability of TRPA1 to be activated by such a broad range of environmental electrophilic irritants that share only minimal structural similarity. Highly electrophilic reactive chemicals known to stimulate TRPA1 are produced within tissues during oxidative stress, a pathological feature of many respiratory diseases associated with dyspnea and cough [55]. Like TRPV1, TRPA1 is also regulated by inflammatory mediators downstream of G_q/PLC signaling [14].

g. Transient Receptor Potential M-member 8 (TRPM8): Menthol, a chemical long known to possess soothing and potentially anti-tussive activities, is a TRPM8 agonist. An agonist of TRPM8 may therefore be predicted to inhibit unpleasant respiratory sensations. There have been relatively few studies specifically evaluating TRPM8 and its effects on vagal afferent nerves. TRPM8 is rarely expressed on vagal afferent nerves innervating the airways of mice [49]. And menthol is unimpressive in its ability to stimulate or inhibit vagal afferent nerves in mouse or guinea pig airways. TRPM8 has been localized to vagal sensory nerves innervating rat airways, where it is activated by cold temperatures [56]. Some of the soothing and anti-tussive effects of menthol may be more related to stimulation of trigeminal afferent nerves in the nasal mucosa than vagal nerves.

B) Potassium Channel Openers

Potassium channels play an important role in regulating resting membrane potential and neural excitability. Opening potassium channels in the nerve terminal membrane would serve to hyperpolarize the nerve away from action potential threshold, and by decreasing the membrane resistance would inhibit the amplitude of a generator potential for any given unit of stimulus. A potassium channel agonist could potentially circumvent the selectivity problem inherent in targeting specific GPCRs and various ligand-gated ion channels. NS1619, an opener of the maxi-K channel inhibits cough in experimental animals [57] [58]. NS1619 also inhibited action potential discharge in tracheal C-fibers and A δ -fibers by a mechanism that was antagonized by the maxi-K channel blocker iberiotoxin [58].

C) Chloride Transporters

G-amino butyric acid (GABA) evokes membrane depolarization in a majority of vagal neurons that innervate the airways [59]. This effect is secondary to increasing current through chloride channels, indicating the reversal potential for chloride ions is positive to the resting potential. Consistent with this hypothesis, isotonic solution containing low chloride concentrations evoke action potential discharge in a subpopulation of A-fibers and C-fibers in the guinea pig isolated airway and activates laryngeal afferent fibers in dogs [60] [61]. Low chloride solutions also cause cough in humans [62]. Stimulation of bradykinin B2 receptors depolarizes vagal afferent neurons in part via opening of calcium-activated chloride channels [17] [15].

The opening of chloride channels in most neurons leads to a hyperpolarization and a decrease in excitability. The ability of chloride channels to depolarize the membrane of primary afferent nerves is likely due to the expression of a Na⁺-K⁺-2Cl⁻ (NKCC1) cotransporter. NKCC1, which is sensitive to furosemide, serves to concentrate chloride within the nerve terminals, and such a mechanism appears to be relevant in vagal afferent nerves innervating the airways [63] [64]. In theory, inhibiting such a pump would reduce intracellular chloride concentrations such that when chloride channels open (say with a Gq-coupled GPCR), not only would the generator potential be inhibited, but may actually serve to decrease the excitability of the nerve via membrane hyperpolarization. Furosemide modestly inhibits action potential discharge in airway afferent fibers and the cough reflex caused by low chloride solutions, but the mechanism underlying those effects has not yet been worked out [60] [61].

V. Drugs Targeting Action Potential Discharge

Up to this point we have discussed numerous drug targets that in theory would diminish the amplitude of various stimulus-induced generator potentials. By limiting the amplitude of the generator potential the peak frequency of action potential discharge would be inhibited and stimulation of second order neurons in the brainstem would be reduced. In this section we consider drug targets that could interact with the primary afferent nerves to diminish the transduction process between the generator potential and action potential formation and conduction. A major advantage of this approach is that it could have a general, rather than a selective inhibitory effect on tussigenic and dyspneagenic nerve activity, irrespective of the stimulus.

A) Voltage-gated sodium channels

Voltage gated sodium channels (Na_v) are the key ion channels leading to the initiation and conduction of action potentials. Local anesthetics are Na_v inhibitors such as lidocaine can completely anesthetize vagal afferent nerves, and if given in sufficient concentration will block reflex cough as well as vagus nerve associated dyspnea. The problem associated with

the use of local anesthetics in chronic cough and dyspnea is the potential for central nervous system and cardiac side effects; a limitation that could be overcome with Na_V subtype-selective blockers.

Based on expression of $\text{Na}_V \alpha$ subunits, there are 9 well characterized voltage gated sodium channels (Na_V 1.1–1.9). Vagal afferent A-fiber and C-fiber neurons innervating the respiratory tract express Na_V s 1.7, 1.8 and 1.9, and to a lesser extent Na_V 1.2 and 1.3 [65] (Muroi et al., 2011, under review). Na_V 1.7, 1.8 and 1.9 are not expressed to any great extent in either the central nervous system, cardiac, or skeletal muscles [66] [65]. There is evidence that these three Na_V subtypes may each play critical, non-redundant roles in regulating excitability of irritant-sensing neurons. Moreover, several studies have shown that these ion channels are upregulated by inflammation.

1. Na_V 1.7—Loss of function mutations in Na_V 1.7 channels have been described in humans [67]. Individuals possessing these relatively rare mutations behave normally with the exception that they do not perceive high intensity noxious stimuli as being unpleasant and they have a decrease in their olfactory sense [67]. [68]. The sensitivity of these individuals to various types of cough- or dyspnea-inducing stimuli has, to our knowledge, not been assessed.

The response of Na_V 1.7 to changes in membrane potential differs from that of most Na_V channels. Na_V 1.7 channels can open in response to slow ramp-like changes in membrane potential designed to mimic generator potentials at afferent nerve terminals [69]. This distinctive property suggests that Na_V 1.7 channels in sensory neurons respond to small depolarizing inputs and act to amplify them. The ability of Na_V 1.7 channels to affect vagal sensory nerve excitability and cough reflexes has recently been examined in guinea pigs (Muroi et al., 2011, under review). Selectively knocking-down Na_V 1.7 gene expression in nodose ganglion neurons inhibits their ability to respond to small depolarizing currents with action potential discharge (Figure 3). In addition, Na_V 1.7 proved to be essential for action potential conduction in vagal afferent nerves. It is therefore not surprising, that when the expression of this specific ion channel was bilaterally inhibited in guinea pig vagal ganglia, the animal's cough response to inhaled citric acid was severely curtailed (Muroi et al., 2011, under review).

2. Na_V 1.8— Na_V 1.8 is a tetrodotoxin (TTX)-insensitive channel with biophysical features considerably different from Na_V 1.7 and other tetrodotoxin-sensitive neuronal Na_V channels. In particular, Na_V 1.8 currents have a more depolarized voltage dependence of activation and fast-inactivation [70]. These properties allow Na_V 1.8 to contribute to continuous action potential discharge during periods of sustained depolarization. An additional feature of Na_V 1.8 is that its normally high threshold for activation can be lowered following exposure of sensory nerves to inflammatory mediators [71]. This observation has led to the hypothesis that modulation of Na_V 1.8 may contribute to heightened sensitivity of irritant sensing nerves during inflammation. Na_V 1.8 is expressed in airway sensory C-fiber and A-fiber neurons [65] and a recent study showed that PGE_2 increases the conductance of an Na_V 1.8-like Na^+ current in capsaicin-sensitive pulmonary sensory neurons which led to an increase in the overall excitability of these neurons (Kwong and Lee 2005).

3. Na_V 1.9— Na_V 1.9, like Na_V 1.8 is a TTX-insensitive Na^+ current; however it has a low threshold for activation [72]. This property gives it the ability to operate within resting membrane potential has led to the hypothesis that Na_V 1.9 may influence the resting membrane potential. Similar to Na_V 1.8, Na_V 1.9 current is subject to regulation by neurotrophic factors and inflammatory mediators and has been suggested to participate in

inflammation-induced increases in sensory nerve excitability. Consistent with these findings, Nav1.9 knockout mice are protected from many forms of inflammatory hyperalgesia [73].

4. Combining Sodium Channel blockers with TRPV1 openers—Recently, a novel idea has emerged that theoretically allows one to selectively anesthetize all TRPV1 expressing C-fibers. The idea takes advantage of the observation that the TRPV1 pore is of suitable size to allow for the transfer of relatively large charged molecules. Most local anesthetics are lipophilic molecules that penetrate the neurons membrane to block, nonselectively, Nav via an intracellular binding site. Binshtock and colleagues showed that a polar impermeant sodium channel blocker could gain access to the inside of cell via an opened TRPV1 channel [74]. In an idealized situation one might imagine inhalation of large concentrations of a polar impermeant and therefore inert lidocaine derivative. Under normal conditions inhalation of large concentrations of a polar impermeant sodium channel blocker would be expected to have no effect on action potential conduction. At sites of inflammation, where TRPV1 is opened due to various aspect of the inflammatory response (acid, heat, lipid mediators etc), the drug would gain access to the intracellular domain of the C-fiber terminals and inhibit Nav, to block action potential formation and conduction. Alternatively it might be possible to deliver such a drug along with capsaicin. These types of strategies are presently ongoing in pain therapeutics [75].

B) Sodium Potassium ATPase

Various stimuli that evoke cough and dyspnea stimulate repetitive action potential discharge that is reproducibly conducted along the axon to central nerve terminals. Vagal A-fibers can fire burst of action potentials at frequencies that can easily exceed 100 impulses/sec; in C-fibers lower frequencies of 30–50 Hz are often observed. It is likely that simply decreasing the peak frequency or bursting of action potentials would serve to inhibit urge-to-cough or dyspneic sensations. In human skin, intraneural stimulation with electrical stimulation at a constant low frequency of action potentials leads to markedly less sensation and pain compared to same number of impulses given at a more irregular higher frequency pattern [76]. More understanding of action potential encoding of cough and dyspnea is needed, but at least one study indicates that for coughing sustained high discharge frequency is required [77].

The Na-K pump via Na-K-ATPase is required to re-establish the membrane potential during high frequency action potential discharge. When Na-K-ATPase is inhibited with ouabain the airway afferent nerve fibers cannot fire at high frequencies, and the cough reflex is diminished [78]. Na-K-ATPase is important in virtually all cell types and consequently nonselective inhibitors such as digoxin and related alkaloids have narrow therapeutic windows. It may be possible to take advantage of the fact that the Na-K-ATPase in vagal afferent nerves is of a relatively restricted isoform. Na-K-ATPase is a heterodimeric protein with α and β subunits, and there are 4 isoforms of the α subunit. The $\alpha 3$ form is largely expressed in vagal afferent nerves, but based on histological assessment this is not the case for other cells in the airways [78]. Topical application of a selective $\alpha 3$ Na-K-ATPase inhibitor may therefore widen appreciably a therapeutic window for a novel anti-tussive or anti-dyspnea drug.

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Highlights

- We discuss potential drug targets aimed at cough and dyspnea
- We review the concept of generator potential-action potential transduction
- We review drug targets aimed at decreasing generator potential amplitude in airway afferent nerves
- We review drug targets aimed at limiting action potential discharge and conduction in airway afferent nerves

Figure 1.

Top: A visual conceptualization of the generator potential and action potentials in a primary afferent nerve. An afferent nerve stimulus (e.g. autacoids, mechanical force, temperature etc) leads to graded membrane depolarization termed the generator potential. If the generator potential reaches a critical threshold, action potential discharge occurs. In yellow is an actual terminal of a vagal A δ fiber situated just beneath the epithelium in a guinea pig trachea (stained with FM-210 dye). The “generator potential” and action potentials are from actual patch clamp recordings of a nodose neuron cell body injected with depolarizing current. Bottom: A description of the quantitative relationship between stimulus intensity, generator potential amplitude, and action potential discharge that is often observed in specialized sensory structures where generator potentials have actually been measured.

Figure 2.
Schematic illustrating numerous potential targets for drugs aimed at limiting the activity of primary afferent nerves in the airways.

Figure 3.

Representative whole cell patch clamp recordings (in current clamp mode) of a sensory neurons isolate from a guinea pig nodose ganglion. On the left is a neuron from an untreated ganglion, showing the response to a 100-pA injection of current over 1 sec. On the right is a recording of neuron isolate the contralateral nodose ganglion previously treated in vivo with $\text{Nav}_V 1.7$ shRNA delivered via local injection of adeno-associated virus. Note that without $\text{Nav}_V 1.7$ the current injection leads to membrane depolarization (analogous to a generator potential) but no action potentials (see Muroi et al, 2011 for details).