

## Review

# Pharmacology of airway afferent nerve activity

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### Abstract

Afferent nerves in the airways serve to regulate breathing pattern, cough, and airway autonomic neural tone. Pharmacologic agents that influence afferent nerve activity can be subclassified into compounds that modulate activity by indirect means (eg bronchial smooth muscle spasmogens) and those that act directly on the nerves. Directly acting agents affect afferent nerve activity by interacting with various ion channels and receptors within the membrane of the afferent terminals. Whether by direct or indirect means, most compounds that enter the airspace will modify afferent nerve activity, and through this action alter airway physiology.

**Keywords:** autonomic, pulmonary, respiratory, sensory, vagus nerve

### Introduction

Airway afferent (sensory) nerves express a variety of receptors and ion channels that, when acted upon by various pharmacologic agents, modulate the activity of these nerves. The induced changes in the activity of airway afferent nerves, in turn, inform the central nervous system (CNS) of a change in their immediate environment and, via reflex pathways, modulate the parasympathetic (cholinergic and noncholinergic) and sympathetic outflow to the airways. In addition, airway afferent nerve activation is responsible for initiating the cough reflex and serves to modulate breathing pattern. Through these actions, the afferent nervous system plays an important role in regulating the physiology of the airways. Abnormalities in afferent nerve function arguably contribute directly to the cause of certain airway pathologies, and undeniably to the symptoms of virtually all airway diseases. It may seem surprising, therefore, that our understanding of the pharmacology of airway afferent nerves is at best rudimentary. This is probably explained by the difficulty

in reducing, in a scientific sense, airway afferent nerve endings to an experimental design that is amenable to classical pharmacologic investigation.

In the present review studies are discussed that investigated the direct actions of chemical compounds on airway afferent nerves. In an attempt to focus the review, we limit the discussion to literature that pertains to the pharmacology of the afferent function of nerves (ie modulation of excitability and action potential discharge). This means that three other important areas of airway afferent nerve pharmacology are excluded from this review: the pharmacology of neuropeptide secretion from C-fibers in the airways [1]; the pharmacology of the synapse between the central terminals of airway afferent nerve endings and secondary neurons in the CNS; and the pharmacology of the developmental aspects and neuroplasticity of afferent neurons. In addition, we do not consider the important topic of nasal-pharyngeal afferent nerves in the present review [2].

## Experimental approaches in the study of airway afferent pharmacology

In assessing the pharmacology of the afferent nerve *per se*, it would be useful to evaluate directly the nerve endings within the airway wall. Regrettably, these nerve endings cannot be directly accessed with current micro-electrophysiologic techniques. Information on the pharmacology of airway afferent nerve endings has been obtained indirectly using the four basic approaches described below. Each of the four designs has certain advantages and disadvantages that must be considered before the pharmacologic data can be interpreted appropriately.

### Electrophysiologic studies of neuronal cell bodies

Although the airway afferent nerve endings are inaccessible to study by electrophysiologic methods, their cell bodies can readily be investigated using standard patch-clamp and intracellular electrophysiologic recording techniques. The cell bodies of afferent fibers that innervate the airway wall are located primarily in vagal ganglia (nodose or jugular ganglia) [3]. Electrophysiologic investigation of vagal ganglion neuron cell bodies has the advantage of allowing for study of the pharmacology of afferent nerves, free from secondary influences.

This technique, like any other, has limitations. First, the extent to which the pharmacology observed at the cell body reflects the pharmacology of the nerve ending is not known. It is worth keeping in mind that the distance between an afferent nerve ending in the human bronchi and its cell body located in a vagal afferent ganglion is the equivalent of over 10,000 cell body diameters. The extent to which the myriad signaling molecules, ion channels, and receptor proteins are transported from the cell body to the nerve endings in the airways is not known. Moreover, the spatial relationships of signaling pathways is likely to be different in nerve terminals as compared with the cell body. The second disadvantage with this technique is that cell bodies situated in the vagal ganglia are diverse with respect to the ionic currents they express, and not only include those cell bodies with axons that innervate the airways, but also those with axons that innervate other thoracic and subdiaphragmatic structures. This disadvantage can be overcome to some extent by using retrograde tracing technology, such that those cell bodies with axons that project to the airways can be identified and studied [4].

### Extracellular recording in isolated tissue

A close approximation of directly studying the nerve ending in the airway wall makes use of the classical isolated tissue design. In these studies, the trachea/bronchus is isolated from the animal with the vagus nerves intact. Using standard extracellular electrophysiologic recording techniques, action potentials that arise from defined receptive fields in the airway wall can be monitored as they are conducted along the nerve fiber.

This approach has the advantage that chemical or non-chemical stimuli can be applied directly to that part of the airway wall that contains the nerve endings (receptive field). Another advantage of the isolated tissue design is that the concentration of compounds bathing the nerve endings within the tissue can be controlled.

A limitation of this design is that it is not possible to assess directly the effect of chemicals on the membrane properties of the afferent nerve that fall short of impulse generation. For example, the influence of a compound that hyperpolarizes the nerve ending, inhibits membrane hyperpolarization, or depolarizes the membrane to a level that is subthreshold for action potential generation would not be directly observed with this preparation. However, the net influence of subthreshold changes can be indirectly assessed by evaluating their influence on the frequency and number of action potentials evoked from the nerve endings.

### Extracellular recording in anesthetized animals

The most common experimental approach to studying airway afferent nerve activity makes use of extracellular recording techniques to detect action potentials traveling in the vagus nerve in anesthetized animals *in vivo*. This approach offers the advantage of studying reflex outputs along with afferent activity while the nerves are in their normal physiologic environment. Although this technique is particularly well suited to the study of airway afferent nerve physiology, the potential for indirect effects limits the utility of this approach in studies of the direct pharmacology of airway afferent nerves.

### Reflex outputs

The least direct approach makes use of reflex outputs as the sole monitors of airway afferent activity. For example, substances that cause cough or changes in breathing pattern, independently of other changes in lung function, can be deduced to act via afferent nerve pathways in the airways. The disadvantage with this technique is that no information on the nature of the afferent fiber type(s) that initiate the reflex is obtained. This method is the only one that has been used to obtain information on human airway afferent nerve pharmacology.

### Indirect pharmacology of airway afferent nerves

Most if not all afferent nerves in the airway wall are sensitive to mechanical perturbations [5–7]. Many of these are low threshold mechanosensors (LTMs). The LTMs can be segregated into those that rapidly adapt to prolonged suprathreshold stimulation (rapidly adapting receptors [RARs]) and those that slowly adapt to mechanical stimulation (slowly adapting receptors [SARs]) [7]. Any substance that alters the normal mechanical forces in the lung can potentially alter the activity of these fibers. Bronchoconstriction is an example of an event that indirectly

leads to activation of LTMs [8–12]. In virtually all mammals studied thus far inhalation of a smooth muscle spasmogen, such as histamine, leads to action potential discharge in RARs, and less commonly in SARs. If the bronchoconstriction is minimized by pretreatment with a bronchodilator, histamine-induced discharge in the LTMs is prevented [11]. This suggests that histamine does not evoke action potential discharge by directly acting on histamine receptors in the airway afferent nerve endings. This conclusion is supported by studies using the isolated, innervated airway preparations [13,14].

It is often tacitly understood by pharmacologists that a component of histamine-induced bronchospasm is secondary to afferent nerve-stimulated parasympathetic reflexes. This is based on the observation that cholinergic muscarinic receptor antagonists can inhibit histamine-induced bronchoconstriction, an effect that is consistent with the indirect stimulation of RARs. It should be recognized, however, that the indirect activation of RARs and increased parasympathetic reflex activity is not unique to histamine. It is reasonable to assume that any bronchoconstrictive agonist will lead to activation of RARs and a consequent change in autonomic activity. Methacholine is commonly used as a 'direct smooth muscle spasmogen' to study airway reactivity. It is often assumed that the effect of methacholine on airflow resistance is independent of the nervous system; however, as expected, several studies [10,15] support the hypothesis that a component of methacholine-induced bronchospasm is due to acetylcholine released from postganglionic parasympathetic neurons that innervate airway smooth muscle. The contribution made by neuronal reflexes may be increased in inflammatory airway disease states, in which neuronal activity is likely to be elevated [16].

Compounds need not contract airway smooth muscle to activate afferent nerves indirectly. Substances that directly affect the vasculature or lead to changes in lung compliance can also alter the activity of afferent nerves [17]. Moreover, substances that lead to the release of autacoids may indirectly lead to changes in the activity of chemosensitive afferent fibers in the airway (see below). Considering the potential indirect manner in which the activity of afferent nerves in the airway wall can be altered, it may be a truism that any substance that affects lung function will influence afferent nerve discharge and reflex control of the airways.

## Direct pharmacology of airway afferent nerves

### Ligand-gated ion channels

Synaptic neurotransmission typically occurs via the release of a neurotransmitter that activates 'receptors' on postsynaptic dendrites. Most often the receptors are actually ligand-gated ion channels. Opening of the ion channel

results in an increase in inward cation current and a membrane depolarization. The prototypical example of this in the peripheral nervous system is presynaptically released acetylcholine acting on postsynaptic nicotinic receptors. Although the peripheral processes of afferent nerves are not activated directly by synaptic neurotransmission, anatomically they have long been recognized as specialized dendrites [18]. It may not be surprising, therefore, that they contain a variety of ligand-gated ion channels (Table 1).

### Acetylcholine

The cholinergic nicotinic receptor is a pentameric protein assembly that forms an ion channel by spanning the membrane four times. The channel is relatively nonselective for cations. There is histochemical evidence of nicotinic receptors on a subpopulation of human vagal afferent ganglion neuron cell bodies [19]. In addition, stimulation of nicotinic receptors on rabbit vagal afferent neuron cell bodies is associated with an increase in nonselective cation current, which is analogous to that seen in autonomic ganglion neurons [20]. Evidence in favor of nicotinic receptors specifically on airway afferent nerve endings is provided by the observations that inhaled cigarette smoke-induced action potential discharge in canine pulmonary C-fibers is inhibited by pretreatment with the nicotinic receptor antagonist hexamethonium [21]. As predicted from these findings, respiratory reflexes such as rapid shallow breathing or apnea evoked by cigarette smoke are also inhibited by hexamethonium [22]. Also consistent with the hypothesis of nicotinic receptors on airway afferent nerves are results from psychometric studies in humans [23] that showed that blockade of nicotinic receptors in the airways inhibits the sensation of irritation caused by cigarette smoke in normally nonsmoking volunteers. The extent to which nicotine acts directly or indirectly to stimulate airway afferent fibers cannot readily be discerned from these *in vivo* studies.

### 5-Hydroxytryptamine

Among the large number of 5-hydroxytryptamine (5-HT) receptor subtypes, the 5-HT<sub>3</sub> subtype stands alone as a ligand-gated ion channel. Like the nicotinic receptor, the 5-HT<sub>3</sub> receptor spans the plasma membrane four times and forms an ion pore that is relatively nonselective for cations. Activation of 5-HT<sub>3</sub> receptors causes substantial membrane depolarizations and increases in ion conductances in most small-diameter (nociceptor-like) neuron cell bodies in vagal ganglia [24]. 5-HT does not evoke action potential discharge in afferent fibers in the guinea pig isolated airway preparation [14]. However, that at least some airway-specific neurons express 5-HT<sub>3</sub> receptors is supported by electrophysiologic studies [4] that showed that 10 out of 12 neurons that were retrogradely labeled by fluorescent dye injection into guinea pig airways depolarized an average of over 8 mV in response to 10 μmol/l 5-HT. 5-HT and phenylbiguanide derivatives

**Table 1****Pharmacologic modulation of airway afferent nerve activity**

Pharmacologic target	Compounds	Effect on activity or excitability
Ligand-gated ion channels		
5-HT <sub>3</sub> receptor	5-HT, phenylbiguanide	↑
P2X receptor	ATP	↑
Nicotinic receptor	Nicotine, acetylcholine	↑
VR1	Capsaicin, resiniferatoxin, various acids	↑
GPCRs		
Histamine H <sub>1</sub> receptor	Histamine	↑
Bradykinin B <sub>2</sub> receptor	Bradykinin	↑
Adenosine A <sub>1</sub> receptor	Adenosine	↑
Prostanoid receptors	PGE <sub>2</sub> , PGD <sub>2</sub> , PGF <sub>2α</sub> , TXA <sub>2</sub> , PGI <sub>2</sub>	↑
Opioid μ receptors	Endomorphins, DAMGO	↓
Cys-LT1 receptors	Cysteinyl leukotrienes	↑
NK1, NK2 receptors	Substance P, neurokinin A	↑
Voltage-gated ion channels		
Sodium channels		
	Tetrodotoxin	↓
	Lidocaine	↓
	Veratridine	↑
	Amiloride	↓
Potassium channels		
	4-Aminopyridine	↑
	α-Dendrotoxin	↑
	NS1619	↓
	Iberiotoxin	↑
Unknown targets		
	Ozone	↑
	Sulfur dioxide	↑
	Acetone	↑
	Water	↑
	Low-chloride solutions	↑
	Furosemide	↓↑

Summary of compounds for which there is evidence for a direct effect on airway afferent nerve endings, or cell bodies. See text for more detail and references. DAMGO, D-Ala<sup>2</sup>-Me-Phe<sup>4</sup>-Gly-ol<sup>5</sup>-enkephalin; NK, neurokinin; PG, prostaglandin; TX, thromboxane.

have long been recognized as effective stimulants of action potential discharge in bronchial and pulmonary C-fibers when delivered to the lungs *in vivo* [25]. Although there has been little attention given to the nature of the receptor responsible for this effect, it is worth noting that phenylbiguanide derivatives are selective for the 5-HT<sub>3</sub> receptor subtype [26].

#### ATP and related purine nucleotides

Purines such as ATP can activate both metabotropic receptors and ionotropic receptors. The ligand-gated ion channels (ionotropic receptors) are referred to as P2X receptors, and comprise subunits with only two transmembrane domains. There are at least seven subtypes of P2X receptors, but interestingly P2X3 appears to be localized nearly exclusively to a subset of small-diameter (nociceptive-like) afferent neurons [27]. Neuronal cell bodies in vagal ganglia express P2X2 and P2X3 receptors [28], and P2X receptors have been identified on afferent nerve processes that innervate airway neuroep-

ithelial bodies [28,29]. Stimulation of P2X receptors in subpopulations of nodose ganglion neuron cell bodies causes an increase in cation current across the plasma membrane and brisk elevations in intracellular calcium concentrations [30]. The characterization of P2X receptors in nodose ganglion neurons is consistent with both P2X2 and P2X3 receptors, and possibly with P2X2/3 heterodimers. The extent to which P2X receptor activation affects the membrane potential of airway-specific neurons in vagal ganglia is not known.

Studies *in vivo* [31] have demonstrated that ATP and related purines stimulate action potential discharge in canine pulmonary C-fibers by a mechanism that was inhibited by P2X receptor-selective antagonists. ATP at concentrations as high as 100 μmol/l, however, does not evoke action potential discharge in rapidly adapting LTMs or C-fibers (unpublished observation) when applied directly to their receptive fields in the guinea pig isolated trachea/bronchus preparation.

*Capsaicin (vallenoids)*

Capsaicin stimulates airway C and A $\delta$  fibers that are high threshold nociceptive-like mechanosensors in the airways [13,14,32]. In guinea pigs and rats, capsaicin causes the release of large quantities of neuropeptides in the airways that effectively constrict guinea pig bronchial smooth muscle [1] and cause plasma extravasation [33]. These neuropeptide-induced effects probably lead to changes in activity of low-threshold RAR or SAR nerve activity in an indirect manner [34], as discussed above.

The first cloned receptor for capsaicin is termed vallinoid receptor (VR1) [35]. VR1 protein has been localized to small-diameter (nociceptor-like) afferent neurons in dorsal root, trigeminal, and vagal sensory ganglia. It is an ion channel that is permeable to sodium and calcium ions and is opened by heat, but the temperatures required for this effect (>42°C) are not likely to be relevant to nerve endings in the lower airways. There is a positive interaction between protons and temperature [36], however, that may result in activation of this channel in afferent nerves in the airway wall. Thus, at a pH of 6.0, the channel is opened at 30°C. Consistent with this concept, the discharge of action potentials induced by a applying a pH 5 solution to receptive fields of afferent C-fibers in the guinea pig isolated airway preparation was blocked by the VR1 antagonist capsazepine [37]. Electrophysiologic studies on afferent cell bodies, and on the cloned VR1 expressed in non-neuronal cells support the hypothesis that various lipid mediators may serve as endogenous VR1 ligands. These include the endogenous cannabinoid receptor agonist anandamide [38], which was recently reported to stimulate C-fibers in guinea pig airways [39], and 5-, 12- and 15-lipoxygenase products of arachidonic acid [40].

The therapeutic potential of VR1 agonists such as capsaicin or resiniferatoxin is based on the ability of these substances to 'desensitize' afferent neurons that express VR1. These ligands not only desensitize the afferent neurons to further activation via VR1 channels, but also lead to nonselective 'heterologous' desensitization such that the nerve becomes unresponsive to other stimuli that would normally evoke action potential discharge. The mechanism of heterologous desensitization through VR1 has not been elucidated in detail, but probably involves an inhibition of voltage-gated sodium channels [41].

**G-protein-coupled receptor agonists**

There are a number of G-protein-coupled receptor (GPCR) agonists that, through various second messenger systems, can affect the function of airway afferent nerves (Table 1). Although not specifically studied in airways afferent neurons, several agonists may affect both ligand-gated ion channel and GPCRs. For example, in addition to cholinergic nicotinic receptors on afferent nerves (as discussed above), there is evidence of cholinergic muscarinic

M2 and M3 receptors on afferent neuronal cell bodies in the dorsal root ganglia [42]. Likewise, in vagal afferent neuronal cell bodies, 5-HT can interact with both 5-HT<sub>3</sub> ion channels and G-protein-coupled 5-HT receptors [43].

*Bradykinin*

Bradykinin is one of the few GPCR agonists that consistently lead to action potential discharge when applied directly to the receptive fields of guinea pig airway afferent neurons [14,44]. This effect of bradykinin is blocked by the B<sub>2</sub>-receptor-selective antagonist HOE140, and is selective for C and A $\delta$  nociceptive-like fibers [44]. The ionic mechanism that underlies B<sub>2</sub>-receptor-mediated activation of airway afferent nerve fibers is unknown. Bradykinin B<sub>2</sub> receptor stimulation depolarizes the membrane potential of nodose ganglion neuron cell bodies, and inhibits a calcium-dependent potassium current that is responsible for an after-spike hyperpolarization [45]. Both of these effects are mediated by bradykinin B<sub>2</sub> receptors, although the latter effect on the after-spike hyperpolarization appears to be secondary to prostacyclin production by the neuron. Bradykinin stimulated action potential discharge in airway C-fiber and RAR afferent nerves in several species studied *in vivo* [32,46]. In some studies, however, the effect of bradykinin on RAR fibers appeared to be secondary to prostaglandin-mediated changes in lung mechanics. Bradykinin B<sub>2</sub> receptors have been localized autoradiographically in human nodose ganglion cell bodies [47], and bradykinin causes sneezing and coughing when applied to appropriate sites in human airways [48,49].

*Histamine*

Histamine H<sub>1</sub> receptor activation results in membrane depolarization of a subpopulation of vagal afferent neuron cell bodies from a variety of species. Unlike ligand-gated ion channel agonists and bradykinin, the membrane depolarization evoked by histamine is typically associated with a decrease in ion conductance [50,51]. Histamine inhibits the resting or so-called 'leak' potassium current in nodose ganglion neuronal cell bodies, and in vagal ganglion cell bodies from some species it inhibits voltage-gated calcium currents and the calcium-activated potassium current that subserves after-spike hyperpolarizations [51,52]. These types of effects on afferent neurons indicate that histamine alone may not directly evoke action potential discharge in airway afferent endings. Consistent with this prediction, histamine does not evoke action potential discharge in vagal afferent fibers innervating guinea pig isolated airway preparations [13,14]. Histamine effectively stimulates RAR and SAR fibers when studied *in vivo*, but the preponderance of evidence supports the hypothesis that this effect is secondary to the effects of histamine on the vasculature or lung mechanics (see above). Studies on vagal ganglion neuron cell bodies that revealed an inhibition of various potassium currents suggest that histamine may increase

the excitability of airway afferent nerves. Indeed, histamine has been shown to increase the mechanical and chemical (capsaicin) sensitivity of afferent C-fibers in dog airways [53].

Although substances that affect afferent nerve activity can be categorized as those that act directly and those that act indirectly, it should not go unnoticed that many substances act in both a direct and indirect manner. Histamine may be considered a prototypical example. By causing bronchospasm, histamine H<sub>1</sub> receptor activation can indirectly lead to activation of mechanosensitive afferent fibers in the airway wall. In addition, the excitability of some of these same fibers may be increased by the direct inhibitory action of histamine receptor activation on various ion channels that are present in afferent nerve terminals. Thus, for any given amount of bronchospasm, the action potential discharge may be greater for a substance such as histamine that has both indirect and direct actions on the nerve than for an agent that only indirectly influences afferent neuron activity by contracting the airway.

#### *Eicosanoids*

Prostaglandins have long been recognized to activate or increase the excitability of afferent nerves. Electrophysiologic studies on vagal afferent ganglion neuron cell bodies have demonstrated excitatory effects of several prostaglandins, including prostaglandin E<sub>2</sub>, D<sub>2</sub>, and I<sub>2</sub> (prostaglandin) [50,54]. Little attention has been given to prostaglandin receptor pharmacology in these studies, although EP3 receptors have been immunohistochemically identified on rat nodose ganglion neuron cell bodies [55]. Prostaglandins have been found to inhibit calcium-activated potassium currents that are involved in the after-spike hyperpolarization, and can lead to an increase in the hyperpolarization-activated cation current (I<sub>h</sub>) [56]. If these effects occur at the nerve terminals, they would probably lead to an increase in the peak frequency of action potential discharge. Consistent with this, *in vivo* studies [58] revealed that low concentrations of prostaglandin E<sub>2</sub> did not cause action potential discharge in rat airway afferent nerves, but effectively sensitized pulmonary C-fiber afferents to capsaicin or mechanical stimulation during lung inflation. Others have noted that thromboxane, and prostaglandin E<sub>2</sub>, I<sub>2</sub> and F<sub>2α</sub> increased the rate of baseline discharge in airway RAR and C-fibers [34,58–61]. In support of a role for prostaglandins in increasing the excitability of human airway afferent C-fibers are the findings that inhalation of prostaglandin E<sub>2</sub> and prostaglandin F<sub>2α</sub> increased the sensitivity of the cough response in human volunteers [62].

There has been little investigation into the potential role of cysteinyl leukotrienes (cys-LTs) on airway afferent nerve activity. Inhalation of leukotriene C<sub>4</sub> causes activation of RAR fibers in guinea pig airways, but this may be sec-

ondary to bronchoconstriction [12]. Cyst-LTs inhibited the after-spike hyperpolarization in vagal sensory ganglion neuron cell bodies, and caused membrane depolarization of identified airway neuron cell bodies isolated from vagal afferent ganglia [50,63]. This latter effect is due to an inhibition of a resting potassium current, and is blocked by the cys-LT1 receptor antagonist zafirlukast. Other lipoxygenase products, as discussed above, may interact directly with the VR1 channel on nociceptive-like nerve terminals in the airways.

#### *Adenosine*

Adenosine increases action potential discharge in rat pulmonary C-fibers [64]. This effect did not appear to involve adenosine A<sub>2</sub> receptors, but was inhibited by the A<sub>1</sub> receptor-selective antagonist 1,3-dipropyl-8-cyclopentylxanthine. In human volunteers inhalation of AMP was found to cause a greater dyspneic response than methacholine when normalized to change in forced expiratory volume in 1 s, suggesting increased afferent activity in sensory nerves [65].

#### *Neurokinins*

Depending on the species, neurokinin 1 receptor agonists have been found to depolarize [66] or hyperpolarize [67] the membrane potential of nodose ganglion neuron cell bodies. The hyperpolarization of ferret nodose ganglion neurons is secondary to activation of a calcium-gated potassium current [67]. Neurokinin 2 receptor agonists depolarize guinea pig nodose ganglion cell bodies secondary to an increase in a nonselective cation current [68]. Interestingly, this effect is 'unmasked' by inflammatory mediators. Thus, none of 156 nodose ganglion neuron cell bodies from control guinea pigs depolarized in response to neurokinin 2 receptor activation. In contrast, within a day of allergen challenge of the airways, >80% of the nodose ganglion cell bodies responded to neurokinin 2 agonists with membrane depolarization [68].

Substance P leads to discharge of RAR fibers in rabbit [69] and guinea pig airways [70]. This does not appear to be a direct effect of the neurokinin on the RAR fiber. In rabbits, the increase in RAR activity was associated with microvascular leakage in the lungs, and in the guinea pig the substance P-induced discharge in RAR activity was inhibited by nitric oxide synthase inhibitors.

#### *Opioids*

The μ opioid receptor agonists inhibit voltage-gated ion calcium currents in a subpopulation of vagal afferent neurons [71]. In guinea pig nodose ganglion neuron cell bodies, the μ opioid agonist Tyr-D-Ala-Gly-MePhe-Gly-ol enkephalin had no effect on resting membrane current or on the I<sub>h</sub>, but inhibited the ability of other inflammatory mediators to enhance I<sub>h</sub> [72]. Thus, opioids may inhibit the increased excitability of afferent nerves induced by

mediators such as prostaglandin  $E_2$  [73]. That opioids may modulate airway afferent nerves is supported by studies on reflex physiology, in which  $\mu$ ,  $\delta$  and  $\sigma$  opioid agonists acting in the airways can lead to an inhibition of respiratory reflexes [74,75]. The extent to which their inhibitory effects are due to action on the airway afferent endings is not clear.

### **Ion channel modulators**

#### *Sodium channel pharmacology*

All afferent nerves require voltage-gated sodium channels for the conduction of action potentials from the regenerative region of their nerve terminals to the CNS. The most commonly studied inhibitors of voltage-gated sodium channels in the airways has been lidocaine and related local anesthetics. Early studies with these compounds led to the hypothesis that the generator potential caused by mechanical activation of the nerve terminal was not affected (or only modestly affected) by local anesthetic action, whereas the ability of the generator potential to evoke regenerative action potentials was blocked [76].

Tetrodotoxin is a potent inhibitor of a subset of voltage-gated sodium channels. Blocking tetrodotoxin-sensitive sodium channels prevents conduction in all airway afferent nerve fibers studied thus far. There are voltage-gated sodium channels that are not blocked by tetrodotoxin, which are termed tetrodotoxin-resistant (TTX-R) channels. At least two types of TTX-R sodium channels have been found to be preferentially localized to afferent nerves [77]. These 'sensory nerve-specific' (SNS) channels are found mainly in small-diameter (nociceptor-like) neurons in afferent ganglia, supporting the idea that they play more of a role in nociceptive fibers than in LTM fibers. The nomenclature of SNS sodium channels is rather confusing, with SNS1 also referred to as PN3, NaNG or  $Na_v1.8$ , while SNS2 is sometimes referred to as the NaN, SNS2, PN5, NaT, SCN12A or  $Na_v1.9$  channel. Nociceptive-like afferent nerves that innervate guinea pig airways are derived from cell bodies that are located primarily in the jugular (superior vagal) ganglia [13]. Christian and Togo [78] noted that the vast majority of neurons in the jugular ganglia have sufficient TTX-R sodium channels to support action potential generation. Although there are no reports of TTX-R channel specifically on airway afferent nerves, preliminary data from our laboratory [79] have shown that jugular neuron cell bodies retrogradely labeled by dye injection into the guinea pig trachea have sufficient TTX-R current to support action potential formation. The TTX-R sodium channels may be relevant to airway afferent pharmacology, because in the somatosensory system substances such as prostaglandin  $E_2$ , that increase excitability of C-fiber neurons, also increase the current through the TTX-R channels [80]. Although drugs have been developed that block both tetrodotoxin-sensitive sodium channels and TTX-R sodium channels [81], there are no

pharmacologic tools available that allow for the selective inhibition of the TTX-R current in sensory neurons.

Veratrum alkaloids were used to activate bronchial and pulmonary afferent nerves, long before their mechanism of action was understood [82]. Veratridine is now known to interact selectively with voltage-gated sodium channels, although it neither opens the channel directly nor blocks the channel. Rather, it is believed to act by inhibiting the inactivation of the channel [83]. In any event, this class of drugs is one the few that leads to activation of all types of airway afferent nerves.

It is generally believed that mechanically sensitive afferent fibers, including those that innervate the airways, express mechanically gated ion channels at their peripheral terminals, and that these channels serve as mechanotransducers (ie convert mechanical energy into a form that can be encoded into action potentials). Although the exact identity of the mechanosensor(s) is unknown, a variety of evidence suggests that epithelial sodium channels (ENaCs) may act as mechanotransducers in primary afferent neurons [84–87]. In particular, the ENaC channel blockers amiloride and benzamil were shown to inhibit pressure-evoked baroreceptor afferent nerve activity [85] and renal nerve activity evoked by increases in renal pelvic pressure [88]. With respect to the airways, amiloride and benzamil were shown to reduce the mechanical activation of guinea pig trachea/bronchus afferent neurons, but this did not appear to occur by a selective blockade of ENaCs or ion channels involved in mechanotransduction, but rather appeared to be secondary to a reduction in neuronal excitability caused by blockade of voltage-gated sodium currents [89].

#### *Potassium channel pharmacology*

There is surprisingly little information published on the effect of potassium channel-modifying drugs on the afferent activity of airway sensory nerves. Voltage-gated potassium channels that are involved in 'A' currents (rapidly inactivating potassium currents) can be inhibited by 4-aminopyridine and  $\alpha$ -dendrotoxin. These compounds have been found to depolarize the resting membrane potential of identified guinea pig airway nodose ganglion neurons [90]. Moreover, applying these A-current inhibitors to the guinea pig isolated airway evokes a burst of action potentials in LTMs, as well as nociceptive-like airways afferent fibers. These findings suggest that a potassium current carried by these channels is responsible for keeping the membrane potential of airway afferent nerves below the threshold for action potential generation. Selective inhibitors of the maxi-K current, such as iberiotoxin, do not affect the resting membrane potential of airway afferent nerves [90]. However, in guinea pig airways the maxi-K channel opener NS1619 did inhibit the hyperosmolar-induced activation of vagal  $A\delta$ -fibers and bradykinin-

induced activation of C-fibers [91]. Consistent with these *in vitro* observations, NS 1619 effectively inhibited the cough reflex induced by bradykinin in guinea pigs [91].

#### *Chloride and calcium channel pharmacology*

$\gamma$ -Aminobutyric acid caused a membrane depolarization in 10 out of 12 identified guinea pig airway nodose ganglion neurons [4]. This effect probably occurs through increasing current through chloride channels. These studies suggest, at least at the cell body, that the reversal potential for chloride ions is positive to the resting potential, such that opening the channels leads to chloride efflux from the cell and membrane depolarization. Consistent with this hypothesis is that isotonic solutions containing low chloride concentrations evoked action potential discharge in a subpopulation of guinea pig airway LTM A-fibers and C-fibers [92], and activated canine laryngeal afferent fibers [93]. Low chloride solutions also cause cough in humans [94]. Furosemide modestly inhibits action potential discharge in airway afferent fibers and the cough reflex caused by low chloride solutions, but the mechanism underlying this has not yet been elucidated [92,93,95]. Similarly, the mechanism(s) of furosemide-induced alleviation of experimentally induced dyspnea [96] or furosemide-induced sensitization of SARs and desensitization of RARs in rat airways [97] is unknown.

The voltage-gated calcium current in guinea pig vagal afferent jugular ganglion cell bodies is due to a composite of N-, L-, and P-type calcium channels [52]. Compounds that block N-type calcium channels, such as  $\omega$ -conotoxin, inhibit neuropeptide secretion from primary afferent nerves in guinea pig bronchi [98], but the effect of this compound or other calcium channel antagonists on action potential discharge or pattern in airway afferent nerves is not known.

#### **Environmental stimuli**

A variety of environmental irritants lead to action potential discharge in airway afferent nerves (Table 1). The nociceptive class of fibers and RAR fibers are particularly sensitive to activation by various inhaled pollutants. Ozone has been found to increase the excitability of airway RARs [99] and C-fibers [100], such that the threshold for their mechanical and chemical activation is reduced. There have been no studies published on the direct effect of ozone on the electrophysiologic properties of sensory neurons.

Allergen challenge *in vivo* leads to activation of airway afferent nerves [101,102]. Allergen exposure *in vitro* increases the sensitivity of A $\delta$  nerve endings in the guinea pig isolated trachea/bronchus to mechanical stimulation [103]. Exposing the nodose ganglia isolated from immunized guinea pig to allergen leads to activation of resident mast cells, and decreases in the resting potassium current and certain calcium-activated potassium currents [104].

Low pH solutions can induce action potential discharge in airway afferent nerve fibers [37]. With respect to nociceptive-like fibers, this effect is most likely due to increasing cation current through VR1 channels (see above). Other vagal afferent neurons may also respond to decreases in pH [105] via activation of various acid-sensing ion channels. It is likely that an increase in proton concentration near the airway sensory terminals, and the consequent increase in cation current through acid-sensing ion channels, is the mechanism by which compounds such as citric acid and sulfur dioxide initiate cough and other respiratory reflexes [94,106].

#### **Conclusion**

A composite image of airway afferent neuropharmacology is emerging from classical studies on reflex physiology and single-unit recording of vagal afferent nerves, in combination with electrophysiologic studies of vagal ganglion neuron cell bodies. The vast majority of afferent nerves that innervate the airway wall are mechanosensory, in that they respond with a discharge of action potentials to deformation of the receptive field. Therefore, any substance that changes the mechanical environment (eg bronchoconstrictors, bronchodilators, and vasoactive substances) will influence afferent nerve activity arising from the airways. Substances that affect the osmolarity or pH in the environment of the sensory nerve endings will also change activity in a subset of acid-sensing and osmolarity-sensing afferent fibers. Agonists such as 5-HT, acetylcholine, ATP, and capsaicin can directly interact with ionotropic receptors in airway afferent nerve fibers, leading to membrane depolarization and action potential discharge. Other agonists can interact with GPCRs on airway afferent nerves in a manner that does not activate the fiber, but modulates its excitability in response to mechanical or chemical stimuli. Finally, ion channel-modifying compounds can increase or decrease ionic current through voltage-gated ion channels in airway afferent nerves, to affect afferent activity.

Based on these observations one may conclude that most substances that enter the airways and affect lung function will affect afferent nerve activity directly, and/or indirectly, thereby altering the communication between the airways and the CNS. This, in turn, will lead to changes in autonomic and respiratory reflex activity.

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