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Neural control of the lower airways: Role in cough and airway inflammatory disease

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Abstract

Airway function is under constant neurophysiological control, in order to maximize airflow and gas exchange and to protect the airways from aspiration, damage, and infection. There are multiple sensory nerve subtypes, whose disparate functions provide a wide array of sensory information into the CNS. Activation of these subtypes triggers specific reflexes, including cough and alterations in autonomic efferent control of airway smooth muscle, secretory cells, and vasculature. Importantly, every aspect of these reflex arcs can be impacted and altered by local inflammation caused by chronic lung disease such as asthma, bronchitis, and infections. Excessive and inappropriate activity in sensory and autonomic nerves within the airways is thought to contribute to the morbidity and symptoms associated with lung disease.

INTRODUCTION

The airways are densely innervated by both sensory/afferent nerves and autonomic (motor/efferent) nerves, which regulate both respiratory rhythms and organ function within the airways. In this chapter, we provide a review of the sensory and autonomic innervation of the lower airways (from the trachea and bronchi through to the intrapulmonary bronchioles, alveoli, and blood vessels) and their physiological role in health, as well as the contribution of these nerves to the pathophysiology of airway disease including inflammation and cough. In the context of this chapter, we use the term “lower airways” to refer to the airway between the larynx and alveoli. The literature pertaining to nasal airways will not be reviewed.

SENSORY AFFERENT INNERVATION OF THE LOWER AIRWAYS

In mammals, the majority of airway sensory innervation is supplied by primary afferent nerves projected from neurons residing in the vagal ganglia (Xth cranial nerve) (Fig. 14.1). This heterogeneous population of sensory nerves expresses a variety of receptors for mechanical, thermal, and chemical stimuli, and serves to detect the local environment of the lower airways and initiate local and central reflexes that can evoke defensive reflexes and also serve to regulate respiratory rhythm and cardiovascular function (Coleridge and

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Coleridge, 1984; Mazzone and Udem, 2016). The sensory innervation of the airways is supplied by branches of the vagus nerve; the branches that innervate the trachea are referred to as the superior laryngeal nerve (SLN) and the recurrent laryngeal nerve (RLN). In guinea pigs and larger mammals, the vagal ganglia are comprised of two distinct structures: the nodose or inferior vagal ganglion and the jugular or superior vagal ganglion (Ricco et al., 1996; Udem et al., 2004). In mice and rats, these two structures are partially fused into the jugular–nodose complex (McGovern et al., 2015b; Kim et al., 2020b). Regardless of mammalian species, neurons within the nodose ganglia are embryologically derived from the epibranchial placodes, whereas the jugular neurons are embryologically derived from the neural crest (Taylor-Clark, 2021). The embryological source of these neurons is a major determinant of their gene expression, sensitivity to growth factors, anatomical connectivity and function. Vagal afferents project their central axons to the brainstem medulla, where they synapse with second-order neurons in the nucleus tractus solitarius (nTS) and, in some cases, the paratrigeminal complex (Pa5) (Kubin et al., 2006; Behrens et al., 2021; Taylor-Clark, 2021). In this way, afferent activity regulates CNS networks that control breathing rhythms and autonomic efferent outflow to the lungs and cardiovascular organs.

The majority of vagal sensory nerves express receptors for stimuli that are considered noxious, such as nonphysiological temperatures, pH, osmolarity or mechanical forces, or inflammatory mediators (e.g., bradykinin) or irritants (e.g., capsaicin, the pungent ingredient in chili peppers) (Coleridge and Coleridge, 1984; Mazzone and Udem, 2016). The activation of these so-called “nociceptive” sensory nerves (or “nociceptors”) evokes defensive responses/reflexes such as apnea, tachypnea, bronchospasm, mucus secretion, cough and the sensation of dyspnea, as well as changes in heart rate and blood pressure. These reflexes serve to protect the airways but if they are triggered excessively or chronically can contribute to the morbidity of airway diseases such as asthma and chronic obstructive pulmonary disease (see below). Nociceptors are typically quiescent during eupneic breathing (Ho et al., 2001), indicating such mechanical forces fail to reach threshold for nociceptor activation. Instead, lung stretch during eupnea activates nonnociceptive stretch-sensitive mechanoreceptor fibers (Widdicombe, 1954; Ho et al., 2001), whose activation finely regulates inspiration and expiration.

Afferent activation

Afferent nerves express ion channel receptors on their peripheral nerve terminals within the airways that are sensitive to specific local stimuli (see Table 14.1) (Mazzone and Udem, 2016). For example, most nociceptive afferents express transient receptor potential (TRP) vanilloid 1 (V1) (Ho et al., 2001; Udem et al., 2004), which is activated by capsaicin, extracellular acidification and excessive heat (Caterina et al., 1997). Binding of capsaicin to TRPV1 causes the channel’s cation-permeable pore to open, leading to Na⁺ and Ca²⁺ influx. These fluxes evoke a graded depolarization of the afferent terminal that, if of sufficient magnitude, can reach the activation threshold of voltage-gated sodium channels (Na_v). Depolarization-evoked activation of Na_v induces rapid influx of Na⁺, resulting in the upstroke of the all-or-nothing action potential (AP). This sets off a wave of depolarization along the axon—i.e., AP conduction. In this way, the sensory stimulus is encoded by AP discharge frequency. Electrical excitability of airway afferents is in part determined by the

number and biophysical properties of Na_V and potassium channels (both voltage-gated and Ca^{2+} -gated) which contribute to the various parts of the AP waveform (Schild et al., 2005; Kwong et al., 2008a; Gover et al., 2009; Sun et al., 2019; Sun, 2021). These parameters are sensitive to cell signaling cascades that can be initiated by inflammatory mediators (Undem et al., 1991, 1993; Kwong and Lee, 2005). Thus inflammation can increase AP discharge independently of the receptor-mediated graded depolarization.

Many afferents also express a host of nonchannel forming receptors for inflammatory mediators that are commonly produced during airway inflammation (Mazzone and Undem, 2016). These receptors are typically G-protein-coupled receptors, such as the bradykinin B2 receptor, histamine H1 receptor, adenosine A1 and A2 receptors and the spingosine-1-phosphate S1PR3 receptor. In addition, some cytokine receptors (e.g., IFN γ receptor) can modulate afferent activity. Activation of metabotropic receptors can modulate afferent excitability via second messenger signaling cascades that target channels involved in the AP. For example, activation of Gs-coupled receptors can cause protein kinase A-dependent increases in Na_V membrane expression and Na_V voltage sensitivity (Kwong and Lee, 2005). However, in order to directly induce AP discharge the inflammatory mediator-sensitive metabotropic receptor must directly regulate ion channels responsible for graded potentials in the afferent terminal. Many of the specifics are currently unclear, but multiple ion channels have been implicated in these “receptor-operated channel” pathways (Undem and Sun, 2020). For example, bradykinin-evoked activation of airway nociceptors has been shown to be mediated by TRPV1 (possibly via Gq-mediated hydrolysis of the inhibitory phosphatidylinositol bisphosphate) (Carr et al., 2003) and by Ca^{2+} -sensitive chloride channels (whose activation causes depolarization of vagal afferents as their reversal potential for chloride is more positive than the resting membrane potential) (Lee et al., 2005).

Afferent subsets

Vagal afferents are heterogeneous and considerable efforts have been made to characterize this heterogeneity at the functional, electrophysiological, pharmacological, anatomical, biochemical and genetic levels (Coleridge and Coleridge, 1984; Mazzone and Undem, 2016). Electrophysiological recordings of single fibers indicate that vagal afferents innervating the airways range from slow conducting (~ 1 m/s) unmyelinated C fibers to fast conducting myelinated A fibers. Myelination of afferents correlates with the size of the afferent cell body within the vagal ganglia, and with neurofilament expression in these neurons. Expression of neuropeptides such as substance P, calcitonin gene-related peptide (CGRP) and neurokinin A is more prevalent in small diameter, neurofilament-negative neurons. Below is a list of the major afferent subsets innervating the lower airways.

Intrapulmonary A fibers

Approximately 10% to 20% of vagal afferents innervating the mammalian lung are myelinated afferents (conduction velocity ~ 12 – 50 m/s, mostly A β range) that are highly sensitive to mechanical stimulation (Widdicombe, 1954; Bergren and Peterson, 1993; Ho et al., 2001; Schelegle and Green, 2001; Mazzone and Undem, 2016). Eupneic breathing induces lung stretch that is sufficient to activate these fibers, and their responses have been characterized based upon their adaptation: slowly adapting receptors (SARs), also known

as pulmonary stretch receptors (PSRs), and rapidly adapting receptors (RARs). RARs tend to conduct slower than SAR, but there is substantial overlap. The rapid adaptation of RARs is unlikely to be due to intrinsic electrophysiological properties (McAlexander et al., 1999), but rather to an adaptation of the stimulus. Activation of the mechanoreceptors regulates central respiratory networks within the nTS. SARs central projections terminate within the nTS at about the rostracaudal level of the area postrema, in particular the ventral and lateral subnuclei, where they synapse with pump cells (whose activation parallel SAR activity) and inspiratory- β cells (Kubin et al., 2006). These second order neurons then project to the parabrachial nucleus and Kölliker–Fuse nucleus in the dorsolateral pons and the ventral respiratory column, synapsing with multiple levels of the respiratory pattern generator network. SAR activation leads to the inhibition of inspiration and the facilitation of expiratory drive (Hering–Breuer reflex) (Widdicombe and Nadel, 1963; Chang et al., 2015). In mice, lung stretch-induced apnea is dependent on the activation of the Piezo2 channel expressed on myelinated nodose afferents innervating the lung (Nonomura et al., 2017). SAR activation also causes a decrease in parasympathetic drive to the airways and heart, resulting in bronchodilation and tachycardia (Widdicombe and Nadel, 1963; Kubin et al., 2006; Mazzone and Udem, 2016). Intrapulmonary RAR fibers project to more caudal and medial nTS regions than SARs, where they synapse with so-called “RAR cells” (Lipski et al., 1991). RAR cells provide inputs into the respiratory premotor networks within the nucleus ambiguus, parabrachial nucleus and Kölliker–Fuse nucleus (Otake et al., 2001; Kubin et al., 2006). RAR activation increases inspiratory effort and rate, as well as increasing parasympathetic drive to the airways, resulting in bronchospasm (Widdicombe, 1954; Mazzone and Udem, 2016). In addition to their antagonistic effects on respiration and autonomic output, SAR/pump cell activity leads to direct inhibition of RAR cells within the nTS (Kubin et al., 2006).

SARs and RARs do not typically express nociceptor-associated receptors such as TRPV1 and the bradykinin B2 receptor, nor do they typically express neuropeptides (Bergren and Peterson, 1993; Ho et al., 2001; Mazzone and Udem, 2016). Therefore these mechanoreceptors are not directly activated by nociceptive stimuli. However, some mechanoreceptor afferents, particularly RARs, are indirectly activated by agents that cause bronchospasm or edema or decreases in lung compliance, and this phenomenon induced some studies to refer to these fibers as “irritant receptors.” Thus, local bronchospastic autacoids can modulate respiratory reflexes: histamine evokes tachypnea in anesthetized guinea pigs, which is reduced by the bronchodilator albuterol, suggesting the involvement of the mechanosensitive RAR fibers (Chou et al., 2008).

Many mechanoreceptors express P2X2 and P2X3 and display sensitivity to ATP and α,β methylene ATP (α,β mATP), the selective ligand of P2X2/3 heteromeric channels, indicating that they are derived from nodose neurons, which express, as expected, high levels of neurofilament (Canning et al., 2004; Hooper et al., 2016).

Extrapulmonary nodose A δ fibers

In guinea pigs, the majority of nodose afferents innervating the extrapulmonary airways are partially myelinated A δ fibers that conduct APs at 2 to 8 m/s (Ricco et al., 1996;

Canning et al., 2004). These fibers, which project to the trachea and bronchi via the RLN, are exquisitely sensitive to punctate stimulation but are insensitive to stretch. Nodose A δ fibers innervating the trachea and bronchi are also activated by acid, but are insensitive to other nociceptive stimuli such as bradykinin and capsaicin (Ricco et al., 1996). A δ fiber responses to acid are not mediated by TRPV1, which they lack. Instead, acid sensing ion channels (ASICs) have been implicated (Canning et al., 2006), but their role has yet to be definitely demonstrated. Consistent with their nodose (i.e., placodal) origin, nodose neurofilament-positive neurons traced from the trachea lack neuropeptide content. Recent evidence suggests that these afferents express both tetrodotoxin-sensitive Na v 1.3 and Na v 1.7 and tetrodotoxin-insensitive Na v 1.8 and Na v 1.9 (Kollarik et al., 2018). Nevertheless, AP discharge from the activated afferent terminal is abolished by tetrodotoxin or by an Na v 1.7-selective inhibitor.

Punctate mechanical stimulation of the trachea and bronchi evokes cough under anesthetic conditions in multiple mammalian species, including guinea pigs, rabbits and cats (Canning et al., 2004, 2014; Canning, 2008). There is substantial evidence, best characterized in the guinea pig, that extrapulmonary nodose A δ fibers are responsible for the cough evoked by either punctate mechanical stimulation, electrical stimulation or citric acid in anesthetized animals (Ricco et al., 1996; Canning et al., 2004; Mazzone et al., 2009; Muroi et al., 2011, 2013): firstly, section of the RLN but not the SLN ablates such tracheal cough responses; secondly, only stimuli (e.g., punctate mechanical, citric acid, electrical) shown in single fiber electrophysiological recordings to activate nodose A δ fibers evoke cough when applied to the trachea of anesthetized guinea pigs, whereas other stimuli such as capsaicin, bradykinin and ATP fail to evoke cough; and thirdly, selective reduction of electrical activity in nodose neurons (induced by knockdown of the tetrodotoxin-sensitive Na v 1.7) eliminates cough evoked by citric acid or mechanical stimulation in anesthetized guinea pigs. Extrapulmonary nodose A δ fibers project axons to an area just rostral of obex and lateral to the commissural subnucleus of the nTS, where they synapse with second-order neurons that regulate cough-associated networks (Canning and Mori, 2010). The signaling at this first synapse is mediated by glutamate.

Nodose A δ fibers innervating the trachea respond to punctate stimuli with rapidly adapting AP discharge, and this has sometimes led to these extrapulmonary A δ fibers being functionally grouped with the faster conducting intrapulmonary RARs (conduction velocity 10–20m/s) (Mazzone and Udem, 2016). Nevertheless, evidence suggests that these are probably two distinct subsets (Canning et al., 2004): (1) activation of extrapulmonary A δ fibers causes cough but activation of intrapulmonary RARs fails to evoke cough, and (2) intrapulmonary RARs are activated by α , β mATP, but extrapulmonary A δ fibers are insensitive to P2X2/3 agonists (despite the fact that they are nodose afferents).

Bronchopulmonary C fibers

Vagal C fibers arise from every level of the lower airways from the trachea through to the alveoli (Dey et al., 1990; Baluk et al., 1992; Watanabe et al., 2005, 2006; Su et al., 2021). C fibers are unmyelinated axons that conduct APs at ~0.3 to 1.2 m/s, and they comprise the majority of vagal airway afferents (Coleridge and Coleridge, 1984; Ricco et al., 1996;

Ho et al., 2001; Udem et al., 2004). C fibers are derived from neurons in both nodose and jugular ganglia. C fiber innervation of the trachea is largely derived from jugular neurons (many via the SLN), whereas the intrapulmonary airways are preferentially innervated by nodose C fibers. Almost all C fibers innervating the lower airways of guinea pigs and rats are activated by the classic nociceptive stimuli capsaicin, due to their expression of TRPV1. The vast majority of guinea pig and rat C fibers are also activated by bradykinin (via B2 receptors) and allyl isothiocyanate or cinnamaldehyde (selective agonists for TRP ankyrin 1 (A1)) (Ho et al., 2001; Udem et al., 2004; Hooper et al., 2016). As such C fibers are robustly activated by stimuli perceived as noxious by mammals and thus can be considered to be nociceptive, according to Sherrington's definition (Sherrington, 1906). Interestingly, only slowly conducting C fibers (0.3–0.7 m/s) innervating the mouse airways are activated by these classic nociceptive stimuli; faster C fibers are insensitive (Kollarik et al., 2003). Airway C fibers in all mammals studied are also mechanically sensitive to either punctate or stretch stimuli, although their threshold for mechanical stimulation is high enough that they are typically quiescent during eupnea (Coleridge and Coleridge, 1984; Ho et al., 2001).

Nodose and jugular C fibers have distinct stimuli sensitivities, and this has been shown to be dependent on their distinct gene expression profiles (Taylor-Clark, 2021). While both nodose and jugular C fibers express P2X3, only nodose C fibers also express P2X2, and it is the expression of the P2X2/3 heteromeric channel which is necessary for robust activation of C fibers by ATP or $\alpha_3\beta$ mATP (Udem et al., 2004; Kwong et al., 2008b; Nassenstein et al., 2010; Kim et al., 2020a). Furthermore, nodose C fibers are activated by adenosine (A1 and A2A receptors) and 2-methyl-5HT (5HT3 receptors), whereas jugular C fibers are insensitive to these agents (Chuaychoo et al., 2005b, 2006). The cell bodies of airway-specific nociceptors (typically identified by TRPV1 expression and/or a lack of neurofilament expression) in the nodose ganglion lack substance P expression, whereas those in the jugular ganglion widely express substance P (Ricco et al., 1996; Udem et al., 2004; Kim et al., 2020b). This is consistent with the robust innervation of the tracheal subepithelial layer by neuropeptide-expressing terminals (Dey et al., 1990; Baluk et al., 1992).

Nodose C fibers terminate within the medial and caudal subnuclei of the nTS (Kim et al., 2020a,b; Su et al., 2021). There is some conflicting evidence for the central terminations of jugular C fibers (Behrens et al., 2021; Taylor-Clark, 2021). Multiple studies have shown using chemical tracers and viral vectors that both vagal neuropeptide-expressing afferents in mice and jugular afferents of rats and guinea pigs terminate within the Pa5 (Driessen et al., 2015, 2020; McGovern et al., 2015a, b; Kim et al., 2020b). Although tracing from the airways itself has identified airway-specific terminations within the Pa5 of rats and guinea pigs, this was not seen in mice (Kim et al., 2020b; Su et al., 2021). Furthermore, it is not clear if the jugular-Pa5 connectivity is exclusive—there is evidence that jugular/peptidergic afferents also terminate within the nTS (Mazzone and Canning, 2002; Kim et al., 2020b). Importantly, a recent study demonstrated that capsaicin inhalation (activates both nodose and jugular C fibers) causes neural activity in both the nTS and Pa5 regions of human subjects, whereas ATP (selective nodose stimulant) only increased activity in then TS, suggesting that these pathways are present in humans as well as in animal models (Farrell et al., 2020).

Capsaicin, the canonical C fiber stimulant, evokes nocifensive reflexes from the lower airways including modulation of respiratory rhythms (apnea, bradypnea, tachypnea, and cough), modulation of central autonomic efferent output (bronchospasm, mucus secretion and bradycardia) and local neurogenic-mediated bronchospasm (Coleridge and Coleridge, 1984; Mazzone and Udem, 2016). The specific role of nodose and jugular C fibers in these nocifensive reflexes is incompletely understood. This is in part due to the lack of selective jugular C fiber stimuli. Nevertheless, some elegant studies have implicated specific reflex function in some cases, particularly in the guinea pig (Muroi et al., 2013; Chou et al., 2018), which has a robust cough and bronchospastic reflex compared to the mouse and rat (Mazzone and Udem, 2016).

As mentioned above, C fiber stimulants such as capsaicin, bradykinin or allyl isothiocyanate fail to evoke cough in anesthetized guinea pigs, despite their robust modulation of breathing rates (Canning et al., 2004). However, these noxious stimuli do evoke cough in conscious guinea pigs, rabbits, cats and humans (Taylor-Clark, 2015). Two lines of evidence suggest such responses are mediated entirely by jugular C fibers: firstly, selective agonists of nodose C fibers (e.g., adenosine, 2-methyl 5HT) fail to evoke cough (Muroi et al., 2013); and secondly, selective reduction of electrical activity in nodose neurons ($Na_v1.7$ knockdown) has no effect on capsaicin-evoked cough (Muroi et al., 2013). Interestingly, in anesthetized guinea pigs, capsaicin- and bradykinin-evoked C fiber activation sensitizes the cough reflex initiated by extrapulmonary nodose A δ fiber activation (Mazzone et al., 2005). This C fiber-mediated sensitization is sensitive to inhibition of neuropeptide signaling in the medulla, suggesting a role of jugular C fibers. Whereas selective activation of nodose C fibers (with adenosine or 2-methyl 5HT) dramatically inhibited nodose A δ fiber-mediated cough (Chou et al., 2018), indicating distinct functional effects of nodose and jugular C fibers on the cough reflex.

Capsaicin and other C fiber stimulants evoke central reflex modulation of autonomic output to the airways and heart (Coleridge and Coleridge, 1984; Mazzone and Udem, 2016). Capsaicin and bradykinin induce atropine-sensitive bronchospasm, mucus secretion and bradycardia, indicating a predominant role of parasympathetic signaling (Davis et al., 1982; Schultz et al., 1985; Canning et al., 2001; Mazzone and Canning, 2002; Hooper et al., 2016). Nevertheless, the airways have a complex autonomic innervation, which will be discussed in sections below. Importantly, the net effect of airway C fiber stimulation (bronchospasm) is abolished by central administration of neurokinin receptor antagonists, suggesting that neurotransmission of neuropeptide-containing jugular afferents play an important role in this reflex (Mazzone and Udem, 2016). Nevertheless, such evidence is not definitive as some nTS neurons also express neuropeptides (Kim et al., 2020b), and there is evidence that the 5HT₃ agonist phenylbiguanide (nodose selective stimulant) also evokes reflex bronchospasm in some species (Szarek et al., 1995; Taylor-Clark, 2021).

What is clear is that the activation of jugular C fibers in the airways also induces bronchospasm via a local neurogenic axon-reflex mechanisms in some mammals such as rats and guinea pigs (although the evidence is lacking in humans) (Ellis and Udem, 1990, 1994; Ellis et al., 1993). Capsaicin-induced depolarization of jugular C fiber terminals evokes release of neuropeptides such as the tachykinins substance P and neurokinin A that

activate NK1 and NK2 receptors on bronchial smooth muscle, inducing contraction. Such responses are independent of the autonomic innervation of the airways. Nodose C fibers lack neuropeptides and so do not contribute to this neurogenic bronchospasm (Undem et al., 2004; Nassenstein et al., 2010). Neuropeptides released from jugular C fibers also activate NK receptors on airway glands and parasympathetic ganglionic neurons, increasing mucus secretion (Hayes et al., 1995; Rogers, 1995) and synaptic transmission (Myers and Undem, 1993), respectively.

C fiber modulation of respiratory rhythm suggests a complex interaction of multiple pathways, which can be preferential activated by different agents and by distinct routes of administration (e.g., inhalation vs. topical vs. intravenous injection) (Coleridge and Coleridge, 1984; Palecek et al., 1989; Chou et al., 2008, 2018). Capsaicin evokes tachypnea when inhaled, bradypnea when applied to the trachea, and tachypnea followed by apnea/bradypnea when given intravenously in guinea pigs, rats and dogs. In the anesthetized guinea pig, both adenosine and 2-methyl 5HT (activate nodose C fibers selectively) evoke tachypnea when inhaled or given intravenously, and these responses were abolished by vagotomy, suggesting that nodose C fibers are responsible (Chou et al., 2008, 2018). This is consistent with tachypnea responses during optogenetic stimulation of nociceptors in mice (Chang et al., 2015). However, adenosine and/or selective 5HT₃ receptor agonists have been reported to evoke apnea in anesthetized rats, mice and cats (Coleridge and Coleridge, 1984; Hsu et al., 2019). As such, the specific role of nodose and jugular C fiber in modulating respiratory rhythms is currently unresolved.

Many of the studies investigating the differential role of nodose and jugular C fibers in respiratory reflexes have been performed in guinea pig and, now with genetic-based approaches, in mice. How these studies relate to earlier studies of afferent neurophysiology in larger animals which identified C fiber subsets based upon preferential stimulation by application of stimuli via the pulmonary and bronchial circulations remains to be seen. Of note, fast C fibers innervating the mouse bronchopulmonary tree lack responsiveness to most noxious stimuli (Kollarik et al., 2003) and the function of these of afferents is currently unknown.

Extrapulmonary jugular A δ fibers

Approximately 50% of jugular afferents innervating the trachea are partially myelinated A δ fibers (Ricco et al., 1996). Like the extrapulmonary jugular C fibers, these jugular A δ fibers have a high threshold for mechanical activation and are sensitive to capsaicin and bradykinin. Unlike jugular C fibers, their cell soma expresses neurofilament but do not express neuropeptides. There are no studies that have selectively activated extrapulmonary jugular A δ fibers, thus their reflex function is currently unknown.

Dorsal root ganglia afferents

There is a minor sensory innervation of the airways from the dorsal root ganglia (C8 to T5) (Dinh et al., 2004; Oh et al., 2006) (Fig. 14.1). DRG neurons are derived from the same embryological source as jugular afferents: the neural crest (Taylor-Clark, 2021). Consistent with this, many DRG airway afferents express neuropeptides but few express P2X₂ (Dinh

et al., 2004; Nassenstein et al., 2010; Kim et al., 2020a). Few DRG airway afferents express neurofilament, suggesting that most project unmyelinated C fiber axons. However, the expression of TRPV1 in DRG airway afferents varies across species: from <12% in mice to >61% in guinea pig. Stimulation of airway DRG nociceptors increases sympathetic drive to the cardiopulmonary system (i.e., evokes reflex tachycardia) (Oh et al., 2006; Shanks et al., 2018). These responses are more evident following vagotomy.

AUTONOMIC EFFERENT INNERVATION OF THE LOWER AIRWAYS

Both parasympathetic and sympathetic nerves project to the lower airways and regulate organ function, including airway patency, mucus secretions and blood flow (Karlsson et al., 1988; Widdicombe, 1993; Rogers, 2001; Canning, 2006; Udem and Potenziari, 2012). Parasympathetic nerves are the predominant regulator of airway patency and mucus secretions, whereas blood flow is regulated by both autonomic branches. Anatomically, autonomic pathways consist of two neuron populations in series: a preganglionic neuron, which resides in the CNS and projects axons to the peripheral autonomic ganglia, where it synapses with the postganglionic neurons that projects axons to effector cells within the lungs (e.g., smooth muscle cells, goblet cells, etc.).

Parasympathetic innervation

As shown in Fig. 14.1, preganglionic cholinergic neurons within the nucleus ambiguus and dorsal motor nucleus of the vagus (DMX) project axons, via the vagus nerve, to airway parasympathetic ganglia (Kalia and Mesulam, 1980; Kalia and Sullivan, 1982; Canning, 2006; Udem and Potenziari, 2012). Studies in cat, dog and guinea pig demonstrate that many axons projected from the nucleus ambiguus are myelinated A fibers, whereas most projected from the DMX are unmyelinated C fibers (McAllen and Spyer, 1978; Haselton et al., 1992; Canning and Udem, 1993a). The majority of ganglia are found in the extrapulmonary airways, but there is substantial variety in the number of neurons within each ganglia, ranging from 2 to 100, depending on the species. Ganglia are associated in plexi superficial to and within the smooth muscle layers (Baker et al., 1986; Bałuk and Gabella, 1989; Dey et al., 1996, 1999). Guinea pig airways also receive input from parasympathetic ganglia within the myenteric plexus of the esophagus (Canning and Udem, 1993a; Mazzone and McGovern, 2010). In the lungs, ganglia are found in the large peribronchial plexus and the smaller perivascular plexus (Honjin, 1956; Fontán et al., 2000; Pérez Fontán and Velloff, 2001). Despite ganglia only being located within the first 2 or 3 generation of intrapulmonary airways, postganglionic fibers project down to the smallest bronchioles. Parasympathetic ganglion neurons have substantial dendritic structures, and these are the sites which receive synaptic input from the preganglionic cholinergic nerves (Mitchell et al., 1987; Myers et al., 1990, 1996). Activity in preganglionic nerves induces fast excitatory postsynaptic potentials (fEPSPs) via acetylcholine-mediated nicotinic channel activation. Ganglionic neurons receive input from multiple preganglionic axons, and the evoked fEPSPs converge onto the axon hillock, eliciting APs if of sufficient magnitude. Often moderate stimulation of preganglionic APs produces fEPSPs in ganglionic neurons that fail to evoke APs—i.e., ganglionic neurons robustly filter preganglionic input (Myers et al., 1990; Myers and Udem, 1991; Kajekar et al., 2001). Ganglionic neurons

also adapt to prolonged depolarizing currents, although heterogeneity in this property has been observed across guinea pig and rat neurons. Many ganglionic neurons are cholinergic, but some instead express vasoactive intestinal peptide (VIP) and nitric oxide synthase (NOS) (Baker et al., 1986; Bařuk and Gabella, 1989; Dey et al., 1996, 1999). Very few choline acetyltransferase (ChAT)-positive neurons express VIP/NOS, indicating there are distinct parasympathetic postganglionic subsets. VIP/NOS-positive neurons are predominantly found in the superficial muscle plexus in the ferret and in the esophageal myenteric plexus in the guinea pig. Postganglionic fibers project to tracheal and bronchial smooth muscle, mucous and serous cells in submucosal glands, goblet epithelial cells and blood vessels.

Chemical stimulation of airway preganglionic parasympathetic neurons or electrical stimulation of the distal end of the vagus nerve (sectioned at the cervical level) causes robust bronchospasm and mucus secretion in all mammals studied (Karlsson et al., 1988; Rogers, 2001; Canning, 2006; Udem and Potenzieri, 2012). These responses are abolished by hexamethonium (nicotinic acetylcholine receptor antagonist) and atropine (muscarinic acetylcholine receptor inhibitor), and are mimicked by muscarinic agonists—indicating the predominant role of cholinergic parasympathetic signaling (Ueki et al., 1980; Tokuyama et al., 1990; Udem et al., 1990; Fung et al., 1992; Canning and Udem, 1993a). Both airway smooth muscle and secretory cells express the Gq-coupled muscarinic M3, whose activation is responsible for the cholinergic-mediated contraction and mucus secretion (Mak and Barnes, 1990; Roffel et al., 1994; Watson et al., 1995; Rogers, 2001). These cells also express Gi-coupled M2 receptors, whose physiological role is less clear. Electrical vagal stimulation-evoked smooth muscle contraction is abolished by the Na_v blocker tetrodotoxin in ex vivo studies of human, mouse and guinea pig tissue (Kocmalova et al., 2017; Kollarik et al., 2021). Responses in human and guinea pig tissue were also abolished by selective Na_v1.7 inhibition, but in mouse tissue both Na_v1.7 and Na_v1.1 and/or Na_v1.3 were capable of conducting APs.

Interestingly, in the presence of atropine, electrical stimulation of vagal efferents causes relaxation of precontracted airway smooth muscle (Canning and Udem, 1993a,b). This muscarinic-independent mechanism is mediated by the nonadrenergic, noncholinergic (NANC) parasympathetic fibers expressing VIP and NOS. In human airways, this branch of the parasympathetic nervous system is the only neural pathway that relaxes airway smooth muscle (Canning, 2006; Udem and Potenzieri, 2012). NANC-mediated relaxation is abolished by hexamethonium (blocks ganglionic transmission) (Canning and Udem, 1993a,b), is reduced by inhibition of VIP or nitric oxide (NO) signaling (Belvisi et al., 1992; Ellis and Udem, 1992; Hasaneen et al., 2003), and is mimicked by either exogenous VIP or NO (Ellis and Udem, 1992; Schmidt et al., 2001; Berisha et al., 2002). VIP is a neuropeptide that binds VPAC1 and VPAC2 receptors, both of which are found in the lung (Busto et al., 2000; Schmidt et al., 2001) and both of which increase adenylate cyclase activity and cyclic AMP levels (Groneberg et al., 2006). Cyclic AMP causes relaxation of airway smooth muscle, and there is evidence that VPAC2 predominates in VIP-evoked relaxation. NO is a gas that is synthesized on demand by NOS following AP-dependent Ca²⁺ influx, diffuses from the NANC efferent and stimulates guanylate cyclase activity in airway smooth muscle cells, inducing cyclic GMP-dependent relaxation

(Esplugues, 2002). There is some evidence in guinea pig and human airway that NANC-mediated relaxations are more dependent on NO than VIP, particularly in the peripheral airways (Li and Rand, 1991; Ellis and Udem, 1992; Bai and Bramley, 1993; Groneberg et al., 2006). NANC-mediated relaxation is slower and more long-lasting than cholinergic-mediated contraction, and requires much higher frequency of vagal electrical stimulation (Canning and Udem, 1993a,b). Consistent with the observation that airway-projecting VIP/NOS-positive parasympathetic neurons are found in the esophageal myenteric plexus in the guinea pig, NANC-mediated relaxations were abolished by esophageal removal (Canning and Udem, 1993a).

Electric field stimulation of ferret tracheal rings under cholinergic and adrenergic blockage causes mucus secretion that is abolished by an antibody targeting endogenous VIP (Liu et al., 1999). Similarly, vagal stimulation in anesthetized cats evokes secretion that is only minimally reduced by cholinergic and adrenergic blockade (Peatfield and Richardson, 1983). Micromolar concentrations of exogenous VIP increases mucus secretion, likely via the activation of VPAC2 receptors on secretory cells (although VPAC1 is also expressed) (Liu et al., 1999; Rogers, 2001). However, in the ferret nanomolar concentrations of VIP have been shown to inhibit cholinergic-mediated mucus secretion, and cholinergic-mediated secretions evoked by low stimulus electric field stimulation are potentiated when VIP is scavenged (Liu et al., 1999). These studies suggest that VIP can act prejunctionally to inhibit acetylcholine release from cholinergic postganglionic neurons. NO is not thought play a substantial role in NANC-mediated modulation of mucus secretion.

Bronchial (i.e., systemic) arteries receive innervation from both cholinergic and NANC postganglionic parasympathetic neurons (Widdicombe, 1993; Coleridge and Coleridge, 1994). Vagal stimulation in large mammals (dogs, cats, pigs) induces vasodilation of bronchial arteries, predominantly in an atropine-sensitive manner (Laitinen et al., 1987b; Pisarri et al., 1999). Consistent with this, exogenous acetylcholine also dilates these vessels via the activation of muscarinic M3 on vascular endothelial cells, which leads to NO generation and subsequent NO-mediated relaxation of vascular smooth muscle cells (Laitinen et al., 1987a; O'Rourke and Vanhoutte, 1990).

Sympathetic innervation

The airways receive postganglionic sympathetic innervation from neurons within the stellate and thoracic sympathetic ganglia (Widdicombe, 1993; Canning, 2006; Udem and Potenzieri, 2012). Most expressed tyrosine hydroxylase, indicating they are adrenergic, and the neurotransmitter neuropeptide Y (NPY) (Kummer et al., 1992). Some ganglionic neurons only express NPY. Ganglionic sympathetic neurons are activated by cholinergic input from preganglionic neurons within the intermediolateral nucleus of the spinal cord. As in the parasympathetic system, acetylcholine release from active preganglionic fibers activates nicotinic receptors on the ganglionic neurons, evoking fEPSPs that will, if of sufficient magnitude, evoke APs along the postganglionic axon (McLachlan, 2003). Unlike parasympathetic ganglionic synapses, there is little filtering of preganglionic input by sympathetic ganglionic neurons.

Humans, primates and rabbits have no direct sympathetic innervation of airway smooth muscle cells (Canning, 2006; Udem and Potenzieri, 2012). However, guinea pig, cat and dog have direct innervation of airway smooth muscle and electrical stimulation of the sympathetic trunk induces relaxation of precontracted airway muscle (Canning and Udem, 1993b). Such responses are abolished by propranolol and are mediated by smooth muscle β adrenoceptors in a cAMP-dependent manner. All mammals express β adrenoceptors on smooth muscle that can be activated by circulating epinephrine released from the adrenal medulla, evoking bronchodilation (Barnes, 1986, 2004). In humans, catecholamine-evoked bronchodilation is mediated exclusively by smooth muscle β_2 adrenoceptors.

There is anatomical evidence that sympathetic nerves innervate submucosal glands, but not epithelial goblet cells. Adrenergic agonists can evoke mucus secretion, although the response is limited compared to parasympathetic stimulation (Ueki et al., 1980; Rogers, 2001). In both cats and ferrets, sympathetic stimulation increases mucus secretion and this is blocked by α and β adrenoceptor antagonists in the ferret, but by only β adrenoceptor inhibitors in the cat.

There is substantial sympathetic innervation of pulmonary arteries and bronchial arteries. Innervation occurs throughout the arterial vasculature down to ~ 10 to $30 \mu\text{m}$ in larger mammals (Widdicombe, 1993; Coleridge and Coleridge, 1994). Electrical stimulation of sympathetic nerves causes vasoconstriction in these vessels, predominantly via activation of smooth muscle α_1 adrenoceptors, although a slower and more longer lasting α_1 -independent vasoconstriction also occurs, which is mediated by NPY (Laitinen et al., 1987a). Sympathetic innervation is considered the predominant branch of the autonomic nervous system controlling arterial blood flow within the airways. There is limited sympathetic innervation of pulmonary veins or bronchial veins.

Basal and reflex autonomic control

The airways are under basal autonomic control (Widdicombe, 1966; McAllen and Spyer, 1978; Roffel et al., 1994; Kesler and Canning, 1999; Canning, 2006). Sectioning of the vagal parasympathetic efferents in vivo causes airway smooth muscle dilation and increased airflow. This is also the case when the afferent nerves innervating the lower airways are selectively resected, indicating that baseline parasympathetic drive stems, at least in part, from activation of afferent subsets, most likely low-threshold mechanosensitive A fibers (Kesler and Canning, 1999). This sectioning also interrupts preganglionic fibers connected to NANC postganglionic fibers. As such the net effect of basal parasympathetic activity on airway smooth muscle at rest is partial contraction. Consistent with the role of cholinergic fibers in maintaining airway patency, muscarinic inhibitors evoke bronchodilation in vivo (Canning, 2006; Peters et al., 2010). Interruption of vagal efferents decreased blood flow in bronchial arteries in dogs (Horisberger and Rodbard, 1960) but pharmacological studies suggest that both cholinergic and NANC contributions to basal dilation of these vessels is variable and inconsistent (Baile et al., 1987; Laitinen et al., 1987a,b; Widdicombe, 1993; Coleridge and Coleridge, 1994; Pisarri et al., 1999; Zimmerman and Pisarri, 2000). Despite the robust vasoconstrictor responses of bronchial and pulmonary arteries to α adrenoceptor-dependent sympathetic stimulation, most studies suggest a lack of basal sympathetic-

mediated vasoconstriction of either vessel type (Williams and Drummond, 1983; Baile et al., 1987; Widdicombe, 1993; Hennessy et al., 1993; Coleridge and Coleridge, 1994; Zimmerman and Pizarri, 2000), although this could be due to particular anesthetic use (Hennessy 93). Measurement of basal mucus secretion in vivo is challenging (Undem and Potenzieri, 2012). Nevertheless, there is evidence that muscarinic inhibitors reduce basal secretions in the cat and dog (Ueki et al., 1980; Johnson et al., 1983; Padua et al., 2001), indicating a role of cholinergic parasympathetic signaling—although other studies have failed to demonstrate this effect (Tokuyama et al., 1990; Fung et al., 1992).

Airway-projecting preganglionic parasympathetic neurons within the nucleus ambiguus and DMX receive input from multiple sources including the nTS (input from sensory afferents), other nuclei in the ventral respiratory group, pons, periaqueductal gray and the hypothalamus (Haselton et al., 1992; Haxhiu et al., 2005). Activation of lung afferents modulates parasympathetic preganglionic neuron activity. Thus activation of C fibers and RAR fibers increases parasympathetic drive, whereas SAR fiber activation decreases parasympathetic drive (Kubin et al., 2006; Mazzone and Undem, 2016).

AIRWAY INFLAMMATION-INDUCED NEUROMODULATION

Pulmonary inflammation can lead to changes in airway neural function at all levels of the reflex circuit; from the afferent nerve terminals, to processing in the CNS, to activity in the efferent autonomic nervous system (summarized in Fig. 14.2). This modulation can directly contribute to the symptoms of airway diseases. Such symptoms include chronic coughing, excessive secretions, bronchospasm, and sensations of dyspnea.

Modulation of afferent nerves

Most investigations regarding airway inflammation and afferent nerve terminals have focused on the vagal C fibers (Coleridge and Coleridge, 1984; Mazzone and Undem, 2016). Although the receptors for only a minority of inflammatory mediators are expressed by vagal sensory neurons, receptors are expressed for several inflammatory mediators that are prevalent in airway inflammatory diseases (Wang et al., 2017; Mazzone et al., 2019). Chemical mediators can interact with their receptors to lead to an overt activation of C fibers; i.e., result in a membrane depolarization (generator potential) that exceeds the voltage threshold of action potential discharge. Chemical mediators can also lead to a “sensitization” of the nerve terminal without overt activation; i.e., they render the nerve terminal more sensitive to activating stimuli such that the nerve responds at lower intensities of stimulation, or responds to the same level of stimulation with a greater action potential discharge frequency.

Some mediators interact with ionotropic receptors such as ATP acting on P2X3 receptors, 5-HT acting on 5-HT3 receptor, and various stimuli that activate TRPV1 and TRPA1 receptors. Mediators that act on GPCRs also activate and sensitize airway C fibers. These mediators can include various eicosanoids, histamine, protease activated receptor agonists, bradykinin, etc. (Coleridge and Coleridge, 1984; Mazzone and Undem, 2016). Finally, activation of certain cytokine receptors has been found to activate and sensitize vagal airway

C-fiber terminals. These receptors include type 1 and type 2 interferons and Tumor Necrosis Factor α (TNF α) receptors (Hsu et al., 2017; Patil et al., 2020).

In addition to producing mediators that directly interact with primary afferent nerves, inflammation can set up conditions where the afferent nerves are indirectly activated. For example, tissue edema associated with plasma extravasation can lead to mechanical stimulation of afferent nerves and drops in pH in the biophase of the afferent terminals can lead to afferent stimulation secondary to activation of acid-sensitive ion channels. Afferent nerves in the airways may also be activated indirectly via accessory cells. Both simple unmyelinated nerve terminals and more complex myelinated terminals are often found around the basal pole of collections of neuroendocrine cells known as neuroepithelial bodies (NEBs). The NEBs can release chemicals such as serotonin and ATP that can in turn activate the afferent nerve terminals (Brouns et al., 2012). The stimuli by which NEBs are activated and their role in airway physiology and disease remains poorly understood. Similarly, in the larger airways afferent nerve terminals have been found to be anatomically associated epithelial brush cells; an arrangement somewhat analogous to taste buds. It is envisaged that activation of the brush cells can release chemicals that activate the juxtaposed afferent nerve terminals. Little is known about the relevant brush cell activators or the specific chemical transmitter(s) involved in brush cell-mediated afferent nerve activation, although cholinergic mechanisms have been proposed (Krasteva et al., 2011).

Airway inflammation can also lead to phenotypic changes in airway afferent neurons. Allergic inflammation as well as respiratory virus infections induces the expression of genes in airway A δ and A β fibers that are normally associated with C fibers. For example, airway inflammation induces de novo expression of genes for tachykinins and certain TRP channels (TRPV1 and TRPA1) in vagal airway A-fibers (Chuaychoo et al., 2005a; Zaccone and Udem, 2016). This leads to a situation where stretch receptors such as SARs become activatable via TRPV1 channel stimulation (Zhang et al., 2008). In the somatosensory system, this has been referred to as inflammation-induced *phenotypic switching* of sensory nerves and is thought to play an important role in hyperalgesia and allodynia (Neumann et al., 1996).

Modulation in the CNS

Airway inflammation is associated with an increase in the excitability of secondary neurons situated in the nTS, i.e., *central sensitization* (Behrens et al., 2021). Allergic inflammation associated with house dust mite in nonhuman primates can lead to a decrease in activation threshold and an increase in peak action potential discharge frequency in nTS neurons (Chen et al., 2009). Activation of afferent C fibers within the lungs, or esophagus of guinea pigs is associated with an increase in the sensitivity of coughing evoked by tracheal A δ fiber activation (Canning and Chou, 2009). The C fiber-induced increase in A fiber cough is a manifestation of central sensitization (Singh et al., 2020).

Modulation of autonomic nerves

By increasing the activity of nociceptive afferent nerves, airway inflammation can indirectly lead to an increase in efferent autonomic outflow via vagal-vagal reflexes. Airway

inflammation can also directly interact with parasympathetic nerve function within the airways. As described above, the filtering by parasympathetic ganglionic neurons results in decreased postganglionic APs compared to their input from preganglionic fibers. Allergic inflammation increases the synaptic efficacy in the airway ganglia thereby reducing the filtering activity (Myers et al., 1991; Kajekar et al., 2003). Thus, there is more postganglionic nerve activation for a given amount of presynaptic input.

As the action potentials invade the postganglionic terminals, acetylcholine, and other parasympathetic transmitters such as nitric oxide and VIP are released onto effector cells. Acetylcholine has been found to negatively feedback on the terminals via the interaction with muscarinic M2 receptors to inhibit further transmitter release. This M2-receptor inhibitory system is downregulated by airway inflammation (Pincus et al., 2021). These mechanisms, acting in concert, can lead to an exaggeration of parasympathetic activities including bronchoconstriction and mucus secretions.

Structural modulation

In addition to neuromodulation relating to electrophysiology, synaptic efficacy and gene expression, airway inflammatory diseases have also been associated with changes in nerve density and architecture (Shapiro et al., 2021). In general, morphometric studies point to an increase in airway nerve density in biopsies of airways obtained from those suffering from airway diseases like chronic cough and asthma (Drake et al., 2018). This is likely due to the prevalence of eosinophils and various nerve growth factors. Little is known about how airway inflammation alters the architecture of specific subtypes of afferent and efferent nerves within the airway, but an increase in sprouting and branching has been observed in nerves within the mucosal layer; a location where nociceptive afferent C fibers predominate (Drake et al., 2018).

ROLE OF AIRWAY NERVES IN ASTHMA, AIRWAY HYPERREACTIVITY, COPD, AND CHRONIC COUGH

Asthma/COPD

The idea that the nervous system is intimately involved in asthma is not new. About 150 years ago Henry Hyde Salter penned his classic treatise “On Asthma” (Salter, 1864). Without the benefit of our present understanding of airway physiology and neurology, Salter used his keen mind and his acute sense of observation to conclude a common feature of his asthmatic patients was morbid proclivity of the nervous system:

“A morbid proclivity of the musculo-nervous system of his bronchial tubes to be thrown into a state of activity; the stimulus may be either immediately or remotely applied, but in either case would not normally be attended by any such result.

There is no peculiarity in the stimulus, the air breathed is the same to the asthmatic and non-asthmatic;...nor probably is there any peculiarity in the irritability of the bronchial muscle; the peculiarity is confined to the link that connects these two—the nervous system—and consists in its perverted sensibility in its receiving and

transmitting on to the muscle, as a stimulus to contraction, that which it should take no cognizance.”

This treatise provides eight lines of evidence for a role of nerves in asthma that remain relevant today. Experimental evidence to support a neural role in asthma was filtering in by the early 1900s when surgeons were selectively denervating the lungs and finding a substantial relief in those suffering from severe asthma. These studies were poorly controlled by today’s standards and must be interpreted with an abundance of suspicion; indeed, most of these studies are best ignored. Nevertheless, in 1929 Phillips and Scott wrote what was in essence a meta-analysis of the burgeoning literature in this area that sprouted up during the first 20 or so years of the 20th century (Phillips and Scott, 1929). They reviewed some 300 cases but considered informative only those cases in which asthma was accurately diagnosed and the subject was followed and evaluated for a minimum of 6 months. With this filter in mind, they found 29 of the 300 cases that are of scientific value. They summarize by stating that...” *there are a few brilliant cures in extremely severe forms of asthma. Roughly one half of the patients definitely improved, while frequently the other half, after temporary improvement, are in no better condition than before the operation. The patients who were cured have been followed on average almost two years.*” Among these 29 cases there was little consistency in the surgical procedure, and some of the surgeries described can be questioned based on present knowledge of the physiology and extrinsic innervation of the airways. Later, the surgical technique to selectively denervate the lungs was refined by Reinhoff working at The Johns Hopkins Hospital. He astutely teamed up with a noted allergist/asthmologist in Leslie Gay to provide a careful analysis of the benefit of lung denervation in asthma (Reinhoff and Gay, 1938). The patients were selected on the basis of an unquestionable diagnosis of bronchial asthma, and on severity of disease with only those totally incapacitated by their disease being included. The report covers 11 patients economically, physically, and socially incapacitated by their disease. All the subjects were then followed for 1.75 to 2.75 years postoperatively. They concluded that approximately 2 years postsurgery:

Of the 10 patients discharged from the hospital, 1 was entirely unimproved; 1 improved for 3 months, finally succumbing to what seemed to be cardiac failure; 4 are completely well as the time of writing, having been free of attacks since the operation or a short time later, and are able to resume their former work; 4 have occasional mild attacks of asthma, all of which are amenable to control by means of small doses of ephedrine.

From physiological perspective this should not come as a surprise. Asthma is a disease of airway narrowing. The immediate relief obtained upon inhaling a β adrenoceptor agonist indicates that much of the airway narrowing in asthma is secondary to smooth muscle contraction. Studies reveal that when a maximally effective dose of a cholinergic muscarinic receptor antagonist is delivered to the airways the resistance to airflow is decreased to the extent that adding a β adrenoceptor agonist provides little additive relief (Ullah et al., 1981). In other words, the chemical responsible for nearly all the airway smooth muscle contraction that can be reversed with a functional antagonist is acetylcholine, derived from the postganglionic parasympathetic nerves that densely innervated bronchial smooth muscle.

Accordingly, in clinical studies newer antimuscarinic drugs provide as much relief as a β adrenoceptor agonist (Peters et al., 2010).

COPD involves dyspnea, incessant coughing, airway narrowing, and excessive secretions. These symptoms are consistent with an overactive nervous system, with the latter two likely involving an enhanced cholinergic drive. Indeed, the mainstay of treatment to maximally dilate the airways and decrease secretions is an anticholinergic drug (Niewoehner et al., 2005). Dyspnea is a complex symptom that can be caused by many disparate neurological mechanisms. It is not clear that the airway sensory nervous system contributes to the dyspnea associated with COPD, but it is worth noting that inflammatory mediators present in the inflamed airways such as adenosine led to sensations of dyspnea in human volunteers in the absence of bronchoconstriction, and this can be reversed by topical (inhaled) delivery of adenosine receptor antagonist (Burki and Lee, 2010). Strategic airway denervation studies are underway for the treatment of severe asthma and COPD (Slebos et al., 2020; Hartman et al., 2021).

Airway hyperreactivity

Airway hyperreactivity is a hallmark of asthma. This is typically quantified as the concentration of an inhaled bronchoconstrictor (usually methacholine) required to increase airflow resistance (FEV1) by 20%. Those suffering with asthma often reach this level of increased resistance at doses <1% of that required in healthy volunteers. The mechanisms underlying airway hyper-reactivity associated with asthma remain largely obscure, but it is known that anti-inflammatory treatments in use today have very little influence on the underlying airway hyperreactivity (Szeffler et al., 2000).

When the airways of laboratory animals are allergically inflamed, they also reveal airway hyperreactivity to an applied bronchoconstrictor. This is most often studied in mice, where it is clear that the nervous system plays a key role. In one study, cutting the vagus nerves just before the cholinergic challenge took place, had no effect on airway inflammation, but eliminated the airway hyperreactivity (McAlexander et al., 2015). In another study, using genetic tools to selectively remove only the C-fiber neurons within the vagal ganglia prevented the hyperreactivity, without noticeably influencing the allergen-induced inflammation (Trankner et al., 2014).

Pathological cough

There are fundamentally two types of induced cough. One type of cough is triggered by stimuli that threaten our airways. This protective cough is typified by the violent cough induced by aspiration. The other type of cough does not necessarily provide any protective role and can be considered a pathological cough. Virtually all adult human beings have experienced both types of coughs, the difference between them being self-evident. The protective cough is very immediate, involuntary, and often a violent cough. The pathological cough is, at least up to a point, a voluntary maneuver aimed at reducing the sensation of an irritable itch in the throat (an itch you cannot scratch). These irritation-induced coughs generally require an awakened state. Cough monitors show this to be true for the coughing associated with diseases like chronic cough and COPD (Satia et al., 2016). Substances

known to activate C fibers, such as capsaicin, bradykinin, and citric acid, especially when delivered to the airways in low concentrations can accurately mimic this itchy urge to cough. When the concentration is increased the irritation is so severe that the ensuing cough becomes all but involuntary.

Chronic cough is an incessant cough that has lasted for at least 2 months. In some cases, treating an underlying disorder such as acid reflux, asthma, rhinitis, solid tumor, can effectively treat chronic cough. In many cases, however, treating all underlying disease does not block the cough. These subjects are said to suffer from chronic idiopathic cough hypersensitivity syndrome (Satia et al., 2016). These subjects are considered hypersensitive, because they cough at doses of a tussive stimuli (e.g., capsaicin) much lower than that required to evoke cough the healthy population. This “hypertussivity” has obvious similarities to airway hyperreactivity, discussed above, as well as hyperalgesia and allodynia associated with chronic pain (O’Neill et al., 2013). These chronic coughers often present to cough clinics with a cough that has lasted many years. Local anesthetics can cause a temporary relief, indicating that the cough most likely arises from inappropriate input from the airway vagal sensory nervous system. This would also be consistent with the finding that a peripheral acting drug named gefapixant, that blocks the purinergic P2X3 and P2X2/3 receptors, has been found in clinical trials to relieve the cough of severe chronic coughers (Abdulqawi et al., 2015; Ford et al., 2021). The expression of purinergic P2X3 and P2X2/3 receptors is limited largely to sensory nociceptors indicating that, at least in some cases, chronic cough is secondary to overactive vagal afferent nerves (Ford et al., 2021).

SUMMARY

Like all visceral organs the airways are richly innervated by sensory (afferent) and autonomic (efferent) nerves. The majority of both sensory and autonomic nerves in the airways are carried in the vagus nerves. These nerves provide key regulatory functions that are important in host-defense and respiratory homeostasis. Uniquely, among visceral organs is the fact that the airway mucosa, where most afferents terminate, is exposed to the ~10,000 L of air an adult breathes every day; air that includes a complex mixture of particles and substances that, depending on the environment, may be harmful and potentially lethal. This can set up conditions of over-activation of vagal afferent nerves, in particular vagal nociceptors. The consequence of their activation, including coughing, dyspnea, parasympathetic reflex bronchoconstriction and secretions, may contribute directly to airway diseases such as COPD, asthma and chronic cough.

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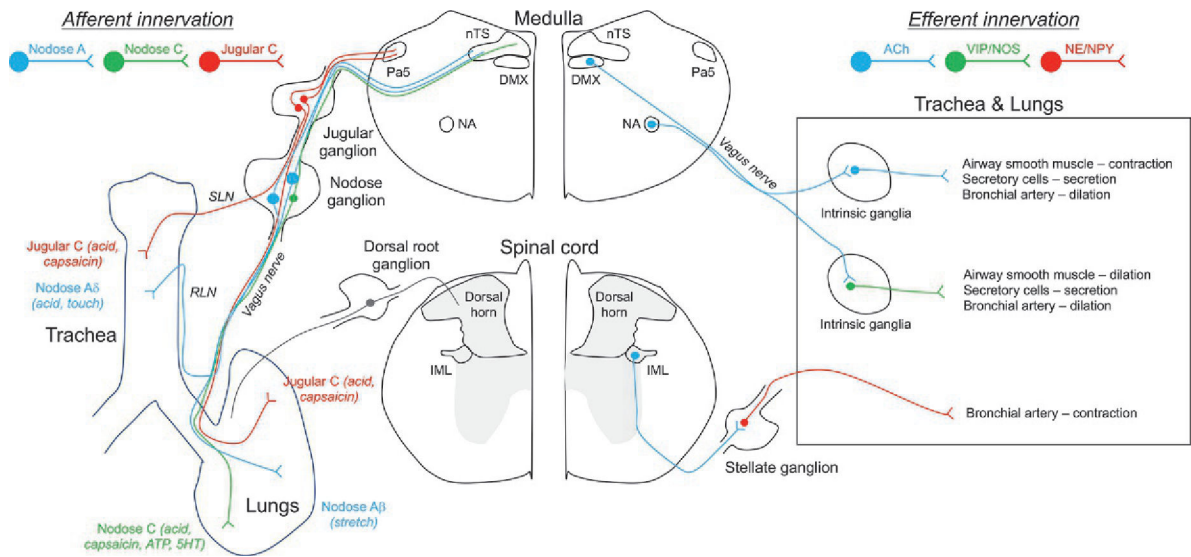


Fig. 14.1.

Schematic illustrating the basic sensory afferent and autonomic efferent innervation of the airways. *Left*, sensory fibers projecting from neurons within the nodose and jugular ganglia project to the trachea and intrapulmonary airways, via the vagus nerve and its branches the superior laryngeal nerve (SLN) and the recurrent laryngeal nerve (RLN). Sensory afferents have been grouped based upon their conduction velocity and their sensitivity to specific stimuli. Nodose sensory fibers project to the nTS in the medulla and jugular fibers project to the Pa5 in the medulla, where they synapse with second order neurons involved in networks regulating cardiopulmonary reflexes. Also shown is the sparse sensory innervation projected from the dorsal root ganglion. Little is known about the sensitivity profile, anatomical pathways and function of these DRG afferents, but it is likely that many are C fibers that project to the dorsal horn lamina. *Right*, extrinsic and intrinsic autonomic innervation of the airways. Cholinergic preganglionic parasympathetic fibers project from the dorsal motor nucleus of the vagus (DMX) and nucleus ambiguus (NA) to the airways where they synapse with neurons within the intrinsic ganglia. Such postganglionic fibers are either cholinergic or NANC fibers (expressing VIP and/or NOS), and they project to airway smooth muscle cells, secretory cells and bronchial arteries. Cholinergic preganglionic sympathetic fibers project from the intermediolateral nucleus of the spinal cord to neurons within the stellate and thoracic sympathetic ganglia. These adrenergic and NPY-expressing ganglionic neurons project to the airways and mainly innervate bronchial arteries.

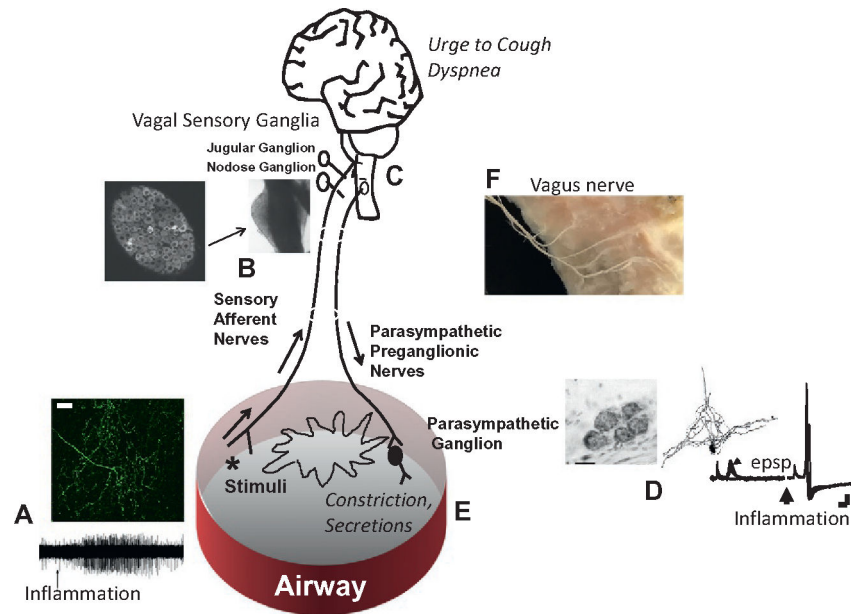


Fig. 14.2.

Inflammation-induced airway neuromodulation. Inflammation can lead to activation and sensitization of C-fiber terminals in the airways; (A) above, a C fiber terminating in the airways of a mouse; below electrophysiological recording of action potentials evoked in a C fiber terminal caused by the application of bradykinin. Inflammation can cause phenotypic changes in the sensory nerves by altering gene expression; (B) photograph of the nodose ganglion along the cervical vagus nerve of a mouse, also a cross-section showing the large number of visceral sensory neurons packed within the ganglion. (C) Inflammation can eventually lead to sensitization of neural activation within the brainstem of the CNS. Inflammation can lead to increases in synaptic efficacy within the parasympathetic bronchial ganglia; (D) shows an image of a small bronchial parasympathetic ganglion containing 5 neurons, a cameral lucida drawing of a single neuron and its dendritic tree, and synaptic recording of excitatory post synaptic potentials before and after inflammation (allergen challenge ex vivo), these images are from a guinea pig bronchial ganglion. (E) Inflammation can also increase transmitter release at the neuroeffector junction leading to increased airway narrowing and secretions. (F) Dissection showing the branches of the vagus nerve innervating a human bronchus.

Table 14.1

Sensitivity of vagal sensory nerve subtypes innervating the lower airways

Fiber	Nodose A β	Nodose A δ	Nodose C	Jugular C
Terminal location	Intrapulmonary	Extrapulmonary	Mainly intrapulmonary	Both extrapulmonary and intrapulmonary
Conduction velocity (m/s)	12–50	2–8	>1.2	>1.2
Mechanical sensitivity	Low-threshold stretch	Low-threshold punctate	High-threshold stretch and punctate	High-threshold stretch and punctate
Chemical sensitivity ^a	ATP, α , β mATP (P2X2/3)	Acid (not TRPV1 channel) Hypotonic saline	Acid, capsaicin (TRPV1 channel) AITC, cinnamaldehyde (TRPA1 channel) Bradykinin (B2 receptor) ATP, α , β mATP (P2X2/3 channel) Adenosine (A1 and A2A receptors) Serotonin (5HT3 channel)	Acid, capsaicin (TRPV1 channel) AITC, cinnamaldehyde (TRPA1 channel) Bradykinin (B2 receptor)
Physiological effect following activation	Inhibition of inspiration, decrease parasympathetic drive (SAR fibers) Increases inspiratory effort and parasympathetic drive (RAR fibers)	Cough	Modulation of respiratory rhythms Increase parasympathetic drive Decrease cough reflex sensitivity	Cough Increase cough reflex sensitivity Increase parasympathetic drive Neurogenic-mediated bronchospasm and secretion Modulation of respiratory rhythms

^aDenotes direct sensitivity of the nerve to the substance. Does not include sensitivity of the nerve to any indirect effects of the substance (e.g., bronchospasm). There are likely species distinctions in the sensitivity to chemical mediators. Much of this summary stems from work on guinea pigs.