HIGHLIGHTED TOPIC | Reflexes from the Lungs and Airways

Reflex regulation of airway smooth muscle tone

Brendan J. Canning
Johns Hopkins Asthma and Allergy Center, Baltimore, Maryland

Canning, Brendan J. Reflex regulation of airway smooth muscle tone. J Appl Physiol 101: 971–985, 2006. doi:10.1152/japplphysiol.00313.2006.—Autonomic nerves in most mammalian species mediate both contractions and relaxations of airway smooth muscle. Cholinergic-parasympathetic nerves mediate contractions, whereas adrenergic-sympathetic and/or noncholinergic parasympathetic nerves mediate relaxations. Sympathetic-adrenergic innervation of human airway smooth muscle is sparse or nonexistent based on histological analyses and plays little or no role in regulating airway caliber. Rather, in humans and in many other species, postganglionic noncholinergic parasympathetic nerves provide the only relaxant innervation of airway smooth muscle. These noncholinergic nerves are anatomically and physiologically distinct from the postganglionic cholinergic parasympathetic nerves and differentially regulated by reflexes. Although bronchopulmonary vagal afferent nerves provide the primary afferent input regulating airway autonomic nerve activity, extrapulmonary afferent nerves, both vagal and nonvagal, can also reflexively regulate autonomic tone in airway smooth muscle. Reflexes result in either an enhanced activity in one or more of the autonomic efferent pathways, or a withdrawal of baseline cholinergic tone. These parallel excitatory and inhibitory afferent and efferent pathways add complexity to autonomic control of airway caliber. Dysfunction or dysregulation of these afferent and efferent nerves likely contributes to the pathogenesis of obstructive airways diseases and may account for the pulmonary symptoms associated with extrapulmonary disorders, including gastroesophageal reflux disease, cardiovascular disease, and rhinosinusitis.

parasympathetic; sympathetic; vagal; autonomic

Selectively stimulating the various autonomic pathways innervating the airways can either near maximally constrict the airways, or reverse a maximal bronchoconstriction. Autacoids that evoke bronchospasm in vivo do so in part or entirely through reflex effects mediated by airway parasympathetic nerves. Dysfunction of the airway autonomic nerves regulating smooth muscle accounts directly or indirectly for many of the symptoms and clinical features of asthma and chronic obstructive pulmonary disease (COPD) (16, 77, 166, 190, 232). Methods and properties relating to the morphology and physiology of airway autonomic and afferent nerves and central pathways regulating airway autonomic outflow have been extensively reviewed (24, 42, 85, 111, 152). The axon reflex, which is prominent in the airways of rats and guinea pigs but of limited importance in the airways of other species, including humans, has also been reviewed in detail elsewhere (12). In this review, the anatomy and reflex physiology of the autonomic nerves regulating airway smooth muscle tone in health and in human disease are discussed. Although written with the primary intention of summarizing the relevant literature, the text is structured to highlight four central themes: the importance of baseline autonomic tone to subsequently evoked reflexes, the profound influence of ongoing vagal afferent nerve activity in regulating this basal tone and subsequently evoked reflexes, the complexity of autonomic control of airway smooth muscle with opposing contractile and relaxant pathways and reflexes that either reduce or augment activity in the...
various autonomic pathways, and, finally, the many important gaps in our understanding in this neglected and essentially unfunded field of research.

ANATOMY OF THE AUTONOMIC NERVES REGULATING AIRWAY SMOOTH MUSCLE

Postganglionic sympathetic and parasympathetic nerves innervate airway smooth muscle (Fig. 1). Relaxations and, under some conditions (and in some species), contractions of airway smooth muscle can be evoked by stimulation of sympathetic nerves. These contractions and relaxations are attributed to norepinephrine acting on α- and β-adrenoceptors, respectively (1, 2, 14, 15, 21, 48, 132, 133, 144, 146, 178, 218, 235). Parasympathetic nerve stimulation has also been shown to evoke both contractions and relaxations of airway smooth muscle (27, 35, 48, 101, 127, 146, 148). The contractions are mediated by acetylcholine, while peptide transmitters such as vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating polypeptide, peptide histidine-isoleucine, or peptide histidine-methionine, and/or the gaseous transmitter nitric oxide (NO) mediate the nonadrenergic, noncholinergic relaxations (5, 13, 24, 28, 39, 109, 117, 135, 158, 180, 195, 211, 237, 242, 244). The distribution and function of parasympathetic-cholinergic nerves is well conserved across species. By contrast, the distribution and function of sympathetic and noncholinergic parasympathetic innervation of airway smooth muscle varies considerably between species. Human airway smooth muscle is largely devoid of sympathetic adrenergic innervation (195). Nonadrenergic, noncholinergic neurotransmitters (likely derived from parasympathetic nerves) mediate the relaxations induced by the only demonstrably functional relaxant innervation of human airway smooth muscle. Dogs have just an adrenergic relaxant innervation of their airway smooth muscle (14, 21, 202), whereas airway smooth muscle from rats and mice appears to have no direct, functional relaxant innervation whatsoever (143, 225).

Studies in cats, guinea pigs, and ferrets have shown that noncholinergic parasympathetic transmitters are not coreleased with acetylcholine from a single population of postganglionic parasympathetic nerves. Rather, an anatomically and functionally distinct parasympathetic pathway regulates nonadrenergic, noncholinergic relaxations of airway smooth muscle (23, 27, 28, 47, 52, 127). Reflexes differentially regulate these cholinergic and noncholinergic nerves (95, 100, 148). Circumstantial evidence suggests that the parasympathetic innervation of human airways is similar to that of cats and guinea pigs (13, 62, 64, 237).

AUTONOMIC REGULATION OF AIRWAY SMOOTH MUSCLE TONE AT REST

Cholinergic and noncholinergic parasympathetic nerves and adrenergic sympathetic nerves are tonically active at eupnea (77, 103, 116, 117, 161, 166, 178, 197, 236, 239). This explains the marked dilatation of the airways evoked by anticholinergics and the constriction/contractions evoked when β-adrenoceptors are blocked or NO synthase is inhibited (53, 87, 93, 116, 151, 161, 178, 218). Ganglionic blockade (all preganglionic neurons regulating airway autonomic nerves are cholinergic; Refs. 27, 85, 178), preganglionic nerve transection, or vagal cooling effectively negates all baseline cholinergic tone in airway smooth muscle, indicating that basal activity in these nerves necessarily requires ongoing preganglionic input arising from the central nervous system (CNS) (89, 110, 116, 117, 122, 151, 160, 223). Basal cholinergic tone is readily demonstrable in all species, whereas basal adrenergic and nondrenergic relaxant tone is highly variable even within species (Table 1). The mechanisms regulating basal autonomic tone in the airways are not fully understood. The few published studies

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Fig. 1. Representative traces showing the effects of sympathetic and parasympathetic nerve stimulation on lung mechanics in the cat. A: vagus nerve stimulation before and after sustained bronchospasm evoked by continuous infusion of serotonin evokes both increases and decreases lung resistance (RL) and compliance (CL). Atropine abolishes the bronchoconstriction evoked by vagus nerve stimulation while having no effect on the vagally mediated bronchodilatation. B: following pretreatment with atropine, sympathetic or parasympathetic nerve stimulation reverses the bronchospasm evoked by sustained infusion of serotonin. The β-adrenoceptor antagonist propranolol abolishes the bronchodilatation produced by sympathetic nerve stimulation but is without effect on the bronchodilatation induced by vagus nerve stimulation. [From Diamond and O’Donnell (48).]
addressing this issue have focused almost exclusively on air-
way cholinergic nerves. Jammes and Mei (103) studied base-
line cholinergic tone in cats (Fig. 2). They found that dissec-
ting the vagus nerve free from its associated nodose ganglia essen-
tially negated all basal airway cholinergic tone. This surgical
intervention destroys the perikarya of vagal afferent neurons
with cell bodies in the ganglia (and thus disrupted their input
to the CNS), but was without effect on bronchospasm evoked
by electrically stimulating the vagus nerves rostral to the nodose
ganglia. Based on these results, Jammes and Mei concluded
that baseline cholinergic tone was driven peripherally by tonic
afferent input arising from the airways and lungs. Mitchell and
colleagues (161) revisited the issue of baseline cholinergic tone
in the airways of cats. Noting an inspiratory rhythm to smooth
tone muscle, these investigators showed that cholinergic nerve
activity increased or decreased as integrated phrenic nerve
activity increased or decreased in response to various interven-
tions (e.g., NaCN, positive end-expiratory pressure, hyperven-
tilation, inhalation of CO2). Mitchell and colleagues thus con-
cluded that cholinergic outflow to the airways was centrally
controlled and coupled to respiratory rhythm generation. It
should be noted, however, that this group studied cats that had
been ventilated to a neural apnea, requiring CO2 administration
to the inspired gas to restore some respiratory drive. Also,
unlike Jammes and Mei, these investigators did not attempt to
selectively disrupt afferent nerve activity while monitoring
cholinergic tone. There are also studies showing independent
regulation of breathing and baseline cholinergic tone in the
airways (10, 50, 82, 220).

We have also studied the origin of baseline cholinergic and
noncholinergic tone in airway smooth muscle (116, 117).
Isometric force transducers were used to measure airway para-
sympathetic tone in the smooth muscle of the extrathoracic
trachea in situ in anesthetized, paralyzed, and mechanically
ventilated guinea pigs. Cholinergic tone was remarkably sen-
sitive to the mechanics of ventilation. Increasing or decreasing
respiratory rate (while adjusting tidal volumes to maintain
minute ventilation) increased and decreased baseline cholin-
ergic tone, respectively. Turning the ventilator off at any phase
of the respiratory cycle produced an immediate and precipitous
fall in cholinergic tone that could be restored almost immedi-
ately by restoring ventilation. These changes in smooth muscle
tone produced by altering ventilation did not occur following
vagotomy or pretreatment with atropine, with or without sub-
sequent recontraction of the trachealis with histamine.

The effects of the interventions described above suggest that
baseline cholinergic tone during tidal breathing in guinea pigs is
largely dependent on afferent nerve activity arising from the
airways and lungs. We addressed this hypothesis by selectively
disrupting afferent nerve activity arising from the airways and
lungs. Cutting the vagus nerves caudal to the recurrent laryn-
geal nerves, thereby disrupting intrapulmonary vagal afferent
(and efferent) nerve activity while preserving entirely the
efferent innervation of the tracheal segment studied, essentially
abolished baseline tone in the trachea. Similarly, nebulizing
lidocaine into the lower airways abolished baseline cholinergic
tone while having little or no effect on vagally mediated
crations evoked by electrical stimulation of the vagus nerves
(116).

Our studies and the studies by Jammes and Mei suggest that
ongoing afferent nerve activity arising from the airways and
lungs drives baseline airway smooth muscle cholinergic tone.
We reported similar results in subsequent studies of both
cholinergic (150) and noncholinergic (117) baseline parasymp-
thetic tone. Jammes and Mei attributed this effect to C fibers,
whereas our data suggest a role for rapidly adapting receptors
(103, 116). The role of rapidly adapting receptors in guinea
pigs but perhaps not in cats may relate to the differences in
respiratory rate in these two species and sensitivity of rapidly
adapting receptors to both the rate and volume of lung infla-
tion and deflation (42, 106, 183, 207). Although all species studied
have some basal cholinergic tone that is likely influenced by
tonic vagal afferent nerve activity (Table 1), it may be prema-
ture to conclude that peripheral factors regulate this tone to a
similar extent in all species. For example, Roberts et al. (197)
found evidence for a basal level of tone in the tracheals of
dogs that was insensitive to vagal afferent cooling. In all
species, however, profound and opposing (excitatory and in-
hibitory) influences of vagal afferent nerve activity on baseline
airway smooth muscle cholinergic tone have been described
(see below).

The functional consequences of baseline activity in the
multiple autonomic pathways innervating airway smooth mus-
cle and the dependence of this autonomic tone on ongoing
afferent nerve activity arising from the airways are severalfold.
Thus contractions and relaxations can be mediated reflexively

Table 1. Muscarinic receptor antagonists, ganglionic blockade vagotomy, or vagal cooling improve basal lung mechanics in most species

<table>
<thead>
<tr>
<th>Species</th>
<th>Intervention</th>
<th>Measurement</th>
<th>Baseline</th>
<th>After Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal humans</td>
<td>Atropine</td>
<td>FEV1</td>
<td>98% predicted</td>
<td>106% predicted*</td>
</tr>
<tr>
<td>Human atopic subjects</td>
<td>0.5–1 g Hexamethonium</td>
<td>Airways resistance</td>
<td>3.8±0.9 cmH2O l−1 s−1</td>
<td>2.7±0.8 cmH2O l−1 s−1</td>
</tr>
<tr>
<td>Human asthmatic subjects</td>
<td>Atropine</td>
<td>Airways resistance</td>
<td>4.3±0.2 cmH2O l−1 s−1</td>
<td>2.6±0.1 cmH2O l−1 s−1*</td>
</tr>
<tr>
<td>Human asthmatic subjects</td>
<td>Atropine</td>
<td>Airways resistance</td>
<td>1.7±0.9 l/s</td>
<td>3.1±1.0 l/s*</td>
</tr>
<tr>
<td>Mild COPD</td>
<td>Atropine</td>
<td>FEV1</td>
<td>63% predicted</td>
<td>76% predicted*</td>
</tr>
<tr>
<td>Sheep</td>
<td>Vagotomy</td>
<td>Airways resistance</td>
<td>2.4±1.0 cmH2O l−1 s−1</td>
<td>1.1±0.2 cmH2O l−1 s−1*</td>
</tr>
<tr>
<td>Dogs</td>
<td>Vagotomy</td>
<td>Airway diameter</td>
<td>31±13% increase*</td>
<td></td>
</tr>
<tr>
<td>Cats</td>
<td>Vagotomy</td>
<td>Airways resistance</td>
<td>3.2±0.3 cmH2O l−1 s−1</td>
<td>2.2±0.3 cmH2O l−1 s−1*</td>
</tr>
<tr>
<td>Rabbits</td>
<td>Vagal cooling</td>
<td>Lung conductance</td>
<td>2.8±0.5 l/min·cmH2O l−1</td>
<td>3.8±0.8 l/min·cmH2O l−1*</td>
</tr>
<tr>
<td>Guinea pigs</td>
<td>Atropine</td>
<td>Airways resistance</td>
<td>0.06±.01 cmH2O l−1 s−1</td>
<td>0.06±0.004 cmH2O l−1 s−1</td>
</tr>
<tr>
<td>Guinea pigs</td>
<td>Vagotomy</td>
<td>Lung conductance</td>
<td>0.31±0.043 l/min·cmH2O l−1</td>
<td>0.32±0.047 l/min·cmH2O l−1*</td>
</tr>
</tbody>
</table>

Comparable results are obtained when airway smooth muscle tone is measured in situ. Drugs were administered intravenously or by inhalation. See text for details and references. COPD, chronic obstructive pulmonary disease; FEV1, forced expiratory volume in 1 s; V40p, flow (l/s) at 40% vital capacity measured in the partial flow-volume curve. *P < 0.05.
by either augmenting or withdrawing activity in one of these opposing afferent and efferent neuronal pathways. Direct evidence for both withdrawal and augmentation of parasympathetic cholinergic tone has been published (10, 17, 26, 42, 43, 111–113, 116, 148, 154, 196, 197, 240) (Fig. 3). In addition, reflex effects initiated by afferent nerve activation or by CNS pathways regulating autonomic outflow must integrate into a tonically active system. This can have a profound influence over the resulting end-organ effects measured in the airways and lungs (50, 82, 150, 220, 221). Attributing reflex effects to activation of any single afferent nerve subtype should be done cautiously, given the many converging and parallel inputs regulating basal and evoked alterations in airway smooth muscle tone. The highly predictable presence and level of baseline tone in the airways of all species studied also suggest the existence of a “set point” for smooth muscle contraction, perhaps optimized such that a withdrawal or augmentation of tone can be rapidly achieved in response to physiological and pathophysiological stimuli. As with the set points regulating mean arterial blood pressure, alterations in afferent or efferent nerve function may contribute to airway hyperresponsiveness and airway obstruction in diseases such as asthma and COPD (see below).

**REFLEX REGULATION OF PARASYMPATHETIC CHOLINERGIC NERVES INNERVATING AIRWAY SMOOTH MUSCLE**

Stimuli initiating reflex-mediated cholinergic contractions of airway smooth muscle include both chemical and physical stimuli delivered to the airways and lungs as well as the heart, arterial baroreceptors and chemoreceptors, and the mucosa of the upper airways and esophagus (24, 42, 111). The chemical stimuli evoking reflex bronchospasm can be subdivided into
autacoids, hormones, and neurotransmitters that act on cell surface receptors, and stimuli such as acid, CO₂, hypertonic saline, and irritant gases like sulfur dioxide that act either mechanically or through ion channels or by unknown mechanisms to initiate reflexes. These chemical stimuli act directly on afferent nerves to initiate reflexes, or indirectly, secondary to effects on smooth muscle or through actions on resident cells in the airways and lungs that release autacoids, which then act on adjacent afferent nerve endings. Mechanical stimuli evoking reflex bronchospasm in the airways include airway smooth muscle contraction, punctuate mechanical stimulation of the airway mucosa, inhaled particulate matter, accumulated secretions, dynamic lung inflations, lung collapse, pulmonary edema, and pulmonary embolism.

Smooth muscle contraction can evoke further contraction of the airway smooth muscle through reflexes (Figs. 4–6). Bronchoconstrictors, including histamine, serotonin, PGD₂, thromboxane, and even methacholine and acetylcholine evoke bronchospasm at least in part by initiating parasympathetic-cholinergic reflexes (24, 26, 42, 46, 51, 54, 55, 71, 110, 111, 134, 148, 216, 236, 243). The reflex component of the response to these stimuli is best studied at threshold concentrations, as the direct effects can obscure the reflex components of the response at higher doses. These indirect effects may be attributable to activation of intrapulmonary rapidly adapting receptors, which are uniquely sensitive to decreases in lung compliance (26, 106, 183, 207). Other autacoids that have only modest, direct contractile effects on smooth muscle but evoke reflex bronchospasm include bradykinin, PGE₂, prostacyclin, platelet-activating factor, and adenosine (24, 26, 42, 54, 117, 150, 174, 198, 199, 228). These autacoids may initiate reflex bronchospasm by acting directly on bronchopulmonary C fibers and rapidly adapting receptors. Alternatively, they may act on other cells in the airways that in turn initiate reflex bronchospasm. Adenosine, for example, can activate mast cells, resulting in leukotriene and histamine and/or serotonin release (18, 145, 229). Platelet-activating factor induces synthesis of the lipoxygenase product 15-HETE by airway epithelial cells (205), an endogenous agonist for the capsaicin receptor transient receptor potential vanilloid type 1 (TRPV1), selectively expressed by C fibers and nociceptors throughout the body, including the airways. TRPV1 activation by capsaicin and 15-HETE has been shown to initiate reflex bronchospasm (94, 150, 203). Bradykinin may also work indirectly to activate C fibers through formation of HETEs and subsequent actions on TRPV1 (31, 121, 204, 213). Acid may also act at least in part through TRPV1 (120, 121, 230).

Precisely how mechanical stimuli, including inhaled particulates, accumulated secretions, punctuate mechanical probing of the airway mucosa, pulmonary edema, pulmonary embolism, smooth muscle contraction, lung inflations, and deflations initiate reflex bronchospasm is unclear. Mechanisms that have been associated with baroreceptor activation and activation of other mechanoreceptors (e.g., gadolinium-sensitive ion channels, epithelial Na channels) have varied roles in the lung (7, 30, 36, 44, 56, 57, 138, 175, 245, 246). ATP has variable
significant inhibition of response to the stimulus studying reflex tracheal contractions evoked by laryngeal capsaicin challenge is mediated by both the vagus nerves caudal to the recurrent laryngeal nerves and the vagus nerves cranial to the recurrent laryngeal nerves (150). These reflexes were mimicked by the TRPV1 receptor agonist 15-HETE and prevented by laryngeal denervation or the TRPV1 receptor antagonist capsazepine. Laryngeal denervation and capsazepine had no effects on baseline cholinergic tone. The reflex was also mimicked by centrally (but not peripherally) administered substance P, all of which indicates the involvement of capsaicin-sensitive, tachykinin-containing tracheal and laryngeal C fibers. Importantly, however, we found that denervating the intrapulmonary airways by cutting the vagus nerves caudal to the recurrent laryngeal nerves completely reversed baseline cholinergic tone but also prevented the reflex effects evoked by laryngeal capsaicin challenge (150). We interpret these data as evidence that laryngeal C-fiber activation is not sufficient for increasing cholinergic tone in the airways but amplifies the ongoing effects regulated by intrapulmonary mechanoreceptors. In effect, the reflex initiated by laryngeal capsaicin challenge is mediated by both the capsaicin but also the mechanical effects associated with tidal breathing. We have shown a comparable interaction between vagal afferent nerves regulating cough (153), and we have compared these interactions to the processes of central sensitization regulating pain sensations in somatic tissues (149, 150).

A number of afferent nerve subtypes induce a withdrawal of cholinergic tone, including baroreceptors, skeletal muscle and diaphragmatic afferents, and pulmonary stretch receptors (10, 17, 24, 26, 42, 43, 111–113, 116, 148, 154, 161, 196, 197, 208, 240). These disparate afferent inputs may be simultaneously recruited during exercise. The dilatation of the airways produced upon exertion may match demands for gas exchange.

Fig. 4. Airway cholinergic nerves regulate airways responsiveness in human (A), dog (B), guinea pig (C), and mouse (D). Blockade of parasympathetic cholinergic reflexes essentially abolishes airway responses to a variety of autacoids and to allergen in all species studied. These reflex effects are easily measured when threshold doses of provocative stimuli are used. When higher doses are used, the direct contractile effects of autacoids can obscure the reflex component of the response. These data are adapted from published data obtained in studies in human subjects, dogs, guinea pigs, and mice (46, 54, 55, 71, 89, 93, 134, 174, 228, 243). Many additional examples of this effect have been published in all species. See text for details. Measurements of lung mechanics included airways resistance, lung conductance, enhanced pause (PenH), and airway pressure-time index (APTI). PAF, plateletactivating factor. *Significant reduction in baseline resistance (P < 0.05). **Significant inhibition of response to the stimulus (P < 0.05).

effects on mechanically sensitive bronchopulmonary vagal afferent nerves (25, 30, 186), so a role for ATP in mechanotransduction in the airways, as has been proposed in other viscera, has not yet been documented but seems unlikely (40, 234). Pulmonary embolism may evoke reflexes following platelet serotonin release (131, 159, 241). Serotonin activates both rapidly adapting receptors and pulmonary C fibers (37, 51, 114, 147).

Extrapulmonary afferents, including vagal afferents innervating the heart, arterial baroreceptors and chemoreceptors, esophagus and larynx, and nonvagal afferents innervating the upper airways, nose, and perhaps the face can also initiate reflex bronchospasm upon activation (20, 42, 43, 50, 65, 66, 102, 108, 117, 123, 141, 142, 148, 172, 188, 208, 221, 224, 231). It is not so obvious what, if any, physiological role each of these reflexes has in maintaining homeostasis. The central pathways controlling these reflexes have not been studied. It is possible that the afferents innervating these extrapulmonary viscera, particularly the vagal afferents innervating the heart, larynx, and esophagus, converge centrally in the nucleus tractus solitarii (nTS), with the bronchopulmonary afferents regulating airway smooth muscle tone, and amplify the actions of bronchopulmonary afferents, resulting in an elevation in baseline tone (19, 153, 184, 189, 217). Alternatively, pathways separate and parallel to the nTS may provide excitatory input to the bronchopulmonary parasympathetic preganglionic nerves.

Convergence of vagal inputs in the nTS may profoundly affect the mechanisms by which reflexes are manifest (Fig. 5). As discussed above, we found that basal parasympathetic cholinergic tone in the guinea pig airways is driven entirely by ongoing afferent input arising from the intrapulmonary airways and lungs, likely from rapidly adapting receptors (116). When studying reflex tracheal contractions evoked by laryngeal capsaicin challenges, we found that the slowly developing and long-lasting increases in cholinergic tone could be prevented or reversed by centrally acting neurokinin receptor antagonists (150). These reflexes were mimicked by the TRPV1 receptor agonist 15-HETE and prevented by laryngeal denervation or the TRPV1 receptor antagonist capsazepine. Laryngeal denervation and capsazepine had no effects on baseline cholinergic tone. The reflex was also mimicked by centrally (but not peripherally) administered substance P, all of which indicates the involvement of capsaicin-sensitive, tachykinin-containing tracheal and laryngeal C fibers. Importantly, however, we found that denervating the intrapulmonary airways by cutting the vagus nerves caudal to the recurrent laryngeal nerves completely reversed baseline cholinergic tone but also prevented the reflex effects evoked by laryngeal capsaicin challenge (150). We interpret these data as evidence that laryngeal C-fiber activation is not sufficient for increasing cholinergic tone in the airways but amplifies the ongoing effects regulated by intrapulmonary mechanoreceptors. In effect, the reflex initiated by laryngeal capsaicin challenge is mediated by both the capsaicin but also the mechanical effects associated with tidal breathing. We have shown a comparable interaction between vagal afferent nerves regulating cough (153), and we have compared these interactions to the processes of central sensitization regulating pain sensations in somatic tissues (149, 150).
Reflex regulation of the postganglionic, noncholinergic, parasympathetic nerves regulating airway smooth muscle tone has been studied in guinea pigs and cats and in human subjects (26, 95, 96, 117, 129, 148, 157, 224). Unlike cholinergic contractions of the airway smooth muscle, which can reach a near maximum within 30 s and can nearly completely reverse at the same rate, noncholinergic parasympathetic nerve-mediated relaxations are both slow in onset and reversal, requiring several minutes to reach equilibrium (26, 27, 35, 48, 101, 117, 127, 146, 148) (Fig. 6). This and other characteristics led Coburn and Tomita (39), who originally described this relaxant innervation in guinea pig trachealis, to conclude that these nerves function to restore or maintain airway patency primarily during or at the conclusion of defensive reflexes, perhaps subsequent to coughing or reflex bronchospasm. There is some evidence that is at least consistent with this hypothesis. Noncholinergic parasympathetic nerve activation is only modestly effective at preventing bronchospasm mediated reflexively or by direct actions on smooth muscle but can gradually reverse an evoked contraction and modulate sustained cho-
noncholinergic tone at eupnea (3–5, 9, 26, 38, 99, 117, 164, 224). Further evidence for a role of noncholinergic parasympathetic nerves primarily in defensive settings is the observation that reflexes differentially regulate the cholinergic and noncholinergic airway parasympathetic nerves (95, 100, 148). Noncholinergic nerves are reflexively activated by stimulants of bronchopulmonary C fibers and rapidly adapting receptors, afferent nerves that are associated with defensive reflexes and responsive to noxious chemical and mechanical stimuli (42, 207). Cholinergic nerves are also reflexively activated by C fibers and rapidly adapting receptors but can also be activated during the diving response and hypoxia and are negatively regulated by pulmonary stretch receptor activation (116, 148, 161, 172, 196, 197, 221, 240). These afferent stimuli generally have no effect on airway noncholinergic nerves (95, 100, 148) (Fig. 6).

Since reflexes differentially regulate the postganglionic cholinergic and noncholinergic parasympathetic nerves controlling airway smooth muscle tone, it follows that distinct populations of preganglionic nerves almost certainly control these opposing end-organ effects (27, 127). Functional and tracing studies have identified preganglionic neurons arising from nucleus ambiguus that regulate cholinergic contractions in dogs and guinea pigs (81, 83, 85, 151, 171). The preganglionic nerves innervating airway noncholinergic, parasympathetic ganglia may be unmyelinated and may originate from a distinct location in nucleus ambiguus or may be derived from the dorsal motor nuclei of the vagus nerves (dmnX) (27, 127). Neurons arising from dmnX have been retrogradely labeled from the airways and from the neuronal plexuses containing the cholinergic and noncholinergic parasympathetic ganglia (80, 84, 85, 107, 187). To date, no function has been ascribed to the preganglionic nerves arising from dmnX (85, 115). A relaxant innervation of the airways arising from dmnX would parallel a relaxant innervation of the stomach and esophagus arising from
abolished by muscarinic-receptor antagonists (16, 24) (Fig. 4). Airway reactivity to many stimuli can be reduced or at least in part to altered autonomic regulation of airway smooth muscle. Several mechanisms controlling airway sympathetic nerve activity. Thus we studied reflex regulation of pulmonary sympathetic nerves innervating the trachealis of guinea pigs (178). The vagus nerves were cut bilaterally to limit the influence of airway parasympathetic nerves on smooth muscle tone. With the trachealis precontracted with histamine, capsaicin inhalation evoked a marked relaxation of the trachealis that was prevented by sympathetic denervation of the trachealis, propranolol, or dorsal rhizotomy (T1-T4). Retrograde tracing and electrophysiological analyses identified a population of capsaicin-sensitive spinal afferent nerves innervating the intrapulmonary airways and lungs. The majority of these spinal afferent nerves expressed substance P. Not surprisingly, then, neurokinin-receptor antagonists prevented the reflex-mediated relaxations evoked by capsaicin inhalation.

We also found that stimulating the central cut ends of the vagus nerves evoked propranolol-sensitive relaxations of the trachealis (178). Vagal afferents are known to regulate sympathetic outflow to multiple organs, including the airways (8, 11, 79, 92). It is difficult to study vagal control of airway sympathetic nerves in guinea pigs and cats, however, as it is not yet possible to fully block noncholinergic parasympathetic nerve-mediated relaxations of the trachealis in guinea pigs or bronchi in cats (5, 117). It may be possible to study these reflexes in the cat or dog trachea, however, as cats have an abundant noncholinergic relaxant innervation of their intrathoracic airways but not in the trachea, while dogs lack noncholinergic relaxant innervation (48, 52, 202).

Interestingly, we found that the sympathetic reflexes evoked in the airways by capsaicin inhalation occurred without any coincident cardiovascular responses (178). This contrasted with responses evoked by asphyxia and electrical stimulation of rostral cut ends of the vagus nerves and adds further evidence against historical notions regarding sympathetic nerve function in homeostatic and defensive settings (104, 170).

**AUTONOMIC NERVE REGULATION OF HUMAN AIRWAY SMOOTH MUSCLE IN DISEASE**

The symptoms of COPD and asthma can be attributed at least in part to altered autonomic regulation of airway smooth muscle. Airway reactivity to many stimuli can be reduced or abolished by muscarinic-receptor antagonists (16, 24) (Fig. 4). Reversible airway obstruction in asthma and COPD is due primarily to an elevated cholinergic tone (16, 77, 166) (Table 1). Upper airway infections and air pollution, both associated with airway obstruction and increased airways responsiveness, initiate these pathological changes in the airways by altering airway parasympathetic tone (60, 72). Potential causes of autonomic dysfunction in asthma and COPD have been reviewed elsewhere (16, 176, 190, 232, 233).

Noncholinergic parasympathetic nerve function may also be altered in asthma and COPD. A failure to dilate on deep inspiration, and not excessive constriction to provocative stimuli, may underlie airway hyperresponsiveness in asthma (219). The dilatation associated with deep inspiration in normal subjects may be reflexively mediated by withdrawal of cholinergic tone or by noncholinergic parasympathetic neurotransmitters such as VIP and NO. The actions or synthesis of VIP and NO may be reduced in asthma and by allergic inflammation (13, 29, 32, 67, 163, 165, 194, 226, 227). Pharmacological inhibition of NO synthase activity increases airway reactivity in mild asthmatic patients, but not in severe asthmatic patients (192, 193). Although several studies have shown little difference in VIP-immunoreactive nerve fibers in biopsies taken from normal and asthmatic patients (34, 91), VIP was not found in histological studies of airway nerves in fatal asthma (179). Genetic analyses show that polymorphisms in the gene encoding the neuronal isoform of NO synthase are associated with a high risk of developing asthma (68, 73, 74, 88, 97, 210). Asthmatic patients with these NO synthase gene mutations have a low concentration of NO in their exhaled breath (238).

Alterations in synthesis and metabolism of nerve-derived NO may also contribute to airways disease. Increased arginine activity has been reported in asthma and allergic inflammation (156, 169, 247). Depletions and/or deficiencies of arginine, the substrate for NO synthase, may impair NO-dependent, nerve-mediated bronchodilation (140). Once synthesized, NO exists in relatively stable and active forms in the lung as S-nitrosothiol (S-NO). Levels of S-NO are markedly depleted in the airways of asthmatic children in respiratory failure (69). S-NO is metabolized by glutathione S-nitrosothiol reductase. An upregulation of glutathione S-nitrosothiol reductase activity during allergic inflammation may also alter airway responsiveness to endogenously produced NO (191).

As mentioned above, sympathetic adrenergic innervation of human airway smooth muscle is sparse and/or nonexistent (24, 195). Functional studies mostly suggest a limited role for sympathetic adrenergic nerves regulating airway function in normal or asthmatic human subjects (75, 76, 78, 126, 167, 177, 223). Hormonal catecholamines and perhaps adrenergic nerves may, however, play a prominent role in regulating the airways in asthma under some conditions (98, 119, 130, 206). Elevated sympathetic nerve activity has also been associated with COPD (86).

**CONCLUSION**

The autonomic nervous system plays an essential role in regulating airway smooth muscle tone. Dysregulation of airway autonomic nerves contributes to the pathogenesis of asthma and COPD and to the acute presentation of other conditions, including pulmonary embolism, pulmonary edema, and anaphylaxis. Airway autonomic nerves may also produce the respiratory symptoms associated with cardiovascular disease, gastroesophageal reflux disease, and rhinosinusitis. Any
effort to understand the role of airway smooth muscle in pulmonary disease should necessarily include an attempt to understand autonomic regulation of airway smooth muscle tone within the context of the disease processes. Surprisingly, however, there are tremendous gaps in our understanding of airway neural control, even in the healthy lung, particularly as it relates to CNS control of autonomic tone, integration of afferent input, the origin, function and neurotransmitters regulating noncholinergic nerve-mediated relaxations of airway smooth muscle, reflex regulation of airway sympathetic nerves, and the effects of inflammation and pulmonary disease on the structure and function of postganglionic autonomic nerves innervating the airways. These physiological analyses will by necessity rely on an integrated, multidisciplinary whole animal experimental design using species other than the mouse, given the technical difficulties associated with studying smaller animals and the poor predictive value of murine airway smooth muscle regulation compared with humans.

REFERENCES

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