Reflex regulation of airway smooth muscle tone

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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.

Airway smooth muscle controls dead space and resistance to airflow to and from the gas-exchanging areas of the lung by regulating the diameter and length of the conducting airways. Although the importance of these functions of smooth muscle in maintaining lung homeostasis has been debated (162, 182, 209, 221, 240), there is general agreement on the importance of smooth muscle in airways disease (61, 90, 105, 118, 128, 181). Constrictions of airway smooth muscle evoked by a variety of neurotransmitters, autacoids, and hormones can effectively constrict the airways to closure in all species, including humans, thereby preventing entire gas exchange and threatening the life of the individual as in asthma and anaphylaxis (21, 22, 24, 33, 54, 55, 71, 110, 125, 136, 139, 144, 160, 173, 200, 216, 222, 243). Conversely, other neurotransmitters, autacoids, and hormones can reverse or prevent maximal contractions of airway smooth muscle, restoring airway patency and thus preserving lung capacity for gas exchange (6, 21, 41, 45, 49, 58, 70, 137, 155, 164, 168, 185, 199, 212). A comprehensive understanding of the mechanisms regulating airway smooth muscle contraction and relaxation is necessary for any attempt at understanding and treating obstructive airways disease.

The autonomic nervous system plays an essential role in regulating airway smooth muscle tone (24, 42, 111, 190). 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

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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 $\alpha$- and $\beta$-adrenoceptors, respectively
(1, 2, 14, 15, 21, 48, 130, 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 ox-
ide (NO) mediate the nonadrenergic, noncholinergic relax-
ations (5, 13, 24, 28, 39, 59, 109, 117, 135, 158, 180, 195, 211,
237, 242, 244). The distribution and function of parasympa-
thetic-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 neurotrans-
mitters (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 function-
ally distinct parasympathetic pathway regulates nonadrenergic,
noncholinergic relaxations of airway smooth muscle (23, 27,
28, 47, 52, 127). Reflexes differentially regulate these cholin-
ergic and noncholinergic nerves (95, 100, 148). Circumstantial
evidence suggests that the parasympathetic innervation of hu-
man 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 $\beta$-adrenocep-
tors 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 demon-
strable in all species, whereas basal adrenergic and nondren-
ergic 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 $\beta$-adrenergic 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 airway cholinergic nerves. Jammes and Mei (103) studied baseline cholinergic tone in cats (Fig. 2). They found that dissecting the vagus nerve free from its associated nodose ganglia essentially 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 muscle tone, these investigators showed that cholinergic nerve activity increased or decreased as integrated phrenic nerve activity increased or decreased in response to various interventions (e.g., NaCN, positive end-expiratory pressure, hyperventilation, inhalation of CO₂). Mitchell and colleagues thus concluded that cholinergic outflow to the airways was centrally 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 restore ventilation. These changes in smooth muscle tone produced by altering ventilation did not occur following vagotomy or pretreatment with atropine, with or without subsequent 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 laryngeal 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 contractions 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 parasympathetic 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 inflation 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 premature 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 trachealis of dogs that was insensitive to vagal afferent cooling. In all species, however, profound and opposing (excitatory and inhibitory) 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 muscle 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>
<thead>
<tr>
<th>Species</th>
<th>Intervention</th>
<th>Measurement</th>
<th>Baseline</th>
<th>After Intervention</th>
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<tr>
<td>Normal humans</td>
<td>Atropine</td>
<td>FEV₁</td>
<td>98% predicted</td>
<td>106% predicted*</td>
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<tr>
<td>Human atopic subjects</td>
<td>0.5–1 g Hexamethonium</td>
<td>Airways resistance</td>
<td>3.8±0.9 cmH₂O⁻¹·s⁻¹</td>
<td>2.7±0.8 cmH₂O⁻¹·s⁻¹*</td>
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<tr>
<td>Human asthmatic subjects</td>
<td>Atropine</td>
<td>Airways resistance</td>
<td>4.3±0.2 cmH₂O⁻¹·s⁻¹</td>
<td>2.6±0.1 cmH₂O⁻¹·s⁻¹*</td>
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<tr>
<td>Human asthmatic subjects</td>
<td>Vaporizer</td>
<td>V̇E(tot)</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>
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<tr>
<td>Sheep</td>
<td>Vagotomy</td>
<td>Airways resistance</td>
<td>2.4±1.0 cmH₂O⁻¹·s⁻¹</td>
<td>1.1±0.2 cmH₂O⁻¹·s⁻¹*</td>
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<tr>
<td>Dogs</td>
<td>Vagotomy</td>
<td>Airway diameter</td>
<td>31±13% increase*</td>
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<tr>
<td>Cats</td>
<td>Vagotomy</td>
<td>Airways resistance</td>
<td>3.2±0.3 cmH₂O⁻¹·s⁻¹</td>
<td>2.2±0.3 cmH₂O⁻¹·s⁻¹*</td>
</tr>
<tr>
<td>Rabbits</td>
<td>Vagal cooling</td>
<td>Lung conductance</td>
<td>2.8±0.5 1·min⁻¹·cmH₂O⁻¹</td>
<td>3.8±0.8 1·min⁻¹·cmH₂O⁻¹*</td>
</tr>
<tr>
<td>Guinea pigs</td>
<td>Atropine</td>
<td>Airways resistance</td>
<td>0.06±0.01 cmH₂O⁻¹·s⁻¹</td>
<td>0.06±0.004 cmH₂O⁻¹·s⁻¹*</td>
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<td>Guinea pigs</td>
<td>Vagotomy</td>
<td>Lung conductance</td>
<td>0.31±0.043 1·min⁻¹·cmH₂O⁻¹</td>
<td>0.32±0.047 1·min⁻¹·cmH₂O⁻¹*</td>
</tr>
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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; V̇E(tot), 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, 156, 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

Fig. 3. Tracheal smooth muscle responses to lung hyperinflation in the dog with and without vagal cooling reveal the opposing reflex effects mediated by bronchopulmonary vagal afferent nerve subtypes. The extrinsic innervation of the trachea was cut, except for the superior laryngeal nerves, which carry a subset of the preganglionic parasympathetic nerves regulating tracheal smooth muscle tone. The vagi were then placed on a cooling coil. The primary response of the tracheal (Tr) smooth muscle (measured as “tension” in grams) to lung inflation in dogs without vagal cooling or with minimal vagal cooling (A–C) is relaxation due to a withdrawal of cholinergic tone. Comparable results have been obtained in studies in guinea pigs and cats, and these effects have been attributed to sustained activation of pulmonary stretch receptors. With vagal cooling to temperatures that preserve action potential conduction in bronchopulmonary C fibers but not in myelinated vagal afferents (D–H), the relaxation response is abolished and a contractile response is revealed. Also note the decrease in cholinergic tone in response to vagal cooling in these 2 dogs (top and bottom, respectively). This suggests that, like that described in cats and guinea pigs, there is a tonic regulatory control of baseline cholinergic tone in dogs provided by vagal afferent nerves. Pt, tracheal pressure; ABP, arterial blood pressure. [From Roberts et al. (197).]
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 airway 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.
REFLEX REGULATION OF NONCHOLINERGIC PARASYMPATHETIC NERVES INNERVATING AIRWAY SMOOTH MUSCLE

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, 101, 117, 127, 146, 148). 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-

Fig. 5. Reflex effects initiated by histamine and bradykinin and synergistic interactions between vagal afferent nerves in the guinea pig. A: increases in tracheal smooth muscle tone (Tt) were measured in situ in the guinea pig trachea. Pulmonary inflation pressure (PIP) and ABP were also measured. The trachea was continuously perfused with warmed, oxygenated Krebs bicarbonate buffer containing histamine and bradykinin receptor antagonists (to prevent the local, direct effects of these autacoids on tracheal tone), and all animals were pretreated systemically with propranolol and the cyclooxygenase inhibitor meclofenamic acid. Bradykinin and histamine, when administered iv, increased tracheal smooth muscle tone to comparable levels. Histamine but not bradykinin simultaneously increased pulmonary inflation pressure (a direct effect on airway smooth muscle). Pretreatment with neurokinin receptor antagonists administered iv (shown) or icv (not shown) abolished the reflex effects evoked by bradykinin but were without effect on the response to histamine. These and other data show that bradykinin and histamine evoke parasympathetic cholinergic reflexes in the airways by selectively activating C fibers and rapidly adapting receptors, respectively. B: representative trace showing that simultaneous activation of C fibers and rapidly adapting receptors with bradykinin and histamine, respectively, greatly enhances the reflex effects evoked when either pathway is activated alone. Atropine or vagotomy completely abolishes the enhanced responses evoked by simultaneous histamine and bradykinin administration as shown in the trachea in situ (C) and in the whole lung (D), using measures of inflation pressure. This enhanced response is also abolished by icv administration of neurokinin receptor antagonists. *Significant reduction in baseline resistance (P < 0.05). **Significant inhibition of response to the stimulus (P < 0.05). [From Canning et al. (26) and Mazzone and Canning (150).]
linergic 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

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**Fig. 6. Reflexes differentially regulate airway cholinergic and noncholinergic parasympathetic nerves.**

*A*: a brief period of hypoxia reflexively increases cholinergic tone measured in situ in the guinea pig trachea (Tr). This reflex is completely abolished by atropine. *B*: after atropine pretreatment and precontraction of the trachealis with histamine, hypoxia fails to alter tracheal smooth muscle tone, suggesting that the noncholinergic parasympathetic nerves are not activated in response to this stimulus (all preparations were pretreated with propranolol). The diving response (evoked by a cold saline challenge applied to the nose/nasal mucosa) also selectively activates airway cholinergic nerves (not shown). *C–F*: the markedly different kinetics of the contractile and relaxant parasympathetic reflexes initiated by intravenous histamine (*C*), inhaled capsaicin (*D*), hypoxia (*E*), and laryngeal capsaicin challenge (*F*). [From Mazzone and Canning (148).]

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dmnX (124, 201). In the guinea pig, at least, the postganglionic, noncholinergic, parasympathetic nerves regulating nonadrenergic relaxations of airway smooth muscle emanate from the adjacent esophagus (27, 28, 63).

**REFLEX REGULATION OF SYMPATHETIC NERVES INNERVATING AIRWAY SMOOTH MUSCLE**

Many studies have shown that the β-adrenoceptor antagonist propranolol increases reactivity to a variety of spasmogens, whereas electrical stimulation of airway sympathetic nerves evokes bronchodilatation (2, 21, 35, 53, 87, 93, 178). Extra-cellular recordings from both pre- and postganglionic sympathetic nerves that might innervate airway smooth muscle have also been reported (8, 79, 214, 215, 239). Until recently, however, no study has directly addressed the reflex mechanisms controlling airway sympathetic nerve activity. Thus we studied reflex regulation of airway 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 tracheal 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 has 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 bronchodilatation (140). Once synthesized, NO exists in relatively stable and active forms in the lung as S-nitrosothiols (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
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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.

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