Brain stem excitatory and inhibitory signaling pathways regulating bronchoconstrictive responses

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This review summarizes recent work on two basic processes of central nervous system (CNS) control of cholinergic outflow to the airways: 1) transmission of bronchoconstrictive signals from the airways to the airway-related vagal preganglionic neurons (AVPNs) and 2) regulation of AVPN responses to excitatory inputs by central GABAergic inhibitory pathways. In addition, the autocrine-paracrine modulation of AVPNs is briefly discussed. CNS influences on the tracheobronchopulmonary system are transmitted via AVPNs, whose discharge depends on the balance between excitatory and inhibitory impulses that they receive. Alterations in this equilibrium may lead to dramatic functional changes. Recent findings indicate that excitatory signals arising from bronchopulmonary afferents and/or the peripheral chemosensory system activate second-order neurons within the nucleus of the solitary tract (NTS), via a glutamate-AMPA signaling pathway. These neurons, using the same neurotransmitter-receptor unit, transmit information to the AVPNs, which in turn convey the central command to airway effector organs: smooth muscle, submucosal secretory glands, and the vasculature, through intramural ganglionic neurons. The strength and duration of reflex-induced bronchoconstriction is modulated by GABAergic-inhibitory inputs and autocrine-paracrine controlling mechanisms. Downregulation of GABAergic inhibitory influences may result in a shift from inhibitory to excitatory drive that may lead to increased excitability of AVPNs, heightened airway responsiveness, and sustained narrowing of the airways. Hence a better understanding of these normal and altered central neural circuits and mechanisms could potentially improve the design of therapeutic interventions and the treatment of airway obstructive diseases.

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Chronic airway diseases such as bronchial asthma and chronic obstructive bronchitis share the salient features of inflammation, hyperresponsiveness to various inhalants, increased cholinergic outflow to the airways, and sustained airway narrowing. Although these two conditions result in enormous morbidity and the neural mechanisms are thought to be play an important role (13, 203), the central mechanisms involved in airway hyperreactivity remain poorly understood.

Airway bronchoconstrictive responses initiated by inhaled agents or psychogenic factors suggest the existence of bidirectional central nervous system (CNS)-airway communication that serves to protect the respiratory system. Repeated exposure to environmental pollutants (like ozone, cigarette smoke, allergens), through parallel or convergent inflow (68), may modulate afferent airway sensory pathways (38, 108, 175, 200–202, 216), setting the stage for airway hyperresponsiveness. Under these conditions, the excitability of airway-related vagal preganglionic neurons (AVPNs) is increased and weak stimuli may trigger responses that last for hours, suggesting that the CNS is involved in causing airway constrictive changes.

A better understanding of the role that CNS pathways play in regulating airway functions in normal and diseased states undoubtedly will provide novel therapeutic approaches in the treatment of diseases whose clinical, biochemical, and pharmacological features indicate a pathophysiological link with the CNS. Therefore, this review is focused on the central determinants of airway control and adds to several recent excellent editorials and reviews on sensory neuroplasticity, autonomic regulation of airway functions, and associated pharmacological implications (42, 43, 55, 111, 113, 137, 176, 201–203, 213, 214).
In this article, we briefly discuss recent studies that clarify two of the basic processes in the central control of the vagal preganglionic neurons that provide cholinergic outflow to the airways: 1) glutamatergic signaling pathways involved in transmission and processing of bronchoconstrictive signals from airway sensory receptors, via the nucleus of the solitary tract (NTS) to AVPNs and 2) GABAergic inhibitory network that downregulates excitability of the AVPNs, consequently decreasing cholinergic outflow to the tracheobronchial system. An imbalance between excitatory and inhibitory inputs to AVPNs could be of considerable importance.

**GENERAL CONSIDERATIONS OF THE NEURAL CONTROL OF THE AIRWAYS**

CNS control of airway functions (Fig. 1, level 4) involves integrated networks along the neural axis that funnel information to tracheobronchopulmonary effector units via the AVPNs in the medulla oblongata. The AVPNs are the final common pathway from the brain to the airways and transmit signals to the intrinsic tracheobronchial ganglia that are part of the network for automatic feedback control (level 3). Each ganglion, located in close proximity to effector systems, possesses a relatively large number of neurons that can be considered as an expanded parasympathetic, preganglionic efferent motor system (level 2). However, intrinsic airway neurons contain neurochemicals other than ACh, including vasoactive intestinal peptide (VIP) and neuronal nitric oxide synthase [nNOS, an enzyme involved in generation of nitric oxide (NO); 54, 219] that are considered to mediate nonadrenergic neuronal airway smooth muscle relaxation (21, 56, 78).

Signals transmitted through the preganglionic nerves are relayed, integrated, filtered, and modulated by intrinsic ganglionic neurons before reaching the airway neuroeffecter sites through postganglionic axons. This structural organization could explain the strong effects of a relatively small number of vagal efferent fibers on coordinated reflex changes in airway smooth muscle tone, submucosal gland secretion, and blood flow along the tracheobronchial tree. A similar arrangement has been described in the parasympathetic control of the enteric tract (215). This unified concept does not exclude the probability that some of the vagal preganglionic neurons also provide, to a lesser degree, direct innervation of airway epithelial cells and the alveolar interstitium of the lung, because ganglia are absent altogether from the bronchial subepithelial space and the most distal gas exchanging units (168). These observations suggest that AVPNs may be involved in regulating the release of the biologically active molecules from epithelial cells, i.e., activation of NOS and release of NO that might oppose excessive cholinergically mediated airway contractile responses at both central and peripheral sites (107, 120). Furthermore, cholinergic mechanisms are involved in controlling the conductivity of the most distal airways (166) and tissue resistance (120, 132), by influencing smooth muscle tone, lung interstitium pericytes, and alveolar myofibroblasts (116).

However, it is not clear whether individual AVPNs provide parallel innervation to airway smooth muscle, submucosal glands, and local blood vessels. A relative simultaneity of airway effector cell responses to stimulation of bronchoconstrictive vagal afferent fibers, such as reflex elevation of smooth muscle tone, increase in submucosal gland secretion, and changes in blood flow, is consistent with the assumption that the same cell bodies of AVPNs that cause smooth muscle constriction also elicit activation of smooth muscle glands or changes in blood flow supplying regional airway structures. Alternatively, it is also possible that groups of functionally selective AVPNs may exert a highly coordinated control over multiple airway functions by central mechanisms that synchronize their output to individual effectors. This assumption is analogous to evidence obtained relevant to coordination of multiple cardiac functions by vagal preganglionic neurons, where functionally distinct, but closely integrated, vagal preganglionic neurons in the nucleus ambiguus (NA) mediate the coordinated control of cardiac rate, atrioventricular conduction, and left ventricular contractility (65, 133, 134). Recent preliminary data in ferrets suggest that presumptive gap junctions between identified AVPNs (Blinder K, Karibi-Ikirkko A, Massari VJ, Haxhiu MA, unpublished data) may serve to synchronize their electrical activity in the rostral nucleus ambiguus (rNA). Alterations in central mechanisms that mediate such synchronization could cause regional differences in airway reflex responses, expressed as inhomogeneity in ventilation. For example, in an asthma attack or induced bronchoconstriction, some branches of the airway may totally close while others remain normal (181). Future studies should address the question of the central mechanisms and origin of synchronization and asynchrony of bronchoconstrictive reflex responses.

An extensive network of vagal afferent fibers of sensory ganglia innervates the bronchopulmonary sensory receptors that are specialized for detecting changes in chemical, mechanical, or thermal stimuli. The bipolar airway vagal afferent neurons are located in the nodose and jugular ganglia and participate in reflex events. Furthermore, the afferent nerve endings (i.e., C fibers) are also believed to be responsible for mediating local axon reflexes (level 1) and neurogenic inflammation via release of neuropeptides such as substance P (141),...
which can be modulated by alterations in activity of neutral endopeptidases (156). In addition, endogenous substance P facilitates synaptic transmission in airway parasympathetic ganglia (34, 154), similar to the effect observed with cyclooxygenase activation and the release of prostaglandins in antigen-induced lung injury (112). The central fibers of these sensory neurons ascend in the vagus nerve and enter the brain stem through the solitary tract (26, 88, 89, 114, 123), making their first synapse with the NTS second-order neurons that are required for full expression of the pulmonary C fiber reflex (94).

In the NTS, the afferent vagal terminals responsible for transmitting information from the airway bronchoconstrictive receptors form a distinct wiring diagram with specific second-order neurons that project to AVPNs (74, 88, 89, 167). However, a variety of inputs from afferent receptors is transmitted to the NTS, including those from cough receptors that are activated by stimuli that may also elicit reflex bronchoconstriction, submucosal gland secretion, and increased blood flow. Cough receptors are preferentially located within the larynx, trachea, and large intrapulmonary airways (25, 35, 213). As is the case with vagal efferents, it is not clear whether the same or different vagal afferent neurons transmit different components of airway defensive reflex responses.

More recently, however, it is reported that a subpopulation of myelinated, polymodal Aδ-fibers that arise from the nodose ganglia respond to punctate mechanical stimulation and acid but are unresponsive to capsaicin, bradykinin, histamine, smooth muscle contraction, longitudinal or transverse stretching of the airways, or distension. Unlike these afferent neurons, considered as the putative cough receptors, the majority of capsaicin-sensitive afferents (both Aδ- and C-fibers) innervating the rostral trachea and larynx have their cell bodies in the jugular ganglia and project to the airways via the superior laryngeal nerves (35). These findings suggest the possibility of presynaptic or postsynaptic interactions of afferent nerves originating from cough and non-cough receptors at the NTS level (25, 35). For example, in conscious guinea pigs, C fiber-dependent cough may require coactivation of bronchoconstrictive airway afferent nerves for the full expression of the cough reflex.

Bronchoconstrictive inputs can be modulated by increasing or decreasing the afferent signals from slowly adapting receptors to NTS second-order neurons, the first site of synaptic contact of primary bronchopulmonary slowly adapting receptors (49). Activation of these receptors causes a reflex airway smooth muscle relaxation (212). Similarly, peripheral chemoreceptors and baroreceptors acting centrally can readily affect cholinergic outflow to the airways. Although stimulation of the carotid bodies reflexly elicits bronchoconstriction (157) and submucosal gland secretion (50) and facilitates bronchoconstrictive responses (205), the activation of baroreceptors leads to opposite changes (157). In this article, signaling mechanisms involved in these and other possible reflex interactions that can centrally influence bronchoconstrictive responses are not considered.

Recently, using conventional and transneuronal labeling techniques and ultrastructural, molecular, and physiological approaches, we identified the central excitatory (82–84, 91, 93) as well as inhibitory pathways and neurotransmitters (88, 92, 94, 96) that regulate the excitability and firing rate of AVPNs. These processes occur via both wiring (synaptic) and volume (nonsynaptic) transmission. We hypothesize that downregulation of central inhibitory influences upon AVPNs result in a shift from inhibitory to excitatory transmission, leading to a hyperexcitable state of the AVPNs and, consequently, cholinergic hyperresponsiveness that can predispose to and worsen bronchial asthma (13, 57).

VAGAL PREGANGLIONIC NEURONS INNERVATING THE AIRWAYS

Vagal preganglionic neurons that generate cholinergic outflow to the airways can be viewed as the central integrators of multiple excitatory and inhibitory inputs that connect the brain with the bronchopulmonary effector system. The critical circuits that regulate these processes include both central glutamatergic and GABAergic pathways controlling cholinergic outflow to the airways (Fig. 2).

Location of AVPNs

Studies using retrograde tracer techniques showed that in mammals (Fig. 3), the cholinergic preganglionic motor neurons innervating the airways arise from the rNA and from the rostral portion of the dorsal motor nucleus of the vagus (DMV). Furthermore, the findings imply that the majority of AVPNs are involved in the innervation of multiple airway segments, thereby assuring the symmetry and simultaneity of bronchomo-

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Fig. 2. Central glutamatergic excitatory and GABAergic inhibitory pathways regulating cholinergic outflow to the airways. In this oversimplified schematic illustration are presented 2 main neural pathways that determine the activity of AVPNs. Excitatory signaling pathway uses glutamate (Glu) as a neurotransmitter in conveying bronchoconstrictive signals from airway sensory receptors to the nucleus of the solitary tract (NTS) and from the NTS to the AVPNs. Inhibitory projections employ GABA as a signaling molecule to downregulate excitability of the AVPNs and cholinergic outflow to the tracheobronchial system transmitted through Ach release. These 2 signaling pathways are emphasized because they comprise the central theme of this review.
Fig. 3. Location of AVPNs innervating airways of the ferret. A: schematic presentation of a coronal section of the medulla oblongata showing that in ferrets, as in other mammals, the preganglionic motor neurons innervating the airways arise from the rostral nucleus ambiguous (rNA) and from the rostral portion of the dorsal motor nucleus of the vagus (DMV). MeV, medical vestibular nucleus; LV, lateral vestibular nucleus. B: confocal photomicrographs showing retrogradely labeled AVPNs within the rNA 5 days after cholera toxin β-subunit (CT-b) injections into the lung. C: same section immunostained for choline acetyl transferase (ChAT). D: higher power of the M image of CT-b and ChAT traits of the region marked by a quadrangle in A. Arrow indicates a representative neuron that is immunolabeled with CT-b and ChAT. *ChAT-positive neuron that does not contain CT-b. Scale bar = 50 μm for B and C and 35 μm for D. Data are from Ref. 119.

In summary, at the ultrastructural level, AVPNs innervating the extrathoracic trachea are clearly distinguished from pharyngeal, laryngeal, or esophageal motoneurons (133, 134). These latter characteristics appear to be unique to the compact formation of the NA and clearly differentiate these neurons from tracheal AVPNs (135). Retrogradely labeled AVPNs in the rNA were readily detectable in the electron microscope. The cell bodies of labeled AVPNs were observed to be 32 ± 1 × 23.0 ± 1.3 μm (means ± SE) in size, with abundant cytoplasm and intracellular organelles. The cell bodies had round uninvaginated nuclei that occasionally contained a prominent nucleolus and displayed both somatic and dendritic spines (Fig. 4). Somatosomatic appositions or somatodendritic appositions without intervening glial processes and dendritic bundling of the tracheal AVPNs were not observed. The axons of these neurons were seldom labeled (Fig. 5).

The localization and the ultrastructural features of the AVPNs differed from neurons innervating the alimentary system that have been examined in the dorsal and ventral columns of the NA (104, 183). By comparison, esophageal motoneurons, despite some similarities, can be distinguished from AVPNs located within the rNA by the presence of extensive somatosomatic and somatodendritic appositions. Furthermore, they also display finger- and leaf-like somatic protrusions that partially envelop longitudinally oriented dendrites and axons, and dendritic bundling is prominent (104, 183). These latter characteristics appear to be unique to the compact formation of the NA and clearly differentiate these neurons from tracheal VPNs and vagal preganglionic neurons innervating the heart (133, 134).

Ultrastructural Characteristics of AVPNs

It has been suggested that the viscerotropic representation, ultrastructure, and synaptology of the different divisions of the NA innervating the alimentary system may be associated with specific physiological functions (22, 104, 183). To define ultrastructural characteristics of AVPNs, recently, brain stems from ferrets, in which cholera toxin β-subunit (CT-b) conjugated to horseradish peroxidase had been used as a retrograde cell body tracer, were examined. Electron microscopy was employed to determine ultrastructural characteristics of these tracheal AVPNs (135).
Chemical Profile of AVPNs

Studies using a double-labeling method that combines immunolocalization of the retrograde tracer CT-b and immunohistochemistry for choline acetyl transferase (ChAT), the enzyme catalyzing the biosynthesis of ACh, indicate that in ferrets, AVPNs innervating the trachea and the intrapulmonary airways are cholinergic in nature (Fig. 3) and use ACh as a neurotransmitter to convey signals to the airway motor systems (119). These neurochemical findings are in agreement with the results of physiological studies showing that chemical stimulation of the vagal preganglionic neurons (80, 82–84) or efferent fibers of the vagus nerve originating from AVPNs produces pronounced contraction of the airway smooth muscle and lung parenchyma that is solely mediated via cholinergic parasympathetic nerves and mechanisms (120, 132, 143, 166). Furthermore, reflexly (42, 43, 171, 218) and centrally (86, 87) induced increases in airway secretion are mediated mainly via cholinergic mechanisms.

Virtually all vagal preganglionic neurons innervating the trachea and intrapulmonary airways coexpress VIP, but not NOS, indicating that ACh and VIP are coexisting messenger molecules in AVPNs (119). In addition, recent studies demonstrated that brain-derived neurotrophic factor (BDNF) mRNA and its protein transcript are expressed by a subpopulation of AVPNs, suggesting that active BDNF synthesis occurs in the cells within the rNA that innervate the airways (Zaidi SI, Jafri A, Doggett T, Haxhiu MA, unpublished data). The possible role of the ACh, VIP, and BDNF-tyrosine kinase (Trk) B signaling pathways in the autocrine/paracrine regulation of AVPNs responses to afferent inputs is briefly mentioned at the end of the article.

CNS Innervation of AVPNs

Activity of AVPNs depends on afferent inputs, although they possess an ability to express synchronous electrical oscillations, unveiled by stimulation of NMDA receptors (83) or blockade of GABAA receptors (150). Recently, it has been shown using conventional and transneuronal labeling techniques that the innervation of vagal preganglionic neurons regulating parasympathetic outflow to the airways arises from cell groups located in the brain stem and from several higher brain regions. By comparing the CNS inputs to the vagal preganglionic neurons that innervate intrapulmonary airways with those controlling extrathoracic trachea, common patterns...
of innervation are seen (74, 88, 167). AVPNs receive inputs from cell groups located in the ventral aspect of the medulla oblongata; NTS; pons; ventrolateral part of periaqueductal gray (PAG) cell group; dorsal, lateral, and paraventricular hypothalamus; and the central nucleus of amygdala (74). Hence, the parasympathetic preganglionic neurons innervating the airways are controlled by networks of brain stem and suprapontine cell groups that lie in regions known to be involved in the central control of autonomic functions (129). Recent studies suggest that some respiratory and airway responses could be produced by single neurons capable of affecting multiple neural pathways, rather than by a complex set of heterogeneous cells regulating individual systems (90, 98).

The AVPNs provide the final common pathway for vagal control of the airways. Although we emphasized the importance of medullary circuits that control the reflex output of these AVPNs, functions of the airways are also powerfully regulated by information from higher CNS centers that are relayed caudally to the AVPNs. These forebrain circuits are critical for expression of behavioral state control and the emotional dimensions of airway disorders. For example, the prefrontal cortex innervates the interconnected amygdaloid complex, the central nucleus of which project subsequently to multiple targets regulating autonomic functions, including the dorsomedial and ventrolateral PAG, the NTS, and the NA (74, 172).

The projections from the amygdala to the PAG are of particular importance because the PAG neurons coordinate functions of multiple visceral organs involved in responses to stress. Active coping responses (fight or flight reactions) are evoked by activation of either the dorsolateral or lateral columns of the PAG; whereas passive coping strategies (e.g., quiescence, immobility, decreased responsiveness to the environment) are usually elicited by activation of the ventrolateral part of the PAG (118). The phenotypic traits of some patients experiencing severe or near-fatal asthma, such as psychosocial barriers, blunted respiratory, and other sensory responses that cannot be explained by different medication regimens (16), resemble the expression of passive coping response seen after stimulation of the ventrolateral PAG region in animals (19).

In humans, little is known about CNS innervation of AVPNs. More recently, in conscious subjects, the CNS sites subserving the experience of loaded breathing and air hunger were studied using the positron emission tomography (PET) and functional nuclear magnetic resonance imaging (fMRI; 71, 77, 165). For example, compared with the unloaded control condition, a moderate inspiratory resistive load is associated with an increased fMRI signal intensity in discrete brain regions, including areas of the dorsal pons that correspond to the locus ceruleus (LC) and parabrachial nucleus (PBN). In animals, stimulation of the LC noradrenergic cell group (97) and activation of PBN (153) induces centrally mediated airway smooth muscle relaxation, a compensatory response that tends to decrease the resistive load. In addition, activity associated with perceived intensity of respiratory discomfort induced by loaded breathing was found within the right posterior cingulate cortex and amygdaloid complex, demonstrating laterality to the challenge (165). Of particular interest are recent findings in obstructive sleep apnea (OSA) showing lower signals in medullary and midbrain areas (77), sites that innervate AVPNs (74, 88). A decrease in activity of these sites may lead to withdrawal of inhibitory inputs to AVPNs and facilitation of cholinergic outflow to the airways (92, 95, 98), suggesting that an association of OSA and bronchoconstriction during sleep could be expected more often than it is recognized.

PATHWAYS AND NEUROTRANSMITTERS INVOLVED IN CONVEYING BRONCHOCONSTRICTIVE INFORMATION FROM THE AIRWAYS TO THE NTS

Bronchopulmonary sensory receptors, innervated by small diameter (A8) myelinated vagal afferents known as rapidly adapting receptors (RARs), and nonmyelinated C fibers (C fiber receptors), are characterized by their well-defined sensitivity to chemical stimuli in the airways, most of which affect both RARs and C fiber receptors (42). The net airway motor response results from the interaction of sensory signals within the NTS, including those from cough and slowly adapting pulmonary receptors (25, 35, 212, 213).

Recently, the NTS second-order neurons activated by afferent inputs from the airways were identified using the expression of the nuclear protein (c-Fos), encoded by the protooncogene c-fos. The c-fos gene, a member of the immediate early genes, and its product, c-Fos, are expressed in restricted numbers of CNS neurons in response to strong stimuli applied to the airway receptors. Thus, c-Fos is considered to be an inducible and high-resolution marker of neurons activated by sensory inputs and extracellular stimuli (63).

The advantage of using c-fos expression as a functionally oriented anatomical approach is that the activity of a large number of cells can be readily identified under conditions that do not affect reflex responses such as anesthesia. Using this approach, we found that after stimulation of pulmonary and bronchial C fibers by capsaicin or stimulation of rapidly adapting receptors and bronchial C fiber receptors by histamine aerosol (41), a significant number of NTS neurons within the commissural, medial, and the ventrolateral NTS subregions expressed c-Fos protein (63, 94), indicating that they had been activated by stimulation of pulmonary C fiber and rapidly adapting receptors (26, 94). In ferrets, the labeling pattern correlated well with studies that defined retrograde tracings of NTS subregions in which afferent fibers from the airways terminate (89). The same subregions contained cell body labeling after pseudorabies virus (PRV) injections into the tracheal wall or into the most distal airways (74, 88, 167), suggesting that the NTS activated neurons are those that transmit information from the airways to the AVPNs. Multiple neurotransmitters, including ACh and/or glutamate, are expressed by the primary sensory neurons that may participate in sensory transmission (17, 99, 160).

Cholinergic Transmission

It was predicted that afferent inputs could be conveyed to the NTS by ACh acting on nicotinic ACh receptors. This postulate was based on findings that cholinergic neurons are present in nodose ganglia (160, 163), whereas 125I-labeled α-bungarotoxin binding sites, indicating the presence of nicotinic ACh receptors (nAChRs) that mediate nicotinic cholinergic transmission, are observed within the NTS (9). Furthermore, nodose ganglionectomy decreases ChAT activity and causes a reduction in nAChR binding sites in NTS (99). The dissociated rat NTS cells were observed to express nicotinic but not muscarinic...
rinic acetylcholine receptors (199). In addition, a subset of second-order NTS neurons expresses ChAT in perikarya and dendrites (6).

Experiments demonstrated that activation of airway sensory receptors (RARs and C fiber receptors) induced c-Fos expression in a subset of NTS neurons that also expressed the α3-subtype nicotinic ACh receptor (nAChR). Furthermore, activation of nAChR within the commissural NTS subnucleus by nicotine increased cholinergic outflow to the airways. These effects were diminished by prior administration of hexamethonium (nAChR antagonist) within the commissural NTS cell group. However, hexamethonium had no significant effects on airway reflex constrictions induced by lung deflation. These findings indicated that endogenously released ACh within the NTS and activation of nAChR are not required for transmission of bronchoconstrictive stimuli from the airways to the NTS (63), suggesting involvement of other excitatory molecules such as glutamate.

**Glutamatergic Transmission**

1-Glutamate, a naturally occurring excitatory amino acid, is a main sensory neurotransmitter (47) mediating communication between neurons within neuronal networks coordinating motor outputs (for review and references see 72, 209). It is present in vagal afferents in the NTS (179, 180, 195, 206) and is required for transmission of fast signals from sensory nerve endings to second-order neurons (5, 94, 211), including baroreceptor inputs (125, 126, 197).

Recently, we studied the role of glutamate in conveying bronchoconstrictive inputs from the airways to the NTS and from the NTS to the AVPNs (91, 93, 94). Glutamate release within the NTS after stimulation of airway sensory receptors was also examined. These studies indicated that stimulating airway sensory receptors increased the spontaneous efflux of glutamate in the commissural NTS subnucleus (94), the site where airway afferent fibers terminate in ferrets (89), and that the release was correlated with airway smooth muscle contraction (94). Typical HPLC chromatograms of microdialysates were collected from the commissural NTS subnucleus in a control state, during repeated excitation of bronchopulmonary sensory receptors, and in the poststimulation period. After airway sensory stimulation, glutamate concentration increased significantly from 42 ± 11 to 109 ± 17 pg/μl (average of 3 trials for each ferret), and pressure in a bypassed tracheal segment (Ptseg) increased from 7.4 ± 1.4 to 26.1 ± 4.6 cmH2O ($P < 0.01$). In the poststimulation recovery period, glutamate and Ptseg returned to control levels. Removal of the vagal afferent and afferent innervations of the tracheobronchial tree eliminated the reflex increase in glutamate release induced by lung deflation (Fig. 6).

Conceivably, the release of endogenous glutamate could be due to baroreceptor loading (125, 126, 197) and/or activation of peripheral chemoreceptors by oxygen deprivation (147). However, in reported experiments, lung deflation had insignificant effects on arterial pressure and ferrets were ventilated with $O_2$, likely excluding the possibility that lung deflation induced release of glutamate by triggering of chemosensory reflex responses. Furthermore, denervation of the airways and the lungs completely blocked the glutamate release induced by lung deflation, suggesting that the release of endogenous glutamate was related to sensory inputs from the bronchopulmonary system.

Over the last two decades, extensive research has revealed a host of glutamate receptor subtypes belonging to the ionotropic (iGluR) and metabotropic glutamate receptor (mGluR) classes, which subserve excitatory synaptic transmission and neurotransmitter release.

* **iGluRs.** iGluRs are ligand-gated ion channels consisting of three known functional receptor subtypes: the AMPA, kainate, and NMDA receptors, which respond to glutamate and glutamate analogs by the opening of a cation channel (4, 10, 27, 44, 138). Quisqualate, AMPA, glutamate, and kainate are potent agonists of the AMPA receptor subunits (GluR1-GluR4) when applied alone or in combination. The quinoxalinediones, such as 6,7-dinitroquinoxaline-2, dione (DNQX) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNOX), are the most potent antagonists of the AMPA receptors. This class of antagonists also blocks the kainate receptors but does not block NMDA receptors. Hence, it can be used to differentiate AMPA receptor-mediated effects from those of the NMDA signaling pathway.

The kainate receptors, composed of five subunits, GluR5-GluR7 and KA1 and KA2, contribute to excitatory postsynaptic currents in many regions of the central and peripheral nervous systems, including the hippocampus, cortex, spinal cord, and retina. In some cases, postsynaptic kainate receptors are codistributed with AMPA and NMDA receptors, but there are also synapses where transmission is mediated exclusively by postsynaptic kainate receptors. Recent analysis of knockout
mice lacking one or more of the subunits that contribute to kainite receptors, as well as studies with subunit-selective agonists and antagonists, have revealed the important roles that kainite receptors play in short- and long-term synaptic plasticity (27, 106). Because AMPA receptors are more abundant than kainite receptors and exhibit faster signaling kinetics than NMDA receptors (70, 169), the AMPA receptors are uniquely suited in mediating cardiopulmonary reflexes (5, 69, 211). Furthermore, synaptic activation of AMPA receptors may elicit not only postsynaptic excitation but also presynaptic inhibition of GABAergic transmission. By suppressing the inhibitory inputs, the activation of AMPA receptors could facilitate bronchoconstrictive inputs to the NTS second-order neurons and from these neurons to the AVPNs. This possibility is supported by findings showing that stimulation of AMPA receptors induces presynaptic inhibition in cerebellar GABAergic synapses (182) but increases ACh release in the hippocampus of young and aged rats (177).

Experiments to determine whether specific AMPA receptors are expressed by the activated NTS neurons were performed in ferrets by using a double-staining technique and confocal microscopy (94). Employing GluR2 mouse monoclonal antibody, it was observed that this AMPA receptor subtype is present on the subpopulation of the NTS neurons activated by sensory inputs from the airways (Fig. 7). In addition, the role of the glutamate-AMPA receptor signaling pathway in transmission of bronchoconstrictive signals from the airways to the NTS was studied using physiological experiments. Blockade of AMPA receptors by bilateral microinjections of CNQX into the commissural subnucleus elicited a significant decrease in reflex tracheal smooth muscle response, markedly slowed the rate of rise of tracheal tone, decreased the peak response, and enhanced the decline of the response after cessation of lung deflation (Fig. 7, bottom). Therefore, bronchoconstrictive inputs from the airways to NTS neurons are transmitted primarily by a glutamate-AMPA receptor signaling pathway.

In addition, studies suggest that NMDA receptors expressed by the NTS neurons that receive afferent inputs from the airways enhance the glutamate-AMPA receptor-mediated responses. These receptors are present in vagal afferents and their dendritic targets in the NTS. Hence they may play a role in autoregulation of the presynaptic release and postsynaptic responses to glutamate at the level of the first central synapse in the NTS (2).

Therefore, it seems that the involvement of AMPA receptors in airway reflex responses is determined by their kinetics, which is much faster than those of the NMDA or kainite receptors. Hence the AMPA receptors are more appropriate for transmission of fast signals, whereas NMDA and kainite receptors may contribute to primary visceral affrent transmission in the NTS, mediating tonic influences.

**G protein-coupled metabotropic glutamate receptors.** G protein-coupled metabotropic glutamate receptors are expressed in autonomic cell groups of the medulla oblongata (79), including NTS second-order neurons (136, 158, 162). Coactivation of iGluRs and G protein-coupled metabotropic glutamate receptors (mGluRs) may affect glutamatergic transmission through regulation of neurotransmitter release. On the basis of amino acid sequence homology, pharmacology, and signal transduction mechanisms, these subtypes have been classified into three groups (103, 136, 170, 196). Group I mGluRs consist of mGluR1 and mGluR5 that are found mainly on postsynaptic terminals and are positively coupled to phospholipase C. Group II mGluRs (mGluR2 and mGluR3) are observed on both pre- and postsynaptic terminals, whereas group III mGluRs (mGluR4, mGluR6, mGluR7, and mGluR8) are predominantly located in or near presynaptic zones. In general, group I mGluRs increase neuronal excitability, whereas group II and group III modulate frequency dependence of synaptic vesicle exocytosis, inhibiting signal transmission (33, 36, 128, 158, 161, 164, 184).

It is accepted that released glutamate acts locally on postsynaptic receptors and is cleared from the synaptic cleft within a few milliseconds by diffusion and by specific reuptake mechanisms. This rapid clearance restricts the spread of neurotransmitter and, combined with the low affinities of many ionotropic receptors, ensures that synaptic transmission occurs in a point-to-point fashion. However, when glutamate release is enhanced
at synapses, the concentration increases and glutamate escapes the synaptic cleft and, via volume transmission, may activate presynaptic inhibitory mGluRs. Hence, at higher frequency stimulation, when the released amount of glutamate exceeds the uptake quanta, presynaptic mGluRs of groups II and III become activated, leading to a rapid inhibition of neurotransmitter release (58, 184) and, consequently, presynaptic depression of synaptic transmission (33) that might have neuroprotective effects (61).

In the rat, activation of mGluRs affects frequency dependence response and depresses vagal and aortic baroreceptor signal transmission in the NTS (66, 127, 128, 144) via activation of one or more phosphoprotein phosphatases such as phosphoprotein phosphatase 2 and/or calcineurin (67). Furthermore, recent findings, determining presynaptic vs. postsynaptic effects, indicate that glutamate released at the first central baroreceptor synapses cannot only regulate its own signaling but can further shape signal transmission by suppressing GABA release via activation of heterosynaptic group II mGluRs (39). The results highlight the complexity of mGluRs functions in modulation of reflex responses within the NTS and suggest the importance of differentiation of presynaptic vs. postsynaptic effects of drugs tested.

In ferrets, recent neuroanatomical and physiological studies showed that subtype 1 of the group I mGluRs is rarely expressed by activated NTS neurons after stimulation of bronchopulmonary receptors. Blockade of the group I mGluRs using a specific antagonist had no significant inhibitory effects on airway reflex responses, when administered into the NTS. Furthermore, blockade of groups II/III mGluRs within the NTS had no significant enhancing effect on reflex airway smooth muscle contraction (Ferguson DG, Haxhiu MA, unpublished results), forming the morphological basis for the powerful influence of excitatory amino acids on the activity of AVPNs (Fig. 8). Effects of glutamate on AVPNs are mediated through different subtypes of glutamate receptors.

**Iontotropic Glutamate Receptors and Transmission of Excitatory Signals From NTS to AVPNs**

Neurochemical studies indicate the presence of AMPA, NMDA, and kainate receptors in vagal preganglionic neurons (45). Neurophysiological investigations have shown that iGluRs play an important role in transmission of excitatory inputs to the AVPNs regulating airway smooth muscle tone. For example, NMDA receptors are expressed by AVPNs and, when activated with a specific NMDA receptor agonist (NMDA), cause airway constriction that is blocked by selective NMDA antagonists (83). The possibility that NMDA receptors may also play a role in reflex airway constriction has been studied by inducing blockade of NMDA receptors with 2-amino phosphonovalerate. Topical application or microinjection of this NMDA antagonist within the rNA, the area in which the AVPNs are located, only slightly affected reflex changes in tracheal tone. However, administration of selective antagonists for the AMPA/kainite subtype of glutamate recep-
tor to the same site caused a dose-dependent decrease in reflex response of tracheal tone induced by 1) lung deflation, 2) stimulation of laryngeal cold receptors, and 3) activation of peripheral or central chemoreceptors (91). These reflexes are known to cause centrally mediated increases in cholinergic tone (42, 51, 52). Inhibitory effects of AMPA receptor blockade were potentiated by prior administration of an NMDA receptor antagonist. Thus an increase in central cholinergic outflow to the airways by a variety of reflex excitatory inputs is mediated mainly via glutamate-AMPA receptors that in turn activate the NMDA receptor signaling pathway.

Physiological studies indicate that increases in airway blood flow and submucosal gland secretion are integral components of pulmonary defensive reflex responses (42, 43, 171, 218). Neural regulation of the bronchial vasculature differs from that of the general systemic circulation in that vasodilator reflexes play a major part in determining blood flow (43). These reflexes originate in the upper or lower airways, in carotid chemoreceptors, or in cardiac chemosensitive nerves. Those arising from the lower airways are the most potent and may increase bronchial blood flow several-fold and cause swelling of the airway mucosa. In addition, neuropeptides released from C fiber terminals provide a local mechanism for vasodilation independent of central reflex control. This so-called axon reflex plays a major role in bronchial vasodilation in rodents but makes only a small contribution in larger animals. In dogs, a centrally mediated vagal reflex vasodilator pathway appears more important. Cooling the cervical vagus nerves eliminates afferent and efferent vagal pathways but preserves local mechanisms (including axon reflex pathways). This treatment diminishes more than two-thirds of the reflex vasodilation; thus a large portion of the vasodilation in dogs, whether caused by activation of sensory C fiber receptors and/or RARs, is due to centrally mediated neural reflexes that include afferent and efferent pathways in the vagus nerves (171). In addition, stimulation of bronchial and pulmonary C fibers or RARs evokes a reflex increase in secretion by tracheal submucosal glands. The responses are abolished on interruption of the afferent and efferent transmissions by cutting the vagus nerves or cooling them to 0°C (42, 218). Furthermore, focal cooling of the rostral ventrolateral medulla between 20°and 15°C significantly decreases the secretion rates produced by capsaicin-induced stimulation of pulmonary C fiber receptors and by mechanical stimulation of the carina and larynx (87).

The involvement of glutamate and glutamate receptors in the transmission of excitatory inputs from the airway sensory receptors to the NTS and from this site to the AVPNs was studied (91). Stimulation of airway sensory fibers by lung deflation induced reflex increases of tracheal blood flow (Fig. 9) and submucosal gland secretion (Fig. 10). These responses were diminished by prior administration of an AMPA/kainate receptor antagonist, CNQX, into the fourth ventricle, or microinjection of AMPA/kainate receptor blockers into the external formation of the rNA, where the AVPNs are located. These findings indicate that the transmission of excitatory inputs from the NTS to the AVPNs is mediated mainly via the

![Fig. 9. Glutamatergic control of reflex-induced changes in airway blood flow. Top: schematic presentation of the extrathoracic tracheal segment that can be used for simultaneous recording of submucosal blood flow, gland secretion, and airway smooth muscle tone. Tracheal segment preparation used to measure trachealis smooth muscle tension was developed by Brown et al. (29), whereas video camera method of measuring mucous secretion was developed by Davis et al. (50). Bottom: tracings from decerebrate, paralyzed, and mechanically ventilated dog after administration of vehicle into the IV ventricle. Vehicle had no effects on resting tracheal circulation. Lung deflation induced an increase in submucosal blood flow (Qt; left) and slightly elevated systemic blood pressure (BP; mmHg). Administration of CNQX, indicated by the arrow, tended to decrease the basal blood flow and abolished the response to lung deflation (right). V (Us), air flow. Modified from Ref. 93.](http://jap.physiology.org/Downloadedfrom)
release of glutamate and activation of the AMPA/kainate subtype of glutamate receptors. Therefore, it is likely that the airway sensory stimulation-evoked airway smooth muscle contraction, vasodilation, and hypersecretion are mediated mainly via cholinergic mechanisms, using the same, glutamate-AMPA signaling pathway, that in turn activate NMDA receptors, as summarized in Fig. 11.

Metabotropic Glutamate Receptors and Transmission of Excitatory Signals From the NTS to AVPNs

In ferrets, mGluRs belonging to group I are expressed by AVPNs (Ferguson DG, Haxhiu MA, unpublished data). Their role in glutamatergic transmission of bronchoconstrictive inputs was investigated using a selective antagonist. Blockade of these receptors within the rNA region had no significant effect on airway basal smooth muscle tone or on reflex bronchoconstriction. Similarly, inhibition of group II/III mGluRs had no demonstrable facilitatory effect on reflex increases in unit activity of the AVPNs (Kc P, Haxhiu MA, unpublished data), suggesting that the physiological relevance of the excitatory and/or inhibitory mGluRs is, at best, limited. However, these findings do not exclude the possibility that these receptors may operate at higher frequency afferent inputs, enhancing glutamatergic responses (group I mGluRs), or suppressing excessive signal transmission at AVPN synapses (groups II and III mGluRs), consequently modulating the transmission of bronchoconstrictive inputs from the NTS to the AVPNs within the rNA.

CENTRAL GABAERGIC CONTROL OF CHOLINERGIC OUTFLOW TO THE AIRWAYS

Processing of central afferent excitatory signals by the AVPNs and conveying integrated output to the airways are highly dependent on synaptic GABAergic inhibitory inputs (152). In general, GABA release and activation of postsynaptic

Fig. 10. Glutamatergic control of reflex changes in submucosal secretion. An example of the effect of CNQX administration into the IV ventricle on secretory response of tracheal submucosal glands to lung deflation. Prior administration of CNQX abolished the response of submucosal glands to lung deflation. A and C: tracheal epithelium 1 min after spraying with tantalum (an inert metal dust that prevents spread of secreted fluid), baselines. B and D: tracheal epithelium 1 min after lung deflation after vehicle (B) or after CNQX administration into the IV ventricle (D). Similar effects were observed when CNQX was bilaterally microinjected into rNA. Modified from Ref. 93.
GABA receptors play an important role in controlling neuronal excitability in adult mammals. This occurs by increasing membrane conductance to chloride ions, thereby causing a potent inhibition of neuronal activity that in turn diminishes the depolarizing effects of excitatory synaptic signals (208). The action of released GABA within or in close vicinity to the synaptic cleft is terminated by its rapid uptake into surrounding neurons and astrocytes by selective GABA transporters (53, 81, 186).

GABAergic Microcircuitry Regulating AVPNs

GABA, the main inhibitory neurotransmitter in mammalian brain, is synthesized mainly via decarboxylation of glutamic acid by two specialized enzymes, the glutamic acid decarboxylases (GADs), designated GAD65 and GAD67 according to their molecular masses (65 and 67 kDa). These enzymes differ in their affinities for their cofactor pyridoxal-5’-phosphate and are encoded by two different genes located on separate chromosomes (32, 60). Both enzymes are often colocalized in the same GABAergic neurons but sometimes differ in their subcellular distribution. GAD67, although also detected in axon terminals, is thought to be preferentially localized to cell bodies, whereas GAD65 tends to be associated with synaptic vesicles in nerve terminals (62, 100). However, more detailed studies revealed a more complex situation, showing that both GAD65 and GAD67 provide important reserve pools of GABA for the regulation of inhibitory neurotransmission, and a deficiency of either isoform of GAD can have significant physiological sequelae. For example, GAD67-deficient animals are born with a cleft palate and die within the first day of life, apparently from respiratory failure (8). By contrast, GAD65-deficient mice appear normal at birth, but the pyridoxal-5’-phosphate-inducible apoenzyme reservoir is significantly decreased, leading to an increased susceptibility to epileptogenic stimuli (7). Hence both isoforms of GAD appear to be physiologically important in the dynamic regulation of neural network excitability.

Recent ultrastructural studies have shown that retrogradely labeled AVPNs receive a significant GABAergic innervation (Fig. 12). Out of a pooled total of 3,161 synaptic contacts with retrogradely labeled somatic and dendritic profiles, 20.2% are GAD-immunoreactive (IR), forming significantly more axosomatic symmetric synaptic specializations than axodendritic synapses (P < 0.02). These ultrastructural findings indicate that central GABAergic modulation of cholinergic outflow is mediated in large part via classically defined inhibitory axoso-
motic synapses and to a lesser degree through axodendritic synaptic transmission. A dense population of GABAergic synaptic contacts on AVPNs provides a morphological basis for the potent physiological effects of GABA on the excitability of AVPNs (150).

Both axosomatic and axodendritic GABAergic innervation may exert modulatory tonic influences. For example, tonic GABAergic signaling regulates the activity of cerebellar granule cells (28, 76). Similarly, the activity and the discharge-frequency patterns of medullary respiratory premotor neurons are subject to potent tonic GABAergic gain modulation (140, 220). The axodendritic synaptic GABAergic modulation of AVPNs structurally distant from the soma/spike initiation zone and proximal dendrites may exert less impact on the resting membrane potential of these neurons than do axosomatic synapses close to the axon hillock or proximal axodendritic GABAergic synapses. However, quantitative assessments of the relative distribution of GABAergic synapses on various parts of individual AVPNs must necessarily be somewhat imprecise because the distal parts of the AVPN dendrites were not as robustly retrogradely labeled with CT-b-horseradish peroxidase as the more proximal portions of the dendrites and the perikarya and therefore these distal synaptic interactions would be seen less frequently. Nonetheless, the ultrastructural data clearly demonstrate that a central GABAergic inhibitory microcircuit uses both axosomatic and proximal axodendritic synapses on AVPNs to modulate cholinergic drive to the tracheobronchial system (150).

GABA Levels Within the rNA

Recently, baseline levels of GABA within the rNA region have been measured by microdialysis and HPLC. After equilibration of the dialysis probe, the average measured concentration of GABA was 29.3 ± 7.1 pg/20 μl during a steady-state condition. After stimulation of the ventrolateral region of the vl PAG, GABA concentration significantly increased (61.4 ± 22.1 pg/20 μl; Fig. 13, top). Although the increase in GABA levels after chemical stimulation is of an exocytotic origin, the basal levels of extracellular GABA could be derived in part from nonexcytotic source, as in other brain regions (53, 198).

Extracellular levels of GABA within the rNA, measured by microdialysis and HPLC techniques, can originate from various sources and depend upon release, diffusion, and uptake mechanisms. GABA released from GABAergic nerve endings can spill over from the synaptic cleft to perisynaptic or extrasynaptic regions (76, 149, 178) and activate extrasynaptic receptors, including those on the edge between effective synapses and uninnervated zones, modulating the gain and maintaining inhibitory tone (148, 187). GABA levels within the synaptic cleft and extrasynaptic regions depend on the activity of high-affinity GABA transporters (i.e., GAT-1) located in axon terminals around the synaptic cleft and/or expressed by surrounding astrocytes (81, 186). GABA uptake mechanisms play a critical role in termination of both synaptic and extrasynaptic GABAergic inhibitory signaling. To a lesser degree, however, GABA spillover through activation of GABA<sub>B</sub> receptors on nerve terminals may control neurotransmitter release at the target site (3, 185).

**GABA Receptor-Mediated Signaling**

GABA receptors are categorized in two distinct types: the ionotropic GABA<sub>A</sub> and GABA<sub>C</sub> and the metabotropic GABA<sub>B</sub> receptors.

**GABA<sub>A</sub> Receptors.** Fast GABA-mediated synaptic inhibitory neurotransmission requires that GABA<sub>A</sub> receptors are expressed and assembled at appropriate postsynaptic sites facing GABA-releasing nerve terminals (124, 130, 149). These chloride ion channel-associated, ligand-gated receptors are heteropentamers that can be formed from a number of subunit classes that include: α (1–6), β (1–4), γ (1–3), δ, ε, θ, π (1 isoform each), and ρ (1–3). The majority of these receptors are composed of two α-, two β-, and one γ<sub>2</sub>-subtype. The coexpression of a γ<sub>2</sub>-subunit with α- and β-subunits produces GABA<sub>A</sub> receptors displaying high-affinity binding for central...
benzodiazepine ligands and anesthetics (15, 23, 149, 174, 189, 190). Changes in GABA_A receptor subtype expression induce plasticity in fast synaptic inhibition. For example, a decrease in the α_1:α_2-subunit mRNA ratio is associated with a decrease in potentiation and increase in decay time constant of GABA_A receptor-mediated inhibitory postsynaptic potentials (30).

The exact composition of the GABA_A receptor subtype expressed by the AVPNs that mediate airway changes induced by GABA remains to be characterized. However, our most recent study demonstrated that clusters of the β_2-isoform of GABA_A receptor subunit are present on the cell bodies and nerve processes of the AVPNs (96) as shown in Fig. 13, bottom. This subunit plays an important role in determining the pharmacology of the GABA_A receptors (75). Furthermore, physiological and pharmacological studies indicated that benzodiazepines, topically applied or microinjected into the rNA region, cause withdrawal of cholinergic outflow to the airways and airway smooth muscle relaxation (85), indicating that this receptor subtype coexpresses a γ_2-subunit (189).

**GABA_B receptors.** In the mammalian CNS, GABA_B receptors exist as a functional GABA_B1/GABA_B2 heterodimer (46). It was thought that the GABA_B receptors function predominantly presynaptically and that they are activated only when GABA is released in large amounts, e.g., on simultaneous activation of multiple GABAergic neurons. Activation of GABA_B receptors produces a presynaptic inhibition and a decrease of neurotransmitter release, including glutamate and ACh (3, 185, 188). Inhibition is mediated either by blocking the action potential invasion into nerve terminals or by reducing the amplitude of the propagated signal. This may occur via multiple mechanisms, including depression of Ca^{2+} channels (3). GABA_B receptors are also expressed by effector cells and can produce hyperpolarization in postsynaptic membranes (46). Furthermore, our preliminary studies demonstrated that GABA_B receptors are expressed by axon terminals innervating the AVPNs and by cell bodies of AVPNs (Kc P, Haxhiu MA, unpublished data) that, in part, may mediate pre- and postsynaptic modulatory effects of GABA on glutamatergic transmission.

**GABA_C receptors.** GABA_C receptors are less abundant and less widely distributed in the CNS compared with GABA_A or GABA_B receptors. These receptors, described in the rodent retina, the optic tectum, hippocampus, pituitary gland, and spinal cord, gate a Cl^- channel similar to GABA_A receptors but exhibit a different pharmacological profile. The main difference between the GABA_C receptors and conventional GABA_A receptors is their bicuculline and barbiturate resistances. Furthermore, GABA_C, in contrast to GABA_A receptors, do not desensitize, rendering them particularly well suited for processing graded potentials (110, 173). Separate lines of recent evidence suggest that the effects of GABA in central neurons can be mediated by coassembly of GABA_A and GABA_C receptor subunits (146). The role of this receptor subtype in mediating GABAergic modulation of AVPNs and cholinergic outflow to the airways needs to be studied.

**Tonic GABAergic influences.** GABA_A receptor signaling may exert tonic and phasic inhibitory effects (148), thereby modulating the gain and the firing threshold of AVPNs. Recently, Moore et al. (150) reported that under baseline conditions, GABA_A-receptor-mediated tonic inhibitory currents are important determinants of AVPN firing rate. Microinjection of a low concentration of the GABA_A receptor blocker bicuculline into the rNA region significantly increased the frequency of spontaneous discharge of the AVPNs (Fig. 14) and led to an increase in airway smooth muscle tone. These responses were not due to bicuculline-induced blockade of apamine-sensitive Ca^{2+}-activated currents (109), indicating that tonically active GABA_A receptors are present and play a role in the control of bronchomotor tone. The tonic GABAergic inhibition could be...
mediated via nonsynaptic transmission (1, 53), through GABA<sub>A</sub> receptors with higher affinity then those needed for synaptic GABAergic signaling, and/or synapses close to the soma (187, 192, 193, 217).

Tonic GABA<sub>A</sub> receptor-mediated inhibitory currents can be influenced by changes in GABA release, enhanced GABA uptake, and alterations in the assembly of GABA<sub>A</sub> receptor isoforms that affect the proportion of high- and low-affinity GABA<sub>A</sub> receptor subunits. These modifications may either augment or decrease the neuronal excitable state. Recently it was shown that the tonic GABA<sub>A</sub> receptor-mediated inhibition can be enhanced by the action of steroid anesthetics (14) or by neurosteroids synthesized by glia and neurons (18) that are involved in ethanol action, tolerance, and dependence (151). An increase in neurosteroid concentration would produce a local, positive modulation of the effect of GABA on GABA<sub>A</sub> receptor gain and thereby can contribute in maintaining inhibitory tone (187).

The GABA modulatory actions of the neurosteroids are dependent on the GABA<sub>A</sub> receptor’s subunit composition (18). GABA-evoked responses mediated by α<sub>1</sub>β<sub>2</sub>γ<sub>2</sub>- and α<sub>1</sub>β<sub>1</sub>γ<sub>2</sub>-receptors are significantly potentiated by a relatively low concentration of neurosteroids, whereas α<sub>4</sub>β<sub>2</sub>γ<sub>2</sub>-receptors are relatively insensitive to these compounds. The isoform of the β-subunit (1–3) was observed to have little influence on the GABA modulatory actions of the pregnane steroids. Whereas the presence of a γ<sub>2</sub>-subunit within the GABA<sub>A</sub> receptor complex is essential for a robust benzodiazepine effect at submicromolar concentrations (190), replacement of the γ-subunit by the δ-subunit enhances steroid sensitivity of the receptor (18). Expression of the δ-subunit in the brain is relatively restricted, region selective, and with an extrasynaptic location, being the major contributor of tonic GABAergic nonsynaptic inhibition. As shown by Moore et al. (150), tonic inhibitory currents may exert a considerable influence on neuronal signaling; therefore, these receptors may be expressed by AVPNs at extrasynaptic sites, a short distance from the subregion innervated by GABA-containing axon terminals (Fig. 15).

Hormonal changes have been suspected to be a cause of increases in airway dysfunction in a subgroup of female asthmatic patients. In these subjects, episodic and potentially fatal increases in the severity of asthma typically occurs during the few days before their menses in the absence of other trigger factors and are associated with a rapid fall in serum progesterone (59). The major metabolite of progesterone, 3α-OH-dihydroprogesterone (3α-OH-DHP), is the most potent endogenous positive modulator of CNS GABA<sub>A</sub> receptor function and has an action similar to that observed for neurosteroids. Hence it can be postulated that in female patients with severe premenstrual worsening of bronchial asthma (59) as well as in postmenopausal asthma (122), beneficial effects of hormone replacement therapy in reducing the severity of airway dysfunctions could be partly mediated by enhanced central GABAergic influences on the AVPNs, which cause withdrawal of cholinergic outflow to the airways. However, it could be expected that long-lasting exposure of GABA<sub>A</sub> receptors to steroids and to other modulators, as well as their withdrawal, may induce marked effects on receptor structure and function. Possible synergic action between endogenous steroids and GABAergic pathways in modulating the functional activity of AVPNs needs to be studied.

In summary, the functional relevance of the GABAergic microcircuit in the central regulation of cholinergic outflow to the airways is presented in Fig. 15. Abnormalities of GABAergic function caused by either genetic or acquired alterations of GABA<sub>A</sub> receptors or ion channelopathies may result in a shift from inhibitory to excitatory neurotransmission. These changes may lead to a hyperexcitable state of AVPNs and to airway hyperreactivity. Hence enhancement of GABA-induced neuronal inhibition may be useful in treating disorders associated with an increased cholinergic outflow to the airways (i.e., obstructive bronchitis and bronchial asthma).

**PARACRINE-AUTOCRINE REGULATION OF AVPNs**

Activity of the vagal preganglionic neurons innervating the airways is extrinsically regulated by the neurotransmitters or neuromodulators that are released from the nerve terminals that innervate them. In addition, these inputs may selectively modulate intrinsic oscillatory processes, unrevealed by activation of NMDA receptors (83) or inhibition of GABAergic inputs of
AVPNs blocking GABA_A receptors (150). Intrinsic synchronization of the activity of AVPNs may provide coordinated motor commands to the airway effector systems.

Excitability of AVPNs may also be enhanced via paracrine-autocrine mechanisms and through suppression of inhibition. Although direct evidence for the activity-dependent release of ACh, VIP, or BDNF from AVPNs into surrounding environment is lacking, it has been shown in other cell groups that these molecules can be released from postsynaptic neurons and their dendritic vesicle clusters, supporting the concept that they can act as messengers, changing basal and stimulated neuronal discharge (12, 101). Furthermore, BDNF could be required and sufficient for long-term facilitation of excitatory inputs, as convincingly shown for spinal respiratory plasticity after intermittent hypoxia (12).

Neutrophins and neutrophin receptors play an important role in the pathophysiology of allergic bronchial asthma (159). Apart of peripheral effects, neurotrophins such as BDNF may affect dendritic growth (105) and facilitate glutamatergic synaptic transmission, partly via suppression of inhibitory inputs. The site of action of BDNF was found to be predominantly postsynaptic, caused by acute downregulation of GABA_A receptor surface expression (31) and decreasing the efficacy of inhibitory transmission by acute postsynaptic downregulation of Cl^- transport (207). Hence, BDNF through increasing excitability of AVPNs may contribute to airway hyperresponsiveness in asthmatic patients.

In summary, recent studies provide a wealth of new information about paracrine-autocrine regulation of AVPN responses and on the interactions between excitatory and inhibitory signaling pathways that may set the stage for airway hyperreactivity. Elucidating processes that affect the balance between the excitatory and inhibitory inputs to the AVPNs will contribute considerably to better understanding mechanisms underlying enhanced AVPN excitability, increased airway responsiveness, sustained obstruction of the airways, and altered smooth muscle relaxation.

FUNCTIONAL RELEVANCE OF THE CENTRAL CONTROL OF CHOLINERGIC OUTFLOW TO THE AIRWAYS

Under normal conditions, an increase or a decrease in chemical drive induces parallel changes in airway smooth muscle tone and breathing pattern, revealing a link between the neuronal networks that control the resistance of the tracheobronchial conduits and the respiratory drive to chest wall pumping muscles (83, 145). Recently, the neuroanatomical basis for this integration has been studied by combining a retrograde tracer technique and a transneuronal labeling method (90). It was shown that a subset of bulbospinal cells that project to the phrenic nuclei also innervate AVPNs, coupling inspiratory activity and parasympathetic outflow to the airways.

Physiological findings indicate that, during normal breathing and changes in chemical drive, cholinergic outflow to the airways changes in parallel with the inspiratory drive of the phrenic nerve (51, 52, 132, 145). However, responses of the phrenic nerve and AVPNs need not parallel each other. For example, chemical stimulation of pulmonary C fiber receptors, or the aspiration reflex, causes an inhibition of inspiratory activity, but induces an increase in cholinergic outflow to the airways that leads to an elevation of airway smooth muscle tone (42). In contrast, activation of the vl PAG induces a release of GABA within the rNA region and airway smooth muscle relaxation, but increases phrenic nerve activity (96). Furthermore, excitation of somatosensory fibers elicits an augmentation in respiratory output but a decrease in bronchomotor tone that cannot be explained by adrenergic or nonadrenergic noncholinergic (NANC) inhibitory influences (117, 191). Taken together, these results indicate that AVPNs within the rNA form a distinct and identifiable cell group, the activity of which is regulated by multiple CNS nuclei using common and/or specific neuronal pathways.

During normal breathing and after changes in chemical drive, airway smooth muscle tone and airway resistance manifest rhythmic fluctuations that are superimposed on baseline levels (82, 145). After hypocapnic apnea, in which there is a complete cessation of phrenic nerve discharge, the gradual increase in arterial P_CO2, or step decrease in inspired O_2, causes a progressive increase in airway smooth muscle tone (51, 52) that commences before the reappearance of phrenic nerve discharge. A similar response is observed after stimulation of AVPNs by activation of the NMDA receptors within the rNA region. With the onset of rhythmic phrenic nerve firing, rhythmic oscillations of airway smooth muscle tone and airway resistance can be observed (83). Within the respiratory cycle, the peak airway smooth muscle tone and airway resistance appear early in expiration, in phase with the postinspiration inspiratory activity of the phrenic nerve (82). As a result of the elevation of airway smooth muscle tone, the airway and tissue components of total lung resistance reduce the dead space and oppose distortion of the airways and the most distal ventilatory units. Phasic oscillation, with the peak in early expiration, may serve to optimize gas exchange by reducing large fluctuations in the functional residual capacity and protect the airway from collapsing. Therefore, the central coordination of cholinergic outflow to the airways with changes in the respiratory drive and fluctuations of smooth muscle tone may help in maintaining the airway wall stability, optimizing gas exchange and the work of breathing, on a breath-by-breath basis throughout the respiratory cycle. Loss of central cholinergic outflow may cause significant alterations in respiration and smooth muscle functions.

It has been shown that unilateral infarct involving the medullary reticular formation and NA is sufficient to generate Ondine’s curse, a loss of automatic respiratory control and hypoventilation during sleep (24). Furthermore, congenital failure of autonomic control could be expressed with an abnormal brain stem integration of central and peripheral chemoreceptor inputs and the primary hypoventilation. These disorders are often associated with other neurocristopathies, especially with Hirschsprung’s disease (loss of the gastrointestinal motility), an association known as Haddad syndrome (73). We assume that diminished cholinergic outflow to the airways may contribute to alterations in airway function and ventilation in these disorders. This assumption is supported by recent experiments showing that interruption of the nerve supply to the lungs (for instance after lung transplantation) abolishes the integration of bronchomotor and ventilatory activities, and, by increasing airway deformation due to loss of airway smooth muscle tone, initiates the fibroproliferative responses in the airway walls. This causes structural changes that are analogous...
to those observed in bronchiolitis obliterans after lung transplantation, or in vagally denervated rats (37). Certainly, if the protective nature of centrally mediated vagal tone to the airways is overexpressed and sustained it may cause significant functional changes.

ALTERATIONS IN CENTRAL CONTROL OF CHOLINERGIC OUTFLOW AND AIRWAY DISORDERS

Repeated exposures to noxious substances, allergens, and cigarette smoke cause alterations in the sensory and intrinsic neuronal networks regulating airway responses (38, 112, 200, 201). Furthermore, under these conditions, remodeling of the brain stem circuitry associated with a central increase in cholinergic drive to the airways may occur. Changes, particularly in glutamatergic excitatory transmission, including enhanced synaptic efficacy and the growth of new connections can increase the susceptibility of the airways to bronchoconstrictive stimuli. The resulting transformations may, in turn, increase the expression of BDNF and TrkB receptors that contribute to an upregulation of glutamatergic excitatory and a downregulation of GABAergic inhibitory influences, thereby enhancing the AVPN susceptibility to excitatory inputs. A shifting balance from inhibition to excitation may facilitate initiation and a sustained reflex bronchoconstriction. For example, decreases in tonic GABAergic inhibitory influences may predispose individuals for air hyperreactivity and sustained bronchoconstriction following reflexly induced airway narrowing (150), exercise (142), and premenstrual or postmenopausal accentuated decrease in steroids in asthmatic patients (59, 122). Hence strategies for targeting central components of airway disorders should include interruption of processes that lead to central sensitization and enhanced responsiveness of AVPNs. Two potential strategies, for example, are use of RNA interference (RNAi) to downregulate BDNF expression and tyrosine kinase receptor inhibition to block BDNF signaling (48). This may diminish facilitation of bronchoconstrictive responses, an assumption that is supported by recent work demonstrating that under in vivo conditions, the BDNF-TrKB pathway is required for intermittent hypoxia-induced long-term facilitation of phrenic nerve output (12).

CONCLUSIONS AND FUTURE STUDIES

In conclusion, the integration and processing of bronchoconstrictive inputs by the AVPNs and the level of cholinergic output to the airways is highly dependent on synaptic and perisynaptic glutamatergic excitatory and GABAergic inhibitory inputs to the AVPNs. Conceivably, the outcome of these competing regulatory processes could be influenced by a number of other pathways (95, 98) using different neurotransmitters and/or neuromodulators, suggesting the complexity of the CNS control. Understandably, this expands greatly the scheme presented in Fig. 1, leading to a conceptual framework that includes behavioral state control (sleep-waking cycle), psychological dimensions of airway regulatory mechanisms (fear, anxiety, mood), and possibly reentrant loops. Hence the central control of AVPNs is a complex system of obviously interrelated networks that shape the final AVPN output. The degree and nature of the relationship between different pathways is imperfectly known, a fact that should elevate the enthusiasm for future studies in central regulation of airway functions.

Much clinically useful information may be derived from the studies on CNS control of airway functions. For example, studies on a molecular network and signaling pathways, which lead ultimately to central hyperresponsiveness of AVPNs, will provide a better understanding of the mechanisms through which the lung injury sets the stage for centrally mediated airway hyperreactivity. Furthermore, elucidation of the CNS determinants of sleep-related airway instability and worsening of airway functions during sleep (i.e., nocturnal asthma) are critical and interrelated areas that offer exciting and useful findings. Similarly, identification of hidden links between phenotypic changes in central neural processing and emotional perturbations in asthmatic patients, will allow us to better characterize the interplay among biological, cognitive, genetic and social factors that contribute to airway hyperresponsiveness, sustainability of bronchoconstrictive changes, and altered emotional coping. The progress made in the CNS control of the airways will contribute to the construction of a more complete conceptual framework that will provide deeper insight into the pathophysiology of airway disorders, reorganize our thinking, and allow us to switch effortlessly to more complete and effective treatment of patients with airway dysfunctions.

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