Published in final edited form as:

Respir Physiol Neurobiol. 2010 October 31; 173(3): 213–222. doi:10.1016/j.resp.2010.03.020.

Role of Central Neurotransmission and Chemoreception on Airway Control

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Abstract

This review summarizes work on central neurotransmission, chemoreception and CNS control of cholinergic outflow to the airways. First, we describe the neural transmission of bronchoconstrictive signals from airway afferents to the airway-related vagal preganglionic neurons (AVPNs) via the nucleus of the solitary tract (nTS) and, second, we characterize evidence for a modulatory effect of excitatory glutamatergic, and inhibitory GABAergic, noradrenergic and serotonergic pathways on AVPN output. Excitatory signals arising from bronchopulmonary afferents and/or the peripheral chemosensory system activate second order neurons within the nTS, via a glutamate-AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor signaling pathway. These nTS neurons, using the same neurotransmitter-receptor unit, transmit information to the AVPNs, which in turn convey the central command through descending fibers and airway intramural ganglia to airway smooth muscle, submucosal secretory glands, and the vasculature. The strength and duration of this reflex-induced bronchoconstriction is modulated by GABAergic-inhibitory inputs. In addition, central noradrenergic and serotonergic inhibitory pathways appear to participate in the regulation of cholinergic drive to the tracheobronchial system. Down-regulation of these inhibitory influences results in a shift from inhibitory to excitatory drive, which may lead to increased excitability of AVPNs, heightened airway responsiveness, greater cholinergic outflow to the airways and consequently bronchoconstriction. In summary, centrally coordinated control of airway tone and respiratory drive serve to optimize gas exchange and work of breathing under normal homeostatic conditions. Greater understanding of this process should enhance our understanding of its disruption under pathophysiologic states.

Keywords

Airways; Airway reflex responses; Nucleus tractus solitarius; AVPNs; Glutamatergic pathways: Glutamate; AMPA receptors; NMDA receptors; GABAergic microcircuitry: GABA_A receptors; Serotonergic pathways; Adrenergic pathways; Parallel control of airways and respiration

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1. Introduction

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. Airway bronchoconstrictive responses protect the respiratory system via a bidirectional CNS-airway communication. However, repeated exposure to environmental pollutants may modulate afferent airway sensory pathways (Chen et al., 2001, Joad et al., 1998, Riccio et al., 1996, Undem et al., 2000), setting the stage for increased excitability of airway-related vagal preganglionic neurons (AVPNs) and airway hyperresponsiveness. In this review, we discuss afferent and efferent neuronal pathways that project to the AVPNs, and the central neurotransmitters that are involved in modulating this output. In addition, alteration in chemical drive may induce parallel changes in airway smooth muscle tone and breathing pattern. Gaining a better understanding of the role played by CNS pathways in regulating airway functions in normal and diseased states, may provide novel therapeutic approaches in the treatment of related pathophysiological diseases.

2. Neural control of the airways

CNS control of airway functions involves integrated networks along the central neural axis that modulate tracheo-bronchopulmonary effector units via AVPNs located within the rostral nucleus ambiguus (rNA) in the medulla oblongata. The AVPNs are the final common pathway from the brain to the airways; they transmit signals to the intrinsic tracheobronchial ganglia which lie in close proximity to effector systems (Baker et al., 1986, Coburn, 1984, Dey et al., 1996, Maize et al., 1998). Signals transmitted through the preganglionic nerves are relayed, integrated, filtered, and modulated by intrinsic ganglionic neurons before reaching the airway neuroeffector sites through postganglionic axons. Varicose fibers from these postganglionic axons are distributed along the effector organs. Cholinergic mechanisms are involved in controlling the conductivity of the most distal airways and tissue resistance (Khassawneh et al., 2002, Martin et al., 1995), by influencing smooth muscle tone, lung interstitial pericytes and alveolar myofibroblasts (Kapanci et al., 1992). 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. Intrinsic airway neurons contain neurochemicals other than acetylcholine (ACh), including vasoactive intestinal peptide (VIP) and nitric oxide (NO) (Dey et al., 1996, Zhu and Dey, 2001) which are mediators of the non-adrenergic non-cholinergic system, and produce relaxation of airway smooth muscle (Berisha et al., 2002, Diamond and O'Donnell, 1980, Hasaneen et al., 2003). These observations suggest that AVPNs may be involved in regulating the release of biologically active molecules such as NO from epithelial cells to oppose excessive cholinergically-mediated airway contractile responses (Khassawneh et al., 2002).

Vagal afferent fibers of sensory ganglia innervate the bronchopulmonary sensory receptors that are specialized for detecting changes in chemical, mechanical, or thermal stimuli. The cell bodies of the bipolar airway vagal afferent neurons are located in the nodose and jugular ganglia, and participate in reflex events. Lung sensory receptors with afferent fibers coursing in the vagus nerves are broadly divided into three groups: bronchopulmonary C fibers, slowly adapting stretch receptors (SARs) and rapidly adapting stretch receptors (RARs). C-fiber receptors are sensory nerve terminals connected to nonmyelinated vagal fibers with cell bodies mainly in the jugular ganglia, and are found both in the lung parenchyma and in the airway wall (Widdicombe 2003). C-fiber receptors contain neuropeptides, especially substance P (SP), neurokinin A (NKA), and also calcitonin gene-related peptide (CGRP) and, are released when the receptors are activated, causing a local

tissue neurogenic inflammation (axon reflex). RARs and SARs are mechanosensitive receptors. RARs have thin (A δ) myelinated vagal afferent nerves, with cell bodies in the nodose ganglia. RARs are sensitive to mechanical stimuli such as probing, lung inflation and deflation (McAlexander et al., 1999). The RAR and SAR fibers project to distinct regions within the nTS and are associated with different reflex outputs. In general, activation of SARs leads to inhibition of inspiration and inhibition of parasympathetic activity, and a consequent relaxation of airway smooth muscle, whereas activation of RARs leads to increased inspiratory effort, increased rate of respiration, and increased parasympathetic outflow (i.e. contraction of airway smooth muscle and secretion by airway submucosal glands). A δ -fiber nociceptors are airway sensory receptors connected to thin vagal myelinated afferent fibers, with cell bodies mainly in the jugular ganglia and whose terminals innervate within and under the airway epithelium. Unlike RARs, they contain neuropeptides, and may contribute to neurogenic inflammation (Widdicombe 1954). These sensory fibers are further differentiated based on their sensitivity to mechanical stress or chemicals. Both A δ - and C-fiber receptors carrying afferent information have their cell bodies in the jugular ganglia and are insensitive to mechanical stimuli, but are activated by capsaicin, bradykinin, hypertonic saline and acid, whereas A δ fiber carrying afferent information from A δ receptors have their cell bodies in the nodose ganglia and are insensitive to capsaicin, bradykinin, and to hypertonic saline, but highly sensitive to mechanical stimuli and acid (Riccio et al, 1996; Kajekar et al, 1999; Kollarik and Undem, 2002). In addition, a subpopulation of myelinated and capsaicin-insensitive polymodal A δ afferent fibers that arise from cell bodies in the nodose ganglia and project to the proximal trachea and larynx are primarily responsible for regulating the cough reflex (Widdicombe 1998). These afferent neurons, the putative cough receptors, are quite unique to this subset of afferent neurons and are thus not classified as RARs, SARs or C-fibers. However, Cough, initiated following activation of bronchopulmonary C-fibers is distinct from the cough reflex initiated by stimulation of the cough receptors. Unlike these cough receptors, the majority of capsaicin-sensitive A δ - and C-fiber receptors innervating the proximal trachea and larynx have their cell bodies in the jugular ganglia and project to the airways via the superior laryngeal nerves (Canning et al., 2004). These findings suggest the possibility of presynaptic or postsynaptic interactions of afferent nerves originating from cough and non-cough receptor neurons at the nucleus of the solitary tract (nTS) level (Canning et al., 2004).

The sensory neurons ascend in the vagus nerve and enter the brainstem through the solitary tract (Bonham and Joad, 1991, Haxhiu et al., 1993, Haxhiu and Loewy, 1996, Kalia and Mesulam 1980b, Kubin and Davies, 1988), making their first synapse in the nTS second order neurons that are required for full expression of the pulmonary C-fiber reflex (Bonham and Joad, 1991) and bronchoconstrictive airway reflex responses (Haxhiu et al., 2000b). Central terminations of an each largely nonoverlapping group are found in regions of the caudal half of the nTS (Kubin et al., 2006). These afferents converge centrally in the nTS and regulate airway smooth muscle tone. Alternatively, pathways which are separate and parallel to the nTS may provide excitatory input to the bronchopulmonary parasympathetic preganglionic nerves. Convergence of vagal inputs in the nTS profoundly affects the mechanisms by which reflexes are manifested (Davies et al. 1986, 1987). A variety of inputs from afferent receptors are transmitted to the nTS, including those from cough receptors that are activated by stimuli that may also elicit reflex bronchoconstriction, submucosal gland secretion and vasodilation. Conversely, activated SARs send afferent signals to nTS second order neurons causing reflex airway smooth muscle relaxation (Davies et al., 1987; Widdicombe and Nadel, 1963). Similarly, peripheral chemoreceptors and baroreceptors acting centrally may modulate cholinergic outflow to the airways; stimulation of the carotid bodies reflexly elicits bronchoconstriction (Nadel and Widdicombe, 1962; Vidruk and Sorkness, 1985), and submucosal gland secretion (Davis et al., 1982), whereas, activation of baroreceptors leads to opposite changes (Nadel and Widdicombe, 1962). Parasympathetic

preganglionic nerve activity is reflexly regulated by afferent input to the nTS; activation of airway RARs and C-fibers increases both cholinergic (via released acetylcholine) and non-cholinergic nerve activity (via released NO and VIP), whereas chemoreceptors and SARs regulate cholinergic nerve activity only.

AVPNs provide parallel innervation to airway smooth muscle, submucosal glands, and the vasculature. A relative simultaneity in the effector response suggests that cell bodies of AVPNs that cause smooth muscle constriction could also elicit activation of submucosal glands or changes in blood flow supplying the airways. Conceivably, groups of functionally selective AVPNs may exert a highly coordinated control over multiple airway functions by central mechanisms which synchronize their output to individual effectors. Studies have shown that the majority of AVPNs have multilobar projections and, via intrinsic ganglia, are involved in the innervation of multiple airway segments (Baker, 1986). Innervation of multiple airway segments assures the symmetry and simultaneity of bronchomotor and parenchymal neurochemical control (Bennett et al., 1981, Hadziefendic and Haxhiu 1999, Haselton et al., 1992, Haxhiu et al., 1991, Helke et al., 1983, Kalia and Mesulam 1980a, Martin et al., 1995, McAllen and Spyer, 1978, Perez Fontan and Velloff, 2001, van, d. V and Hulsman, 1999). In addition, AVPNs may provide direct innervation to airways, without interposition of intrinsic neurons (Hadziefendic and Haxhiu, 1999, Perez Fontan and Velloff, 2001). Retrograde tracer techniques indicate that in different mammalian species, the vagal preganglionic neurons innervating the airways arise primarily from the rostral nucleus ambiguus (rNA) and to a lesser degree from the rostral portion of the dorsal motor nucleus of the vagus (DMV). AVPNs, in addition to projecting to the airways, innervate submucosal glands and blood vessels (Haxhiu et al., 2000a). The preganglionic cells of the DMV predominantly project to tracheobronchial secretory glands and blood vessels (Haselton et al., 1992, Haxhiu et al., 1993, Haxhiu and Lowey, 1996, Kalia and Mesulam, 1980b).

3. Airway-related vagal preganglionic neurons

Vagal preganglionic neurons are the central integrators of multiple excitatory and inhibitory inputs that connect the brain with the bronchopulmonary effector system. The critical circuits which regulate these processes include central glutamatergic, GABAergic, noradrenergic and serotonergic pathways controlling cholinergic outflow to the airways (Haxhiu et al., 1987, 1991, 1997, 1998, 2000a, 2000b, 2002, 2003a, 2006). Using neuronal tracing techniques as well as ultrastructural, molecular and physiologic approaches, the central excitatory (Haxhiu et al., 1986, 1987, 1989a, 1997, 1998), as well as inhibitory pathways, and neurotransmitters (Haxhiu et al., 1986, 1998, 2000b, 2002) that are involved in regulating the excitability and firing rate of AVPNs have been determined. These processes occur via both synaptic and non-synaptic transmission, and down-regulation of central inhibitory influences upon AVPNs may result in a shift from inhibition to excitation, leading to a hyperexcitable state of AVPNs and, consequently, hyperresponsiveness of the airways, potentially predisposing to and worsening bronchial asthma.

AVPNs innervating the trachea and the intrapulmonary airways are cholinergic in nature, and use ACh as a neurotransmitter to convey signals to the airway motor systems (Kc et al., 2004). These neurochemical findings are in agreement with the results of physiological studies showing that chemical stimulation of the vagal preganglionic neurons (Haselton et al., 1992, Haxhiu et al., 1986, 1987, 1989a) or efferent fibers of the vagus nerve originating from AVPNs produce pronounced contraction of airway smooth muscle and lung parenchyma that is solely mediated via cholinergic parasympathetic mechanisms (Khassawneh et al., 2002, Martin et al., 1998, McWilliam and Gray, 1990). Furthermore, stimulation of airway receptors (Coleridge and Coleridge, 1994a, 1994b, Haxhiu et al.,

2000a, Pisarri et al., 1999, Yu et al., 1989) and/or activation of brain regions (Haxhiu et al., 1991a, 1991b) increases airway secretion and blood flow mediated mainly via cholinergic mechanisms. Virtually all vagal preganglionic neurons innervating the trachea and intrapulmonary airways co-express VIP, but not NOS, indicating that ACh and VIP are co-existing messenger molecules in AVPNs (Kc et al., 2004). AVPN have also been shown to express neurotrophin and its receptor, Trk B (Zaidi et al., 2005). Future study might focus on the role of neurotrophins on central regulation of airway caliber, as BDNF acting postsynaptically has been shown to cause downregulation of GABAA receptor expression (Brunig et al., 2001; Wardle et al., 2003).

4. Bronchopulmonary signaling from the airways to the nTS

Bronchopulmonary sensory receptors are innervated by small diameter ($A\delta$) myelinated vagal afferents also known as RARs as well as non-myelinated C-fibers. These receptors are sensitive to chemical stimuli in the airways (Coleridge and Coleridge, 1994a). The net output to the airways is influenced by the cumulative sensory signals received in the nTS, including those from cough receptors and SARs (Canning et al., 2004, Widdicombe and Nadel, 1963, Widdicombe, 1998). Studies have shown that within the nTS, subpopulations of neurons in the commissural, medial, and the ventrolateral subregions (Ferguson et al., 2000, Haxhiu and Loewy, 1996, Haxhiu et al., 2000b) are activated following stimulation of pulmonary and bronchial C fiber receptors by capsaicin, histamine or stimulation of rapidly adapting receptors (Coleridge and Coleridge, 1977). Furthermore, studies combining neuronal tracer with immunohistochemistry have confirmed that the same nTS subregions that are activated following stimulation of airway receptors are co-labeled following microinjection of neuronal tracer into the tracheal wall or into the most distal airways (Hadziefendic and Haxhiu, 1999, Haxhiu et al., 1993). These data suggest that activated nTS neurons are those that transmit information from the airways to the AVPNs. Multiple neurotransmitters including acetylcholine and/or glutamate are expressed by the primary sensory neurons that may participate in such sensory transmission (Helke et al., 1983, Okada and Miura, 1992).

4.1. Cholinergic transmission

The presence of nicotinic acetylcholine receptors (nAChRs) in the nTS indicates that afferent inputs could be conveyed to the nTS by acetylcholine. The fact that nodose ganglia express cholinergic neurons (Okada and Miura, 1992, Palouzier et al., 1987), and nodose ganglionectomy decreases nAChR binding sites in the nTS (Helke et al., 1983) supports the notion that cholinergic mechanisms may be activated during afferent signaling from the airways. Furthermore, $\alpha 3$ subtype nAChRs are expressed by commissural nTS neurons, activation of which by nicotine increases cholinergic outflow to the airways (Ferguson et al., 2000). However, administration of the ganglionic blocker, hexamethonium, within the commissural nTS, decreased cholinergic output, but had no significant effect on airway reflex constriction induced by lung deflation. This indicates that ACh-nAChR activation is not required for transmission of bronchoconstrictive stimuli from the airways to the nTS (Ferguson et al., 2000), suggesting involvement of other excitatory molecules such as glutamate.

4.2. Glutamatergic transmission

L-glutamate, a naturally occurring excitatory amino acid, is a main sensory neurotransmitter and is present in vagal afferents in the nTS (Ruggiero et al., 1994, Saha et al., 1995, Sykes et al., 1997). Stimulation of airway sensory receptors increases glutamate release in the commissural nTS, producing airway smooth muscle contraction (Haxhiu et al., 1997, 2000a, 2000b). Quantitative analysis using high pressure liquid chromatography (HPLC) of

microdialysates from the commissural nTS during repeated activation of bronchopulmonary sensory receptors resulted in significant glutamate release, which corresponded to increased tracheal pressure (Figure 1) (Haxhiu et al., 2000b).

A host of glutamate receptor subtypes belonging to the ionotropic (iGluRs) and metabotropic (mGluRs) glutamate receptor classes subserve excitatory synaptic transmission and neurotransmitter release. Ionotropic AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors are more abundant than kainate receptors, and exhibit faster signaling kinetics than NMDA (N-methyl-D-aspartic acid) receptors (Gouaux, 2004, Petralia and Wenthold, 1992), making them uniquely suited in mediating cardiopulmonary reflexes (Andresen and Yang, 1990, Wilson et al., 1996). Furthermore, synaptic activation of AMPA receptors may elicit not only postsynaptic excitation, but also presynaptic inhibition of GABAergic transmission. By suppressing inhibitory inputs, the activation of AMPA receptors could facilitate bronchoconstrictive inputs to nTS second order neurons, and from these neurons to the AVPNs. Immunohistochemical studies indicate that a subpopulation of commissural nTS neurons activated by airway reflexes also expresses the AMPA receptor subtype, GluR2 (Haxhiu et al., 2000b). In addition, blockade of AMPA receptors by CNQX injected into the commissural nTS, significantly decreases tracheal smooth muscle tone in response to lung deflation and the rate of rise of tracheal tone (Haxhiu et al., 2000b), indicating that bronchoconstrictive inputs from the airways to nTS neurons are transmitted primarily by a glutamate-AMPA receptor signaling pathway. However, vagal afferents and their dendrites in the nTS neurons also express NMDA receptors, thereby suggesting that these receptors may play a role in autoregulation of the presynaptic release of, and postsynaptic responses to, glutamate (Aicher et al., 1999). These NMDA receptors in the nTS enhance glutamate-AMPA bronchoconstrictive inputs from the airways to the nTS and may contribute to mediating tonic influences. Metabotropic glutamate receptors are also expressed by nTS neurons (McWilliam and Gray, 1990, Nakanishi, 1994, Pawloski-Dahm and Gordon, 1992). Binding to glutamate at presynaptic metabotropic glutamate receptors leads to inhibition of neurotransmitter release and consequently presynaptic depression of synaptic transmission (Burke and Hablitz, 1994, Cartmell and Schoepp, 2000, Pawloski-Dahm and Gordon, 1992). Therefore, glutamate-AMPA receptor signaling pathways play a key role in transmitting bronchoconstrictive inputs from the airways to the nTS, where signals are processed, modulated and relayed to the vagal parasympathetic neurons innervating the airways.

5. Central innervation of AVPNs

The AVPNs provide the final common pathway for vagal control of the airways. Activity of AVPNs depends on afferent inputs, although they possess an ability to express synchronous electrical oscillations, unveiled by stimulation of NMDA receptors (Haxhiu et al., 1987) or blockade of GABA_A receptors (Moore et al., 2004). Conventional and transneuronal labeling techniques have shown that AVPNs receive inputs from cell groups located in the ventral aspect of the medulla oblongata, nTS, pons, and ventrolateral part of the periaqueductal gray (vl PAG) cell group. In addition to the medullary circuits, which control the reflex output of these AVPNs, functions of the airways are also regulated by inputs from higher CNS centers including the suprapontine cell groups, namely, dorsal and lateral paraventricular nucleus of the hypothalamus, and the central nucleus of the amygdala (Haziefendic and Haxhiu 1999). These forebrain circuits including the amygdaloid complex are critical for expression of behavioral and emotional states, and project to multiple targets that are involved in regulating autonomic functions, including the dorsomedial and vl PAG cell group, the nTS and the rNA (Haziefendic and Haxhiu 1999, Price, 2003). The projections from the amygdala to the vl PAG are of particular importance since vl PAG neurons coordinate functions of multiple visceral organs involved in responses to stress. In

addition, activation of the locus coeruleus (LC) noradrenergic cell group (Haxhiu et al., 2003a) and parabrachial nucleus (Motekaitis et al., 1996) induces airway smooth muscle relaxation suggesting their inhibitory input to AVPNs.

5.1 Glutamatergic pathways from the nTS to the AVPNs

A majority of nTS neurons contain glutamate (Kihara and Kubo, 1991) and therefore a glutamate-AMPA receptor signaling mechanism was thought to be involved in transmitting bronchoconstrictive signals from the nTS to the AVPNs mainly via hard-wired synaptic pathways (Haziefendic and Haxhiu 1999, Haxhiu et al., 1993). Neurochemical studies indicate the presence of glutamatergic AMPA, NMDA and kainate receptors in vagal preganglionic neurons (Corbett et al., 2003). Administration of selective antagonists for the AMPA/kainite glutamate receptor to the rNA where AVPNs are located causes a dose dependent decrease in reflex response of tracheal tone induced by lung deflation, stimulation of laryngeal cold receptors, and activation of peripheral or central chemoreceptors (Haxhiu et al., 1997). These reflexes are known to cause centrally mediated increases in cholinergic tone (Coleridge and Coleridge, 1994a, Deal et al., 1986, 1987). Microinjection of NMDA receptor antagonist into the rNA did not significantly affect reflex changes in tracheal tone. However, prior administration of an NMDA receptor antagonist in the rNA region potentiated the effect of AMPA receptor antagonist. A possible mechanism could be via activation of AMPA receptors, which increases Na^+ influx eliciting excitatory postsynaptic potentials that in turn activate NMDA receptors leading to Ca^{2+} entry into a postsynaptic cell. These results suggest that increases in cholinergic outflow to the airways are mainly mediated by glutamate-AMPA receptors that in turn activate the NMDA receptor signaling.

Airway blood flow and submucosal gland secretion are integral components of pulmonary defensive reflex responses (Coleridge and Coleridge, 1994a, 1994b, Pisarri et al., 1999, Yu et al., 1989). In addition to axon reflex pathways, which provide a local mechanism for vasodilation, stimulation of central reflex pathways elicited via bronchial and pulmonary C-fiber receptors or RARs evoke an increase in tracheal blood flow and secretion by tracheal submucosal glands. The responses are abolished upon interruption of afferent and/or efferent vagal transmission (Bennett et al., 1981), by prior administration of an AMPA receptor antagonist, CNQX, into the fourth ventricle or by microinjection 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 glutamate-AMPA receptor signaling mechanism. Therefore, it is likely that airway sensory stimulation-evoked airway smooth muscle contraction, vasodilation, and hypersecretion are mediated mainly via a cholinergic mechanism, using a glutamate-AMPA signaling pathway that in turn activates NMDA receptors.

5.2 Central GABAergic control of cholinergic outflow to the airways

Integrated output to the airways depends on the balance between excitatory and inhibitory modulation. Processing of central afferent excitatory signals by AVPNs and conveying them to the airways is highly dependent on synaptic GABAergic inhibitory inputs. Ultrastructural studies have shown that retrogradely-labeled AVPNs receive significant GABAergic innervation partly via axosomatic synapses and to a lesser degree through axodendritic synaptic transmission, and that they may modulate cholinergic drive to the tracheobronchial system (Moore et al., 2004). GABA-GABA_A receptor signaling controls neuronal excitability of the AVPNs. Extracellular levels of GABA within the rNA can originate from multiple pathways. Stimulation of the vl PAG cell group significantly increases GABA levels within the rNA region (Figure 2), indicating that vl PAG GABAergic terminals innervate the AVPN region. Furthermore, blockade of the GABA_A receptor subtype expressed by AVPNs by bicuculline diminishes the inhibitory effects of vl PAG stimulation

on airway smooth muscle tone (Figure 2) (Haxhiu et al., 2002). These data indicate that activation of the vl PAG cell group elicits airway dilatation by GABA release and via activation of GABA_A receptors expressed by AVPNs.

The effect of GABA may be via a direct synaptic contact, or GABA released from GABAergic nerve endings may spill over to extrasynaptic regions (Hamann et al., 2002, Mody and Pearce, 2004, Rossi and Hamann, 1998), and activate extrasynaptic receptors (Mody, 2001). GABA levels within the synaptic cleft and at extrasynaptic region are dependent on the activity of GABA transporters (such as GAT-1) located in axon terminals near synaptic cleft and/or in the surrounding astrocytes (Haydon 2001; Schousboe et al., 2004). GABA uptake mechanisms also play a critical role in terminating synaptic and extrasynaptic GABAergic signaling. Although to a lesser degree, activation of metabotropic GABA_B receptors via GABA spillover may control neurotransmitter release (Alford and Grillner, 1991; Scanziani 2000)

GABA receptors are categorized in two distinct types, namely the ionotropic GABA_A and GABA_C and the metabotropic GABA_B receptors. GABA_A receptors are most abundant and widely distributed in the CNS in comparison to GABA_C ionotropic receptors. The majority of these receptors are composed of two α , two β , and one $\gamma 2$ subtype. The co-expression of a $\gamma 2$ subunit with α and β subunits produces GABA_A receptors which display high binding affinity for central benzodiazepine ligand (Barnard et al., 1998, Mody and Pearce, 2004, Siegel and Buhr, 1997, Sieghart and Sperk, 2002), indicating that changes in GABA_A receptor subtype expression induce plasticity in fast synaptic inhibition. 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, the $\beta 2$ -isoform of the GABA_A receptor subunit is present on cell bodies and nerve processes of AVPNs (Haxhiu et al., 2002). Furthermore, physiological and pharmacological studies indicate that benzodiazepines, topically applied or microinjected into the rNA region, cause withdrawal of cholinergic outflow to the airways and produce airway smooth muscle relaxation (Haxhiu et al., 1989b), indicating that this receptor subtype co-expresses a $\gamma 2$ subunit (Sieghart and Sperk, 2002). The GABA_B receptor, on the other hand, is predominantly found in presynaptic terminals, and is activated only when GABA is released in large amounts. Activation of GABA_B receptors produces presynaptic inhibition and a decrease of neurotransmitter release, including glutamate and acetylcholine (Scanziani, 2000, Shirakawa et al., 1987). Our preliminary studies have demonstrated that GABA_B receptors are expressed by axon terminals innervating AVPNs and by cell bodies of AVPNs (Kc P, Haxhiu MA, unpublished data) that may, in part, mediate pre- and post- synaptic modulatory effects of GABA upon glutamatergic transmission. GABA_C is less widely distributed in the CNS and its role in mediating GABAergic modulation of AVPNs is not known.

GABA_A receptor signaling may exert tonic and phasic inhibitory effects (Mody, 2001), thereby modulating the gain and the firing threshold of AVPNs. Under baseline conditions, GABA_A-receptor-mediated tonic inhibitory currents are important determinants of AVPN firing rate (Moore et al., 2004). Microinjection of a low concentration of the GABA_A receptor blocker, bicuculline, into the rNA region, significantly increases the frequency of spontaneous discharge of AVPNs and increases airway smooth muscle tone. Tonic GABA_A receptor-mediated inhibitory currents can be influenced by multiple factors including enhanced GABA uptake, location of receptors, or alterations in the assembly of GABA_A receptor isoforms, that may in turn affect the proportion of high and low-affinity GABA_A receptor subunits (Belelli et al., 2002). In addition, tonic inhibitory currents may exert a considerable influence on neuronal signaling via receptors located at extrasynaptic sites on AVPNs (Moore et al., 2004). Therefore, since GABAergic microcircuitry controls cholinergic outflow to the airway, alteration in GABA modulation may shift from inhibitory

to excitatory neurotransmission, leading to a hyperexcitable state of AVPNs and subsequently airway hyperreactivity.

5.3 Central noradrenergic control of cholinergic outflow to the airways

The vagal preganglionic motor cells are innervated by a network of brain stem catecholaminergic neurons, in particular the norepinephrine-containing A5 cell group, the locus coeruleus (LC) and subcoeruleus cells (Berisha et al., 2002, Carver et al., 1997, Davies et al., 1987, Haxhiu et al., 1993). Ultrastructural studies suggest that modulatory effects of norepinephrine on cholinergic outflow to the airways are mainly exerted by nonsynaptic actions (Haxhiu et al., 2003a). The adrenergic receptor (AR) family that mediates the effects is composed of three subfamilies ($\alpha 1$, $\alpha 2$, and β); each containing a minimum of three distinct subtypes (Civantos and Aleixandre, 2001, Hein et al., 1999, Hudson et al., 1999). As opposed to the $\alpha 1$ -ARs (Morin et al., 2000), activation of $\alpha 2$ -ARs by norepinephrine inhibits excitatory synaptic transmission and decreases neurotransmitter release (Bertolino et al., 1997, Boehm, 1999, Travagli and Williams, 1996). The $\alpha 2$ -ARs are divided into four subtypes, based primarily on radioligand binding characteristics in native tissue homogenates (Civantos and Aleixandre, 2001). The $\alpha 2_A$ -ARs, characterized by relatively high affinity for yohimbine and rauwolscine, are present in brain stem neurons, including catecholaminergic and serotonergic cells innervating the spinal cord (Guyenet et al., 1994), and AVPNs (Travagli and Williams, 1996). Furthermore, the $\alpha 2_A$ -ARs are expressed on glutamatergic nerve terminals, where their activation inhibits glutamate release and excitatory synaptic transmission (Bertolino et al., 1997). Physiological data have shown that stimulation of the LC and subcoeruleus region elicits a significant increase in norepinephrine release within the rNA (Figure 3) (Haxhiu et al., 2003a), further indicating that endogenously released norepinephrine acting via adrenergic receptors could affect the activity of AVPNs (Travagli and Williams, 1996). LC stimulation caused decrease in tracheal pressure and blockade of $\alpha 2_A$ -ARs by bilateral microperfusion of yohimbine into the rNA diminished this decrease in tracheal smooth muscle tone elicited by activation of LC neurons (Figure 3). These data strongly support the concept that central noradrenergic inhibitory pathways participate in the regulation of cholinergic drive to the tracheobronchial system, mainly via volume (nonsynaptic) transmission and to a lesser extent through synaptic connectivity. Our recent study in ovalbumin exposed and repeatedly challenged ferrets showed suppressed central noradrenergic inhibitory influences on AVPNs and, consequently, increased cholinergic outflow to the airways (Wilson et al., 2007). Allergen-challenged ferrets showed decreased message and protein expression for $\alpha 2_A$ -AR as compared to control animals exposed to vehicle. Furthermore, microperfusion of an $\alpha 2_A$ -AR agonist, guanabenz, in close proximity to AVPNs elicited decreases in unit activity, and reflexly evoked responses of AVPNs with a corresponding decrease in cholinergic outflow to the airways in control versus ovalbumin-sensitized and challenged ferrets (Wilson et al., 2007). These data suggest that allergic airway disorders may affect central noradrenergic inhibitory pathways, resulting in a shift from inhibitory to excitatory influences, a hyperexcitable state of AVPNs, and centrally mediated airway hyperreactivity.

5.4 Central serotonergic control of cholinergic outflow to the airways

Midline serotonin (5-hydroxytryptamine; 5-HT)-producing raphe neurons play an important role in the central regulation of autonomic functions and overall homeostasis (Anwyl, 1990, Azmitia, 1999). Immunolabeling studies have demonstrated that 5-HT-immunoreactive fibers exist within the rNA in close proximity to AVPNs (Haxhiu et al., 2006). Similarly, the retrograde transneuronal labeling method showed that AVPNs are innervated by the medullary raphe complex which consists of the raphe magnus, raphe obscurus and raphe pallidus (Haxhiu et al., 1993). Microinjection of glutamate to stimulate the serotonin-containing raphe neurons caused a significant increase in 5-HT levels within the rNA,

resulting in inhibition of cholinergic outflow to the airways as demonstrated by decrease in airway smooth muscle tone and lung resistance (Figure 4) (Haxhiu et al., 1998). This response was diminished following blockade of serotonin receptors in the rNA by microinjection of methysergide, a non-selective serotonin receptor (mixed 5-HT₁/5-HT₂ receptor) antagonist (Figure 4). Among the 14 different 5-HT receptor subtypes so far characterized (Martin et al., 1998), the 5-HT_{1A} receptor is expressed in high density by the AVPNs, and is found on a somatodendritic postsynaptic site (Haxhiu et al., 2006). 5-HT_{1A} receptors are inhibitory in nature, whereas 5-HT₂ receptors, as found for example in hypoglossal motoneurons, exert stimulatory effects (Haxhiu et al., 1998, Kubin et al., 1992). Future studies will be needed to document a definitive role for the serotonergic system in airway caliber regulation via central mechanisms. In humans, airway caliber undergoes cyclic oscillations; with increasing caliber during the day and decreasing caliber at night and these oscillations are greatly amplified in patients with nocturnal asthma (Ballard et al., 1989; Bellia et al., 1989; Martin et al., 1990). During sleep, bronchoconstrictive responses are heightened and airway conductivity is decreased as compared to the wakeful state. The decrease in conductivity of lower airways parallels the sleep-induced decline in the discharge of brainstem monoaminergic cell groups that provide inhibitory inputs to the AVPNs. Although the mechanisms by which such alterations occur are unknown, it is conceivable that increases in cholinergic outflow to the airways during sleep could be partly related to withdrawal of inhibitory influences that might trigger a cascade of events leading to airway narrowing and nocturnal worsening of asthma.

6. Parallel central control of airway tone and respiration

Under normal conditions, alteration in chemical drive induces parallel changes in airway smooth muscle tone and breathing pattern, indicating a link between the brainstem neuronal networks that control the resistance of airway conduits and the respiratory drive to the diaphragm (Haxhiu et al., 1986, 1987, Mitchell et al., 1985). The neuroanatomical basis for this integration has been demonstrated using neuronal tracer combined with immunohistochemistry, where subsets of medullary neurons that project to phrenic motoneurons also innervate the airway-related vagal preganglionic cells, demonstrating coupling of inspiratory activity and parasympathetic outflow to the airways (Haxhiu et al., 1996). This forms the morphological basis for respiratory rhythm modulating cholinergic outflow to airway smooth muscle. Physiological findings also indicate that during normal breathing and changes in chemical drive, cholinergic outflow to the airways changes in parallel with inspiratory drive as recorded from the phrenic nerve (Deal et al., 1986, 1987, Martin et al., 1995, Mitchell et al., 1985). However, parallel responses of the phrenic nerve and AVPNs may not need to be maintained at all times; for example, chemical stimulation of pulmonary C-fiber receptors induces an increase in cholinergic drive which leads to increase in airway smooth muscle tone, but it causes an inhibition of inspiratory activity (Coleridge and Coleridge, 1994a). In contrast, activation of the midbrain ventrolateral periaqueductal gray area evokes release of GABA within the rNA region and causes relaxation of airway smooth muscle, whereas it increases respiratory output (Haxhiu et al., 2002). Taken together, these data indicate that AVPNs within the rNA form a distinct and identifiable cell group in the medulla, the activity of which is regulated by multiple CNS nuclei, utilizing common and/or specific neuronal pathways.

Excitatory outputs to airway smooth muscle are driven by the same pattern generators that drive the phrenic and inspiratory intercostal motoneurons (Haxhiu et al., 1986, Mitchell et al., 1985). Changes in chemical drive, such as hyperoxic hypercapnia, affect both airway tone and respiration (Deal et al., 1986). Following hyperoxic hypocapnia, in which there is a complete cessation of phrenic nerve discharge, the gradual increase in arterial PCO₂, or decrease in inspired O₂ causes a progressive increase in airway smooth muscle tone which

reappears prior to phrenic activity (Deal et al., 1986, 1987). Similarly, with the onset of rhythmic phrenic nerve firing, rhythmic oscillations of airway smooth muscle tone and airway resistance can be observed (Haxhiu et al., 1987). Within the respiratory cycle, peak airway smooth muscle tone and airway resistance appear early in expiration, in phase with post-inspiratory activity (Haxhiu et al., 1986). Increase in airway smooth muscle tone and lung resistance at this phase of the respiratory cycle may serve to reduce the dead space, oppose distortion of the airways and most distal ventilated units, and optimize gas exchange by reducing large fluctuations in functional residual capacity.

Diminished cholinergic outflow to the airways may contribute to alterations in airway function and ventilation. Airway denervation (as following lung transplantation) does not appear to markedly affect ventilatory pattern. However, such denervation is associated with a fibroproliferative response in the airways. Deposition of increased amounts of collagen in lungs, as seen in bronchiolitis obliterans after lung transplantation may impair airway and vascular adaptability, potentially impacting homeostatic ventilation and pulmonary blood flow (Carver et al., 1997). Defects in cholinergic mechanisms may have significant physiologic consequences, the severity of which may depend on a reduction in the size of cholinergic capacity. A partial deficit in central cholinergic mechanisms following ablation of a subpopulation of vagal preganglionic neurons innervating the extrathoracic trachea only had a slight effect on room air breathing, but significantly affected ventilatory responses to hypercapnia and hypoxia (Wu et al., 2006). These findings suggest that intact functional communication between AVPNs and respiratory neurons within the ventrolateral medulla is an important pathway in regulating respiratory output to ventilatory challenges such as hypercapnia and hypoxia. This concept is further supported by findings that interruption of cholinergic transmission by blockade of muscarinic acetylcholine receptors reduces the ventilatory response to hypercapnia (Monteau et al., 1990, Nattie and Li, 1990).

7. Chemoreception and airway control

Serotonergic and noradrenergic cells appear to have chemosensitive properties. CO₂ inhalation increases c-Fos protein, used as a marker of increased activity, in both serotonergic and noradrenergic cells (Haxhiu et al., 2001). Serotonin expressing raphe neurons innervate airway-related vagal preganglionic neurons (AVPNs) and appear to modulate airway tone. On the other hand, noradrenergic-containing locus coeruleus (LC) neurons innervate the hypoglossal nucleus, with resultant modulation of upper airway muscle activity. Activation of raphe neurons increases serotonin levels in the hypoglossal nucleus and in AVPNs, and LC stimulation increases noradrenergic neurotransmitter levels in the AVPN region. Inhalation of CO₂ also increases serotonin release in the dorsal motor medulla (Kanamaru and Homma, 2007). Serotonin acting on the hypoglossal nuclei, and serotonin and noradrenaline acting at the AVPN elicit hyperventilation with airway dilatation (Kanamaru and Homma, 2007), and decrease airway tone (Haxhiu et al., 1998 and 2003). During rapid eye movement sleep (REM) sleep, activity of the hypoglossal nerve that innervates the genioglossus, an upper dilator muscle, decreases. In obstructive sleep apnea patients, upper airway dilating muscle activity becomes insufficient to oppose negative inspiratory pressure, and these obstructive episodes are more severe during REM sleep. Microinjection of carbachol in the pontine region to induce REM-like sleep following microinjection of serotonin and noradrenaline antagonists into the hypoglossal nucleus produces REM-like atonia of the genioglossus (Fenik et al., 2005). Together, these observations suggest that chemoreceptive serotonergic and noradrenergic cells modulate airway tone and airway resistance.

8. Conclusions

In conclusion, the integration and processing of bronchoconstrictive inputs by AVPNs and the level of cholinergic output to the airways are highly dependent on synaptic and extrasynaptic excitatory glutamatergic and inhibitory GABAergic, noradrenergic and/or serotonergic inputs to the AVPNs. Conceivably, the outcome of these competing regulatory processes could be influenced by a number of other pathways, suggesting the complexity of CNS control. Central inhibitory pathways participate in the regulation of cholinergic drive to the tracheobronchial system. Downregulation of these influences may result in a shift from inhibitory to excitatory influences, leading to a hyperexcitable state of AVPNs, and resultant airway hyperreactivity. Central coordination of cholinergic outflow to the airways with changes in respiratory drive may serve to modulate gas exchange, work of breathing, airway smooth muscle tone and respiratory drive.

Acknowledgments

This paper is dedicated to life work of Musa A. Haxhiu in CNS control of the airways. This work is supported by the National Heart, Lung and Blood Institute Grants 4R00HL087620-03 (to P. Kc) and R01HL056470-10A2 (to R. J. Martin).

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Figure 1.

Reflex-induced glutamate release within the nucleus tractus solitarius (nTS). (A) Average concentrations (mean $\pm$ SEM; pg/ μ L) of L-glutamate (Glu) in a control state (baseline), during excitation of bronchopulmonary sensory receptors (stimulation), and in a post-stimulation period (recovery). Filled bars: ferrets with intact innervation of the airways and lungs (vagus intact; $n = 5$). Open bars: ferrets after afferent and efferent denervation of the airways and lungs (vagotomized and superior laryngeal nerves cut; $n = 3$). (B) Tracheal smooth muscle tone measured as pressure in a bypassed tracheal segment (PTseg, cmH₂O) before, during, and after stimulation in ferrets with intact innervation of the airways. Stimulation of afferent sensory fibers significantly increased glutamate release and subsequently augmented pressure in the tracheal segment. * = $p < 0.05$. (Modified from Haxhiu et al., 2000a).

Figure 2.

(A) An example of the effect of chemical stimulation of the ventrolateral periaqueductal gray (vl PAG) cell group by microinjection of L-glutamate (4 nmol) on tracheal segment pressure (PTseg, cmH₂O) in a paralyzed and mechanically ventilated ferret. (B) Average results (mean $\pm$ SEM; $n = 6$) of GABA obtained from microdialysates collected from the airway-related vagal preganglionic neurons (AVPNs) within the rostral ventrolateral medulla in a control state (Baseline) and after vl PAG stimulation (PAG stim). (C) Average data (mean $\pm$ SEM; $n = 9$) on the effect of vl PAG stimulation on tracheal smooth muscle tone in response to L-glutamate-induced activation of vl PAG before and after bicuculline microperfusion into the AVPN region of paralyzed and oxygen-ventilated ferrets. Stimulation of vl PAG significantly increased GABA release within the AVPN region which decreased in tracheal pressure. This response was abolished following bilateral microperfusion of bicuculline into the AVPN region. * = $p < 0.05$ (Modified, from Haxhiu et al., 2002).

Figure 3.

Effects of endogenous norepinephrine release on cholinergic outflow to the airways in ferrets. (A) Average norepinephrine levels (mean $\pm$ SEM; fg/ml; $n = 8$) obtained from microdialysates collected from airway-related vagal preganglionic motor neurons (AVPNs) within the rostral nucleus ambiguus (rNA) in a control state and at different time points after cessation of chemical stimulation of locus coeruleus (LC) neurons (horizontal bar). In three control animals, no stimulation was performed. (B) Tracings of tracheal segment pressure (PTseg, cmH₂O) from a paralyzed and oxygen-ventilated ferret. In a control period (A), activation of LC neurons by L-glutamate (4 nmol/80 nl) induced a decrease in tracheal tone, expressed as a decrease of PTseg. Bilateral microperfusion of yohimbine into the rostral nucleus ambiguus (rNA) region diminished the tracheal smooth muscle response to LC stimulation. (C) Average decrease in PTseg (mean $\pm$ SEM; PTseg, cmH₂O; $n = 8$) induced by LC stimulation before (A) and after (B) microperfusion of α 2A-adrenoreceptor blocker into the rNA regions. Microperfusion of α 2A-adrenoreceptor blocker inhibited the decreases in tracheal pressure to LC stimulation. * = $p < 0.05$. (Modified from Haxhiu et al., 2003a).

Figure 4.

(A) An example of the effect of raphe neuron stimulation (L-glutamate, 4 nmol/80 nl) on serotonin release (μmol) in the rostral nucleus ambiguus (rNA) of an anesthetized cat. (B) The medullary raphe stimulation-induced changes (mean ± SEM; PTseg, cmH₂O) in tracheal tone (Δ PTseg) and lung resistance (Δ RL; cmH₂O/L/s) in a control period, and after 5-HT receptor blockade with topical application of methysergide (100 mg/site), a broad-spectrum serotonin receptor antagonist. Release of serotonin decreased airway smooth muscle tone and lung resistance ($p < 0.05$). This response was diminished after blockade of 5-HT receptors within the ventrolateral medulla (Modified, from Haxhiu et al., 1998).