The Role of Nerves in Asthma

Bradley J. Undem, PhD and Michael J. Carr, PhD

Address
The Johns Hopkins Asthma and Allergy Center, Johns Hopkins School of Medicine, 5501 Hopkins Bayview Circle, Baltimore, MD 21224, USA.
E-mail: bundem@mail.jhmi.edu

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Asthma is a syndrome characterized by reversible episodes of wheezing, cough, and sensations of chest tightness and breathlessness. These symptoms are secondary to changes in the activity of the nervous system. The mechanisms by which the nervous system is altered such that the symptoms of asthma occur have not yet been elucidated. Airway inflammation associated with asthma may affect neuronal activity at several points along the neural reflex pathway, including the function of the primary afferent (sensory) nerves, integration within the central nervous system, synaptic transmission within autonomic ganglia, and transmission at the level of the postganglionic neuroeffector junction. We provide a brief overview of these interactions and the relevance they may have to asthma.

Introduction

What is asthma? Woolcock and Barnes [1], in the introduction to a 2000-page textbook on asthma, answered this query with, "In spite of the immense amount of material presented here, we do not know the answer." All can agree with them, nevertheless, when they point out at the end of the paragraph that "…asthma is a syndrome characterized by a set of symptoms." The symptoms of asthma, as delineated in the Global Initiative for Asthma Workshop Report, include recurrent episodes of wheezing, breathlessness, chest tightness, and cough [2]. To pose the question of what role the nervous system plays in asthma is, therefore, to ask how the nervous system contributes to recurrent episodes of wheezing, breathlessness, chest tightness, and cough. We address this question after a basic overview of airway innervation is presented.

Airway Innervation

The afferent innervation of the airways is carried predominately by pseudounipolar neurons with fibers that travel in the vagus nerves [3]. The cell bodies of these neurons are located in vagal sensory ganglia and give rise to single, relatively short processes that branch into two fibers, one of which projects to the airways while the other projects to the brain stem, where it synapses with secondary neurons located within the nucleus of the solitary tract in the medulla. Some airway-specific neurons within the vagal sensory ganglia have relatively small-diameter cell bodies that give rise to unmyelinated C fibers, while others with larger cell body diameters give rise to faster conducting myelinated A fibers [4].

Classic studies of the afferent innervation of the airways have led to subclassification of airway afferent A fibers based on their response (pattern of action potential discharge) to prolonged suprathreshold mechanical stimuli [5]. When the lungs are inflated for a prolonged period, some of the fibers, termed "slowly adapting receptors" (SARs), respond constantly as long as the pressure is maintained, whereas other fibers, termed "rapidly adapting receptors" (RARs), respond with bursts of action potentials only during the dynamic phase of inspiration. SAR fibers are thought to terminate within the airway smooth muscle layer and participate in respiratory reflexes regulating breathing pattern [6]. In contrast, RAR fibers often have been found to be stimulated by various inhaled irritants, and because of this, they are often referred to as "irritant receptors" [7].

The peripheral terminals of RARs and C fibers are situated near and within the airway epithelium and are thought to represent one of the first lines of defense of the airways. When stimulated, RARs and C fibers transmit action potentials to the brain stem, leading to defensive reflexes, including cough, stimulation of mucus secretion, and bronchoconstriction [8]. In addition, neuropeptide-containing C fibers may participate in local axon reflexes leading to end-organ responses, including vasodilatation, plasma extravasation, and leukocyte adherence to the microvascular endothelium, mucus secretion collectively known as "neurogenic inflammation." Most studies of neurogenic inflammation have been carried out in rats or guinea pigs. The hypothesis that neurogenic inflammation can be elicited in the airways of humans has not been tested.

Neuropeptide-containing C fibers, in addition to participating in central and local axon reflexes, may participate in peripheral reflexes. A peripheral reflex occurs when activation of a C fiber peripheral terminal initiates the release of transmitter via an axon reflex at synapses in peripheral autonomic ganglia. In human, ferret, and guinea pig airways, neuropeptide-containing C fibers are found in close association with parasympathetic ganglia

[9], and parasympathetic ganglion neurons contain receptors for neurokinins that, when stimulated, lead to excitatory electrical potentials [10]. Thus, an afferent nerve-mediated autonomic respiratory reflex may occur independently of the central nervous system. Functional and electrophysiologic studies support the idea that peripheral reflexes occur in both cholinergic and nonadrenergic, noncholinergic (NANC) parasympathetic pathways in the airways [9].

Postganglionic parasympathetic cholinergic and NANC fibers innervate airway smooth muscle, where they provide the dominant control of airway caliber. They also innervate airway glands and microvasculature in the respiratory tract. Preganglionic parasympathetic nerve fibers arise primarily from cell bodies located in several discrete medullary nuclei and project to the airways via the vagus nerves [11]. Within the airways, they form cholinergic synapses with postganglionic neurons in parasympathetic ganglia. Although airway parasympathetic ganglia are predominately physically associated with larger airways, the postganglionic fibers to which they give rise innervate structure throughout the airway tree [9]. At least a portion of the postganglionic neurons that innervate the airways of mammalian species are cholinergic, and virtually all mammals, including humans, have cholinergic-mediated smooth muscle tone in their airways as a consequence of baseline parasympathetic reflex activity [9].

Older textbooks often state that the sympathetic nervous system provides relaxant innervation to airway smooth muscle via adrenergic transmission and activation of β-adrenoceptors, but this is not the case in human airways, where relaxant innervation is provided by the NANC component of the parasympathetic nervous system. Although the transmitters released by these parasympathetic nerves have yet to be completely delineated, transmission at smooth muscle synapses appears to involve nitric oxide and vasoactive intestinal peptide. As noncholinergic parasympathetic pathways, at least in guinea pigs, arise from a neural pathway separate from that of the cholinergic pathways, it may be that these two pathways can act independently. Indeed, it was recently demonstrated that airway reflex cholinergic activity could occur independently of airway reflex noncholinergic parasympathetic activity in guinea pigs [9].

Although sympathetic nerves are seldom found innervating human bronchial smooth muscle, the airway vasculature receives sympathetic input, likely arising from the superior cervical stellate and rostral thoracic sympathetic ganglia.

The Role of Nerves in Asthma Symptoms

Wheezing and breathlessness (dyspnea)

There is little doubt that bronchial smooth muscle contraction underlies the episodic wheezing, breathlessness, and chest tightness that defines asthma. The dominant control of airway smooth muscle tone, and thus airway caliber, is derived from the parasympathetic branch of the autonomic nervous system. As described above, this system includes both cholinergic contractile and noncholinergic relaxant pathways. The bronchi are tonically constricted at rest [12]. This baseline smooth muscle tone is due in large part, if not exclusively, to reflex cholinergic activity [13,14]. With each inspiration, mechanosensory afferent nerve fibers in the airways are activated to send impulses to the brain stem, where they initiate parasympathetic reflex bronchial smooth muscle contraction. In a sense, then, with each breath, we receive an endogenous "acetylcholine challenge" that results in baseline tone.

A pathognomonic abnormality of asthmatic airways is their exaggerated narrowing in response to bronchospastic stimuli. This so-called airway hyperreactivity or hyperresponsivenes is most often quantified by the concentration of an inhaled stimulus required to increase the resistance to airflow by 20%. The difference in this parameter between nonasthmatic and asthmatic airways often is quite staggering. Nonasthmatic airways, for example, typically do not respond to an inhaled solution of histamine, even at concentrations greater than 16 mg/mL, whereas less than 1 mg/mL is typically sufficient to constrict asthmatic airways [15].

The mechanisms underlying airway hyperreactivity are not known. Airway hyperreactivity appears to be associated with airway inflammation, but cause-effect relationships remain obscure. A recently published childhood asthma study revealed that long-term treatment with inhaled corticosteroids decreased asthma-related hospitalizations in children but had almost no effect on their airway hyperreactivity [16•]. This is consistent with the vast literature on this subject, which reveals that corticosteroid treatment over a long period may dampen hyperreactivity but seldom, if ever, reverses the phenomenon. Numerous studies support the concept that abnormal neuronal reflex activity contributes to hyperreactive airways, but again, specific cause-effect relationships are unknown. Most (arguably all) agonists used to evaluate airway reactivity modulate airflow resistance in part or *in toto* by stimulating parasympathetic cholinergic reflexes. In addition to classic reflex stimuli, such as irritant gases, histamine, nonisotonic aerosols, and cold air, this also likely includes so-called direct-acting smooth muscle agonists, such as methacholine [17]. It is, therefore, not surprising that, in nearly all cases, cholinergic muscarinic receptor antagonists can substantially inhibit or even abolish the hyperreactive response to inhaled stimulants in asthmatic airways [9]. The fact that the phenomenon of airway hyperresponsiveness (>100-fold increases in agonist sensitivity) is not observed in studies on bronchi isolated from deceased asthmatic airways when studied in vitro is also consistent with the hypothesis that the hyperreactivity results from exaggerated reflex responses rather than exaggerated end-organ (*ie,* smooth muscle) responsiveness.

Perhaps the most heuristic studies on airway hyperreactivity are those that induce the phenomenon in nonasthmatic subjects. In one such study, upper respiratory tract viral infection was shown to lead to airway hyperreactivity to histamine, and this hyperreactivity persisted well beyond the infection (much like postviral cough syndromes) [18]. In another group of studies, hyperreactivity was found in normal subjects who avoided taking deep inspirations or "sighs" for 20 minutes. When nonasthmatic subjects avoided deep inspiration, they responded to concentrations of methacholine or histamine aerosol that, under normal conditions, have no effect [19,20•]. The heightened sensitivity to histamine induced by upper respiratory tract infection or by deep breath avoidance is prevented by pretreatment with an anticholinergic drug [18,21]. This supports the hypothesis that the induced airway hyperreactivity was due to a "hyperreflexivity."

The episodic bronchospasm associated with asthma likely contributes to sensations of dyspnea, but it is unlikely to be the only contributor to the sensation of breathlessness. Increases in vagal afferent activity caused by inflammation, independent of bronchospasm, may also play a role. The neurophysiology of dyspnea has not yet been elucidated. There are likely to be multiple neuronal pathways underlying dyspnea, some of which do not depend on intact vagal pathways [22]. Nevertheless, several lines of evidence support the hypothesis that dyspnea may be brought about by perturbations in vagal sensory nerve activity. First, electrical stimulation of the vagus nerves, using stimulation paradigms that do not cause bronchoconstriction or changes in heart rate, leads to dyspnea in humans [23]. Second, inhalation of histamine, a mediator known to lead to activation of airway sensory nerves, causes dyspnea. Interestingly, histamine inhalation leads to a more profound dyspnea than methacholine inhalation, despite similar degrees of bronchoconstriction [24•]. The dyspnea associated with histamine is inhibited by lidocaine [25]. Finally, prostaglandin E_2, a mediator that increases sensory nerve excitability, exacerbates the dyspnea associated with exercise, despite being a bronchodilator [26]. Considered together, these findings suggest that discharge of action potentials in airway sensory nerves may lead to dyspnea and that this is not necessarily dependent on bronchoconstriction.

Cough

Cough is a common, and sometimes the predominant, symptom of asthma [27]. Cough-inducing sensations in asthma may be elicited secondary to bronchospasm. They may also be caused by the presence of mucus in the airways. Mucus secretion, like bronchospasm, may be increased as a consequence of altered autonomic reflex activity. Often, an indefinable and persistent itch or irritation in the airway often provokes a dry, unproductive cough reflex in asthmatic subjects. Regardless of the stimulus, all cough reflexes are initiated by activation of primary afferent fibers in the larynx, trachea, and larger bronchi. Studies in animals indicate that RAR-type fibers are the principle fibers involved in initiation of cough, although C fibers may also play an important role [28].

Nonasthmatic subjects may, under appropriate circumstances, experience cough, dyspnea, and chest tightness when their airways are constricted. The problem in asthma, therefore, is often one of an exaggeration of normal reflex behavior. Analogies to this type of process may be found in other systems. In the study of pain, it has long been recognized that inflammation may lead to a state of hyperalgesia such that the threshold for painful stimuli is decreased [29]. Hyperalgesia thus has similarities to airway hyperreactivity. Inflammation may also lead to painful sensations in response to normally nonpainful stimuli, such as gentle brushing of the skin or hair. The term given to this inappropriate pain sensation is "allodynia" [30]. A similar phenomenon occurs with respect to inappropriate itch sensations, termed "alloknesis." In some cases of asthma, one might argue that when severe shortness of breath is experienced despite only a mild compromise in lung function, there is an inappropriate hunger for air, *ie*, an "allodyspneic" sensation. Similarly, asthmatics may experience irritating itch sensations in their airways leading to an urge to cough, despite the lack of physical objects in the airway provoking the irritation. This "allotussive" effect may be analogous to alloknesis.

Neuromodulation by Airway Inflammation

Asthma is associated with a particular type of airway inflammation that is often triggered by allergens and typified by the presence of eosinophils and T_H2-type immune processes. It is likely that the inflammatory process contributes to the aberrant neurophysiology of asthma. In the past decade, a vast literature has accumulated that describes the characteristics of this inflammation in impressive detail. Regrettably, little progress has been made on the key question of how the often rather mild inflammation associated with asthma perverts the nervous system such that the symptoms of asthma emerge.

Neuronal control of airway function is carried out by means of reflex arcs. By modulating the activity at different points along the reflex pathways, airway inflammation may quantitatively and qualitatively affect airway neurophysiology. The reflex is initiated by stimulation of afferent nerve fibers. This leads to the transmission of information (action potentials) to the central nervous system (CNS), where it is integrated and ultimately expressed as changes in respiration pattern, alterations in autonomic preganglionic neuronal activity, or, in some cases, conscious sensations (*eg*, dyspnea, chest tightness, cough-inducing irritations). The autonomic preganglionic nerve fibers synapse in bronchial ganglia, and if the synaptic transmission is successful, it leads to action potential discharge in the postganglionic fibers and release of acetylcholine and other

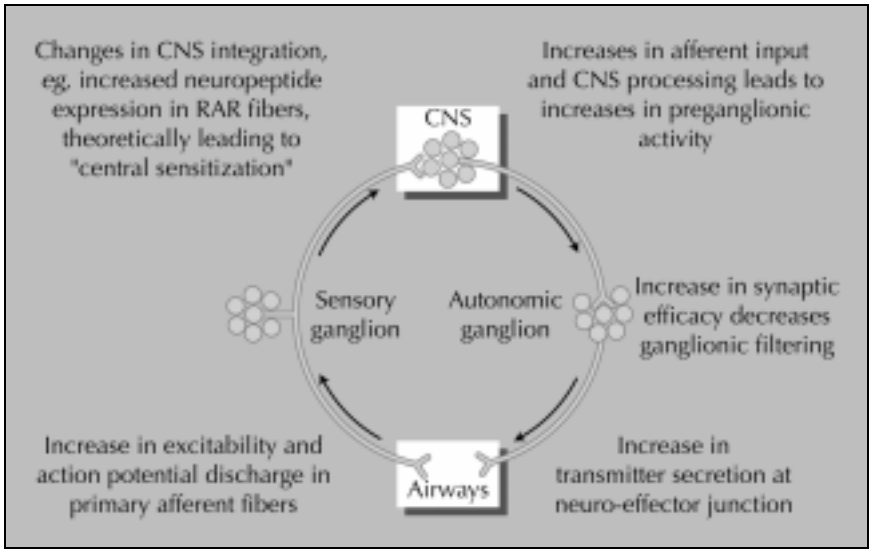

Figure 1. Effect of allergic inflammation on reflex activity. Inflammation may increase reflex activity by affecting all points in the reflex arc. CNS—central nervous system; RAR—rapidly adapting receptors.

NANC transmitters at the junction of the airway smooth muscle, vasculature, glands, and so on. That airway neuronal reflex arcs are modulated by allergic inflammation may be most obviously exemplified by studies on neural reflex activity in the human upper airway [31,32]. Applying an irritant such as bradykinin to the nasal mucosa of nonallergic people or to seasonally allergic subjects out of their allergy season results in little or no response. If the same dose of bradykinin is applied to the nasal mucosa of symptomatic subjects (*eg,* during the relevant allergen season or to perennial allergic subjects), it causes excessive sneezing and autonomic reflex–mediated secretions.

Various mechanisms by which inflammation affects the neurophysiology of airway reflexes have been discussed in some detail elsewhere [33]. Available evidence indicates that allergic airway inflammation may affect neuronal reflex activity at multiple sites in the reflex pathway (Fig. 1). Allergic inflammation can affect the initiation of airway reflexes by increasing the activity of the primary afferent nerve fiber [34]. Mediators associated with inflammation, such as bradykinin and various eicosanoids, can directly activate C fibers in the airway wall [8]. In addition, the activity in low-threshold mechanosensors may be increased by inflammatory mediators via increases in electrical excitability. This is exemplified by a study on single RAR nerve endings in trachea/bronchi isolated from actively sensitized guinea pigs [35]. Adding the sensitizing antigen to the airway tissue did not overtly lead to action potential discharge in the mechanosensors, but it substantially reduced the amount of mechanical force needed to activate the fibers. This likely is explained by mediators acting on cell surface receptors to alter the electrophysiologic properties of the nerve membrane [36]. These data support in vivo studies with allergen inhalation that show increases in action potential discharge in mechanosensitive afferent fibers [37]. The low-threshold mechanosensors in the airways (*ie,* SARs and RARs) respond to the physical displacement of their nerve terminals. This mechanical transduction process likely is influenced by the manner in which the nerve terminals are integrated into the airway tissue [38]. It is tempting to speculate, therefore, that the submucosal remodeling associated with more chronic airway inflammation may change stress-strain relationships of the mechanical fibers during respiration, leading to either exaggeration or dampening of afferent nerve discharge. This speculation has yet to be addressed experimentally.

Little attention has been given to how airway inflammation affects synaptic transmission between the primary afferent nerve and second-order neurons in the brain stem (NTS). One mechanism by which integration of afferent information in the CNS can be altered is by changing the neurochemistry of afferent fibers. The low-threshold mechanosensors in the airway are thought to use excitatory amino acids (EAAs) as their principal neurotransmitter [39]. When EAAs, such as glutamate, are released onto second-order neurons in the brain stem, they cause fast, excitatory postsynaptic potentials. The efficacy by which an EAA results in synaptic transmission in the CNS may be increased by the presence of neurokinins [40]. This enhancement of synaptic transmission in the CNS has been termed "central sensitization." Central sensitization likely contributes to the augmentation of neuronal reflex activity during C fiber activation. The concept of central sensitization has been studied extensively in the somatosensory system, where it appears to be a major mechanism underlying certain types of hyperalgesia and allodynia [29]. By increasing the expression of the preprotachykinin gene in airway sensory neurons, allergic inflammation can increase the efficacy of synaptic transmission in the brain stem, thereby modulating the CNS integration [41]. It is typically thought that nociceptive C fibers are required to be activated before neurokinins are released in the brain stem. It is interesting to note, however, that during allergen- or viral-induced airway inflammation, the preprotachykinin gene may be unregulated not only in nociceptive C fibers but also within the low-threshold mechanosensors themselves [42]. This, in theory, could

lead to central sensitization during respiration, independent of C fiber nerve stimulation. A similar hypothesis has been derived from experimental data in the somatosensory system, where inflammation of the rat paw leads to neuropeptide expression in large Aβ brush fibers [43].

The neuromodulation associated with airway inflammation is not limited to primary afferent nerve activity and CNS integration. Airway inflammation also directly affects autonomic neuronal activity. Allergen challenge in vitro has been associated with increases in electrical excitability of bronchial ganglion neurons [44]. This increase in electrical excitability leads to an increase in synaptic efficacy and, consequently, a decrease in the capacity of the ganglia to filter preganglionic input. Thus, a larger percentage of the preganglionic volley associated with each inspiration may lead to action potentials in postganglionic nerve fibers, resulting in a generalized increase in parasympathetic tone. Airway inflammation also may lead to an increase in the amount of acetylcholine released per action potential from the postganglionic fibers at the level of the neuro-effector cells [45].

In addition to acutely modulating airway neurophysiology, inflammation early in life may have a more insidious effect on airway innervation. It is well established that development of sensory systems often requires use-dependent activity early in life [46]. During postnatal sensory nerve development, there is a defined window of time during which the nerves are susceptible to this experience-dependent plasticity. Before or after the defined window of time, the same experience does not alter the neuronal development. Thus, for example, if a young animal is deprived of vision by eyelid closure (or various other techniques), changes occur in the neural circuitry of the visual cortex leading to severe and permanent loss of visual acuity. This occurs only if the vision deprivation occurs during a critical period of time. Even prolonged vision deprivation after the critical period is without effect on visual acuity. Since these pioneering studies, critical periods have been defined in audio and somatosensory systems and have been noted in virtually all species from humans to songbirds to *Drosophila* [46].

Two recent reports support the concept that inflammation-dependent sensory nerve plasticity during a critical period may lead to persistent changes in somatosensory and vagal-sensory neural circuits. Ruda *et al.* [47•] inflamed one hind paw of rat pups using complete Freund's adjuvant. The pups exhibited stereotypic behavior indicating pain in the paw. More importantly, there was a substantial increase in the density of primary afferent nerves in the ipsilateral dorsal horn of the spinal cord. This change in the density of neuronal circuits persisted beyond the resolution of the inflammation and lasted into adulthood. Behavioral studies revealed that adult rats that experienced paw inflammation as neonates were significantly more hyperalgesic in response to subsequent inflammatory stimuli than were control rats. The authors concluded that "peripheral inflammation experienced during neonatal periods has long-standing consequences on nociceptive neuronal circuitry."

More directly relevant to visceral inflammatory disease is the study by Al-Chaer *et al.* [48•] on gastrointestinal hypersensitivity. It has long been known that inflammation of the colons of laboratory animals leads to a neuronal hypersensitivity and exaggerated and abnormal reflex physiology of the gut. In this study, the colons of rats were chemically or mechanically irritated 8 to 21 days postnatally. As expected, this led to inflammation and afferent nerve hyperexcitability and a state of heightened visceral reflexia. These changes in sensory nerve hyperexcitability persisted beyond the gut inflammation, lasting for at least 3 months (the longest time point analyzed). By contrast, if the colons of rats were inflamed after postnatal day 21 (beyond the critical period) no persistent sensory hyperreactivity developed. Thus, in both the somatosensory pain model and the visceral-sensory model of hyperreflexia, inflammation during a critical period in postnatal development caused abnormalities in the sensory near-circuitry that persisted well beyond the initiating stimulus or inflammation.

Activation of nerve growth factor (NGF) receptor Trk-A has been implicated in several models of critical-period sensory nerve plasticity [49]. It is worth noting that both mRNA for NGF and immunoreactive NGF protein, as well as other neurotrophins, are found in guinea pig and human airways, located principally in the epithelium but also in lymphocytes and resident mononuclear cells [50]. It may be relevant in this regard that NGF, when applied to the airway wall, is effective at inducing neurokinin expression in airway afferent RAR fibers [51•]. In recent years, there has been much discussion on the potential role played by early life events on the development of the immune system as it pertains to the immunology of allergy and asthma. One might hypothesize that early life events occuring during critical periods in sensory nerve development may also contribute to asthma susceptibility affecting the neural reflex circuitry.

Conclusions

Allergic rhinitis and asthma are the two most common allergy-associated airway diseases. When the allergic rhinitic subject inhales specific allergen, upper airway irritation occurs and is accompanied by sneezing, nasal congestion, and excessive secretion. Asthmatic subjects experience episodic and reversible bronchoconstriction, mucus secretion, excessive cough, and shortness of breath (*ie,* an attack of asthma). It should not go unnoticed that in both pathologies, the nervous system is the pivot between the immune activation by allergen and the symptoms of airway disease. With respect to asthma, the coughing, sneezing, and sensations of dyspnea are the direct results of altered sensory neuronal activity. Increases in reversible

bronchoconstriction, vascular congestion, and mucus secretion are likely to be, at least in part, secondary to increases in autonomic neuronal activity. In general terms, experimental evidence indicates that allergic inflammation can potentiate neuronal function at all key points along the reflex arc, from the primary afferent nerve terminals, to integration of signals in the CNS, to synaptic transmission in autonomic ganglia, to transmitter release from postganglionic nerve varicosities. As more is learned about the specific molecular and biophysical mechanisms of this neuromodulation, a better understanding of the pathophysiology of asthma likely will emerge.

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