Physiol Rev 96: 975–1024, 2016
Published June 8, 2016; doi:10.1152/physrev.00039.2015

VAGAL AFFERENT INNERVATION OF THE AIRWAYS IN HEALTH AND DISEASE

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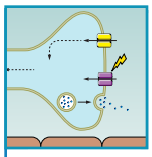

Mazzone SB, Undem BJ. Vagal Afferent Innervation of the Airways in Health and Disease. *Physiol Rev* 96: 975–1024, 2016. Published June 8, 2016; doi:10.1152/physrev.00039.2015.—Vagal sensory neurons constitute the major afferent supply to the airways and lungs. Subsets of afferents are defined by their embryological origin, molecular profile, neurochemistry, functionality, and anatomical organization, and collectively these nerves are essential for the regulation of respiratory physiology and pulmonary defense through local responses and centrally mediated neural pathways. Mechanical and chemical activation of airway afferents depends on a myriad of ionic and receptor-mediated signaling, much of which has yet to be fully explored. Alterations in the sensitivity and neurochemical phenotype of vagal afferent nerves and/or the neural pathways that they innervate occur in a wide variety of pulmonary diseases, and as such, understanding the mechanisms of vagal sensory function and dysfunction may reveal novel therapeutic targets. In this comprehensive review we discuss historical and state-of-the-art concepts in airway sensory neurobiology and explore mechanisms underlying how vagal sensory pathways become dysfunctional in pathological conditions.

I. INTRODUCTION

Sensory nerve terminals communicate information about the local environment to the central nervous system. Our knowledge is deepest about those sensory nerves that recognize and communicate information about the external environment (e.g., vision, hearing, olfaction, and somatosensory sensations of touch and pain). We know less about the sensory nerves that have evolved to recognize and communicate information regarding our internal environment, i.e., the visceral sensory nervous system. The visceral sensory nerve fibers are often colocalized within autonomic sympathetic and parasympathetic nerves. This has led to a confusing nomenclature with terms such as "autonomic sensory nerves" or "sympathetic and parasympathetic sensory nerves." This abuses the original intent of autonomic nerves describing the efferent visceral nervous system (232). One could therefore argue that more appropriate terms would be spinal-visceral and vagal afferent nerves. Although afferent nerves within the vagus innervate virtually all visceral organs, nearly 20% terminate within the airways and lungs. This review provides an up-to-date overview of these respiratory vagal afferent nerves.

An average adult inhales over 6,000 liters of air a day from which the lungs extract the vital oxygen. The air we breathe however is neither constant nor homogeneous. The temperature, osmolarity, pH, and gas composition can change with changing environments. In addition, there can be substantive differences in the amount and type of particulate matter and environmental irritants in the air we breathe. To sense these differences, the airways utilize a dense afferent innervation that is derived mainly, but not exclusively, from neurons in the vagal sensory ganglia.

The information arriving in the CNS from the vagal afferent nerves is largely subconsciously interpreted and integrated into efferent actions including alterations in the rate and depth of breathing as well as increasing or decreasing autonomic flow to the airways smooth muscle, glands, and vasculature. In addition, activation of certain vagal afferent nerves in the respiratory tract can lead to the conscious sensations of dyspnea and urge to cough. In health, the afferent nerves assist in the fine-tuning of lung function, and perhaps more importantly, provide a critical defense mechanism aimed at keeping the airspaces sterile. In respiratory pathology, the sensory nervous system can become dysregulated. This can lead to autonomic reflexes (bronchospasm, secretions), urge to cough, and dyspnea that are out of balance and exaggerated with respect to the activating sensory stimulus. This would appear to be espe-

Table 1. *Characteristics of airway vagal afferent neurons*

	Jugular Ganglia		Nodose Ganglia			
Defining molecular profiles	Runx1/Wnt1 (during development only), neuropeptides (SP/CGRP)[a], P2X3, TRKA, GFRα3		Phox2a/Phox2b (during development only), 5-HT3 receptor, P2X2, P2X3, TRKB			
Fiber type	C-fibers	Aδ-fibers	C-fibers	Aδ-fibers	Aβ-fibers	
Common names	Nociceptors, chemosensors	Aδ-nociceptors	Nociceptors, chemosensors	Cough receptors	RARs	SARs
Conduction velocity, m/s	~1	~6	~1	~5	~15	~18
Terminations						
Extrapulmonary	Many	Some	Few	Many	Few[c]	Few[c]
Intrapulmonary	Some	Some	Many	Some[b]	Many	Many
Responsivity						
Punctate mechanical[d]	No	No	No	Yes	Yes	Yes
Tissue stretch[d]	No	No	No	No	Yes	Yes
Bronchoconstriction	No	No	No	No	Yes	Yes
Capsaicin	Yes[e]	Yes	Yes[e]	No	No	No
Acid	Yes	Yes	Yes	Yes	Unknown	Unknown
ATP	No	No	No	No	Yes	Yes
Physiological responses	Apnea, cough	Unknown	Tachypnea, bronchoconstriction	Cough	Tachypnea, bronchoconstriction	Hering-Breuer, bronchodilation

[a]Jugular Aδ-fibers do not express substance P (SP). [b]Intrapulmonary cough receptors are confined to the large airways. [c]Some species have a small population of RARs/SARs in the trachea. [d]Low-threshold mechanical stimulation, as all afferents respond to mechanical stimuli if the intensity is high. [e]In the mouse, a sizeable population of capsaicin-insensitive nociceptors exists.

cially obvious for those disorders that involved inflammation including rhinitis, bronchitis, asthma, and chronic obstructive pulmonary disease (COPD).

In this manuscript we have attempted to provide a thorough review of the physiology of the vagal afferent innervation of the respiratory tract. In addition, we have included some insights regarding how this system may contribute to the pathophysiology of respiratory diseases. Although effort has been made to provide a balanced overview, some areas of vagal respiratory afferent physiology will not receive the emphasis that they deserve. The reader is encouraged to seek other excellent reviews that may fill in any such gaps (87, 90, 240, 385, 449).

II. CLASSIFYING AIRWAY AFFERENT NERVES

A. Characterization Based on Embryological/Developmental Aspects

The vagal sensory neurons innervating the respiratory tract are situated in two distinct ganglia referred to as the nodose ganglion and the jugular ganglion (also referred to as the inferior and superior vagal ganglion or nodose and supranodose ganglion) (19). As discussed in more detail throughout this review, the nodose and jugular afferent nerves innervating the airways have distinct phenotypes, anatomical projections to the respiratory tract and brain stem, and thus are likely to subserve distinct functions (summarized in **TABLE 1**).

1. Nodose neurons

During development the lateral borders of the neural plate form neural folds in a process referred to as neurulation. These folds fuse together forming the neural tube, and the dorsal midline of the neural tube is referred to as the neural crest. The vast majority of peripheral neurons are derived from neural crest cells. When the neural crest is ablated, however, nodose neurogenesis and gangliogenesis proceed normally. This is because the nodose neurons are embryologically derived not from neural crest cells, but from cells within the placodes (19). The placodes are bilateral thickened patches that form on the surface of the ectoderm. There are a series of placodes that form above the pharyngeal clef. These are collectively referred to as the epibranchial placodes and comprise the geniculate, petrosal, and nodose placodes. In these embryonic structures birth is given to the neurons that eventually form the geniculate, petrosal, and nodose ganglia (20).

Neurogenesis and the migration of neurons to the nodose ganglia are complex and highly orchestrated events guided by a set of molecular signals. Neurogenin 1 and neurogenin 2 are a family of basic helix loop helix (bHLH) transcription factors that are essential for the development of nodose neurons. The paired-like homeodomain transcription factors Phox2 are expressed in epibranchial placode-derived neurons. These factors are not essential for neurogenesis, but appear to be required for survival (132, 267). Phox2a is genetically upstream

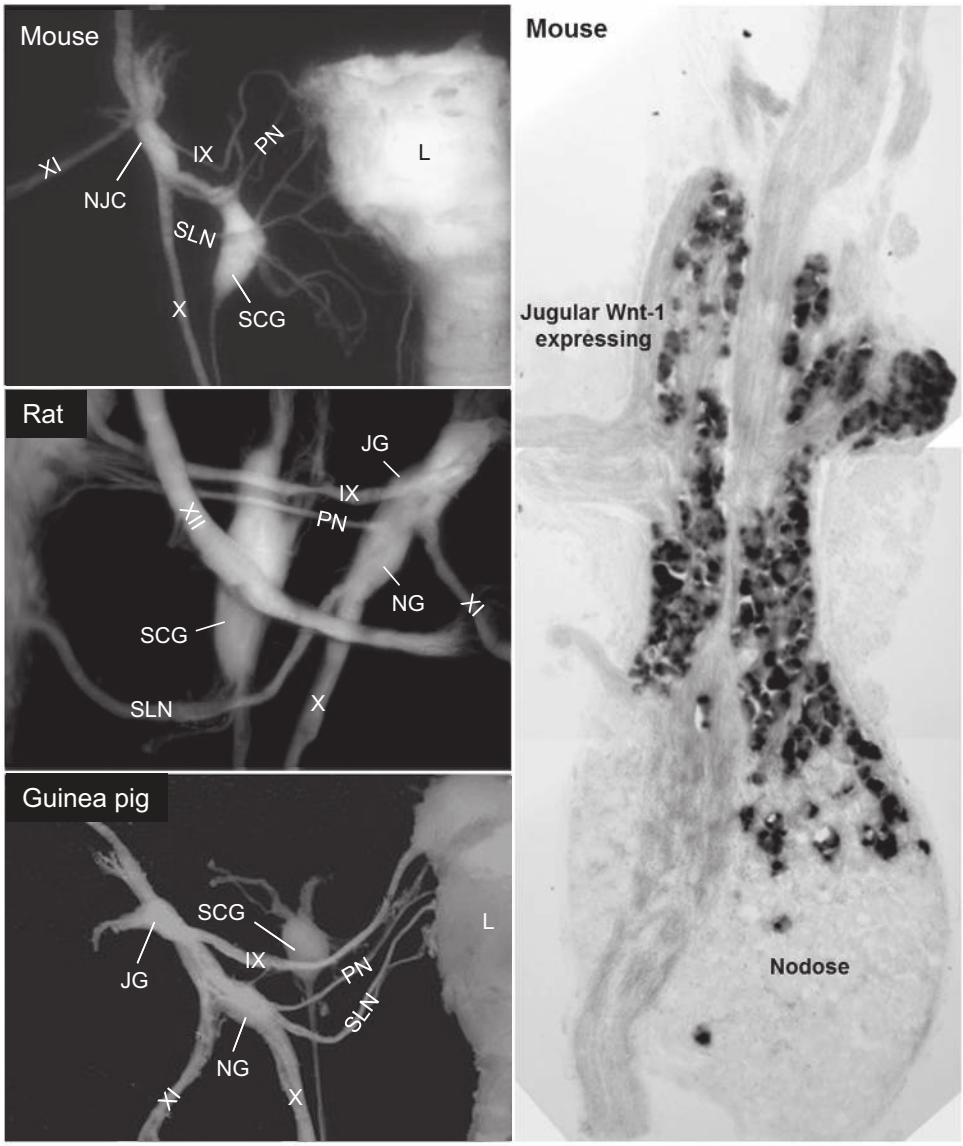

FIGURE 1. Vagal sensory ganglia. *Left*: dissections of mouse, rat, and guinea pig vagal sensory ganglia revealing their anatomical location relative to the superior cervical ganglion (SCG), superior laryngeal nerve (SLN), and larynx (L) (note: the right vagus is shown for mouse and guinea pig, and left vagus shown for rat; dissections performed by Dr. Fei Ru, Johns Hopkins Asthma and Allergy Center). Cranial nerves are labeled with Roman numerals. JG, jugular ganglion; NG, nodose ganglion; JNC, nodose-jugular complex; PN, pharyngeal nerve. *Right*: X-Gal staining of vagal ganglion complex in Wnt1Cre/R26R mice. Neural crest-derived cells formed the rostral pole of the ganglion, whereas the body and caudal pole of the ganglion comprised the placodal cells. [From Nassenstein et al. (334).]

from Phox2b which is then required for *ret* formation. Ret is a co-receptor for the neurotrophic factor GDNF that is important in nodose neuronal survival. In mice lacking Phox2a or Phox2b, the nodose and petrosal ganglia are virtually absent (132, 267).

2. Jugular neurons

In contrast to the nodose neurons, jugular neurogenesis and gangliogenesis are entirely dependent on the neural crest (97, 332). The neurons within the jugular ganglion were originally neural crest cells that emigrated from the hindbrain at the level of the first three somites. The neural crest cells emigrating from this region are fated for more than just jugular sensory neurons in that they also comprise the sensory neurons in the most rostral dorsal root ganglia, parasympathetic neurons, sympathetic neurons, the carotid body, as well as Schwann cells and glial cells. The ultimate fate among this panoply of cells depends on the molecular timing of various key transcriptional signals. This is reviewed in detail here (156).

One such key signaling molecule is Wnt1. Wnt1 is not expressed in fully developed neurons, but neural crest neurons are derived from precursor cells that express Wnt1. Neural crest derived cells can be identified experimentally by transgenically taking advantage of the Wnt1 promoter (117) **(FIGURE 1)**. In the rat and mouse it often appears morphologically as if there is a single vagal sensory ganglion (9, 334). In these cases neurons in the rostral component of the ganglion express Wnt1 during development and are therefore jugular neurons (derived from neural crest), whereas the bulk of the body of the ganglion comprises nodose neurons **(FIGURE 1)** (334). In other words, it is often the case that in the mouse the vagal sensory ganglion is a fusion of nodose and jugular

neurons. In other mammals including human beings, the jugular ganglion is seen as a distinct ganglion, but only ~20% the size of the nodose ganglion.

B. Characterization of Anatomical/Morphological Properties

1. Ganglionic origin

The respiratory passages and lungs span from the nasal cavity in the cranium through extrathoracic and intrathoracic conducting airways and ultimately to the alveoli, the functional units for gas exchange. In doing so, the airways undergo substantive changes in their histological make-up, reflective of the diversity in physiological functions that occurs at different sites along the airway tree. Thus the airways in their entirety not only occupy a large area across multiple body cavities, but are functionally complex (as distinct from a simple conduit for gas movement). The size and functional attributes of the airways are therefore reflected in the organization of the sensory neural innervation that supplies the respiratory tree with afferent sensors for monitoring airway environment. This innervation is neither homogeneous, nor is it derived from a single sensory neural origin, instead emanating from multiple sources including several cranial and spinal nerve origins. Understanding the ganglionic neural origin of airway sensors provides some fundamental insights into airway sensory neurobiology.

Although this review is focused on vagal afferent pathways, for completeness a brief discussion of the innervation to the most proximal airways follows, mainly to highlight the distinction between airways with vagal versus nonvagal innervation. The sensory innervation of cutaneous tissues and the mucosal membranes in the mammalian head, including the nasal passages, is derived from the trigeminal ganglia with the majority of sensory fibers reaching the nasal mucosal via the anterior ethmoidal nerve. The structural organization of the trigeminal ganglion has been studied in some detail and in many species sensory neurons within the ganglia are topographically arranged. Early studies employed sectioning of discrete peripheral branches of the trigeminal nerves to assess associated chromatolytic changes in ganglion neurons that supply the nerve branch in question (281). The results of such studies, for example, in rats, have shown distinct topography of cells that project to nasal, mental, alveolar, labial, and other trigeminal-innervated tissues. Comparable findings have been reported in electrophysiological experiments mapping terminal field locations and in studies employing more modern neuroanatomical tracing techniques (99). We are not aware of any studies showing innervation of the nasal mucosa by any of the spinal dorsal root ganglia.

Trigeminal innervation is replaced by glossopharyngeal and vagal innervation at the level of the nasopharynx, pharynx and soft palate, and vagal innervation of the larynx and more distal airways, the latter projecting to the airways via pharyngeal, superior laryngeal, recurrent laryngeal, and pulmonary branches of the vagi. Glossopharyngeal afferents have their soma in the petrosal ganglion while as described above vagal afferents are derived from either the jugular or nodose vagal ganglia. In some species, these three ganglia are not discrete anatomical entities but rather semifused or completely fused as a single ganglion complex, albeit with some somal topographical organization as described above with jugular and nodose neurons preferentially located in the rostral and caudal poles of the ganglion complex, respectively **(FIGURE 1)**. DRG innervation of pharyngeal structures has not been reported to our knowledge; however, DRG neurons in the C2-T6 spinal segments can be retrogradely labeled from the epiglottis, laryngeal mucosa, and more distal airway segments in many species, albeit the density of this innervation may differ between species (300, 462).

2. Termination sites and terminal structures

Sensory nerve terminals can be found widely distributed throughout all levels of the airway tree and in association with many different end-organs or tissue types within the airway wall. Generally speaking, terminal structures are found in close apposition to epithelial cells or innervating structures such as neuroepithelial bodies (NEBs), mucosal glands, submucosal vasculature, airway smooth muscle, and intrinsic autonomic ganglia **(FIGURE 2)**. However, terminals may also be present in adventitial, submucosal, and parenchymal tissues without apparent association with a distinct cell type (43, 231, 393, 448).

Throughout the airways, the mucosal lining is amply supplied with a dense plexus of fibers that typically exists in intimate association with the epithelium, inasmuch as disruption of the epithelium also disrupts this nerve plexus (22, 23, 243, 412). Although the density of the fibers is highest in the large (conducting) extrapulmonary and intrapulmonary airways, fibers can nonetheless be found throughout the entire airway tree (234). Comparable findings have been reported in many species including rat, pig, guinea pig, sheep, cat, and human airways (23, 91, 106, 231, 448). In all species, the majority of these fibers can be seen at the light microscopy level as having a relatively simple terminal structure/profile with a varicose or beaded appearance **(FIGURE 2)** and their axons often do not stain with markers for myelin but rather express one or more neuropeptides [notably substance P, calcitonin gene-related peptide (CGRP) and/or vasoactive intestinal polypeptide (VIP)] as well as the enzyme needed for producing the gaseous transmitter nitric oxide and the capsaicin receptor, transient receptor potential vanilloid 1 (TRPV1) (106, 108, 146, 231, 243,

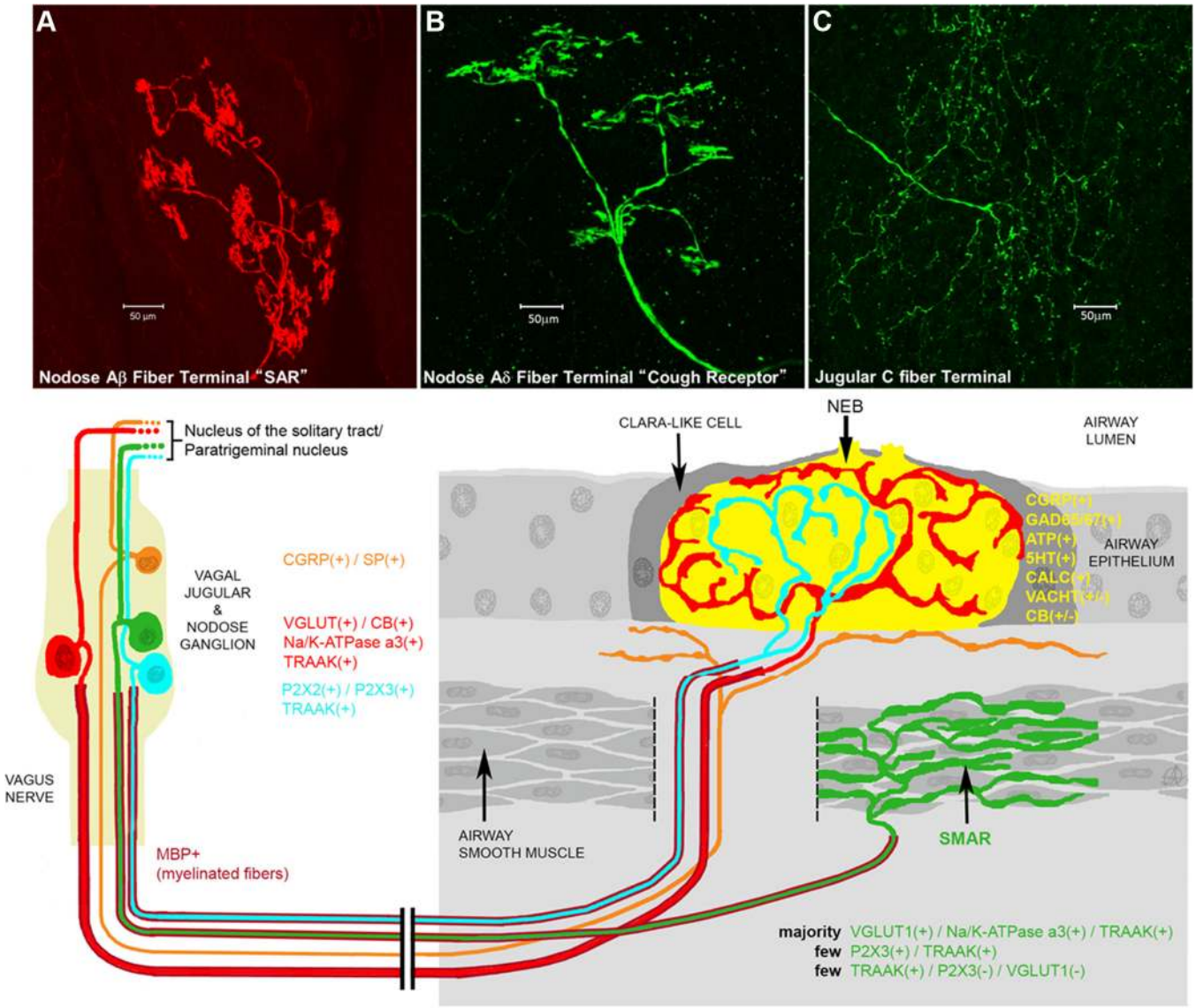

FIGURE 2. Microanatomy of airway vagal sensory nerve terminals. *Top*: examples of vagal afferent nerve terminals in airway tissue. *A*: afferent nerve termination in rat lung. This nerve was first identified electrophysiologically as the termination of an Aβ SAR fiber. The nerve terminal was then immunohistochemically defined using an antibody against Na^+-K^+-ATPase. (Figure kindly provided by Dr. Jerry Yu, Univ. of Louisville.) *B*: nerve terminal of a "cough receptor" in guinea pig trachea arising from a nodose neuron. The terminal was identified after in vivo transfection of ganglion neurons with AAV-GFP (for more structural information about these terminals, see Ref. 294). *C*: the terminal of a presumed jugular C-fiber in the guinea pig trachea. This terminal was also identified after in vivo transfection of ganglion neurons with AAV-GFP. For information on the AAV-GFP transfections, see Ref. 212. *Bottom*: schematic representation of the vagal afferent nerve fiber populations found to innervate neuroepithelial bodies (NEBs) in mouse intrapulmonary airways. [Modified from Adriaensen et al. (15a) and kindly provided by Dr. Dirk Adriaensen, Univ. of Antwerp.]

294, 442). Retrograde tracing experiments using diffusible versus nondiffusible neuronal tracers have confirmed the sensory origin of epithelial fibers, with those in the large airways (as many as 95% in guinea pigs, rats, and mice) originating from the jugular vagal ganglia (176), albeit a population of DRG derived fibers may also exist in the large airways of some species (221, 300). In the lower airways, many comparable epithelium-associated fibers can be found originating from the nodose ganglia (as well as from the jugular ganglia and DRG), although many of these fibers can be devoid of neuropeptide expression (65, 79, 176, 334). Jugular ganglia neurons typically project to the proximal airways (larynx and upper trachea) principally via the superior laryngeal branch of the vagus nerve, while nodose neurons reach the same airway segment via the recurrent laryngeal nerve branches. The lower airway segments receive innervation via the recurrent and/or pulmonary branches of the vagi. At the electron microscopic level, epithelial nerve fibers appear unmyelinated, densely packed with mitochon-

dria, and the axon membranes are surrounded by the membranes of adjacent epithelial cells, with nerve terminal structures sometimes penetrating as far as the luminal surface (185, 228).

Specialized collections of neuroendocrine cells within the airway epithelium known as NEBs have been studied in detail with respect to their sensory innervation **(FIGURE 2)**. NEBs have a nonuniform distribution throughout the airways of many species, including humans, with the highest density in early life and a progressive decline thereafter (185, 434). They are characterized by their expression of several releasable mediators including a wide variety of neuropeptides, serotonin, and ATP. Intriguingly, both simple unmyelinated sensory neuron terminals (comparable to those innervating other epithelial sites) as well as more complex myelinated terminal structures terminate within or around the basal pole of NEBs, and these sensory profiles are derived from vagal (exclusively nodose) and spinal (T1–T6) ganglionic origins (43, 45, 245).

Beneath the epithelium, axons both with and without myelin can be found. Unmyelinated fibers have the same anatomical and neurochemical appearances as those described innervating the epithelium. Their simple terminal structures can be readily found in association with mucous glands, the microvasculature, striated or smooth muscle (depending on the airway level) and within autonomic ganglia, airway lymph nodes and in specialized tissues such as bronchial associated lymphoid tissues (BALTs) (22, 58, 179, 192, 286, 288, 294, 372, 448). In contrast, myelinated fibers have a more restricted distribution and a more complex terminal anatomy **(FIGURE 2)**. In the laryngeal skeletal muscles, many myelinated fibers are likely associated with specialized sensory apparatus for detecting muscle length and touch, including muscle spindles, Pacini-like corpuscles, and Ruffini-like corpuscles. As these are not derived from vagal origins, they are beyond the scope of the present review. The myelinated sensory fibers described in most detail are those which innervate the laryngeal mucosa, tracheobronchial airways, and/or lung parenchyma. At these sites, the vast majority of myelinated fibers lose their myelin sheath before ramifying into an exquisite terminal structure of varying complexities. Such terminals can be found beneath the epithelium, presumably tethered to the subepithelial matrix or associated with NEBs, in the lung parenchyma or associated with airway smooth muscle (in which case they are referred to as "smooth muscle associated receptor," SMARs) (44, 45, 469, 471). Unlike in the myenteric plexus of the gastrointestinal tract where myelinated intraganglionic laminar endings that have been described, there are no myelinated sensory fibers that have been described innervating the intrinsic airway autonomic ganglia, although a subset of intraganglionic endings have been described in the tracheal ganglia, which appear not to be sensory in nature but rather are derived from neurons in the adjacent esophageal myenteric plexus (105, 288).

The main axon trunk, and sometimes several of the first-order branches, of myelinated sensory nerves stain positively for markers of myelin, large molecular weight neurofilaments or the sodium potassium chloride exchanger (NKCC1) (53, 289, 448), but not for neuropeptides (at least none that have been studied to date). The terminal structures themselves have been visualized by several means, including anterograde axonal dye filling, immunohistochemical staining (for example, for the alpha3 subunit of Na^+-K^+-ATPase, the calcium binding proteins calretinin and calbindin, K^+ channels or P2X receptors), histochemical staining with osmium tetroxide or staining with fluorescent styryl dyes such as FM2-10 (22, 102, 212, 246, 289, 294, 461-463). The results of such studies reveal a terminal structure that is remarkably conserved across species, characterized by a single large-diameter primary axon giving rise to a number of ramifying branches with terminal boutons that are typically aligned parallel with the arrangement of the underlying or associated smooth muscle spindles. Some ramifying myelinated afferents in the laryngeal mucosa express purinergic P2X2 and P2X3 receptors but are distinct to the alpha3 Na^+-K^+-ATPase expressing terminals at the same location (412). At the electron microscopy level, the loss of terminal Schwann cells is confirmed, and the terminal structures are densely packed with mitochondria, lysosomes, and synaptic vesicles (218, 464). Most, if not all, of these myelinated neurons are derived from the vagal (principally the nodose) ganglia (294, 344, 464). A population of myelinated jugular ganglia and dorsal root ganglia derived nerve fibers (likely Aδ-nociceptors) has been shown to innervate the airways of some species (344, 376); however, the terminal structure of these fibers has not been conclusively resolved.

C. Characterization Based on Fiber Conduction Velocities

The velocity by which an action potential is conducted along the nerve axon is a function of the axon diameter and degree of myelination (170). Large myelinated axons conduct action potentials with much higher velocities than small unmyelinated axons. The action potential conduction velocity is commonly used in classifying afferent nerve subtype.

There is however some confusion when it comes to nomenclature based on conduction velocities of afferent nerves. The conduction velocities of the axons within a nerve like the vagus are evaluated by quantifying the compound action potential and generally classified based on the scheme of Erlanger and Gasser (see Ref. 362). A compound action potential is obtained by electrically

stimulating a compound nerve like the vagus and recording the potentials with an extracellular electrode positioned at a defined distance from the point of stimulation **(FIGURE 3*A*)**. There are typically three or four discrete waves in peripheral nerves reflecting three or four conduction velocity classes. The axons conducting in the slowest wave of the compound potential (the wave arising at the recoding electrode last) are referred to as C-fibers (or in some cases type iv fibers). In some cases the second to slowest wave is referred to as the B (or type iii) wave and the fastest waves are referred to as A waves. The B wave is often considered by convention to represent autonomic fibers, so afferent nerves within the compound potentials are often referred to the C wave and three A waves designate Aδ, Aγ, and if present Aβ going from slowest to fastest, respectively. When an Aγ wave is discussed, it is the wave faster than Aδ but slower than Aβ. As with the B wave, the Aγ is by convention limited to characterization of efferent autonomic nerves. The conduction velocities of axons comprising the sensory nerve compound potential in the vagus are thus referred to as C-fibers (slowest), Aδ intermediate, and the fastest comprising Aβ fibers. It should be kept in mind that the classification of a nerve based on conduction velocity is not based on the velocity per se, but on the conduction velocity of the axon relative to other fibers within the compound nerve. A given type of A-fiber in the mouse may have a different conduction velocity than the same type of nerve in other species. In the thoracic vagus nerve of the guinea pig, the C wave comprises fibers averaging ~0.4–3 m/s, the Aδ wave averaging between 3 and 10 m/s and the Aβ wave averaging ~20 m/s (62). A fourth wave (Aα) is seldom observed in vagal compound potentials unless those innervating the laryngeal muscles are included. In dogs, the C wave averaged 1 m/s; Aδ wave, 10.5 m/s; and the Aβ wave, 18 m/s (465). In the mouse vagus nerve, the C wave was 0.5–0.9 m/s and the fastest A wave only 6 m/s (213).

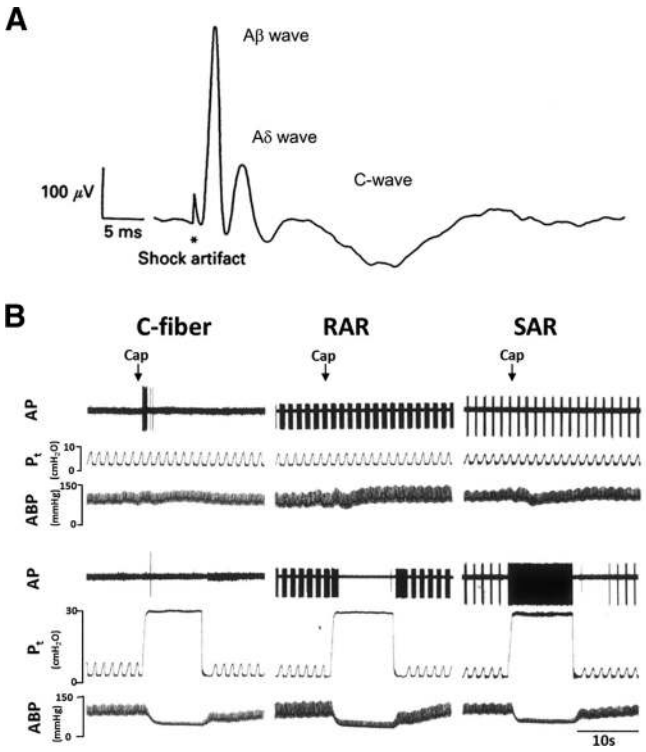

FIGURE 3. Airway vagal afferent electrophysiology. *A*: example of a compound action potential recorded from the guinea pig recurrent laryngeal nerve. The vagus nerve was stimulated at asterisk with an electrical impulse of sufficient magnitude to stimulate all axons. The action potentials arrive at the recording electrode on the recurrent laryngeal nerve in three waves corresponding to the Aβ (~20 m/s), Aδ (~10 m/s), and C (0.3–3 m/s) waves. [From Canning et al. (62).] *B*: representative experimental records illustrating three different vagal afferent nerve phenotypes innervating the lungs of a rat. The first panel shows a pulmonary C fiber; conduction velocity of this fiber was 1.05 m/s. The second panel shows a RAR fiber; conduction velocity, 21.4 m/s. The last panel shows an SAR fiber; conduction velocity, 23.5 m/s. Capsaicin (Cap) was slowly injected at arrows, and hyperinflation was generated by maintaining a constant tracheal pressure (Pt.) at 30 cmH_2O for 10 s, while the respirator was turned off. AP, action potentials; ABP, arterial blood pressure. [From Ho et al. (169), with permission from Elsevier.]

1. C-fiber subclasses

C-fibers outnumber A-fibers in the sensory vagus nerves by a factor of ~8:1 (305). In the respiratory tract, C-fibers are often subclassified as pulmonary C-fibers or bronchial C-fibers, depending on whether the terminations receive blood supply from the pulmonary or bronchial circulation, respectively. This classification is based on pioneering studies by Coleridge and Coleridge (85, 87). Operationally a C-fiber is considered to be a pulmonary C-fiber if it responds to a chemical stimulant with short latency when delivered by right atrial injection to the pulmonary circulation. A C-fiber is termed "bronchial" if it is located in the large airways or if it responds with short latency to a chemical stimulant injected directly into the systemic circulation, i.e., into the bronchial artery. Pulmonary C-fibers are thought to terminate largely in the lung interstitium close to the pulmonary capillaries. For this reason, Paintal (351) referred to these fibers as juxtacapilliary receptors or J-receptors. Nerve fibers with conduction velocities in the A-range can also be found terminating in this region so technically the pulmonary C-fibers may be a subset of the J-receptors (17).

More recently, vagal C-fibers innervating the respiratory tract have been subclassified based on whether the cell body is situation in the jugular (neural crest derived) or nodose (placodal derived) ganglia (334, 432). Extensive studies in mice and guinea pigs reveal the C-fiber nerve phenotype is distinct between nodose and jugular C-fibers. The jugular C-fibers are more apt to contain sensory neuropeptides than nodose C-fibers. The nodose C-fibers can be stimulated by a wider range of chemical stimuli than jugular C-fibers (80, 224, 334, 432).

The majority of C-fibers terminating in the large extrapulmonary airways of guinea pigs are jugular C-fibers, with nodose C-fibers comprising only 10–20% of tracheal C-fibers (376). In contrast, similar numbers of nodose and jugular C-fibers terminate in the intrapulmonary tissues (432). Importantly, where it has been studied, the C-fiber phenotype of a nodose and jugular C-fiber remains constant regardless of where it terminates within the respiratory tract. Therefore, when describing phenotypic subsets of C-fibers, the embryonic history has advantages over location of the terminations. That embryonic history is important is verified by the observation that the jugular C-fiber phenotype is similar to the C-fibers that arise from neurons within the dorsal root ganglia (like jugular neurons, they too are derived from the neural crest) (224, 408).

Nodose and jugular C-fibers respond to potentially damaging mechanical forces in a graded fashion. They also respond to inflammatory mediators and tissue acidification **(FIGURE 3*B*)**. It can be therefore argued that these nerves provide the tissue "with a sense of its own potential injury," a feature that Sherrington used in his characterization of afferent nerves that he termed "nociceptors" (398). This does not however mean that they may not also subserve physiological functions. For example, studies support the hypothesis that a subset of pulmonary C-fibers respond to decreases in lung compliance in a manner that may lead to subconscious sighs or deep inspirations (265).

2. A-fiber subclasses

The vast majority of respiratory vagal afferent fibers that conduct action potentials in the "A" range are derived from nodose neurons. These nerves generally respond with low thresholds to mechanical forces **(FIGURE 3*B*)**.

A) Aδ-FIBERS (COUGH RECEPTORS). In guinea pigs there is a group of A-fibers that lead to cough and terminate almost exclusively in the extrapulmonary bronchi, trachea, and larynx (55). These nerves conduct action potentials at ~5 m/s (within the slowest vagal A-fiber compound potential, i.e., Aδ fibers). This velocity is about five times faster than C-fibers and five times slower than the Aβ class. These fibers are unique in their lack of response to tissue distension, airway smooth muscle contraction, and inflammatory mediators. They are, however, exquisitely sensitive to punctate mechanical stimulation (touch) of the epithelium. These terminals are also sensitive to acid, but only when there is a rapid drop in pH (215). When the decrease in pH occurs gradually, the acid sensing mechanism adapts before action potential discharge occurs. The terminals only respond during the dynamic phase of a ramp and hold mechanical force applied vertically to the epithelium just above their terminations (296). As long as the force is changing, the nerve response is nonadaptive, but once the force is static regardless of the degree of epithelial indentation, the nerve ceases firing. Therefore, the adaptation is likely at the level of the stimulus and not a property of the nerve terminal membrane.

The unique structures of these terminals have been described in guinea pig trachea, and nerves with similar structures have recently been described in human bronchi (294) (448). Interestingly, mice have a less developed cough reflex and are lacking these fibers, and rats, which cough less than guinea pigs, have quantitatively fewer of these fibers in their trachea. Physiological studies have revealed that stimulation of these fibers cause a strong cough response, even when an animal is anesthetized (55). It stands to reason that these bona fide "cough receptors" provide a selective advantage by reducing the potential lethal complications of aspiration.

B) Aβ-FIBERS (RAR, SAR, STRETCH RECEPTORS). The vagal afferent fibers terminating in the respiratory tract that conduct action potentials in the Aβ range are by in large sensitive to the lung distention evoked by inspiration (240, 385) **(FIGURE 3*B*)**. A subset is also sensitive to the mechanical forces caused by lung deflation (257). Recording of vagal afferent nerves responding to lung inflation were included in the pioneering studies of afferent nerve recording by Adrian nearly a century ago. In fact, some of the first single unit recordings of action potentials in any nerve were described by Adrian in his evaluation of vagal nerve responses to lung inflation in the cat in 1933 (6).

3. Adaptation rate

The mechano-sensitive Aβ fibers are conventionally subcategorized based on action potential adaptation as slowly adapting receptors (SARs) and rapidly adapting receptors (RARs). The mechanical forces of a deep inspiration can be subdivided into the dynamic component and the static (maintained) component. It has long been recognized that some vagal afferent A-fibers respond strongly to the dynamic component but not to the sustained component, whereas others respond both to the dynamic and sustained component (211). Those nerves that respond primarily to the dynamic component of a suprathreshold inspiration are referred to as RARs, whereas those that respond to both components are referred to as SARs. Although SARs respond during the maintained static component of the inspiration they generally respond with a higher frequency of action potential discharge during the dynamic component (385) **(FIGURE 3*B*)**. The SARs are more sensitive to lung inflation than RARs. For example, in one study the SARs on average responded to transpulmonary pressures of ~6 cmH_2O, whereas RARs required pressures of about 13 cmH_2O (203).

Traditionally, going back to the studies of Knowlton and Larabee, a low-threshold stretch receptor is given an "adaption index" (211). The adaption index is defined as the

percentage of discharge occurring during the first second of the dynamic phase of inspiration relative to that occurring during the first second of both the dynamic and sustained phase. If the adaption index is >70, it is referred to as an RAR, whereas <50 is referred to as an SAR. This operational definition of RAR and SAR invites ambiguities where a nerve may respond robustly during the dynamic component and also respond during the sustained component but very weakly with low-frequency discharge such that the index is in the range of 50–70. The potential ambiguity notwithstanding, the adaptation index has been useful as a method that allows for nerve characterization among different laboratories.

The action potential adaptation is less of an objective phenotypic marker of a nerve than the conduction velocity. The adaptation of RARs is not likely to be an intrinsic electrophysiological feature of the terminals such that they cannot sustain repetitive discharge. A given RAR fiber that responds in a rapidly adapting manner to prolonged distension may respond in a nonadapting fashion to other stimuli, e.g., continuous mechanical stimulation or bronchoconstriction caused by histamine or methacholine **(FIGURE 4)** (32, 186, 296). In the case of the RAR fibers, the adaptation in action potential discharge may be a case of adaptation of the generator potential due to a decrease in the effective mechanical stimulus caused by the viscoelastic properties of the microenvironment of the terminals, rather than true ionic electrophysiological adaptation as seen for example in phasic firing neurons (319, 331). One might speculate that a given nerve that responds to lung distension in a rapidly adapting manner

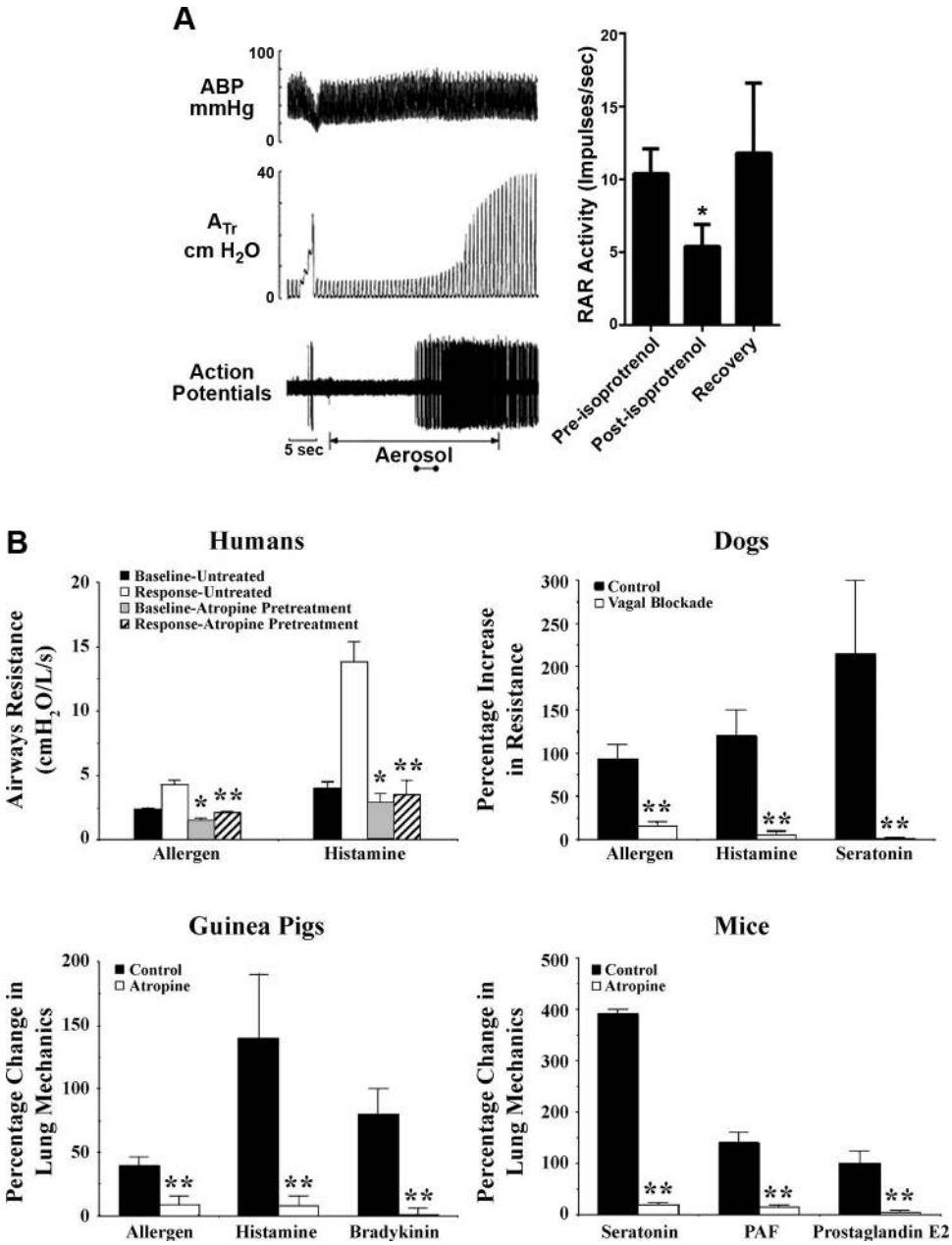

FIGURE 4. Airway vagal afferent physiology. *A*: example of an RAR fiber in the guinea pig lung indirectly activated by bronchoconstriction. Histamine was administered by aerosol, and the arterial blood pressure (ABP) tracheal pressure Ptr and action potential in a vagal RAR fiber was recorded. The histogram on the right shows that inhibiting smooth muscle contraction with isoproterenol inhibited the histamine-induced RAR activation. [From Bergren (32), with permission from Elsevier.] *B*: parasympathetic cholinergic reflexes regulate airways responsiveness in human (*top left*), dog (*top right*), guinea pig (*bottom left*), and mouse (*bottom right*). Blockade of parasympathetic cholinergic reflexes inhibits airway responses to a variety of autacoids and to allergen in all species studied. These reflex effects are easily measured when threshold doses of provocative stimuli are used. When higher doses are used, the direct contractile effects of autacoids can obscure the reflex component of the response. Measurements of lung mechanics included airways resistance, lung conductance, enhanced pause (PenH), and airway pressure-time index (APTI). PAF, platelet activating factor. [From Canning (52).]

might respond to lung distension in a slowly adapting manner if it were to terminate in a different location.

D. Characterization Based on Physiological Responsiveness

1. Mechanosensitive afferents

Mechanosensitive afferent fibers almost certainly correspond to the myelinated afferent structures described anatomically above. These have been studied at all levels of the airway tree, but their physiological attributes are perhaps best characterized within the intrapulmonary airways and lungs. Experimental work conducted in the early to mid-19th century was consistent with the existence of laryngeal sensors with mechanical activation properties, and the pioneering studies of Breuer in the mid-19th century predicted the existence of pulmonary mechanosensors, activated by lung inflation and/or deflation and intimately involved in the reflex control of respiration (see the translated works in Ref. 42). However, true characterization of such fibers as afferent mechanosensors was ultimately predicated on the introduction of electrophysiological recording techniques which were developed many years later. Indeed, as discussed above, single/teased fiber vagal recordings, typically studied in response to sustained lung inflation and/or lung collapse, differentiated intrapulmonary mechanosensors as possessing rapidly or slowly adapting stretch activated terminals, which of course is reflected in the common nomenclature employed today (RARs and SARs) (55, 169, 257, 451, 467, 469).

Rapidly adapting receptors (RARs) were named reflecting their rapid (1–2 s) adaptation to sustained lung inflations (and hence their adaptation index), but can equally be distinguished from other airway afferents by activation in response to lung deflation (including lung collapse), changes in dynamic lung compliance and bronchospasm, and to some extent their conduction velocity (4–18 m/s), the latter a reflection of their myelinated axons (32, 55, 59, 169, 186, 188, 216, 280, 387, 451). Notably, dynamic alterations in lung volume (rather than static or sustained changes) do not reveal the rapidly adapting phenotype of RARs. This suggests that adaptation may reflect a complex interplay between the mechanical events occurring within the airway tissues and their transference to the nerve terminal, rather than electrophysiological adaptation per se. Given their responsiveness to lung stretch, it is not surprising therefore that some RARs are active throughout the respiratory cycle **(FIGURE 3)**, dependent upon the rate and volume of lung inflation (169, 349, 350). In smaller animals such as guinea pigs and rats, eupneic RAR activity of 16–27 impulses/s is routinely reported, whereas this may be fewer than 5 impulses/s in larger animals. In monkeys (*Macaca mulatta*), RAR fibers were found to terminate mainly in the lobar bronchi. They conducted action potentials consistently around 20 m/s and were not activated by the mechanical force of eupneic respiration (373).

Slowly adapting stretch receptors (SARs) differ from RARs in some species in terms of their action potential conduction velocities (which tend to be faster for SARs) and by a different airway distribution compared with RARs (26, 55, 218, 296, 449, 467, 469, 471). However, the best defining feature of an SAR is their slow rate of action potential discharge adaptation during sustained lung inflations. Similar to RARs, SARs are also highly sensitive to the mechanical forces that occur in the lungs during normal tidal respiratory cycles. Accordingly, the inspiratory phase is accompanied by a sharp increase in SAR activity that peaks at the point of inspiratory termination **(FIGURE 3)** (169, 390).

Pulmonary mechanoreceptor activity can also be increased secondary to bronchospasm or obstruction resulting from mucus secretion or edema (32, 39, 59, 153, 197, 313, 318). Thus a wide variety of endogenous and exogenous substances (for example, histamine, acetylcholine, capsaicin, substance P, and bradykinin) that act upon airway smooth muscles, glands, or the vasculature can activate RARs or SARs, and this in turn can be prevented by inhibitors of the local end organ effects that these stimuli produce **(FIGURE 4)**. Indeed, this indirect chemosensitivity of RARs has resulted in some researchers applying the name "irritant receptor" to define this afferent nerve subtype (110, 111, 310, 311, 395, 396). However, this name is somewhat misleading (and not widely used anymore) as these afferents likely play an important role in the normal physiological control of breathing, and few chemical stimuli have been shown to directly cause intrapulmonary mechanoreceptor (either RAR or SAR) discharge, independent of a mechanical action on airway structural cells. An exception to this is ATP, which activates intrapulmonary mechanoreceptors (both RARs and SARs) via P2X2/3 receptors that are expressed by most nodose neurons (133, 224, 334, 444). Indeed, pulmonary mechanoreceptor activation may occur secondary to tissue release of ATP during breathing or associated with bronchoconstriction or obstruction. In the mouse, pulmonary mechanoreceptors may also express the P2Y1 ATP receptor, although it is unknown if ATP can activate mechanosensitive afferents via this receptor (72). Even though few chemicals can directly cause discharge in RARs or SARs, a variety of proinflammatory mediators may sensitize mechanoreceptors and lower their threshold for activation by mechanical stimuli (32, 39, 198, 215, 239, 280, 391).

The anatomical arrangement of RAR and SAR terminal units in the airway wall is not well described. Functional studies suggest that RARs terminate within or beneath the epithelium and are localized to both intra- and extrapulmonary airways, while SARs may be associated with the airway smooth muscle. Although a variety of studies have assessed the profile of unidentified nerve endings in the air-

ways of many different species, few have successfully married physiological recordings to characterize afferent subtypes with morphological studies to correlate this with nerve fiber terminal structures. In the rabbit and rat, intrapulmonary SAR terminal structures were successfully visualized by first functionally identifying them in vivo with electrophysiological recordings in which the local receptive field was discretely mapped, following which lung tissue microdissection was performed for post hoc immunohistochemical staining of terminal endings (440, 469) **(FIGURE 2*A*)**. Such studies not only point to the arborized terminals arising from myelinated afferents described above as belonging to lung mechanoreceptors, but also that the terminal structure is required for mechanosensation since afferent firing during lung stretch is lost following dissection of the terminal receptive field. Strikingly, these studies also suggest that SAR terminals assume varying positions within the airways and lungs and whilst most (in the rabbit) are found in the peripheral airways they are not uniformly associated with the bronchiolar smooth muscle. Indeed, SAR terminals in the lung parenchyma are not uncommon. In the dog, however, SARs are reportedly intimately associated with the smooth muscle and are activated during bronchoconstriction (384), even in the large airways such as the trachea. In contrast, cats, guinea pigs, rabbits, and rats have few if any SARs in their extrapulmonary airways (390).

The heterogeneity of pulmonary mechanoreceptors may not be as simple as a binary division between RARs and SARs, but rather subtypes within each functional category may exist (33, 169, 257, 349, 350, 387, 390, 466). Some of this additional heterogeneity might reflect the precise tissue organization of the terminal endings, rather than any distinct neurobiological features of the afferents per se. Inconsistencies in how different laboratories define RARs and SARs as well as the different preparations employed (open versus close chested animals, in vitro preparations etc.) may also account for some of this reported heterogeneity. Yet, even within a single experiment, heterogeneous populations of each afferent type have been identified. Indeed, this has led some to propose that pulmonary mechanoreceptors exist within a function spectrum in which classic RARs and SARs represent the extremes of the spectrum with many other mechanoreceptors displaying functional phenotypes laying somewhere between the two. To add to this complexity, individual mechanosensitive afferent axons can have multiple encoders (i.e., multiple terminal structures with distinct receptive fields within the lung) and in some instances one encoder can functionally respond to lung inflation while another encoder of the same afferent nerve is active during lung deflation (470). Again, this points to the tissue environment supporting the terminal structure as the main determinant of functional responsivity. This concept of a functional spectrum, however, needs to be reconciled with data showing distinct central termination patterns identified for RARs versus SARs as well as the distinct reflexes that these afferents have been shown to mediate (both of which are discussed below).

Neuroepithelial bodies may comprise an important component of the functional unit mediating SAR and/or RAR mechanosensory units. This suggestion has largely arisen because neuroepithelial bodies are innervated by vagal sensory neuron terminals that display a morphological phenotype that is comparable to a pulmonary mechanically sensitive terminal (43, 44, 245, 246). In this sense, it has been proposed that mechanical perturbations in the lung may release ATP, or another signaling molecule, from the neuroepithelial body that in turn acts as the stimulus for the vagal afferent mechanically sensitive terminal, thereby indirectly initiating the neural component of the mechanically induced responses. However, in rats, electrophysiological characterization of intrapulmonary SARs followed by microdissection of the terminal receptive field and histochemical staining of the associated nerve terminals, has shown that few, if any, classically defined SARs are associated with neuroepithelial bodies (471). Whether the same is true for classically defined RARs is not clear.

Touch sensitive airway mechanosensitive afferents (the cough receptors as described above) are distinct from RARs and SARs inasmuch as they display a low propensity to be activated by tissue stretch but rather respond vigorously to light punctate mechanical stimuli. Such receptors have been best studied in guinea pigs, although comparable afferents likely exist in the airways of other species, including humans (55, 289, 294, 448). Cough receptors are principally found within the large airways (larynx, trachea, and main bronchi) and represent a population of polymodal nodose ganglia-derived low-threshold mechanoreceptors, which in the guinea pig represent the only significant population of nodose derived A-fibers in the large airways (55, 294, 376). In addition to responding to punctate mechanical stimuli of the epithelium overlying the afferent terminal, these afferents are also highly responsive to rapid changes in luminal pH (acidification) and hypotonic solutions (e.g., distilled water), which are dependent on the expression of acid sensing ion channels (ASICs) and Ca^{2+}-activated chloride channels (215, 241, 289). However, they are unresponsive to many other chemical stimuli (55, 193, 376) and to airway distending, even well beyond the physiological range, and myriad bronchospatic agents, which are known to indirectly activate other pulmonary mechanosensors (55). Cough receptor afferents are myelinated, but typically conduct action potentials slower (~5 m/s) than classic intrapulmonary RARs and SARs, indicative of an Aδ-fiber profile. They do, however, rapidly adapt to stimuli (for example, sustained punctate stimulation or acidification; Ref. 296), but their molecular profile and the reflexes that they initiate indicate that these afferents are not simply RARs that innervate the extrapulmonary airways.

The anatomical organization of cough receptor afferents in guinea pigs has been described in detail (289, 294). Like other mechanosensors in the lung, a single myelinated axon can give rise to one or several unmyelinated arborized terminal structures of varying complexity. Terminals typically lay above the airway smooth muscle but below the epithelium basement membrane, a position that may in part explain their lack of sensitivity to bronchoconstricting agents. The guinea pig trachea contains on average 180 individual terminals that increase in density at the level of the carina, the left and right vagus nerve contributing approximately equal numbers of receptors ipsilaterally (in the tracheal wall) to their vagal origin. The main axon of each terminal stains positively for medium-sized neurofilament proteins (160KD) and NKCC1, both of which disappear soon after the axon ramifies (53, 289). The entire structure is selectively identifiable by alpha3 ATPase staining, osmium tetroxide staining, or with the vital styryl dye FM2-10 (22, 287, 294). Indeed, anatomical and physiological studies have conclusively shown that this afferent terminal is derived from nodose ganglia neurons and corresponds to the touch-sensitive fibers that mediate mechanically evoked coughing. In human tracheal biopsies, neurofilament positive axons with one or more branch points have been described, although the exquisite terminal structure identified in the guinea pig has not been described to date in the human (448).

2. Chemically sensitive afferents

A second general category of vagal afferents innervating the airways displays sensitivity to a wide range of chemical stimuli but are relatively less sensitive to mechanical perturbations in the airways. Such afferents have been referred to by a variety of terms over the years, including "nociceptors" because of their recruitment during noxious stimuli, "chemosensors" because of their chemical sensitivity, and "high-threshold mechanoreceptors" because of their relative insensitivity to mechanical stimuli **(FIGURES 3** and **4)**. Furthermore, given that such afferents can be unmyelinated (C-type) or lightly myelinated (Aδ-type) fibers, a prenominal of "C-fiber" or "A-fiber" commonly is added to the nomenclature (e.g., C-fiber nociceptor). Chemically sensitive vagal afferents are often defined by their sensitivity to the pungent component of chili peppers, capsaicin, and hence by the expression of the capsaicin-sensitive ion channel TRPV1 (55, 59, 85, 137, 169, 254, 334). However, this definition is not all encompassing, as some species have a significant population of vagally derived capsaicin-insensitive yet otherwise chemically sensitive afferents innervating the airways and lungs (213). Also of note, vagal chemically sensitive afferents are derived from both the jugular and nodose ganglia although, as discussed elsewhere in this review, such afferents are not equivalent as they differ in their molecular phenotypes, their peripheral and central terminations, and the reflexes that they evoke.

Unmyelinated C-fiber afferents comprise the majority of afferent nerves innervating the airways (90, 169, 238). Afferent C-fibers are distinguished from mechanically sensitive afferents by their conduction velocity and their direct responsiveness to a wide variety of chemical substances acting at both ligand-gated ion channels and G protein-coupled receptors (17, 32, 59, 169, 238, 376, 449). The direct sensitivity of this class of afferents to chemical stimuli is inferred by the observation that chemical activation of C-fiber endings in the airways is not inhibited by pretreatment with a bronchodilator. Furthermore, this is supported by expression studies in vagal ganglia preparations which show a wide variety of ion channels and receptors in C-fiber afferents and by the ability of ligands of these receptors to produce action potentials in patch recordings of acutely isolated vagal neurons in culture (reviewed below). Indeed, bronchodilators such as prostaglandin E_2 (PGE_2) and epinephrine actually enhance afferent C-fiber excitability rather than inhibit it (151, 152, 168, 238). C-fiber endings are polymodal, and thus can respond to both chemical and mechanical stimulation; their high threshold for mechanical activation means that C-fibers generally don't fire action potentials throughout the respiratory cycle but rather are recruited in times of tissue injury/inflammation or in the presence of noxious chemicals (17, 32, 90, 169, 238, 376, 439). Indeed, in addition to the long list of chemicals that can activate C-fibers, many inflammatory mediators can additionally sensitize C-fibers and lower their threshold for activation such that more physiological stimuli (e.g., bronchoconstriction) may activate C-fibers in the diseased airways.

A subpopulation of C-fibers synthesize neuropeptides that are subsequently transported to their central and peripheral nerve terminals (23, 176, 263, 287, 289, 294), and this has been exploited to describe the morphology of C-fibers in a variety of species, including rats and guinea pigs. Neuropeptide staining of large airway wholemount preparations or of tissue sections of the lung show a vast plexus of fine varicose fibers innervating the airway epithelium and effector structures such as airway smooth muscle, glands, the vasculature, and autonomic ganglia within the airway wall. Such fibers are impressively simple compared with their mechanosensor terminal counterparts, forming (especially in the mucosa) a web of fibers in which it is difficult to define any discrete organizational units. However, it is important to note that the expression of neuropeptides in C-fibers is both species-dependent (for example, human vagal afferents contain less neuropeptides than do guinea pigs or rats) and dependent on the ganglionic origin of the C-fiber (nodose C-fibers largely do not express substance P or CGRP), and as such, it is not clear if the described morphology is true of all C-fibers and in all species.

The absence versus presence of neuropeptide expression in subsets of C-fibers represents one example of heterogeneity

among chemically sensitive afferent neurons. In dogs, airway C-fibers have been classified as "bronchial" or "pulmonary," a distinction based partly on anatomical termination sites and supported by differences in functional responsiveness to stimuli (90). For example, bronchial C-fibers in dogs, but not pulmonary C-fibers, are responsive to histamine. However, this is not true in guinea pigs, as histamine is without direct effect on any airway C-fibers (32, 59, 88). Nevertheless, C-fiber subtypes have been identified innervating the airways and lungs of mice, rats, and guinea pigs, distinguished based on their ganglionic origin, molecular phenotype, responsivity, and termination sites within the airways (193, 224, 249, 301, 334, 376). In guinea pigs, the vast majority of chemically sensitive vagal afferents in the extrapulmonary airways are derived from the jugular ganglia, while one-half to two-thirds of the afferents in the intrapulmonary airways and lungs are derived from the nodose ganglia. Almost all jugular C-fibers express the neuropeptides substance P and CGRP and are unresponsive to ATP, 5-HT, and adenosine. In contrast, nodose C-fibers do not express substance P or CGRP, but are responsive to ATP, adenosine, and 5-HT. Whilst in guinea pigs, capsaicin and bradykinin activates all C-fibers (regardless of origin), in mice, capsaicin and bradykinin activate only a subset of airway C-fibers. Whether these distinct subsets in mice, guinea pigs, and rats are the same as the bronchial and pulmonary C-fibers identified in dogs is unclear, although this seems plausible.

Aδ fiber chemically sensitive afferents have also been described in the airways of some species (55, 176, 296, 328, 376). Extracellular unit recordings and retrograde neuronal tracing in guinea pigs suggest approximately half of the extrapulmonary chemically sensitive afferents are myelinated Aδ-fibers. As would be expected of extrapulmonary chemically sensitive afferents, these Aδ fibers originate exclusively from the jugular ganglia and are functionally distinct to the nodose-derived touch-sensitive Aδ-fibers that also innervate the extrapulmonary airways of guinea pigs trachea by their 15-fold lower sensitivity to punctate mechanical stimuli but responsivity to bradykinin and capsaicin (376). Chemically sensitive Aδ fibers express the capsaicin receptor TRPV1, but do not synthesize neuropeptides. Their terminal structure within the airways has not been defined nor is it known if subtypes of these afferents exist. The role of Aδ-nociceptors in airway homeostatic and defensive reflexes (i.e., whether similar or distinct to C-fibers) is also unknown, as is the extent to which other species possess similar afferent fibers.

E. Characterization Based on Neurotrophic Factor Receptors

Two major families of neurotrophic factors are particularly relevant to peripheral sensory neurons. The neurotrophin family comprise the prototypical neurotrophic factor nerve growth factor (NGF) along with brain-derived neurotrophic factor (BDNF), neurotrophin 3 and neurotrophin 4 (247). The other major family is referred to as the glial derived neurotrophic factor (GDNF)-related family ligands (GFLs). GFLs include GDNF, neurturin, artemin, and persephin (7). Neurotrophins and GFLs not only influence vagal sensory cell survival during development, they can alter gene expression and cell phenotype in fully developed neurons. The extent to which vagal sensory neurons are influenced by a given neurotrophic factor depends on the neurotrophic factor receptors that are expressed.

The receptors for neurotrophins are referred to as tropomyosin receptor kinase (trk) receptors. The trk receptors comprise trkA, trkB, and trkC. NGF binds preferentially to trkA, BDNF and neurotrophin 4 bind preferentially to trkB, and neurotrophin 3 binds preferentially to trkC. The receptors for the GFLs are termed the GDNF family receptor alpha (GFRα). GDNF, neurturin, artemin, and persephin preferentially stimulate GFRα1, GFRα2, GFRα3, and GFRα4, respectively. The GFRαs use Ret as a co-receptor. Ret is a receptor tyrosine kinase encoded by the protooncogene *ret* (112).

Vagal afferent neurons express both trk and GFRα receptors. NGF interacting with trkA receptors is critical for the proliferation and survival of neural crest neurons. It is therefore not surprising that most of the airway-specific sensory neurons situated in the jugular ganglia express trkA receptors (249, 334). In contrast, BDNF/NT4 and trkB have been shown to be important in the formation of placodal ganglia (118). Accordingly, virtually all airway-specific nodose neurons express trkB (249). Without trkB signaling the number of neurons in the nodose ganglia is dramatically diminished, and severe problems in respiration ensue (118). The absence or presence of trkB expression alone however is not sufficient to characterize a neuron as originating from the jugular or nodose ganglia, as a subset of jugular neurons also express trkB, just as a subset of nodose neurons express trkA (227, 249, 334).

All the vagal sensory neurons innervating the murine and guinea pig respiratory tract were found to express the GFRα co-receptor Ret (249, 334). Most nodose and jugular neurons express GFRα1. GFRα2 is less predominantly expressed in vagal sensory neurons, and GFRα3 is preferentially expressed in jugular neurons (249, 334). In summary, NGF is likely to influence primarily jugular vagal sensory neurons; BDNF is more likely to provide a major influence on nodose neurons. With respect to the GFLs, GDNF will influence all sensory neurons, whereas the GFRα3 agonist artemin may provide selective modulation of jugular neurons.

It is possible that certain neurotrophic factors are preferentially expressed within phenotypically distinct subtypes of

nodose and jugular neurons. The little work that has been done in this area indicates, however, that the neurotrophic factor receptors expressed relate more to ganglion of origin (nodose vs. jugular) than nerve subtypes (e.g., C-fiber vs. A-fibers) (249).

III. TRANSDUCTION MECHANISMS IN ACTIVATION OF AIRWAY AFFERENT NERVES

The neuronal communication between the respiratory tract and the CNS is dependent on action potentials that are generated at the afferent nerve terminals and conducted to the central terminals in the CNS where they lead to transmitter release into the synapse with second-order neurons within the brain stem nuclei in receipt of vagal afferent inputs. The pattern and frequency of this "digital" input is then integrated within the CNS ultimately leading to sensations (urge to cough, dyspnea) and reflex changes in breathing pattern and changes in the activity of sympathetic and parasympathetic outflow to the respiratory tract and other organs.

To evoke an action potential, a stimulus must depolarize the nerve terminal. This stimulus-evoked membrane depolarization is referred to as the generator potential. If the generator potential is of sufficient rate and magnitude, voltage-gated sodium channels are stimulated leading to action potential formation. The ionic basis of the generator potential depends on the nature of the stimulus.

A. Mechanical Activation

Virtually all sensory nerves innervating the respiratory tract can be stimulated to evoked action potential by mechanical perturbation of the nerve terminals. When it comes to nodose and jugular C-fibers, however, the mechanical force required is relatively large and beyond what might be expected to occur physiologically so that they would be classified as high-threshold mechanosensors; an exception to this rule may be certain pulmonary C-fibers that have been identified as weakly responding to inspiration (88, 203). Many slow conducting Aδ fibers are also relatively insensitive to tissue stretch and are referred to as high-threshold A-fibers (although some of these may be very sensitive to punctate mechanical stimulation as discussed above). In contrast, the faster conducting Aβ-fibers have evolved a more meaningful "low-threshold" mechanosensitivity to tissue distension.

1. Low-threshold mechanosensitive Aβ-fibers

Vagal A-fibers that conduct in the fast Aβ range are often found to be very sensitive to lung distension and can respond to the distension caused by deep inspiration and even eupneic breathing. As mentioned above, these nerves have been classified as SAR and RAR based on their rates of adaptation. The SAR fibers are generally more sensitive to lung distension than RARs (203).

Very little is known about the ionic basis of the mechanically evoked generator potentials in the touch-sensitive Aδ fibers or stretch sensitive vagal Aβ-fibers. The SAR fibers are generally considered to respond to increases in transpulmonary pressure and circumferential tension. Also cartilage in the trachea and bronchi can yield a transverse stretch of the tissue in which some SARs terminate that may contribute to the mechanical transduction (26, 320). SARs increase their rate of firing both with respect to the increase in pressure but are also sensitive to increases in the rate of distension (26, 350). The lack of adaptation during a breath hold indicates a sensitivity to static pressure, although there is some argument that the SAR are responding to changes in force within the microdomain of the terminals (319). The transducing elements in SARs are unknown, but data have been obtained that support a model of a viscous element arranged in parallel with an elastic element (385). The RAR fibers also respond to increases in transpulmonary pressure. At a constant rate of inflation, the frequency of action potential discharge increases as the transpulmonary pressure increases (349). Unlike SARs, RARs are also sensitive to changes in lung compliance. The less compliant the lung, the more sensitive is the RAR fiber to mechanical activation (188).

It must be kept in mind that SAR and RAR are rather crude subdivisions and the transduction mechanisms may be different in mechanosensors terminating in different structures of the respiratory tract (e.g., bronchioles vs. trachea). Although there have been some attempts at further subdivision of SAR and RAR fibers based on response characteristics, little heuristically has come of it.

More refined characterization awaits more precise detail in the structure-function relationships of the terminals within different regions. For example, it has been argued that some SARs may be stimulated mechanically in an indirect fashion via stimulation of neuroepithelial bodies. In this scenario, the mechanotransduction takes place within the NEB leading to the release of a transmitter that stimulates SAR terminals. A candidate for such a transmitter is ATP, known both to be released from NEBs and to evoke action potentials in SARs via interaction with P2X2,3 receptors (5, 55). A similar indirect mechanism has been argued for the mechanical activation of a subset of nodose C-fibers in response to bronchial smooth muscle contractions (444).

Although indirect mechanisms may contribute, it is likely that low-threshold mechanosensitive A-fibers comprise mechanically sensitive ion channels that directly activate the nerves (71, 217, 379). Since first discovered in bacteria in

the 1970s, the field of mechanically sensitive channels (Mscs) has grown immensely and along with it the number of ion channels gated by mechanical forces. This immense topic is beyond the scope of this review, but suffice it to say two general models of mechanotransduction are commonly pursued in the laboratory (71). The first model is the so-called membrane force model where the mechanical stimulus leads to activation simply by changing the nerve terminal membrane forces. Sophisticated patch-clamp recordings have proven that certain Mscs can be gated by changes in force applied only to a lipid bilayer in which the channel resides. However, molecular genetic investigations in simple organisms strongly support the idea of the mechanical forces acting on tethering molecules between the ion channel and intracellular or extracellular structures (71). It is reasonable to assume that in many cases of low-threshold mechanosensations both intrinsic membrane forces as well as forces upon tethering elements work in concert to express the full extent of the mechanically evoked action potential response.

The number of potential channels that are involved in sensory mechanotransduction is large, but the recent discovery of piezo 1 and piezo 2, representing a new class of channels may be particularly relevant to low-threshold nodose mechanosensors in the respiratory tract (93, 94). These channels are unique in their large size. In the mouse, piezo 1 contains over 2,000 amino acids and is estimated to span the membrane 30–40 times. Piezo channels can be gated independently of cytostructural elements, but full sensitivity of the channel is revealed when potential tethering proteins are involved. Piezo 2 would appear to be largely responsible for light touch and respond to delicate punctate stimuli in the somatosensory system (455). The role of piezo channels in visceral mechanotransduction is unknown, but piezo mRNA is expressed in nodose neurons. On the basis of their sensitivity to light touch, it is tempting to speculate that piezo 2 may contribute to mechanotransduction in the exquisitely touch-sensitive nodose Aδ cough receptors terminating in guinea pig extrapulmonary airways. Amiloride, an ENaC inhibitor which blocks mechanosensory channels in other systems, also blocks mechanosensitivity in cough receptor Aδ afferents, although this appears to be due to an action on voltage-gated sodium channels rather than any specific mechanosensory channel (64). Intrapulmonary stretch receptor activity can be inhibited by the stretch-sensitive ion channel blocker gadolinium, although this does not alter mechanical activation of touch-sensitive afferents (64, 266). Mouse intrapulmonary mechanosensors express the two-pore domain K^+ channel TRAAK, although functional studies supporting the role of this channel in mechanosensation have not been conducted (246).

RAR and SAR low-threshold mechanosensors have been well described in the somatosensory system (4). In the skin these nerves are often associated with secondary structures such as Pacinian corpuscles, Meissner corpuscles, hair follicles, and Merkel cells. Other than a potential association of a subset of A-fibers with NEBs (5), little evidence has been provided for key accessory cell function in vagal RAR or SARs in the lungs. With more sophisticated technologies it should not come as a surprise if such secondary cells/structures relevant to airway vagal afferent mechanotransduction are revealed in the future.

B. Chemical Activation

Vagal afferent nerves in the respiratory tract are not only mechanosensitive, but they also can communicate information to the CNS about the chemical environment in which they terminate. A myriad of chemicals, both inhaled and endogenous, can interact with specific receptors at the afferent nerve terminals that can lead to generator potentials of sufficient intensity to evoke action potential discharge. Inasmuch as low-threshold mechanosensitive A-fibers are exquisitely sensitive to mechanical changes in their environment, many of these nerves will respond to chemicals that cause bronchoconstriction **(FIGURE 4)**, plasma leakage, and mucus secretion, in an indirect manner. For example, histamine stimulates RAR fibers in guinea pigs secondary to bronchoconstriction (32). Substance P can stimulate RAR fiber in guinea pigs, but this is secondary to the production of nitric oxide and changes in lung compliance (187). In the monkey, RAR fibers respond to a host of chemicals including histamine, 5-HT, and acetone, but the extent to which this is direct activation or indirect activation was left unresolved (373). This section reviews the literature focusing on those chemicals where the evidence supports a direct chemical activation of vagal afferent nerve terminal. By and large, the nerve subtypes that respond directly to endogenous autacoids and inhaled irritants are C-fibers, but in some cases slow conducting Aδ-fibers can also respond directly to chemical stimulation.

There are several mechanisms by which chemicals can directly activate afferent nerve terminals. The simplest mechanism involves the activation of ionotropic receptors that are basically ligand-gated ion channels. The other major activating mechanism is via metabotropic G protein-coupled receptors (GPCRs). Most of the ligand-gated ion channels that have been described in vagal afferent nerves are nonspecific cation channels. In the case of the GPCRs, the ligand binds with high sensitivity and selectivity causing a signaling cascade in the terminal that ultimately opens (or possibly closes) channels leading to a depolarizing generator potential.

1. Ionotropic receptor agonists

A) NICOTINIC RECEPTOR AGONISTS. The nicotinic cholinergic receptor is one of the first described and most studied ionotropic receptors. All nicotinic receptors are pentomeric with

each of the five subunits forming four α-helical transmembrane domains (262). Genes have been discovered that encode 17 different subunits referred to as α1–10, β1–4, γ, δ, and ε. The acetylcholine-binding site is within the α subunits (though α8 is not expressed in mammals). The terminology of nicotinic receptors is based on the subunits. For example, the nicotinic receptor at the neuromuscular junction in skeletal muscle is $(\alpha1)_2\beta1\delta\varepsilon$ (262).

In an interesting psychophysical study, Lee et al. (235) showed in human subjects whom had never smoked cigarettes that, not surprisingly, inhaling cigarette smoke causes strong irritation and urge to cough. These sensations were largely dampened when the subjects inhaled the nicotinic receptor antagonist hexamethonium prior to the smoke inhalation. This indicates that nicotine is the primary agent in cigarette smoke that activates respiratory sensory nerves. Whether these sensations were secondary to vagal afferent nerves or spinal (DRG) afferent nerves could not be determined.

Paintal (352) first showed in the 1950s that nicotine injection activated vagal afferent nerves terminating in the cat lungs. These nerves were relatively slow conducting nerves that he later termed J receptors. The activation of these nerves coincided with the stimulation of nocifensive reflexes like apnea and rapid shallow breathing. That nicotine is a relevant inhaled irritant also comes from reflex studies in dogs showing that inhalation of cigarette smoke caused either apnea or rapid shallow breathing, and these respiratory reflexes could be prevented by pretreatment with the nicotinic receptor antagonist hexamethonium (236). The studies were followed up with electrophysiological investigations showing cigarette smoke and nicotine stimulates action potentials in RAR and pulmonary C-fibers in dogs by a hexamethonium-sensitive mechanism (216, 236). Along with the reflex studies, these investigations led to the conclusion that not only can the nicotine in cigarette smoke activate vagal afferent nerves, it is the major vagal afferent stimulant within the smoke.

The in vivo investigations support a direct mechanism of action for the effects of nicotine on subsets of vagal afferent nerves, yet it is possible that activation of nicotinic receptors on nonneuronal cells indirectly leads to the production of afferent nerve activating autacoids. The best evidence for direct activation of vagal afferent neurons via nicotinic receptor activation comes from studies showing that nicotine activates airway-specific individual neurons isolated from vagal sensory ganglia via a hexamathonium-sensitive mechanism (209, 460). In these studies a subset of both capsaicin-sensitive (presumably C-fibers) and capsaicin-insensitive (presumable A-fibers) were sensitive to nicotinic receptor activation, responding with both an inward depolarizing current, as well as increases in cytosolic calcium and neuropeptide secretion. The composition of the nicotinic receptors expressed by airway specific vagal sensory neurons has not been worked out in detail. Nodose neurons express mainly α3 and β4 subunits, but also some α2, 4, and 5 and β2 subunits (275). The α3 and α5 subunits may be enriched in the C-fiber population of sensory neurons (405).

Although nicotinic receptors are clearly relevant to sensations and reflexes evoked by inhaled nicotine, it remains unclear whether they are activated by endogenous acetylcholine. It is unlikely the postganglionic cholinergic nerves innervate the afferent terminals; however, there are potential nonneuronal sources of acetylcholine in the airways, including the epithelial cells that may lead to activation of nicotinic receptor on afferent terminals (222). In addition, choline is an agonist at certain nicotinic receptors and is conceivably found in concentrations in the airways that are relevant for afferent nerve stimulation (262).

B) SEROTONERGIC 5HT-3 AGONISTS. Among the dozen or so 5HT receptors, only 5HT-3 is an ionotropic receptor. This receptor is a member of the Cys-loop family of channels that also include nicotinic receptors. Five 5HT-3 receptor subunits have been identified, but only 5HT-3A and 5-HT3B have been studied in detail. Homomeric assembly of 5HT-3A forms a ligand-gated ion channel, whereas 5HT-3B forms heteromeric channels with 5HT-3A resulting in 5-HT3AB channels that have distinct pharmacological properties (174).

Since the 1950s, phenylbiguanide (sometimes referred to as phenyldiguanide) has been commonly used to interrogate the physiology of cardiopulmonary reflexes (128). Around this time the structure of serotonin was being worked out, and it was recognized that there were some structural similarities between phenylbiguanide and 5-HT. Moreover, phenylbiguanide mimicked the cardiopulmonary effects of serotonin, but other behaviors of serotonin were not at all mimicked by the drug (128). The results from these types of studies are now readily explained by the fact that phenylbiguanide effectively stimulates the ionotropic 5-HT3 receptors, but not the other metabotropic 5-HT receptors, and 5-HT3 receptor activation strongly activates action potential discharge in respiratory vagal C-fibers in most species studied (81, 85, 90, 103, 198, 370, 476). Incidentally, phenylbiguanide is ineffective in stimulating vagal C-fibers in guinea pigs due to the fact the amino acid sequence in the phenylbiguanide binding site renders the 5-HT3 receptor with little affinity for the drug (233). 5HT and other 5-HT3 receptor selective agonists, however, strongly activate vagal C-fibers in guinea pigs lungs (81).

The 5-HT3 receptor is selectively expressed in nodose but not jugular sensory neurons (370). Accordingly, in both mice and guinea pigs, the placodal derived nodose C-fibers in the lungs are activated by 5-HT agonists to a much

greater extent that neural crest derived jugular C-fibers (81). In dogs, the activating effect of phenylbiguanide is selective for bronchial over pulmonary vagal C-fibers (85).

c) purinergic p2x receptor agonists. ATP receptors are broadly subcategorized as metabotropic P2Y receptors and ionotropic P2X receptors (2). Among the ionotropic P2X receptors there are seven subtypes (P2X1-7). The nonselective P2X cation channel is formed by homomeric or heteromeric trimers.

Detailed pharmacological evidence first indicated that most of the ATP-induced depolarizing current in nodose neurons is due to the stimulation of the heteromeric channel P2X2,3. This pharmacological evidence was substantiated by histological studies and studies using P2X2 and P2X3 gene knock out mice (84, 207, 438, 458).

Inhalation of ATP can evoke coughing and dyspnea in human subjects indicative of activation of bronchopulmonary C-fibers (27). Among the first studies that provided direct evidence for P2X receptor activation of vagal afferent C-fibers in the lung came from studies in the dog where ATP stimulated action potentials in capsaicin-sensitive pulmonary C-fibers (360). This effect was not mimicked by adenosine, but was mimicked by P2X selective agonists.

In guinea pigs, ATP strongly activates C-fibers and low-threshold Aβ stretch-sensitive fibers (i.e., RAR/SAR fibers) (55, 432). With respect to C-fibers, ATP stimulates action potentials in all nodose C-fibers irrespective of their terminations, but does not stimulate action potential discharge in jugular C-fibers in the lungs or extrapulmonary airways (224, 432). When RT-PCR was carried out on individual jugular and nodose neurons that were retrogradely traced from the respiratory tract of mice and guinea pigs, virtually every airway-specific neuron in both ganglia expressed P2X3 receptors. Therefore, the lack of action potential discharge in jugular neurons is unlikely explained by a lack of P2X3 receptors. The difference between nodose (C- and A-fiber neurons) and jugular neurons is that the nodose neurons also expressed the P2X2 receptors, whereas this receptor is lacking in jugular neurons (224, 334). This implies that nodose neurons can form the heteromeric P2X2,3 receptor, whereas jugular neurons are limited to homomeric P2X3 receptors.

Patch-clamp electrophysiological studies support this hypothesis in that ATP produces a large slowly inactivating inward current reminiscent of the P2X2,3 channel in nodose neurons, whereas it produces a sharp rapidly inactivating current in jugular neurons that mimics homomeric P2X3 channels (224). Considered together, the data indicate that stimulating P2X2,3 receptors in the terminals of nodose C- and Aβ-fiber neurons leads to large generator potentials capable of reaching action potential thresholds, whereas due to rapid inactivation, stimulation of the homomeric P2X3 receptors in jugular neurons leads to generator potential insufficient for action potential discharge. The observation that placodal neurons selectively express P2X2,3 receptors whereas neural crest jugular (and DRG) neurons express homomeric P2X3 receptors is not limited to the respiratory tract as it has also been observed vagal afferent fibers terminating in the guinea pig and mouse esophagus (408, 472).

The ubiquitous presence of cellular ATP and the fact that it can be secreted from cells by many mechanisms including mechanical perturbation inevitably leads to the concept that some of the mechanically evoked action potential discharge in nodose A- and C-fibers may be secondary to ATP release. This hypothesis has been directly supported in guinea pig isolated lungs where infusing the bronchial smooth muscle constrictors methacholine or histamine leads to the activation of a subset of nodose C-fibers by mechanisms that are inhibited by pharmacological blocking P2X2,3 receptors or by blocking the smooth muscle contractions (444). There is also circumstantial evidence in support of the concept that ATP released from stimulated neuroepithelial bodies may activate nearby nodose A- and C-fibers (45, 245).

d) transient receptor potential channel agonists. Transient receptor potential (TRP) channels are a large family of ion channels comprising some 28 members that collectively are expressed by myriad cell types (356). So far, with respect to respiratory sensory neurons TRP-vanilloid-1 (TRPV1), TRP-ankyrin-1 (TRPA1), and TRP-melastatin-8 have been the most seriously investigated.

i) TRPV1. Capsaicin has long been known to stimulate contractions of guinea pig airways (314), but its mechanism was not fully appreciated until the discovery of TRPV1. Capsaicin has now become the prototypical stimulant for afferent C-fibers. Capsaicin strongly activates most vagal C-fibers innervating the respiratory tract irrespective of their terminal location or ganglionic origin. An exception to this is the mouse where a sizeable percentage of vagal afferent C-fibers are not stimulated by capsaicin (213).

The vanilloid moiety on capsaicin and resiniferatoxin (a potent capsaicin analog) is required to stimulate C-fibers. It was therefore deduced that these chemicals selectively bind to a receptor that was tentatively termed the vanilloid receptor (409). An intensive search for this receptor, using calcium imaging-based expression cloning, heeded fruit in the discovery of the ionotropic receptor vanilloid receptor 1 (67). Further analysis revealed that this receptor belonged to the TRP family of ion channels and was renamed TRP-vanilloid 1 or TRPV1. TRPV1 is a nonselective cation channel that permits the influx of calcium and sodium.

Patch-clamp studies showed that this channel is sensitive to heat stimuli and acidic conditions. These two stimuli are integrated such that the amount of heat needed to activate the channel is reduced in the presence of acidic conditions, and vice versa (426). Certain intracellular messengers of GPCR signaling also interact positively with TRPV1. The multiple mechanisms underlying GPCR-TRP channel interactions have recently been reviewed (436).

The heat threshold for activating TRPV1 is ~43°C, a temperature that is unlikely to be reached in the healthy respiratory tract (337). Hyperthermic temperatures below that required for activation may however increase the sensitivity of the channel to other respiratory relevant stimuli (338, 437). Studies in laboratory animals and with human subjects support the hypothesis that ventilation with hot humid air can lead to stimulation of C-fibers via TRPV1 activation (163, 208, 240, 436).

II) TRPA1. This member of the TRP family of ion channels was first cloned from a fibroblast line, but is expressed extensively in C-fiber afferent neurons (24, 191, 406). In most C-fibers innervating the murine respiratory tract, TRPA1 is colocalized with TRPV1. Therefore, activation of respiratory sensory nerves via TRPA1 is expected to evoke similar reflexes as seen with capsaicin and other TRPV1 stimulants (333). For example, inhalation of either TRPA1 or TRPV1 agonists will lead to coughing in guinea pigs and human subjects (15, 34, 47). The coexpression of TRPA1 and TRPV1 is relevant because they can synergistically interact with each other in airway C-fibers such that the threshold for a TRPA1 agoinst for activating the nerve is reduced when TRPV1 is stimulated, and vice versa (254). TRPA1 is seldom expressed in large-diameter mechanosensitive A-fibers neurons.

TRPA1 is a polymodal chemical sensor that is activated by a large number of structurally distinct chemicals. Structurally dissimilar chemical activators of TRPA1 share an electrophilicity and capacity to modify cysteine residues via Michael adduction (167, 271). The most commonly studied of such chemicals are cinnamaldehyde and ally-isothiocyanate (mustard oil). Particularly relevant to respiratory physiology, however, are the large number of inhaled irritants that can stimulate TRPA1. Inhalation of crotonaldehyde and acrolein that is found in cigarette smoke can stimulate airway C-fibers via TRPA1 (14, 256). Toluene diisocynate, a molecule implicated in certain types of occupational asthma, strongly activates respiratory C-fibers and nocifensive reflexes via TRPA1 (418). Ozone has long been known to evoke defensive respiratory reflexes, but the neuronal mechanisms are unknown. Studies ex vivo have shown that ozone is a very potent activator of C-fiber neurons in mouse vagal ganglia as well as the terminals within the mouse airways. Ozone had no effect on afferent neurons or terminals in TRPA1 knockout mice (420).

There are also endogenous activators of TRPA1 that are potentially relevant to the respiratory physiologist. Molecules associated with oxidative stress, found for example in inflammatory respiratory disease, can evoke strong activation of C-fibers via a TRPA1-dependent mechanisms (421). Such chemicals include the alpha-beta unsaturated carobonyl compound 4-oxo-2-nonenal and to a much lesser extent 4-hydroxy-2-nonenol (419, 428). Hydrogen peroxide can stimulate TRPA1, but impotently. The prostaglandin D_2 metabolite deoxy $\Delta^{12,14}$ prostaglandin J_2 (and related prostanoids) activates respiratory C-fibers via TRPA1 (277, 422), a finding that may be relevant to allergic diseases where prostaglandin D_2 is highly elevated as a consequence of mast cell activation. Nitrated fatty acids that occur downstream from nitric oxide formation are also strong TRPA1-dependent activators of respiratory C-fibers (417). These electrophilic molecules can be produced by inflammatory cells during oxidative stress and potentially activate C-fibers in a paracrine fashion. It has also been argued that electrophilic molecules of oxidative stress may also be generated in mitochondria within hyperractive afferent terminals leading to autocrine actions (336). As with TRPV1, TRPA1 may also serve as a cation channel linking GPCR activation to generator potentials in afferent terminals (143).

III) TRPM8. TRPM8 is a receptor for menthol and can also be stimulated by cold temperatures (304, 359). The number of respiratory-relevant chemical activators is not as extensive as seen with TRPV1 or TRPA1. In a study of vagal afferent nerves innervating the mouse lungs, there was little evidence of TRPM8 expression (333). Vagal afferent nerves innervating the rat trachea which may be preferentially jugular C-fibers were found to express TRPM8 and were activated by cold temperatures (459). In another study a small percentage (~15%) of bronchopulmonary C-fibers were found to be sensitive to cold temperature and menthol, but not a TRPA1 agonist (477). There is some evidence in guinea pigs that TRPM8 may be selectively expressed in jugular more than nodose C-fibers (473).

E) ACID SENSING CHANNELS. Inhalation of acid solutions leads to coughing and nocifensive reflexes in human subjects and laboratory animals (8, 54, 100, 134, 200, 230, 261). It may be argued that the endogenous acidification may also contribute to reflex activity caused by hypercapnea, lactic acidosis, or tissue inflammation (83, 173, 175, 441). An acidic environment can lead to activation of virtually all C-fibers in the respiratory tract. The ionic mechanisms driving this activation have yet to be worked out in detail, but given the promiscuous effect of pH on ion channel structure and function, it is likely to be a complex response. In jugular C-fibers innervating the guinea pig trachea, the acid-induced action potential discharge is partially inhibited by drugs that block TRPV1 (137, 215). In C-fibers of the mouse lungs (mainly nodose C-fibers), acid only activated

capsaicin-sensitive nerves, and this response was largely but not totally inhibited in TRPV1 knockout mice (213). TRPV1 blockers are also effective at inhibiting acid-induced cough in guinea pigs (230). Thus it is clear that TRPV1 stimulation is an important mechanism underlying acid-induced activation of bronchopulmonary C-fibers.

Acidic conditions can also lead to activation of A-fibers in the respiratory tract. The nodose Aδ cough receptors in the guinea pig trachea are in fact more sensitive to acid than are the tracheal C-fibers (215). These nerves do not express TRPV1, and TRPV1 blockers do not inhibit the acid-induced action potential discharge in these cough receptors, nor do they inhibit the cough evoked by acid-mediated activation of these nerves (54, 215).

There are a large number of ion channel candidates other than TRPV1 that may be involved in acid-induced activation of afferent nerves (447). It is of interest that Aδ cough fibers in the guinea pig trachea were very sensitive to rapid decreases in pH, but entirely insensitive to gradual drops in pH. This indicates an acid-sensing mechanism with rapid inactivation. Certain members of the ASIC (acid sensing ion channel) family of channels are rapidly inactivated including ASIC3. Nodose and jugular neurons express a combination of ASIC1, ASIC2, and ASIC3 (149), although the subtypes of ASIC2 were different between nodose and jugular neurons (113). The extent to which ASICs or other acid modulated channels contribute to the acid-induced action potential discharge is at present left to the realm of speculation.

2. GPCR agonists (metabotropic receptors)

There have been relatively few GPCR agonists that have been proven to directly evoke action potential discharge in vagal afferent nerves innervating the respiratory tract. More commonly GPCR agonists serve to modify the sensitivity of vagal C-fibers (239). Nearly all the work on GPCR agonist-induced vagal afferent nerve activation has focused on C-fibers. There is little evidence for direct activation of mechanosensitive A fibers by GPCR agonists, however, when studied in vivo these stimuli may lead to activation of RARs and SARs via indirect means (bronchoconstriction, mucus secretion, etc).

Unlike the ionotropic receptors, the ionic mechanisms by which signaling via GPCRs lead to generator potentials remains unclear, especially at the nerve terminals. Studies at the cell soma implicate the opening of TRP channels (TRPV1, TRPA1) as one possible mechanism (24, 143, 399, 436). A mechanism by which this may occur envisages TRPV1 situated in the plasma membrane in a manner that is under tonic inhibition by phosphatidylinositol-4,5-bisphosphate. A GPCR-G_q receptor agonist such as bradykinin leads to PLC activation and cleavage of the inositol moiety and a disinhibition of the channel (78). An alternative mechanism for GPCR activation of TRPV1 involves the stimulation of PLA_2 and the cleavage of arachidonic acid from membrane phospholipids. The arachidonic acid is oxidized by lipoxygenase enzymes to form eicosanoids that can act as capsaicin mimetics at the channel (399).

A TRP channel-independent mechanism has also been shown to support GPCR-induced depolarizing current in vagal C-fibers neurons that involves the stimulation of chloride channels (345). In contrast to neurons in the CNS, the reversal potential for chloride in adult primary afferent neurons is more positive than the resting potential, so opening chloride channels will lead to the efflux of the anion and a net membrane depolarization. The higher than expected electrochemical chloride equilibrium is due to functional expression of a Na^+-K^+-$2Cl^-$ cotransport mechanism (NKCC1) that actively accumulates Cl^- (10, 289). The stimulation of the ionotropic chloride channel receptor GABAa consistently leads to membrane depolarization of airway specific nodose neurons (77, 289). Stimulation of G_q-coupled GPCRs often leads to increases in cytosolic calcium that may in turn stimulate calcium-activated chloride channels to depolarize the nerves.

Most of the mechanistic work on GPCR-induced depolarizing currents has been carried out at the level of the patch-clamped cell body. It should be kept in mind that the signal transduction at the cell body, replete with organelles used in GPCR signally (rich endoplasmic reticulum, nuclear membrane etc.), may be quite distinct from the nerve terminals rich in mitochondria. There has been little work done on the mechanism of GPCR-induced action potential discharge at the nerve terminals. In guinea pig jugular nerves terminating in the trachea, the bradykinin-induced action potential discharge is largely inhibited by a combination of the TRPV1 and chloride channel antagonists (66). TRPV1 and TRPA1 antagonists inhibited bradykinin-induced cough in guinea pigs (143). In mouse lungs however, the bradykinin-induced activation of nodose C-fiber terminals was only very modestly different between wild-type and TRPV1 knockout animals (214).

A) BRADYKININ. Bradykinin is commonly used to activate respiratory vagal C-fibers. Kaufamn et al. (202) showed that bradykinin effectively stimulates action potential discharge in vagal C-fibers that terminate in the intrapulmonary airways of dogs. Bradykinin was more effective in stimulating the bronchial versus pulmonary C-fibers. This is based on the finding that C-fibers accessible via the pulmonary circulation were relatively insensitive to bradykinin. Bradykinin is enzymatically rapidly inactivated in the circulation so whether the difference is due to phenotypic distinctions in C-fiber subtypes or due to differences in the instability of bradykinin when subjected to vascular enzymes remains unknown. Bradykinin stimulates both jugu-

lar and nodose C-fibers in guinea pig and mouse lungs, and this is due to stimulation of the bradykinin B2 receptors (135, 193, 432). Consistent with C-fiber activation, bradykinin leads to defensive reflexes including parasympathetic reflexes and coughing (60, 76, 89, 204, 293).

B) HISTAMINE. Histamine stimulates action potential discharge in a subset of respiratory vagal C-fibers of dogs, cats, and guinea pigs (85, 103, 444). Histamine aerosol also stimulated action potential discharge in rabbit lungs, but the afferent nerves that responded were not defined by conduction velocity and referred to simply as "irritant receptors" (396). In dogs the activation of C-fibers is relatively weak, and limited to bronchial C-fibers. In guinea pigs the activating effect of histamine was selective for nodose C-fibers, but was not due to a direct effect on the nerve terminals. Rather the effect was secondary to ATP released as a consequence of smooth muscle contraction, and the stimulation of ionotropic P2X2,3 receptors. Similarly, histamine only indirectly leads to activation of RAR fibers in guinea pigs (32) and dogs (391).

C) 5-HT. As mentioned above bronchopulmonary C-fibers in many species can be activated by 5-HT via ionotropic 5-HT3 receptors. In the mouse lungs, 5-HT3 activation is selective for nodose C-fibers. Jugular C-fibers in mouse lungs do not express 5-HT3 receptors, yet respond strongly to 5-HT. The jugular C-fiber stimulation is not inhibited by 5-HT3 receptor antagonists indicating the a metabotropic 5-HT receptors is likely involved (370). Based on gene expression in these neurons, likely candidates include 5-HT1 and 5-HT4 receptors. The endogenous 5-HT released from airway mast cells is sufficient to activate mouse jugular C-fibers (370).

D) ADENOSINE. Adenosine evokes sensations of chest tightness in humans and classical C-fiber reflexes in laboratory animals (50, 374). Adenosine stimulates nodose but not jugular C-fibers in guinea pig lungs (80). The nodose C-fiber neurons innervating the guinea pigs lungs express both adenosine A1 and A2A receptors, and selective activation of either receptor subtype leads to action potential discharge at the terminals. The stimulation by adenosine of nodose C-fibers in guinea pig lungs is blocked only when both A1 and A2A receptors are antagonized (80). Adenosine increases action potential discharge in rat pulmonary C-fibers (152, 172). This effect is unaffected by adenosine A2 antagonists, but is inhibited by A1 antagonists.

E) PROTEASE ACTIVATED RECEPTORS. There are four protease activated receptors (PAR1-4). A single cell analysis of PAR expression in vagal sensory neurons innervating mouse respiratory tract revealed that PAR1 and PAR3 are expressed, but not PAR2 or PAR4 (227). Trypsin and thrombin evoked action potential discharge from intrapulmonary nodose but not jugular C-fibers in the mouse, an effect mimicked by selective PAR1 agonists. The intrapulmonary C-fibers in PAR1 knockout mice fail to respond to selective PAR1 agonists as well as to the less selective PAR agonists thrombin and trypsin (227).

Rat nodose neurons express PAR2 (147). In bronchopulmonary C-fibers, PAR-2 receptors appear to serve more as amplifiers of C-fiber activity evoked by other stimuli than as direct activators (147–149).

F) EICOSANOIDS. Electrophysiological studies have shown that leukotrienes and prostanoids stimulate their respective GPCRs to modulate neural excitability (e.g., inhibiting certain potassium channels, altering voltage-gated sodium channel properties) more than directly evoke activating generator potentials (see below). Nevertheless, prostanoids (PGs) have been found to overtly stimulate action potential discharge in vagal airway C-fibers and also evoke C-fiber-dependent reflex activity in the respiratory tract. PGE_2 and PGI_2 stimulate canine bronchial and pulmonary C-fibers and may in fact lead to reflex bronchoconstriction at a concentration below that needed for direct bronchial muscle relaxation and bronchodilation (378). Inhalation of PGD_2 causes coughing and stimulates capsaicin-sensitive neurons, an effect mimicked by a DP1 receptor selective agonist but not by DP2 selective agonists (273). $PGF_{2\alpha}$ and PGE_2 lead to cough and PGE_2 enhances dyspnea (76, 95, 272, 274, 410). The PGE_2-induced cough in guinea pigs is inhibited by EP3 receptor antagonism (274). Infusion of thromboxane leads to strong action potential discharge in intrapulmonary C-fiber in cats (198) and stimulation of RAR fibers in rabbits (280). The RAR activation by thromboxane was not secondary to the accompanying bronchoconstriction.

3. Voltage-gated sodium channels and action potential generation

Activating mechanical and chemical stimuli lead to a depolarizing generator potential. If this membrane depolarization is not of sufficient rate and magnitude to reach the threshold for action potential generation, it will electrotonically decay back to resting potential and have essentially no physiological influence. To reach the threshold of action potential, the generator potential must activate voltage-gated sodium channels (NaVs). It is the inward rush of sodium ions through NaVs that elicits the upstroke of the action potential that is then conducted to the central terminals in the brain stem.

A breakthrough in our understanding of NaVs came relatively recently with the unraveling of their molecular biology. The pore unit of the NaVs comprises a large α subunit with four homologous domains. The α subunits are encoded by 9 distinct genes and the channels formed are referred to NaV 1.1–1.9 (68). These channels can be blocked nonselectively with the class of drugs known as

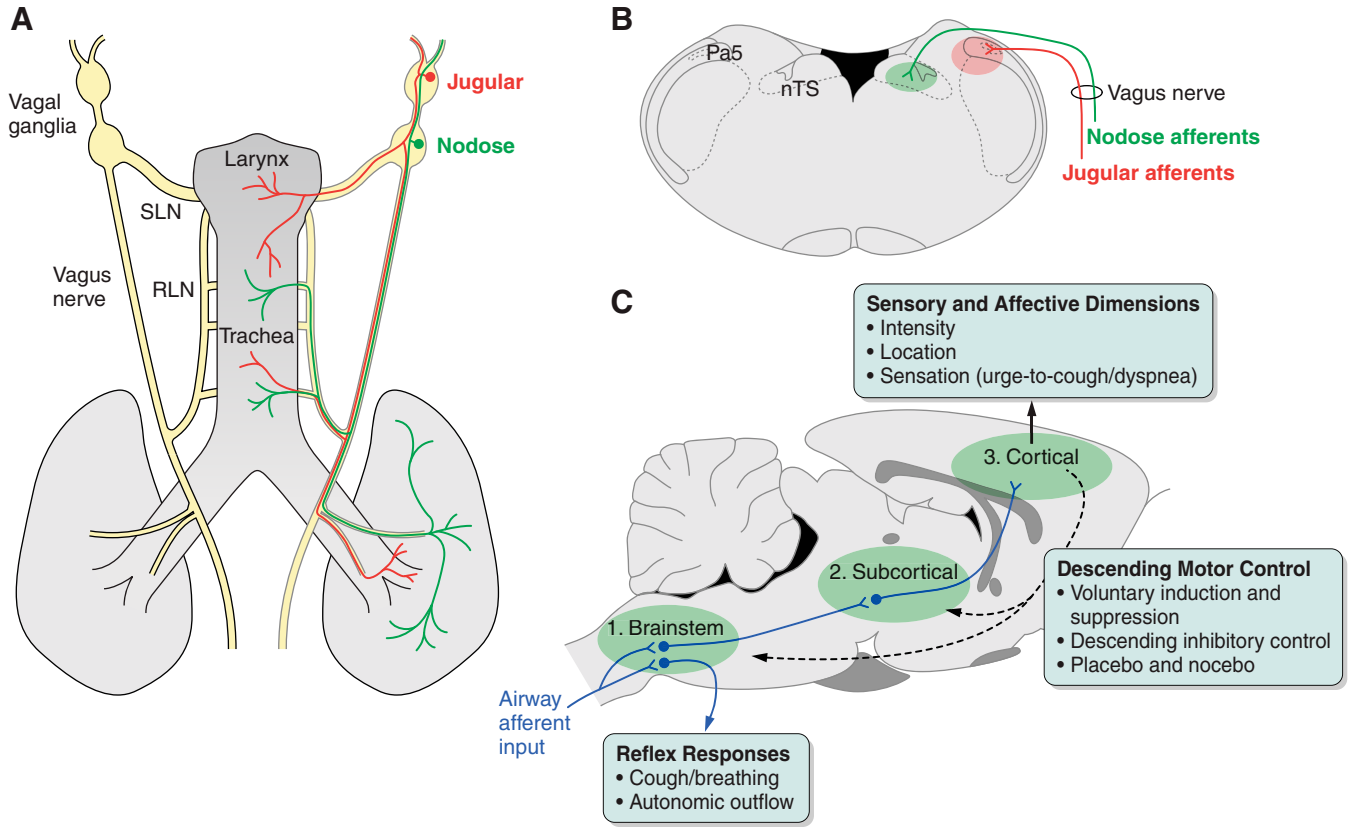

FIGURE 5. Schematic overview of the peripheral and central neural pathways regulating airway afferent processing. *A*: embryologically distinct neurons constituting the jugular (red) and nodose (green) vagal ganglia innervate the airways and lungs. The axons of these neurons reach the airways via distinct vagal branches, including the superior and recurrent laryngeal nerves (SLN/RLN, respectively). *B*: the brain stem terminal projections of jugular (red) and nodose (green) neurons are confined predominately to the paratrigeminal nucleus (Pa5) and the nucleus of the solitary tract (nTS), respectively. *C*: brain stem neurons in receipt of airway vagal sensory input in turn contribute to both reflex and higher order circuits that encode various involuntary and voluntary motor responses and perceivable sensations subsequent to airway sensory nerve stimulation. Descending control circuits help regulate airway sensory processing at multiple levels of the neuraxis.

local anesthetics. They are "local" because systemic blockade of all NaVs is lethal, greatly limiting the utility of local anesthetics for visceral diseases. A plethora of studies have revealed that somatosensory nociceptors ("pain receptors") in DRG express mainly, though not exclusively, NaV 1.7, 1.8, and 1.9 (154). Lagging far behind, however, are investigations into NaVs in visceral nociceptors. Retrograde tracing and single-cell RT-PCR combined with patch-clamp electrophysiology has shown that vagal afferent neurons innervating the respiratory tract also express (nearly exclusively) NaV 1.7, 1.8, and 1.9 (223, 323). In the somatosensory system, NaV 1.8 and 1.9 are selectively expressed in nociceptors; however, in the respiratory system, it appears that even the large-diameter capsaicin-insensitive non-nociceptor low-threshold mechanosensitive nerves express these subtypes (223). When NaV1.7 gene expression was selectively silenced in the vagal sensory ganglia (nodose and jugular), the action potential conduction along the axons was substantively reduced, and classical vagal reflexes such as the cough reflex were blocked (322, 323).

IV. CENTRAL NERVOUS SYSTEM TERMINATIONS AND ASCENDING PATHWAYS

A. Brain Stem

The central projections of all vagal afferents terminate in the brain stem where they innervate second-order neurons that *1*) project to other brain stem nuclei, *2*) ascend to higher brain regions, or *3*) descend to the spinal cord **(FIGURE 5)**. The embryological dichotomy between nodose and jugular ganglia neurons, which results in distinct peripheral termination patterns, appears to extend to the central terminations of these neurons as well. Whereas nodose neurons (including those that innervate the airways) have a relatively well-described pattern of terminations in medul-

lary regions of the nucleus of the solitary tract and adjoining area postrema, neurons derived from the jugular vagal ganglia favor trigeminal nucleus terminations (301, 302). Thus, in rats, injection of a retrograde tracing dye into the nucleus of the solitary tract labels only neurons in the nodose ganglia, while a comparable injection in the spinal trigeminal nucleus (in particular the paratrigeminal nucleus) labels only jugular neurons. This is perhaps not surprising given the visceral versus somatic nature of nodose versus jugular ganglia (9, 97, 98, 334). Also consistent with this, neuronal tracing from the airways of several species reveals populations of afferent fibers in both the nucleus of the solitary tract and the spinal trigeminal nucleus (190, 195, 300–302, 355, 389, 413). In this sense, it is interesting that distinct reflex responses occur subsequently to the activation of different populations of airway C-fibers, indicative of these distinct second-order neuron populations differentially regulating brain stem autonomic and respiratory networks (75).

Nodose derived chemically and mechanically sensitive vagal airway afferents terminate primarily in the commissural, lateral, medial, dorsolateral, and ventrolateral regions of the caudal nucleus of the solitary tract (121, 125, 194–196, 300–302, 312, 348). There is some topographical organization of individual afferent subtypes within these regions. Electrophysiological and morphological studies have identified the second-order neurons in the nucleus of the solitary tract that receive inputs from RARs and SARs (38, 40, 121, 125, 312, 348). RARs project principally to the ipsilateral commissural and medial nucleus of the solitary caudal to the area postrema where they provide excitatory glutamateric input onto so-called "RAR cell" second-order neurons. RAR terminations into the inspiratory pool of premotor neurons in the ventrolateral nucleus of the solitary tract are rare, suggesting minimum direct regulation of inspiration via these afferents. Individual RAR cells are also excitatory in nature and have extensive dendritic branches projecting towards the nucleus ambiguus (where autonomic preganglionic neurons reside) and ventral respiratory cell group, as well as both ascending and descending projections to the rostral brain stem and beyond the spinomedullary junction (119, 220, 348). In contrast, SARs terminate more rostrally in the medial lateral and ventrolateral nucleus of the solitary tract. Two types of second-order neurons known as pump (or "P") cells and inspiratory Iβ cells receive excitatory inputs from SAR terminals, the latter an inspiratory modulated neuron exclusively found within the ventrolateral nucleus of the solitary tract (i.e., comprising the dorsal respiratory group) (31, 101, 120, 121, 123, 125). Pump cells are largely GABAergic and, thus when activated, serve to inhibit their downstream synaptic partners, although additional subtypes of P cells have been proposed as not all express inhibitory neurotransmitters. The projections from individual pump cells extend to neighboring subnuclei of the nucleus of the solitary tract as well as to dorsal and ventral lateral pontine nuclei and the ventral respiratory group (101, 123, 125). Indeed, the local projections of pump cells within the nucleus of the solitary tract have been shown to include the territories occupied by RAR cells, and functional evidence suggests that pump cell stimulation inhibits RAR cell activity (122, 124). Aδ nodose derived cough receptors also terminate within the nucleus of the solitary tract. The second-order neurons of Aδ nodose afferents have not been described in any detail, although studies employing either microinjection strategies to block synaptic transmission of Aδ nodose afferents or microstimulation of the brain stem to initiate responses (all in guinea pigs) have defined their central location within the medial or interstitial regions of the nucleus of the solitary tract (57, 155, 181, 347). The neurochemistry and dendritic projections of the second-order neurons innervated by Aδ nodose afferents are unknown.

Less is known about the neurons in the nucleus of the solitary tract that receive input from nodose derived airway C-fibers, and essentially nothing is known about the neurons in the trigeminal/paratrigeminal nucleus that receive inputs from jugular ganglion derived airway C-fibers (and presumably jugular derived chemically sensitive Aδ fibers as well) **(FIGURE 5)**. C-fiber inputs to the nucleus of the solitary tract terminate mainly in the medial, commissural, and dorsolateral nuclei, and some terminals can also be seen in the area postrema (300–302, 453). These afferents appear to rely predominately on glutamatergic neurotransmission as second-order neuron responses can be blocked entirely with glutamate receptor antagonists. This is seemingly consistent with the lack of neuropeptide expression by many airway C-fiber afferents derived from the nodose ganglia. The dendritic projections of the second-order neurons that are innervated by nodose derived C-fibers have not been described.

The predominant innervation of trigeminal second-order neurons by jugular ganglia afferents has only recently been described, although older studies have detailed barosensitive vagal afferent inputs to trigeminal brain stem regions (21, 403, 404, 474). The paratrigeminal nucleus is a diffuse cluster of neurons within the dorsal spinal trigeminal nucleus, and although there have been no discrete electrophysiological studies of second-order neurons receiving airway inputs, electron microscopic studies combined with sensory nerve fiber tracing suggest that at distinct classes of second-order neurons are synaptically connected with laryngeal afferents in this brain stem region (389). Furthermore, the synaptic terminations of laryngeal afferents overlap with those arising from somatic afferent sources. Whether differences exist in the synaptic organization between jugular C-fiber and Aδ nociceptor inputs is not known. Conventional neuronal tracing studies have shown extensive projections of paratrigeminal neurons to ventrolateral medulla, nucleus of the solitary tract, pons and subcortical brain

regions such as the thalamus (302, 388), supporting a role for this nucleus in autonomic and somatic sensory processing. The interplay between chemically sensitive vagal afferent processing within the nucleus of the solitary tract and the paratrigeminal nucleus is conceptually intriguing and awaits further investigation.

Studies assessing the neurotransmission between primary vagal afferent neurons innervating the airways, regardless of their specific subtype, and nucleus of the solitary tract second-order neurons have shown a predominance of glutamatergic transmission acting via non *N*-methyl-D-aspartate (NMDA) receptors (18, 161, 162, 453). This is consistent with expression studies showing that vagal afferents express one or more subtypes of vesicular glutamate transporter (VGlut1 and/or VGut2) (287, 334). The exception to this is the synaptic integration of extrapulmonary Aδ nodose touch-sensitive afferents in the nucleus of the solitary tract, which may be reliant on NMDA glutamate receptor-dependent neurotransmission (57). However, additional transmitters may contribute to synaptic integration. For example, the tachykinins substance P and neurokinin A are likely released from some bronchopulmonary C-fibers, acting at postsynaptic neurokinin-1, neurokinin-2, and neurokinin-3 receptors (39, 59, 285). Glutamateric transmission may be additionally modulated by retrograde nitric oxide signaling and presynaptic metabotropic glutamate receptors (74). Adenosine, ATP, serotonin, CGRP, carbon monoxide, angiotensin, GABA, norepinephrine, and others may also regulate airway primary afferent transmission in the nucleus of the solitary tract, many of which are not derived from the primary afferents themselves. Transmission between airway primary afferents and second-order neurons in the paratrigeminal nucleus has not been studied to date, although glutamateric and peptidergic (especially CGRP) mechanisms are likely based on the neurochemical phenotypes of jugular ganglia primary afferent neurons.

A variety of primary afferent inputs terminate in common and often overlapping brain stem regions (i.e., not just airway vagal afferents), and therefore, it is frequently questioned whether individual brain stem second-order neurons integrate inputs from multiple afferent sources. This has been best addressed in the nucleus of the solitary tract, and the answer is almost certainly in the affirmative, although debate remains as to whether convergence of cross modal inputs occurs. That is, whilst second-order neurons may receive multiple A-fiber or multiple C-fiber inputs, it remains unclear whether a single second-order neuron receives both A- and C-fiber synaptic inputs. Earlier work in animal preparations would suggest that cross modal convergence is not only possible, but indeed widespread (283, 308, 357, 358, 400), whereas more recent studies employing brain stem slice recordings contradict these findings and support a model of modal segregation (298, 299, 363). Given that a wide variety of different sensory inputs can drive common respiratory and autonomic behaviors, it would seem that widespread convergence occurs at some juncture along these circuits, if not at the primary afferent synapse. In the paratrigeminal nucleus, visceral and somatic afferent input may converge, perhaps facilitating the coordination of autonomic processes during noxious somatosensation (270). An alternative mechanism of cross modal afferent interactions within primary integration sites may involve volume transmission in which neurochemicals (especially neuropeptides from chemically-sensitive afferents) may augment synaptic transmission at adjacent synapses. Indeed, neuropeptide sensitization within the brain stem of airway reflexes driven by mechanically sensitive afferents has been previously described (283, 293, 394).

B. Higher Brain Circuits

Airway afferent inputs to the brain stem synapse with circuits that ascend the neuroaxis, terminating in pontine, midbrain, and a variety of suprabulbar (subcortical and cortical) sites **(FIGURES 5** and **6)**. Older studies showed that vagal stimulation (not specific for the airways) leads to neuronal activity in the pontine parabrachial nuclei, ventral posteromedial and ventral posterolateral parvocellular nuclei of the thalamus, insula cortex, primary sensory cortex, and amygdala (69, 159, 180, 264), indicative of the existence of a visceral sensory neural circuit for ascending vagal inputs to higher brain sensory processing sites. It has only been relatively recently that experiments have assessed whether such a circuit exists specifically for ascending airway sensory pathways. Thus circuit tracing studies in rodents using recombinant neurotropic viruses suggest that multiple ascending airway circuits exist, and whilst similarities with the generic vagal circuits exist, some important distinctions have also been noted (300–302). Viral tracing from the lung (intrapulmonary airways) shows characteristic sensory inputs to the visceral thalamus (ventral posteromedial parvocellular thalamus) via pontine relays, similar to that described for other visceral afferents (69, 301). This circuit almost certainly projects onto the visceral sensory processing areas of the insula cortex. In contrast, the afferent inputs from the upper (extrapulmonary) airways do not terminate appreciably in the visceral thalamus, but rather in the general somatic processing regions of the thalamus. Consistent with this, these somatosensory terminations can be traced back to a trigeminal origin, rather than the nucleus of the solitary tract, and in fact represent the jugular afferent neural pathway that densely innervate the extrapulmonary airways (301, 302). Furthermore, these somatosensory pathways likely provide the inputs to somatosensory processing regions of the cortex described for airway vagal afferents. Collectively, these findings suggest that the afferent innervation to the airways undergoes a transition from somatosensory to viscerosensory at some juncture along the airway tree, not only in terms of the primary afferent neurons themselves, but also the ascending afferent circuits involved in sensory processing (301, 334).

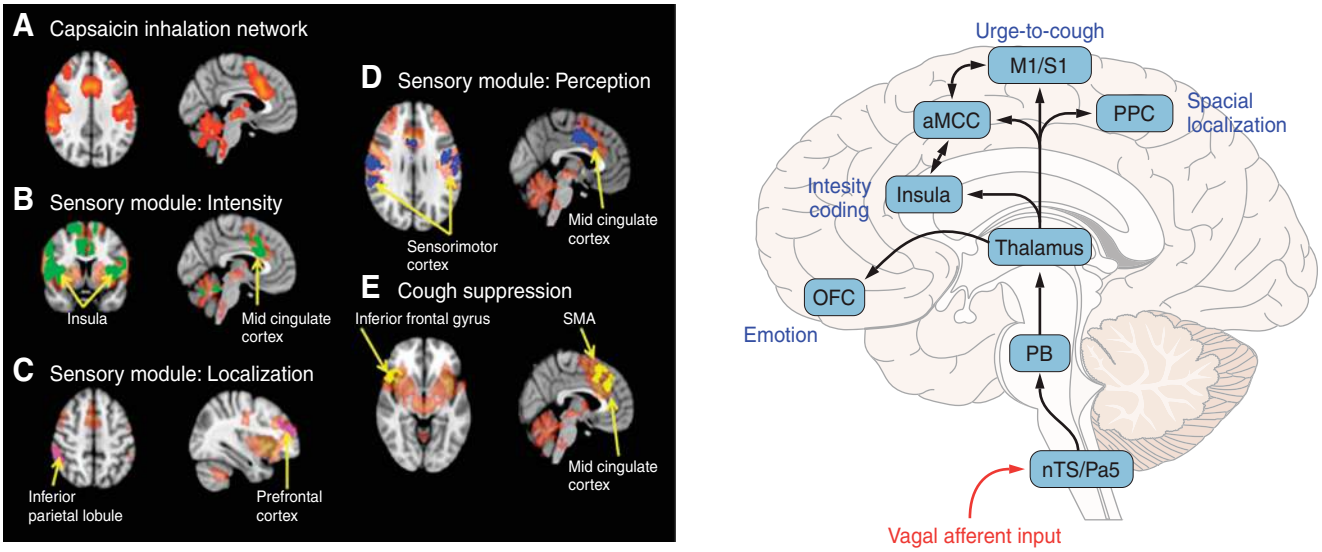

FIGURE 6. Central processing of the urge to cough in humans. Inhalation of the nociceptive afferent stimulant capsaicin evokes cough and related sensations. The central neural correlates of these sensorimotor processes have been studied using functional brain imaging. *Left, A*: capsaicin inhalation activates a distributed network that can be functionally divided in several subnetworks (modules) that relate to the intensity of the stimulus (*B*), the spatial localization of the sensation (*C*), the intensity of the perceivable sensation (urge to cough) (*D*), and the resultant voluntary suppression of cough dictated by the imaging protocol (participants were instructed not to cough) (*E*). [Adapted from Mazzone et al. (291).] *Right*: a summary figure of the putative central networks regulating cough sensory processing. nTS, nucleus of the solitary tract; Pa5, paratrigeminal nucleus; OFC, orbitofrontal cortex; aMCC, anterior mid-cingulate cortex; M1/S1, primary motor and sensory cortices; PPC, posterior parietal cortex.

The distinction between nodose and jugular afferent neural pathways in the brain is not confined to the thalamocortical projections. Airway specific nodose related relays via the nucleus of the solitary tract preferentially terminate in the locus coeruleus, amygdala, lateral hypothalamus, paraventricular nucleus of the hypothalamus, and zona incerta, compared with their jugular afferent counterparts relayed via the paratrigeminal nucleus (302). In contrast, airway jugular-related circuits include a broader representation in the medullary and pontine trigeminal nuclei and the submedius nucleus of the thalamus. These fundamentally different circuits likely contribute distinct functional behaviors associated with vagal afferent stimulation.

The circuitry described above has been identified from animal studies, and it is reasonable to question whether it is representative of that involved in human airway sensation. This has been addressed to some extent using functional brain imaging that has assessed the networks of human brain activations associated with inhalation of an irritating airway stimulus **(FIGURE 6)**. Such studies have shown that airway irritation (by inhalation of the chemically sensitive afferent stimulant capsaicin) produces neuronal activity in the primary sensory, anterior and mid insula, cingulate, premotor, motor and orbitofrontal cortices and in medullary, pontine, and thalamic regions that are difficult to define anatomically due to the spatial resolution constraints of brain imaging (126, 127, 244, 284, 290, 292). These regions are presumed to encode perceptual awareness of airway irritation and the associated emotional, cognitive, and behavioral (motor) consequences. Interestingly, activity in the primary sensory cortex correlates with an individual's perception of airway irritation while activity in the human insula relates to the magnitude of the stimulus delivered to the airways. This is somewhat compatible with the notion of distinct afferent pathways projecting from the airways to the cortex.

V. AFFERENT-INDUCED REFLEXES AND RESPONSES

A. Physiological Regulation of Respiratory Rhythm

Some of the earliest hypotheses about the origin of respiratory rhythm generation were based around the concept that sensors in the lung (perhaps detecting pulmonary CO_2 levels) regulated, if not established, cyclical breathing. In laboratory animals, bilateral vagotomy has dramatic effects on breathing, slowing rate and increasing the volume of tidal breaths (141, 251, 353). However, such predictions typically predated the necessary technological advances required to study pulmonary sensors and was opposed to the results of transection studies that pointed to the brain stem as critically important in rhythm generation. Nowadays, of course, we know that a complex network of pontomedullary brain stem neurons is responsible for establishing and

maintaining basic respiratory rhythmogenesis, and this network is in constant receipt of modulatory peripheral afferent feedback, including from the airways and lungs. The organization of pontomedullary respiratory network is beyond the scope of this review, but is described in several excellent recent publications and reviews (115, 129, 255, 402). Here we restrict the discussion to the contribution of airway vagal afferent inputs that alter breathing control.

In 1868, Josef Breuer and his mentor Ewald Hering reported the first direct evidence for mechanically sensitive afferent regulation of breathing (42). In an elegant series of studies that assessed the inspiration and expiration phases of breathing during inflation or deflation of the lungs, Hering and Breuer showed that lung expansion inhibits inspiration and promotes expiration, while a reduction in lung volume arrests expiratory movements and promotes inspiration. As these responses were prevented by prior vagotomy, they were deemed to be reflexive in nature, giving rise to the classic notion of the Hering-Breuer inflation and deflation reflexes as we know today. A little over 50 years after Breuer's discoveries, with the development of electrophysiological methods, SARs and RARs were first characterized and displayed physiological properties likely responsible for Hering and Breuer's inflation and deflation reflexes.

Many subsequent studies (in many different species) have provided additional support for Hering-Breuer reflex-mediated regulation of breathing, showing that pulmonary mechanoreceptors are responsible for facilitating inspiration and expiration phase transitions. However, in humans, initiation of the Hering-Breuer reflex requires large lung inflations, above that experienced at normal end-tidal volumes (29, 96). This suggests that the Hering-Breuer inflation reflex may be "recruited" to alter breathing under some circumstances rather than having an ongoing role in regulating breathing rhythmogenesis per se. Indeed, studies in rodents suggest that the relative contribution of pulmonary mechanically sensitive afferents to establishing tidal respiratory rhythm may be age-dependent in that young animals display a prominent inflation reflex that diminishes throughout development (114). Nevertheless, regardless of the circumstances during which SAR and RARs become active, the respiratory consequences appear consistent with Breuer's original description.

B. Cough and Defensive Respiratory Reflexes

Cough is a defensive respiratory event that characteristically begins with a brief inspiration, followed by expiration against a closed glottis (the compression phase) which produces large increases in intrapulmonary pressures such that the final phase of opening of glottis evokes a large expulsive airflow for clearing the airways. This stereotypical series of events occurs mechanistically by reconfiguration of the respiratory pattern generator in the brain stem to switch from rhythmical breathing to that of the cough motor pattern (397). The trigger for coughing is often a peripheral stimulus within the airways, and hence, this involves the activation of one or more subsets of airway afferent nerves (although cough can also be initiated voluntarily, but this is beyond the scope of the review; discussed in Ref. 13). Both mechanical and chemical perturbations within the airways can evoke coughing, either by reflexively reconfiguring the brain stem respiratory pattern generator or via ascending pathways to the cerebral cortex described above that then encodes perceivable sensations associated with airways irritation (the urge to cough) that promote behavioral (voluntary) coughing. The following text is restricted to the description of the primary afferent pathways that are thought to initiate coughing.

For many years, cough evoked by mechanical stimuli in the airways was assumed to be mediated by RARs, in part because some stimuli that activate RARs also induce coughing (87, 387, 415, 450). This was supported by studies employing vagal cooling which showed that cough could be blocked at temperatures that also inhibit action potentials in myelinated, but not unmyelinated, fibers (415, 416). This suggestion of RARs mediating cough, however, was difficult to reconcile with the observation that stimuli which effectively evoke bronchospasm, and therefore robustly activate RARs, were in fact poor inducers of cough (25, 139, 189). The spontaneous activity of RARs throughout the respiratory cycle also raised questions about their role in cough. When interpreted in its entirety, the available data collectively suggested that either very specific stimuli for RARs is needed to alter their pattern of activation in a specific manner to encode for coughing or, alternatively, a specific subset or RARs or RAR-like fibers may be recruited in response to stimuli that evoke coughing. Studies performed around a decade ago resolved some of these conflicting observations, when the description of the extrapulmonary Aδ nodose mechanosensitive vagal afferent nerve fiber subtype was made (55). As described above, these mechanically sensitive afferents are rapidly adapting by nature but are not the same as the intrapulmonary rapidly adapting receptors assumed to mediate cough. Convincing studies in guinea pigs showed that punctate mechanical stimuli and rapid changes in luminal pH (acidification) activate Aδ nodose fibers and induce coughing, even in anesthetized animals, and that stimuli that activate RARs did not. Perhaps the best example of this stimulus specificity is ATP, which produces robust firing in RARs, but does not activate Aδ nodose fibers and is relatively ineffective at evoking cough in anesthetized animals. A relatively large body of literature now describes cough in guinea pigs occurring via Aδ nodose afferent pathways, and although this is assumed to reflect mechanically evoked cough in all

coughing mammals, comparable studies in other species are lacking.

Cough can also be evoked by a wide range of chemical stimuli selective for the activation of chemically sensitive vagal afferents, suggesting that at least two airway afferent pathways can mediate cough evoked by different stimulus modalities. Chemical cough evoking stimuli include (but are not limited to) capsaicin, citric acid, bradykinin, and allyl isothiocyanate which evoke cough via their actions on ion channels and/or GPCRs as described above. All of these stimuli evoke cough in conscious animals and in humans with approximately equal efficacy (47, 55, 76, 90, 134, 199). The role for chemically sensitive afferents in cough evoked by these agents comes from experiments using capsaicin desensitization in guinea pigs to destroy or inactivate chemically sensitive afferents, which abolishes cough evoked by both capsaicin and citric acid but is without effect on mechanically evoked cough evoked by probing the airway mucosa (134). Neurokinin receptor antagonists also inhibit bradykinin-, citric acid-, and capsaicin-induced cough in cats and guinea pigs, which is thought to reflect the functional expression of neuropeptide transmitters in some vagal chemically sensitive afferent neurons (36, 293).

The role for chemically sensitive vagal afferents in mediating cough has also been debated, largely because of conflicting observations that have been difficult to resolve. For example, chemical stimuli that are extremely effective at evoking cough in conscious animals typically fail to do so in anesthetized animals, despite the same stimuli effectively evoking other reflexes, including airway smooth muscle bronchospasm secondary to enhanced autonomic vagal outflow. In fact, intravenous delivery of stimulants of chemically sensitive afferents may actually inhibit cough evoked by mechanical stimulation (75, 416). It is plausible that general anesthesia selectively modulates chemically sensitive afferent firing in such a way that the encoding for cough is disrupted without affecting other chemically sensitive afferent reflexes or cough induced by mechanical stimulation. Indeed, there is some evidence that the central pattern generator for breathing and cough requires a unique afferent action potential signature to reconfigure (56). Very deep anesthesia will also inhibit mechanically evoked cough, perhaps consistent with this notion. Alternatively, anesthesia may enhance descending inhibition of chemically afferent evoked coughing, although there is no available evidence to support this notion. It remains entirely possible that chemically evoked afferents do not evoke reflex coughing, but rather promote behavioral coughing secondary to generating perceptual feelings of the need to cough (13). A large body of functional brain imaging and behavioral experimental data exploring voluntary and placebo evoked suppression of capsaicin cough supports this notion (116, 126, 127, 164, 165, 177, 184, 242, 244, 284, 290, 292). However, this notion is not supported by studies assessing cough evoked by intravenous injection of the C-fiber stimulant lobeline in awake versus anesthetized or comatose humans in whom some cough or coughlike responses were observed in the absence of consciousness (371). More likely is that chemically sensitive afferents can evoke both reflex and behavioral coughing or their activity could be utilized to modulate the intensity of reflex coughing, depending on the initiating stimulus intensity.

The role of chemically sensitive afferent fibers in cough is further complicated by the sensory neuronal heterogeneity discussed elsewhere in this review. Thus, while stimuli (such as capsaicin) that activate both jugular and nodose vagal chemically sensitive afferents evoke cough in conscious animals, stimuli that are selective only for nodose chemically sensitive afferents do not evoke cough (75). By deduction, this suggests that the jugular afferent pathway is critical for chemically evoked cough and given the central neuroanatomy of the jugular afferent system, this might support the notion of chemically sensitive afferents inducing behavioral rather than reflex coughing. The fact that jugular afferents (and touch-sensitive nodose afferents for that matter) principally terminate in the larger airways is somewhat consistent with the functional notion that cough induction is restricted to the larger airways. Indeed, it is assumed that if coughing were inducible by stimuli that reached the peripheral airways, then the maximum attainable airflow velocity in those airways would be too low to effectively expel the irritant. For this reason, processes other than cough are thought to be initiated for airway clearance from the peripheral airways, and by extension, it therefore may not be surprising that the afferents thought to mediate cough are not readily found in the peripheral airways.

Although SARs almost certainly do not evoke coughing, they are not without effect in cough evoked by other afferent pathways. The mere fact that a deep inspiration and increases in intrapulmonary pressure precedes a cough expiratory effort suggests that enhanced SAR activity likely occurs and may be involved in the ensuing cough motor pattern, but electrophysiological recordings from SARs in rabbits support the notion that they are not directly involved in cough (279). In fact, the role of SARs in cough might be quite complex as respiratory preloading, which should increase SAR activity, has been reported to both increase and decrease the expiratory efforts during cough (158, 341), whereas inhaled sulfur dioxide (used to block SAR activity) attenuates the cough reflex (157, 279). However, the selectivity of sulfur dioxide for SARs is questionable since sulfur dioxide may activate chemically sensitive afferents, which can inhibit cough. SAR activity is probably not inspiratory-inhibitory during cough as it is during breathing, instead providing a permissive role for the production of cough (35, 157, 386). Thus SAR activity may not have an integral role in the production of cough but rather it facilitates cough, perhaps via a subset of pump cells that

act via a cough gating mechanism in the brain stem (37). However, an excitatory role of pump cells in cough would need to be reconciled with pump cell-mediated inhibition of other vagal reflex pathways (59, 122).

A variation of cough can be evoked by mechanical probing of the glottis, laryngeal vocal folds, and (under some circumstances) from the tracheal mucosa in which the large expiratory effort proceeds without an initial inspiration, termed the "expiration reflex" (369, 414, 435). There appears to be a rostrocaudal transition in response from almost exclusively the expiration reflex from the glottis, a mixture of expiration and cough reflexes from the larynx and almost exclusively cough from the tracheal mucosa. From a teleological perspective, such a transition in responses may occur as it would be undesirable to inhale an offending particle deeper into the respiratory system if it was first detected in the glottis or larynx, hence the absence of an initial inspiratory effort, whereas objects detected deeper in the airways may require inspiration to generate the high airflow velocity needed to aid their physical removal. Regardless of the putative function, the expiration reflex is thought to result from a distinct central neural representation compared with cough, predominately because its latency for induction is much shorter than that for cough (as little as 15–25 ms for the expiration reflex compared with as long as 500 ms for tracheobronchial cough) and because of central administration of codeine blocks cough but not the expiration reflex (369). The timing of the initiating stimulus relative to the phase of the respiratory cycle also appears to be important (339, 435). It is not known whether there are anatomical, physiological, or molecular differences in the glottal/laryngeal mechanosensitive afferents that initiate the expiration reflex, compared with tracheobronchial touch-sensitive afferents that are responsible for mechanically evoked cough.

The activation of vagal afferents can produce defensive respiratory responses other than cough. In the mid-1950s, Mott and Paintal (321) reported reflex respiratory effects elicited by intravascular injections of the 5-HT receptor agonists, including phenylbiguanide. They named the putative afferents mediating these effects as J receptors as they speculated that the afferent terminals were positioned juxtaposed to the pulmonary capillaries. The J receptor-mediated reflex was characterized by rapid shallow breathing, often occurring following an initial apnea, and accompanied by hypotension and bradycardia (12, 321, 351). This triad of responses collectively became known as the pulmonary chemoreflex, and in fact, the response had been first described earlier (in 1867) by von Bezold and Hirt and then again by Jarisch in the late 1930s using intravenous injections of veratrum alkaloids, hence the equally correct nomenclature, the Bezold-Jarisch reflex. Subsequent studies confirmed that pulmonary chemoreflex is in fact mediated (at least in part) by pulmonary chemically sensitive vagal afferent C-fibers, and that their location is not juxtacapillary as hypothesized, but rather broadly distributed throughout the airways and lung parenchyma. Indeed, the Coleridges expanded upon Mott and Paintal's work by delineating bronchial and pulmonary C-fiber reflexes, based on the anatomical terminations of the afferent endings and their responsiveness to different chemical agents and/or different routes of drug administration (85, 87, 89). However, with respect to breathing, their studies suggested that bronchial and pulmonary C-fibers produced somewhat comparable effects. More recently, Canning and colleagues (75) employed stimuli selective nodose versus jugular chemically sensitive afferents in guinea pigs to assess whether differential respiratory effects are mediated by these embryologically distinct afferent subtypes. Stimuli selective for nodose chemically sensitive afferents (2-methyl-5HT and adenosine) evoked only tachypnea while the activation of jugular afferents (by selectively targeting capsaicin delivery to the tracheolaryngeal mucosa which is largely devoid of nodose chemically sensitive afferents in guinea pigs) evoked only respiratory slowing and apnea. These data may argue that the full expression of the chemoreflex reflects the combined (or sequential) activation of both jugular and nodose chemically sensitive afferent subtypes, albeit others have shown that additional afferent processes (such as SAR activation in association with C-fiber evoked bronchoconstriction and cardiac afferents) contribute to the full expression of the pulmonary chemoreflex (407). Optogenetic techniques for stimulating distinct afferent subtypes in transgenic mice have now confirmed much of the older literature showing that nodose C-fiber stimulation evokes rapid shallow breathing (72), although interestingly the injection of capsaicin into the superior vena cava in conscious humans does not appear to elicit the chemoreflex (but does evoke coughing and pulmonary/extrapulmonary sensations) drawing into question the human physiological relevance of much of the animal data in this regard (454).

The activation of chemically and mechanically sensitive vagal afferents in the larynx may also induce swallowing. For example, capsaicin or citric acid infusion onto the laryngeal mucosa in guinea pigs increases swallowing frequency presumably secondary to the activation of chemically sensitive afferents from the jugular ganglia (429). Similarly, distension of the larynx/laryngopharynx also induces swallow presumably via nodose-derived mechanically sensitive afferents. Such laryngeal challenges are accompanied by a complex mixture of cough, apnea, and swallow, suggesting that swallow may act in concert with other protective responses, collectively facilitating the clearance of foreign stimuli from the glottal area. Very little is known about how the various afferent inputs to the brain stem may be integrated to produce the unique and complex motor processes needed to coordinate such responses.

C. Reflex Regulation of Autonomic Outflow

Cranial (vagal) parasympathetic and spinal sympathetic preganglionic neurons are reflexively regulated by vagal afferent nerves innervating the airways. The somata of vagal preganglionic neurons reside in one of two brain stem autonomic cell groups, namely, the dorsal motor nucleus of the vagus (a component of the dorsal vagal complex) and the nucleus ambiguus (part of the ventrolateral medullary respiratory and autonomic cell groups), whereas sympathetic preganglioinic neurons are located in the intermediolateral cell column of the spinal grey matter. Few, if any, airway vagal sensory neurons provide direct monosynaptic inputs to autonomic preganglionic neurons, instead relying on relayed inputs via the nucleus of the solitary tract and (presumably) the paratrigeminal nucleus to both autonomic premotor and motor nuclei (300–302). Indeed, the absence of labeled terminals in association with preganglionic cell populations in neural tracing studies of airway afferents as well as the morphological studies showing efferent projection patterns of SAR and RAR second-order neurons to the ventrolateral medulla is consistent with this notion. Two distinct parasympathetic pathways exist: the first is the classic pathway that utilizes acetylcholine as the neuroeffector (postganglionic) transmitter, and the second is a noncholinergic pathway dependent on nitric oxide and vasoactive intestinal peptide (VIP) for postganglionic transmission (62). Distinct airway vagal preganglionic neurons are thought to regulate these independent parasympathetic pathways (282, 297, 303), although this is not well described.

RAR activation enhances parasympathetic outflow **(FIGURE 4)**. For example, bronchoconstricting agents such as histamine, PGD_2, and thromboxane, which enhances RAR activity due to a mechanical effect in the lung, produce a coincidental reflex increase in both cholinergic and noncholinergic parasympathetic neural activity (e.g., Ref. 59). The observation that bronchodilators reverse the enhanced parasympathetic neural activity evoked by histamine while the inhibition of prostanoid and nitric oxide formation is without effect, coupled with the relative insensitivity of airway chemically sensitive afferents to histamine, argues in favor of the activation of RARs secondary to smooth muscle contraction and a reduction in dynamic pulmonary compliance as the mechanism for enhanced parasympathetic tone. Similar effects have been reported during pulmonary congestion (edema) which again is known to activate RARs, while RAR activation induced by intermittent lung collapse enhances airway secretions, an effect abolished by atropine or vagus nerve transection (468). Furthermore, the injection of a small volume of water into a lobar bronchus provides as osmotic stimulus for activating RARs that coincidentally produces a reflex increase in bronchial artery blood flow. Interestingly, this vascular effect is dependent on both enhanced parasympathetic (cholinergic) and reduced sympathetic neural outflow (367, 368), suggesting that RAR activation might serve to inhibit sympathetic-mediated airway responses, although this has not been studied in any great detail. The fact that bronchospasm (induced by inhalation of neurokinin A) does not induce sympathetically mediated airway smooth muscle relaxation is consistent with RARs not having an excitatory effect on sympathetic outflow (344).

There exists a baseline level of parasympathetic derived airway smooth muscle tone that is regulated on a breath-by-breath basis. The ongoing activity of vagal sensory neurons may in fact provide drive for establishing this tonic level of brain stem parasympathetic outflow. In cats, baseline cholinergic tone can be abolished by selective nodose ganglionectomy, a procedure that does not affect cholinergic mediated airway contractions evoked by stimulating the vagi above the level of the ganglionectomy (i.e., disrupts the sensory but not the motor innervation to the airways) (183). The reduction in baseline cholinergic tone associated with ganglionectomy coincided with the disappearance of the C wave of the vagal compound potential, suggestive that ongoing activity in bronchopulmonary C-fibers plays a significant role in establishing parasympathetic smooth muscle tone in this species (183). Studies in guinea pigs on the other hand suggest that the source of this baseline parasympathetic nerve activity may be derived from central input generated by the ongoing activity of RARs during cyclical inflation and deflation of the lungs (205, 206, 283). Thus the selective interruption of RAR activity during tidal breathing, without disrupting the autonomic motor pathways projecting to the airways, abolishes baseline parasympathetic-dependent airway smooth muscle tone. The airway vascular smooth muscle is also under tonic parasympathetic and sympathetic tone (63, 86, 286), although it is not known if RARs provide any contribution to this. Furthermore, whether the RAR-dependent drive for generating parasympathetic outflow to the airways also extends to the general regulation of global baseline parasympathetic nerve activity to other viscera is not known.

The effect of SARs on autonomic outflow is surprisingly less well studied. Enhanced SAR activity results in an inhibition of cholinergic drive to the airways, leading to decreased airway smooth muscle tone (54, 282, 381, 443). Thus, during mechanical ventilation, a sudden increase in positive end-expiratory pressure (PEEP), which is sufficient to activate SARs due to enhanced lung stretch, results in a rapid fall in baseline cholinergic drive and a blunting of reflex evoked increases in cholinergic outflow to the airways (for example, that evoked by RAR activation following histamine bronchospasm as described above). These observations are entirely consistent with the notion that GABAergic pump cells in receipt of SAR inputs in the brain stem in turn inhibit RAR cell activity at the level of the nucleus of the solitary tract (120, 122). Interestingly, whereas the activation of RARs (and chemically sensitive airway afferents)

enhances both cholinergic and noncholinergic parasympathetic outflow to the airways, SAR activation is reportedly devoid of effect on noncholinergic mediated responses (282), arguing that nuances exist in the central synaptic organization of airway afferent pathways feeding into autonomic circuits in the brain stem. Also of interest is the observation that although SAR activation can evoke systemic vasodilation, it is reportedly without effect on tracheal vascular resistance according to studies in dogs and sheep (381, 443). SAR activation may also not reflexively alter airway mucous secretion (366).

Stimuli of chemically sensitive afferent fibers, including, but not limited to bradykinin, capsaicin, leukotrienes, and a variety of autacoids increase parasympathetic preganglionic activity when delivered intravenously, inhaled, or directly applied to the airway mucosa (59, 206, 283, 344), thereby enhancing both cholinergic and noncholinergic postganglionic output to the airways. Elevated parasympathetic drive is not end-organ specific; thus stimulants of chemically sensitive afferent fibers also influence bronchial and vascular smooth muscle tone and mucous gland secretion, as well as producing effects on blood pressure (typically hypotension) and heart rate (bradycardia) as well as modulating other functions in organs innervated by parasympathetic nerves. Furthermore, electrical stimulation of the central cut end of the vagi, at stimulus intensities sufficient to activate C-fibers, increases spinal sympathetic outflow, presumably by enhancing the activity of sympathetic premotor neurons in the brain stem (344). This would suggest that chemically sensitive afferents can reflexively enhance the activity of both parasympathetic and sympathetic preganglionic neurons. By and large, the cholinergic division of the parasympathetic nervous system dominates the resultant airway end organ responses following airway chemically sensitive vagal afferent stimulation resulting in bronchoconstriction, vasodilation, and enhanced mucous secretion. The coincidental activation of sympathetic and noncholinergic parasympathetic neurons (59, 206, 282, 344) probably serves as a brake on cholinergic responses, and this complex mix of activity likely contributes to the varied systemic (e.g., heart and blood pressure) autonomic responses reported for different airway sensory stimuli. Although not studied in great detail, the available evidence suggests that subtypes of chemically sensitive afferents produce fundamentally the same effect on parasympathetic outflow. Nevertheless, variations in the pattern or kinetics of parasympathetic activity may exist. For example, in the guinea pig, inhaled capsaicin (via a tracheal cannula to bypass the proximal airways) evokes reflex elevation of cholinergic bronchomotor tone that displays rapid on and off kinetics and generally persists only a few minutes longer than the stimulus duration. However, when capsaicin is transiently and discretely applied to the laryngeal mucosa, there is an initial brief withdrawal of cholinergic tone (lasting 1–2 min) followed by a slow and sustained elevation in cholinergic tone that peaks over the course of 20–60 min and is sustained for some time thereafter (59, 282, 283). This unique laryngeal-evoked response is interesting in that the chemically sensitive afferent terminals in the guinea pig larynx are almost entirely derived from the jugular ganglia, whereas those activated by inhaled stimuli are predominately derived from the nodose ganglia. Whether this reflects the different central projections of jugular and nodose neurons (302) is not known.

D. Axon and Peripheral Reflexes

Sensory neurons possess efferent, in addition to their afferent, functions that are known to contribute to the control of peripheral tissues **(FIGURE 7)**. Perhaps the first definitive description of sensory efferent control of end organs was made by Ninan Bruce in studies of the "triple response" to cutaneous irritation. Bruce (48), and soon after Sir Thomas Lewis (248), noted that the flare vasodilator response following scratching of the skin was likely mediated by a local nervous system reflex independent of central processing, the so-called "axon reflex." In this regard, local tissue damage-evoked sensory neuron activation was thought to promote local sensory neurotransmitter release via antidromic action potentials travelling along collateral branches of the sensory neuron axon, leading to the activation of tissue mast cells and vasodilation **(FIGURE 7)**. However, effects attributed to axon reflexes may also occur independently of action potentials along collateral branches. For example, pharmacological inhibitors of neuronal sodium channels involved in action potential propagation (lidocaine or tetrodotoxin) or voltage-gated calcium channels involved in vesicular neurotransmitter release (omega conotoxin GVIA) generally do not prevent capsaicin axon reflex-mediated neurotransmitter release in the airways, whereas electrically evoked responses are abolished (335). This suggests that capsaicin may evoke peripheral transmitter release at the terminal site of activation rather than following conduction to a collateral branch. In this instance, presumably TRPV1 itself carries the calcium current needed for vesicular transmitter release.

Although axon reflexes can occur in the vagal sensory neurons that innervate the airways, the relative importance of these in physiological and pathophysiological functions is likely species dependent. For example, guinea pigs and rats possess many neuropeptide containing vagal afferent terminals which, when activated, can evoke profound bronchospasm, vasodilation, and mucous secretion independent of any central neural processing (59, 282, 283, 286, 335). This can be demonstrated in vitro or in vivo using vagus nerve electrical stimulation, electric field stimulation, or chemical agents such as capsaicin. In guinea pigs, the magnitude of capsaicin-evoked axon reflex dependent bronchospasm approaches the maximal response attainable with methacholine. However, in stark contrast, many species including

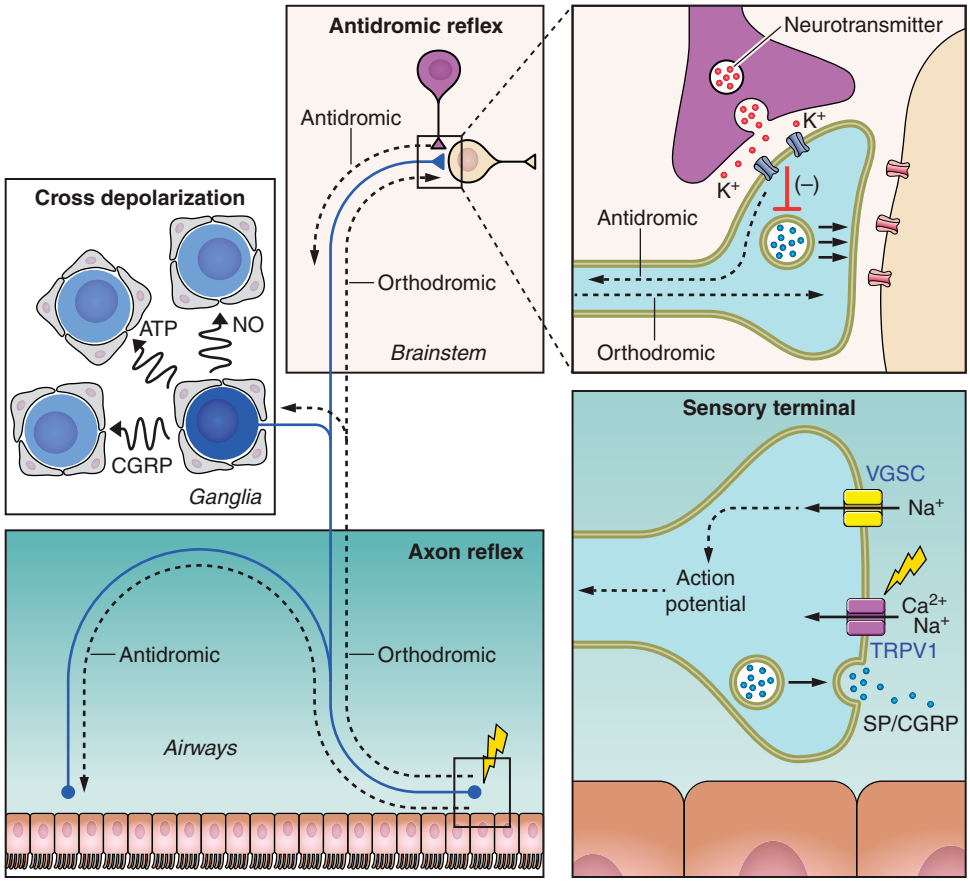

FIGURE 7. Axon reflexes, cross depolarization and antidromic reflexes, alternate modes of sensory dependent responses in the airways. Subsets of airway afferents, namely, the peptidergic nociceptors, can produce local end organ effects within the airways via classic axon reflexes. Thus peripheral stimuli can lead to Ca^{2+} and Na^{+} entry into the nerve terminal that leads to both the local release of neurotransmitters substance P and calcitonin gene-related peptide (SP/CGRP) at the site of stimulation, or at distal sites secondary to the antidromic conduction of action potentials along collateral sensory branches. Orthodromic action potential conduction depolarizes sensory neuron soma in the vagal ganglia leading to the somal release of signaling molecules such as CGRP, ATP, and nitric oxide (NO), which can act in a paracrine fashion to cross depolarize neighboring sensory soma. Within the central nervous system, presynaptic inputs to primary afferents help regulate synaptic transmission by depolarizing primary afferent terminals (serving to inhibit transmission). A build-up of extracellular K^{+} and/or presynaptic transmitters can induce antidromic action potential formation within primary afferents, capable of conduction to peripheral tissues. TRPV1, transient receptor potential vanilloid receptor 1; VGSC, voltage-gated sodium channel.

sheep, dogs, and humans display minimal airway end organ effects following antidromic activation of sensory nerve terminals either in vitro or in vivo. Why some species display prominent axon reflexes and others do not is unclear. Adding further complexity, not all C-fiber sensory stimuli evoke robust axon reflexes, even in species that display prominent axon reflexes. For example, whilst both capsaicin and bradykinin reliably evoke strong othodromic C-fiber activation leading to CNS dependent reflex responses in guinea pigs, bradykinin (unlike capsaicin) is a very poor stimulus for the axon reflex-dependent release of neuropeptides (59, 282). This is a striking observation given that bradykinin has been shown to evoke sensory activation in part via coupling with TRPV1 (66, 143), although TRPV1 may not be obligatory for bradykinin-evoked activity (214). Differential selectivity of capsaicin and bradykinin for particular C-fiber subtypes is unlikely to explain their abilities to induce peripheral neuropeptide release, because the same afferents (including neuropeptide expressing jugular C-fibers) can be activated by both compounds.

Variations of the axon reflex have also been reported, although in some instances there may be few, if any, specific studies of airway vagal circuits in this regard. Nevertheless, these will be presented briefly for completeness. Axon collaterals arising from sensory neurons, in addition to innervating muscles and glands in peripheral tissues, provide synaptic interactions with local tissue parasympathetic ganglia neurons (58, 326, 327, 329). Under these circumstances, sensory nerve activation can reflexively modulate parasympathetic neural activity via "peripheral reflexes" that proceed independently of CNS integration. With respect to the airways, capsaicin-sensitive vagal afferents have been shown to evoke peripheral reflex-dependent relax-

ation of the airway smooth muscle in guinea pigs by collateral branches that innervate esophageal myenteric plexus neurons that in turn provide the relaxant parasympathetic drive to the airways (61). Such responses are inhibited by TTX and can be reproduced in isolated tissues in vitro (i.e., in the absence of a central nervous system).

In spinal sensory pathways, action potentials can be recorded traveling antidromically in axons of the spinal dorsal roots **(FIGURE 7)**. These action potentials were thought initially to be occurring in recurrent branches of primary afferent nerves; however, subsequent studies suggested that they are initiated in the nerve terminals in the spinal cord and then travel antidromically along the parent axons of the primary afferents. The induction of these antidromic action potentials was termed a "dorsal root reflex," and examples of these were recorded most readily in myelinated afferent nerve fibers, although evidence for C-fiber involvement has also been noted (252, 253, 452). Dorsal root reflexes are thought to be initiated by neurotransmitters or a buildup of extracellular potassium acting presynaptically at the central terminals of primary afferents following their activation (219, 452). This in turn depolarizes the central terminals of sensory neurons concomitantly reducing central sensory neurotransmitter release (known as presynaptic or primary afferent inhibition) and initiating antidromic action potentials conducted towards the peripheral nerve terminals (361, 452). Several lines of evidence indicate that dorsal root reflexes may contribute to the peripheral end-organ effects attributed to axon reflexes. For example, the flare response initiated by an intradermal injection of capsaicin may far exceed the maximum branching of afferent terminals in the skin (171). This suggests that either vasoactive substances released from peripheral nerve terminals have a wide sphere of influence or that additional nerves are recruited to regulate local end organ responses. Strikingly, severing the dorsal roots innervating a region of the skin markedly attenuates the vasodilatation and edema initiated by capsaicin challenge (140, 252, 253). Whether comparable antidromic vagal sensory pathways exist is unknown, although central presynaptic inhibition of vagal primary afferents is well described, suggesting that antidromic vagal activity is possible.

Sensory neuron activation can also influence the activity of other sensory neurons through nonsynaptic interactions between neuronal soma in sensory ganglia **(FIGURE 7)**. Thus sensory ganglia neurons, including those in the vagal ganglia, can become depolarized and may even form action potentials secondary to heightened activity in adjacent neuronal soma through processes called cross depolarization and cross excitation (11, 104, 346). Nonsynaptic interactions depend on activity-dependent release of diffusible mediators, including glutamate, tachykinins, ATP, serotonin, acetylcholine, and potassium, from one neuron within the ganglia that subsequently depolarize the membranes of adjacent ganglia neurons (138, 201, 278, 354, 431). Resident glial cells (satellite cells) may also contribute to intraganglionic communication by gap junction connections with neurons or through diffusible mediators (130). Cross excitation and cross depolarization in the ganglia may result in action potentials traveling antidromically to the peripheral terminals of primary afferent nerves or orthodromically to contribute to vagal afferent evoked reflexes. Indeed, given that there is little viscerotopic organization in the vagal sensory ganglia, afferent signals arising from one organ could conceivably modulate the afferent processing of another by virtue of the fact that their vagal sensory neuron soma lay in close proximity.

VI. EVIDENCE OF VAGAL SENSORY NERVE DYSFUNCTION IN AIRWAY DISEASE

The symptoms of the most common respiratory diseases (respiratory tract viral infections, rhinitis, bronchitis, asthma, COPD, chronic cough) are a consequence of alterations in the nervous system. Such symptoms include reflex mucus secretions, painful oropharynx, excessive sneezing, coughing, reflex bronchospasm, and sensations of dyspnea and the urge to cough. It is likely that is some cases the symptoms of these airway diseases are secondary to inappropriate activity within the afferent nervous system.

The sudden reversible bronchospasm that typifies an asthma attack is often linked to environmental stimuli that are known to activate afferent nerves in the respiratory tract (viral infection, allergens, pollutants, changes in temperature or osmolarity, etc.). This connection led Salter in the second chapter of his classic monograph "On Asthma" (382) to the conclusion that the nervous system is the regulator of asthma.

"A morbid proclivity of the musculo-nervous system of his bronchial tubes to be thrown into a state of activity; the stimulus may be either immediately or remotely applied, but in either case would not normally be attended by any such result. There is no peculiarity in the stimulus, the air breathed is the same to the asthmatic and non-asthmatic; . . . nor probably is there any peculiarity in the irritability of the bronchial muscle; the peculiarity is confined to the link that connects these two-the nervous system-and consists in its perverted sensibility in its receiving and transmitting on to the muscle, as a stimulus to contraction, that which it should take no cognizance."

Salter's detailed and rational basis for this conclusion for an altered nervous system in asthma predates our advanced understanding of respiratory nervous system, but nevertheless, the arguments he posed have largely stood the test of time. Indeed, more recent clinical investigations provide objective data in support of the concept of a "sensory hyperactivity" in asthma (309, 423).

Prior to advances in anti-inflammatory therapy, surgical denervation techniques were often used to quell the number and severity of attacks in the severe asthmatic patient. By the 1920s, our understanding of the extrinsic neuroanatomy of the respiratory tract along with advances in surgical techniques rendered the surgical approach to asthma a rational approach in severe cases, especially given the lack of other effective therapies. The literature soon was filled with reports on the efficacy of this treatment, but many are single case reports and poorly described studies that must be interpreted with substantial skepticism. Nevertheless, Phillips and Scott (365) critically evaluated this literature and after reviewing some 300 studies concluded that 29 were scientifically sound (reasonable diagnostic criteria of asthma along with at least 6 mo follow-up) investigations. They were drawn to the conclusion that "there are a few brilliant cures in extremely severe forms of asthma. Roughly on half the patients definitely improved, while frequently the other half, after temporary improvement, are in no better condition than before the operation. The patients who were cured have been followed on average almost two years."

This spurred further interest and improvement in the surgical techniques for denervating the extrinsic innervation to the respiratory tract. The most selective surgical approach was perhaps that carried out by Reinhoff at the Johns Hopkins Hospital where he severed only the posterior pulmonary plexus. With the assistance of his colleague and noted allergist Leslie Gay, they evaluated this technique for the treatment of severe allergy-associated asthma (377). They carried out the study on 11 patients that were virtually incapacitated by their asthma and followed them before and 1.75–2.75 years following the surgery. Their conclusions were thus:

"Of the 10 patients discharged from the hospital, 1 was entirely unimproved; 1 improved for 3 mo, finally succumbing to what seemed to be cardiac failure; 4 are completely well as the time of writing, having been free of attacks since the operation or a short time later, and are able to resume their former work; 4 have occasional mild attacks of asthma, all of which are amenable to control by means of small doses of ephedrine."

The discovery in the 1950s that the severity and number of asthma exacerbations could be limited with systemic cortisone treatment put an end to the denervation treatment of asthma. Nevertheless, this history speaks to the importance of airway innervation in asthma and by inference to the potential efficacy of novel therapeutic approaches that would selectively target nerves within the respiratory tract. Although controversial, a rational case can be made for a neuronal role in the wheezing, hyperreactivity, dyspnea, and coughing associated with asthma and other airway disorders.

A. Wheezing and Airway Hyperreactivity

Contractions of bronchial smooth muscle lead to airway narrowing and in severe cases to the wheezing of an asthma attack. The dominant regulator of bronchial smooth muscle tone and thus airway caliber is the parasympathetic nervous system, which provides contractile cholinergic innervation and relaxant VIP/nitrergic innervation to the smooth muscle (433). There is a resting parasympathetic drive that keeps the bronchial smooth muscle in a slightly constricted state. As discussed above, part of this baseline parasympathetic drive appears to be due to reflex activity initiated by activation of vagal afferent nerves during eupneic breathing. This parasympathetic contractile reflex can be substantially increased by activation of vagal C-fibers and RAR fibers in the respiratory tract.

In studies with asthmatic subjects, blocking the cholinergic contractile activity enlarges the airways and decreases the resistance to airflow (144, 145). What is important to note is that in some subjects once this pathway is maximally inhibited, there is relatively little additional effect of adding a direct bronchodilator such as a β adrenoceptor agonist. Ullah et al. (430) noted that in fully 40% of their subjects, ipratroprium provided the same or better effect than a maximally effective dose of salbutamol (430). Keeping in mind that it is difficult to clearly define a maximum effect in clinical studies, at face value this means that for a sizeable percentage of asthmatic subjects bronchoconstricting autacoids may be present in the inflamed airways, the concentration or location of these agents are insufficient to contribute meaningfully to the afferent nerve driven reflex cholinergic regulation of bronchial smooth muscle tone. This may explain why in recent studies with asthmatic subjects uncontrolled by their glucocorticoid inhaler, adding the potent and selective muscarinic receptor antagonist tiotropium was at least as effective as adding the functional antagonist salmeterol as measured by reductions in airway resistance and increases in asthma-control days/week (364).

A hallmark of asthma is referred to as bronchial hyperreactivity. This is experimentally identified by the concentration of a bronchoconstricting stimulus (most often methacholine) required to increase airways resistance by 20% (260). The asthmatic individual can be 10-1,000 times more reactive than healthy controls, and the degree of hyperreactivity is correlated with the severity of asthma. The underlying cause of hyperreactivity remains enigmatic. It is often argued that hyperreactivity is causally linked to airways inflammation. It is likely that inflammation plays a role in bronchial hyperreactivity, but such a role is not always obvious. Subjects that suffer from an allergy to ragweed typically exhibit all the signs of allergic TH2 type inflam-

mation in both their upper and lower airways, yet many of these subjects do not have bronchial hyperreactivity; they suffer with symptoms of rhinitis but not asthma (28, 41, 46). Conversely, long-term use of inhaled corticosteroids substantively inhibits inflammation, but has relatively little effect on hyperreactivity (1).

Some clinical and experimental observations indirectly support the idea that hyperreactivity in some cases may actually be a hyper-reflexivity. The idea is that in asthmatic subjects, inhaling a stimulant such as histamine or methacholine evokes a strong parasympathetic reflex that is not as present in healthy volunteers. The mouse is a common animal model for the study of asthma. The physiological end point for "mouse asthma" is most often airways hyperreactivity to methacholine. In one study the airways hyperreactivity associated with allergen inhalation was entirely prevented if the vagus nerves were cut prior to the methacholine challenge. There were no differences noted between allergically inflamed mice and control mice with respect to the ability of methacholine to directly evoke bronchoconstriction. In the allergically inflamed mice, however, methacholine appeared to cause a strong reflex cholinergic response, a response not present in the healthy control mice (295). In a more mechanistically elegant study, it was found that simply deleting capsaicin-sensitive vagal sensory C-fiber neurons using (cre-diptheria toxin methodology) prevented allergen inhalation from evoking methacholine hyperreactivity (427). Removing the vagal C-fiber neurons had no effect on the inflammatory response. These data indicate that the hyperreactivity caused by the allergic inflammation was entirely secondary to an augmented vagal C-fiber dependent methacholine-induced reflex bronchoconstriction.

It is more difficult to assess reflex actions in human subjects. Yet, there are some hints that the nervous system is involved in the mechanism (s) underlying bronchial hyperresponsiveness. It is clear that when the human bronchi from lungs of asthmatics are studied as isolated tissues, the reactivity of the smooth muscle to histamine and methacholine is essentially the same between tissues from asthmatic and healthy lungs (16, 70, 142, 178, 427). This would be predicted if neuronal reflexes were causal to the hyperreactive state.

Hyperreactivity to methacholine can be induced in healthy subjects by having them voluntarily keep from taking a deep inspiration. Upon taking a deep breath their airways again become normally reactive (392, 401). Asthmatic subjects have somehow lost this protective effect of deep inspiration. In this context, it is interesting to note that deep inspiration is a strong persistent stimulus for the activation of vagal SARs, and upon such activation there is an inhibition of parasympathetic drive induced within the brain stem. It is tempting to speculate that this SAR-driven negative controller of reflex drive may be less active in asthmatic subjects. Respiratory virus infection also leads to airways hyperreactivity in otherwise healthy subjects. Laitinen et al. (229) noted that this increases the reactivity to inhaled histamine, but the enhanced component of the response was entirely reversed by pretreatment with a cholinergic muscarinic receptor antagonist; the direct effect of histamine on the muscle (the response in the present of the anticholinergic agent) was not influenced by the infection, again arguing that only cholinergic reflex component of the histamine challenge was hyperreactive (229).

B. Dyspnea

Dyspnea, or literally "difficult breathing," is a term used to describe sensations of chest tightness and air hunger. The neural substrates of dyspnea are complex and extend well beyond the vagus nerves. The dyspneic sensations during maximal incremental bicycle exercise, for example, are not diminished in a person following a double lung transplant (effectively vagotomized) (210). Nevertheless, at least four lines of evidence support the hypothesis that vagal afferent nerves can contribute to the sensations of dyspnea, and in inflammatory diseases this contribution may be exaggerated. First, electrical stimulation of the cervical vagus nerves leads to dyspneic sensations in some individuals (160, 342). Second, inhalation of mediators such as adenosine, histamine, and PGE_2 that can activate vagal afferent nerves, particularly C-fibers, either causes dyspnea or intensifies the sensations of dyspnea that are evoked with exercise or a bronchoconstricting agent (276, 410, 424). Third, inhalation of histamine and adenosine leads to dyspnea by a mechanism that is inhibited by inhalation of lidocaine (51, 411). Fourth, clinical studies with inhaled furosemide, a drug that can inhibit afferent nerve activity, was found to quell experimentally induced dyspnea (340). In airway inflammatory disease, therefore, the increased activation of vagal C-fibers may enhance sensations of dyspnea in a manner that discoordinates it from increases in airway resistance.

C. Chronic Cough

Cough, as discussed above, is a critically important and potentially life-saving reflex. Cough can also be pathological and nonproductive. Historically pathological coughing has been considered to be a relatively benign consequence of some underlying primary disorder such as postnasal drip, asthma, respiratory tract viral infection, or gastrointestinal reflux disorder. More recently, however, clinical experts that treat cough are coming to the realization that as with chronic pain syndromes, there may be individuals in which a distorted and hypersensitive cough reflex pathway is a primary disorder (316, 317). Individuals suffering from idiopathic chronic cough can be found coughing some 50–100 times per hour most of their awake lives.

There are many similarities and analogies between the mechanisms underlying chronic cough and chronic pain

(82, 343). In pain, the differentiation of hyperalgesia and allodynia has proven to be informative. Hyperalgesia is defined as a leftward shift in a pain stimulus-response curve. A given pain stimulus results in more intense pain in the individual suffering from hyperalgesia. Allodynia, on the other hand, describes situations where typically nonpainful stimuli such a lightly brushing one's hair becomes painful. In chronic cough there are also conditions of "hypertussivity" and "allotussivity." The hypertussivity is commonly seen as a leftward shift in the tussive stimulus (usually inhaled capsaicin)-cough response curve (317). Chronic cough patients also suffer allotussivity, i.e., non-tussive stimuli such as talking, singing, laughing, etc. leads to strong urge to cough sensations (166). It is likely that alterations in the activity and central connectivity of vagal afferent nerves underlie important aspects of the pathological disorder of chronic cough.

D. The Urge to Cough

A perceivable sensation, termed the urge to cough, typically accompanies the motor act of coughing in patients with pulmonary disease. In fact, the urge to cough is a sensory experience that is more akin to the sensation of pain, whereas coughing (the motor event) can be likened to the act of withdrawing away from a painful stimulus. Accordingly, the generation of an urge to cough is highly dependent on vagal afferent input to higher brain centers that encode sensory, cognitive, and/or affective dimensions of airway irritations. In this sense, the urge to cough is an interoceptive experience that informs the brain of the internal environment of the pulmonary system. Like dyspnea, the urge to cough has proven difficult to study because investigations are limited to human subjects, as only they can report the psychophysical nature of the sensation. Nevertheless, as discussed above, functional brain imaging has provided insight into the central processing networks likely important in aspects of the urge-to-cough experience. In patients with respiratory disease, the stimulus threshold for evoking an urge to cough is significantly reduced, and patients with chronic cough report an ongoing perception of an urge to cough that is not experienced by healthy people (107, 166). Collectively, these clinical observations are indicative of disease-induced sensory hypersensitivity and enhanced basal vagal afferent activity.

VII. MECHANISMS OF SENSORY INVOLVEMENT IN RESPIRATORY DISEASE

There are at least four interrelated general mechanisms by which vagal afferent nerves can be incorporated into the pathologies and symptoms discussed above. First, airway inflammation leads to the production of mediators that overtly activate nociceptors in the respiratory tract. Second, inflammatory processes can change the excitability of the afferent nerve terminals such that other activating stimuli are more efficacious in evoking high-frequency afferent nerve discharge. Third, processes in the diseased airways can lead to phenotypic changes in the vagal afferent neurons. Fourth, the increased information reaching the brain stem can lead to neuroplasticity and changes in central synaptic transmission, events that are collectively referred to as central sensitization.

A. Overt Activation of Afferent Nerves in Disease

Inflammatory airway diseases are associated with increases within the airways of a myriad autacoids including various biogenic amines, acid, eicosanoids, purines, cytokines, and chemokines. As discussed earlier, many of these chemicals can overtly stimulate action potential discharge in vagal afferent nerves that terminate in the respiratory tract, most commonly C-fibers (e.g., **FIGURE 9*A***). The therapeutic approach of anticipating and blocking one particular nerve activator may be an inefficient strategy given the potential for the redundancy of the various activating stimuli. Nevertheless, ATP is a mediator that is elevated in airway disease such as COPD (259), and a drug that can effectively block that purinergic P2X3 and P2X2,3 receptor has been developed and studied in human subjects. This compound, AF-219, effectively inhibits ATP-induced stimulation of airway nodose C-fibers (444). In a phase 2 clinical trial with chronic cough patients that find little or no relief with existing therapies, AF-219 was found to radically decrease coughing frequency (3).

Coughing is a common side effect of antihypertensive drugs that block angiotensin-converting enzyme (ACE). Studies in guinea pigs have found that this too may be secondary to the action of a single mediator. Treating guinea pigs with the ACE inhibitor captopril led to an increase in the acid-induced coughing. This increased tussive response was blocked by icatibant, a drug that blocks bradykinin 2 receptors (136). Studies such as these suggest that in some disorders, a single type of mediator may drive excessive afferent activity.

B. Changes in Excitability

Many mediators present in inflamed airways (e.g., histamine, eosinophilic cationic protein, proteases, adenosine, PGE_2) may increase the excitability of afferent nerves without evoking generator potentials and overtly stimulation action potential discharge (147, 150, 225, 239) **(FIGURE 8)**. This results in situations where the threshold for activation by other stimuli is reduced, and the peak action potential frequency evoked by other stimuli is increased. For example, intrapulmonary nociceptive C-fibers are relatively in-

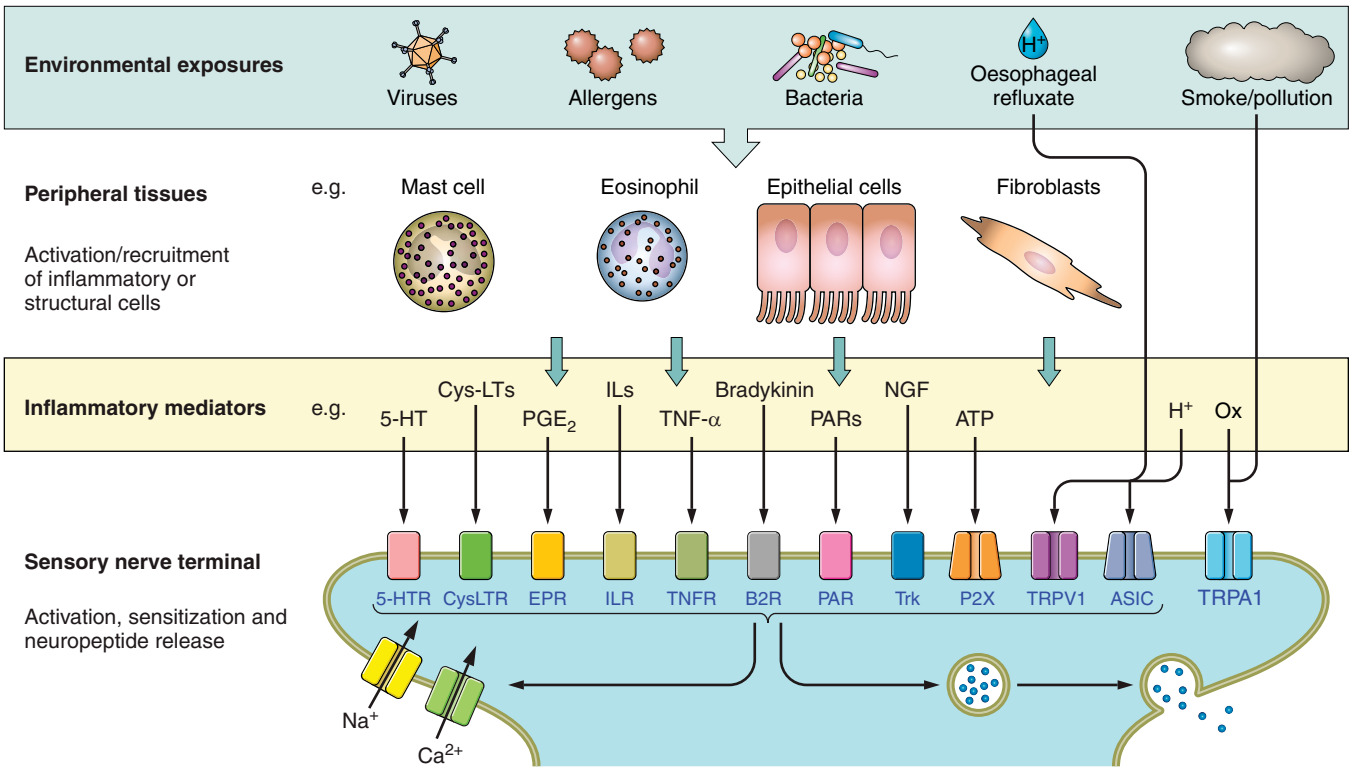

FIGURE 8. Mechanisms of peripheral sensitization of airway afferent nerve fibers. A variety of environmental triggers, including viruses, allergens, bacteria, esophageal refluxate, and smoke/pollution, are capable of inducing primary afferent hypersensitivity. These triggers drive complex inflammatory and oxidative (Ox) processes, characterized by the generation of many inflammatory mediators, all capable of acting upon airway sensory nerves to produce generator potentials that serve to modify the activation threshold needed to evoke action potential formation (peripheral sensitization) and/or to induce neural activation directly. Peripheral sensitization increases primary afferent-dependent response via increased sensory-dependent central output to the airways and via peripheral (axon reflex) mediated processes. 5-HT, 5-hydroxytryptamine (serotonin); CysLTs, cysteinyl leukotrienes; PGE_2, prostaglandin E_2; Ils, interleukins; TNFa, tumor necrosis factor-α; PARs, protease receptor agonists; NGF, nerve growth factor; H^+, acid; EPR, E prostanoid receptor; B2R, bradykinin subtype 2 receptor; Trk, tropomyosin receptor kinase; P2X, purinergic receptor; TRP, transient receptor potential.

sensitive to the mechanical forces associated with inspiration. After treating the lungs with histamine that by itself did not activate the C-fibers, the excitability of C-fibers was consistently increased in some cases to the point that inspiration now leads to their activation (237). Inflammatory mediators can also lead to increases in the excitability of A-fibers in the airways **(FIGURE 9)**. The guinea pig tracheal Aδ cough fiber is very sensitive to light touch of the mucosa. Immunological activation of tracheal mast cells and the release of allergic mediators fails to activate these nerves, but leads to impressive increases in the excitability of the terminals such that their sensitivity to subsequent touch is dramatically and persistently increased (375) **(FIGURE 9*B*)**.

The ionic mechanisms leading to increases in vagal afferent excitability are manyfold **(FIGURE 8)**. Inflammatory mediators often stimulate classical GPCR signaling pathways, leading to phosphorylation of ion channels that are involved in setting the threshold and frequency of action potential discharge. In the somatosensory system, stimulation of G_q or G_s signaling pathways can lead to phosphorylation of NaV channels, in particular NaV 1.8, thereby increasing peak action potential frequency of discharge, and decreasing the activation threshold. Similarly, PGE_2 can stimulate G_s pathways to sensitize pulmonary C-fibers by mechanisms that involve NaV channel phosphorylation (225, 226). Other mediators can lead to the inhibition of certain potassium leak currents that provide a break on action potential firing. By blocking these channels the mediators cause an overall increase in input resistance and amplify the membrane depolarization for a given amount of generator current (92, 445, 446).

As more specifics are learned about the molecular mechanisms underlying changes in excitability, it is anticipated that therapeutic strategies may be developed that will normalize the state of excitability of afferent nerves.

C. Phenotypic Changes and Critical Periods

Airway inflammation not only leads to a quantitative increase in action potential discharge in afferent nerve via

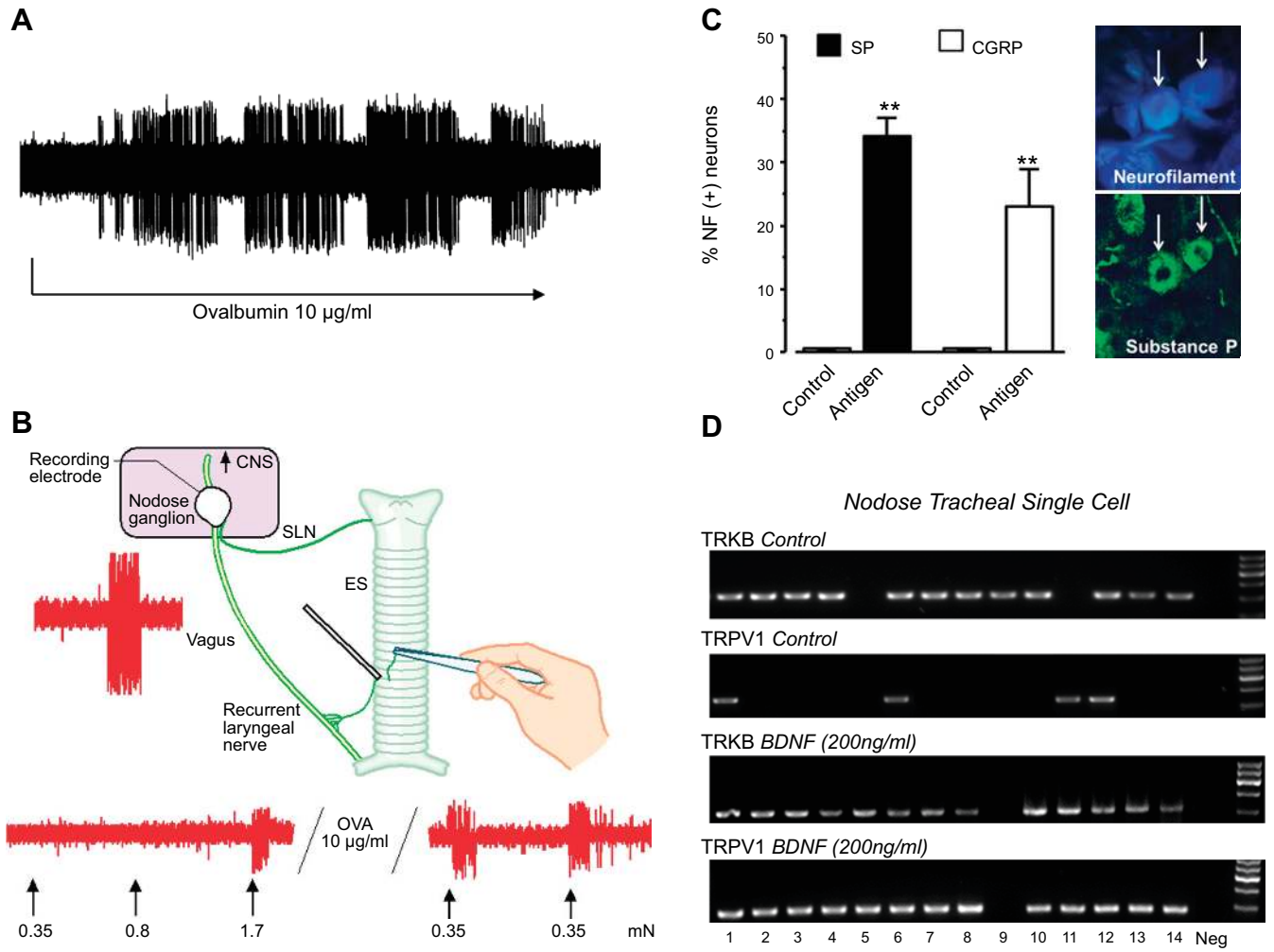

FIGURE 9. Examples of inflammation-induced modulation of guinea pig airway vagal afferent nerves. *A*: representative example of a nodose C-fiber responding with action potential discharge to allergen (ovalbumin) provocation (guinea pig was previously actively sensitized to ovalbumin). *B*: allergen (ovalbumin) challenge does not overtly activate nodose Aδ-fibers in the guinea pig trachea, but increased the excitability of the fiber to mechanical activation. [From Riccio et al. (375).] *C*: allergen (ovalbumin) inhalation leads to phenotypic changes in the neurons expressing substance P and CGRP. In control animals, large neurofilament-positive (NF+) nodose neurons innervating the respiratory tract do not express these neuropeptides, but 1 day following allergen challenge, ~25% of these neurons become neuropeptide positive. [From Myers et al. (328).] *D*: another example of neuroplasticity showing a phenotypic switch in TRPV1 gene expression. In control animals, nodose fibers innervating the trachea do not express TRPV1, but 1 day following exposure to BDNF, TRPV1 is induced in a majority of the neurons. [From Lieu et al. (250).]

overt activation and increases in excitability; it can also qualitatively change the nerve phenotype **(FIGURE 9)**. Airway inflammation is associated with increases in the production of various neurotrophic factors that can interact with receptors at the nerve terminals in a manner that ultimately leads to changes in gene expression in the cell bodies situated in the distant vagal ganglia. Allergic inflammation of rat airways leads to a phenotypic change in SAR and RAR neurons such that they begin to express de novo TRPV1. These nerves then become responsive to capsaicin (and to other endogenous TRPV1 activating stimuli) (475). Likewise, airway inflammation in guinea pigs leads to increases in functional TRPV1 channels in tracheal Aδ cough neurons that normally do not express these channels (250). Allergen inhalation also phenotypically changes nodose C-fiber neurons such that they develop responsiveness to neurokinins (315). By evoking these phenotypic changes airway inflammation can expand the activation profile of the terminals such that stimuli that would normally be sensed as inert will potentially now lead to coughing, dyspnea, and nocifensive reflexes (bronchospasm, secretions).

Activating ion channels are not the only relevant genes that can be turned on by the processes of airway inflammation. The genes responsible for the production of sensory neuropeptides are enhanced in vagal sensory neurons following airway inflammation evoked by allergen challenge or viral infections. Again, a phenotypic switch is observed where

large-diameter RAR/SAR neurons as well as tracheal Aδ cough neurons that normally do not express neuropeptides begin to express and transport peptides like neurokinins and CGRP towards the nerve terminals (65, 79, 109, 131, 328). When neuropeptides like substance P are released from the central terminals, increases in synaptic transmission may occur via central sensitizing processes (293). Normally this is reserved for the activation of bona fide nociceptors (nerves that provide the organ a sense of its own potential injury); however, airway inflammation may set up conditions where the simple process of respiration and the activation of RAR/SAR type fibers leads to increases in neuropeptides in the central nervous system and the potential consequence of central sensitization (see below).

The neurotrophic factor-driven changes in gene expression within vagal afferent nerves is reversible over the course of a few weeks (65, 250). However, during certain periods of postnatal development, such neurotrophic-mediated events can become persistent, and even irreversible. These periods are referred to loosely as critical periods (30). In animal models of inflammation-induced hyperalgesia, it was found that when the inflammation was evoked neonatally the neuronal circuitry and mechanisms underlying hyperalgesia persisted beyond the inflammation and into adulthood (380). In animal models of inflammatory bowel disease, gut inflammation of adult rats leads to a hyperreflexic state that is reversible with time. However, when the inflammation was evoked during early life critical periods in gut nerve development, the hyperreflexia outlasted the inflammation and persisted into adulthood (258). One might hypothesize, by analogy, that airway inflammation early in life due to say a respiratory infection during a critical period might lead to long-lasting changes in afferent neurobiology setting up conditions of an airways hyperreflexic state that persists into adulthood.

D. Central Sensitization

Central sensitization is broadly defined as an increase in the excitability of central neurons that are in receipt of peripheral sensory related information, and typically occurs subsequent to excessive sensory neuronal activity resultant from peripheral tissue inflammation or neuropathic injury. In a functional sense, central sensitization reflects an activity-dependent increase in synaptic gain, rendering the central nervous system hypersensitive to sensory inputs such that sensations and reflexes are amplified with respect to the amount of sensory neuron input that is received **(FIGURE 10)**. Altered coupling of sensory input to behavioral output results in innocuous stimuli evoking behaviors that are characteristic of noxious sensory processing. Upon establishment of central sensitization, the state of heightened sensitivity is often maintained long after the original tissue injury has resolved, and furthermore, the body region from which heightened sensations can be evoked expands to incorporate tissues outside of the primary injury zone. Although most commonly investigated at the level of the second-order neurons that are in direct receipt of primary afferent inputs, central sensitization can also occur at higher order processing sites within the sensory system networks of the brain and can manifest as an increase in excitatory drive and a withdrawal of inhibitory neurotransmission.

The mechanisms underpinning the enhanced central neuronal excitability have been investigated in most detail in the spinal dorsal horn. Repetitive activation of nociceptive C-fibers produces a long-lasting hyperexcitability of spinal integrative neurons (268). Indeed, pioneering experiments in the 1960s identified a peculiar feature of spinal integrative neurons receiving input from cutaneous nociceptive C-fibers in that repetitive, low-frequency stimulation of C-fibers (at a constant stimulus intensity) resulted in a progressive build up in the activity (or action potential discharge) of spinal integrative neurons receiving this input (306, 307). This observation was termed "windup," and it provided new insights into how the recruitment of C-fibers during inflammation could affect the excitability of a broad range of spinal sensory pathways. Indeed, it was recognized that the recruitment of normally quiescent C-fibers could lower the threshold for activation of spinal integrative neurons by subsequent input from other afferent sources, such as mechanoreceptors (268, 269, 456), in part because of an anatomical convergence of different afferent populations onto common integrative circuits in the spinal cord.

Subsequent investigations into the molecular mechanisms underlying the development of central sensitization have highlighted a very complex series of central inflammatory and adaptive changes that occur in response to persistent C-fiber inputs. Spinal integrative neurons express several types of receptors capable of evoking slow postsynaptic potentials. Most notably, these include tachykinin and metabotropic excitatory amino acid (EAA) receptors as well as the ionotropic NMDA glutamate receptor (49, 330, 425). In the absence of C-fiber input, these receptors are inactive, in part due to a lack of synaptic agonist and also due to the fact that at resting membrane potential NMDA channels are blocked by magnesium ions (even in the presence of glutamate). However, during persistent C-fiber input, neuronal depolarizations evoked by the activation of neurokinin and/or metabotropic EAA receptors are sufficient to initiate the removal of the voltage-dependent magnesium block of the NMDA receptor (330, 425). This is further facilitated by a rise in intracellular calcium (due to the release of calcium from internal stores) and the activation of calcium-dependent protein kinases which then phosphorylate the NMDA receptor channel and facilitate glutamate-evoked channel conductance (457). As the NMDA receptor is permeable to both sodium and calcium ions, NMDA receptor recruitment contributes to both the slow postsynaptic potential as well as the sustained rise in intra-

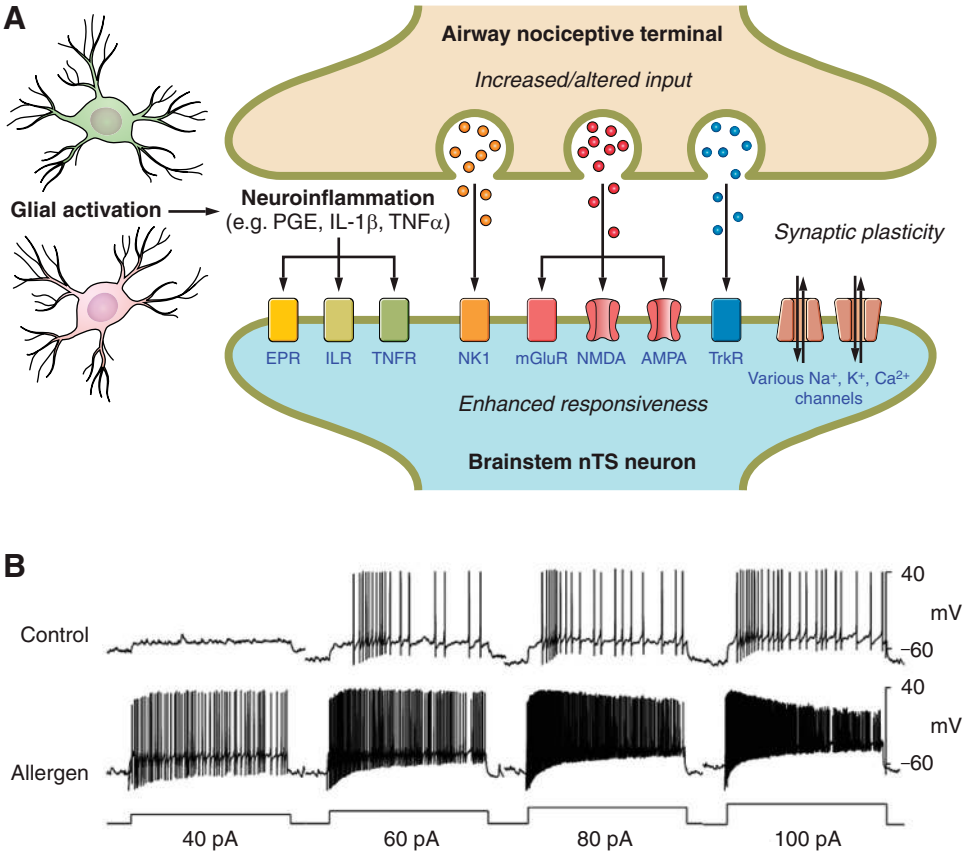

FIGURE 10. Mechanisms of central sensitization of brain stem neurons in receipt of airway afferent inputs. *Top*: schematic showing how aberrant sensory input to the central nervous system leads to a series of events that culminate in persistent hypersensitivity of the central neural circuits receiving sensory input. Sensory neurotransimitters substance P (SP) and glutamate (Glu) and neurotrophins (NTs) act postsynaptically to induce wind up and/or to lower the activation threshold of second-order brain stem neurons. In addition, activation of the brain's immune cells (glia) facilitates the upregulation of synaptic processes by orchestrating neuroinflammatory events. Collectively, these mechanisms drive synaptic plasticity, fundamental to generating heightened sensory nerve-dependent responses. *Bottom*: an example of central sensitization evoked by pulmonary antigen sensitization and challenge. Electrophysiological recordings of two neurons within the nucleus of the solitary tract (nTS) in vitro, one from a control animal and a second from an animal after prior in vivo allergic sensitization and challenge in monkeys. In the control cell, injection of increasing current (40–100 pA) produces stimulus-dependent action potential formation (spikes). Prior allergic inflammation in the airways lowers the current threshold needed for brain stem neuron action potential formation and dramatically increases the number of spikes per current step. PGE, prostaglandin E_2; IL-1b, interleukin-1β, TNFa, tumor necrosis factor-α; EPR, E prostanoid receptor; NK1, neurokinin 1 receptor; mGluR, metabotropic glutamate receptor; NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; TrkR, tropomyosin receptor kinase. [From Chen et al. (73), with permission from Elsevier.]

cellular calcium and therefore aids in the recruitment of additional permeable NMDA channels (425).

C-fiber-evoked increases in intracellular calcium and kinase-evoked phosphorylation of key membrane channels produce sensitization of spinal neurons lasting up to tens of minutes. The increase in intracellular calcium, however, also leads to the transcription of a variety of genes and the synthesis of new proteins, most notably several proinflammatory enzymes such as nitric oxide synthase (NOS) and cyclooxygenase-2 (COX2) (383). The products of NOS and COX2 activation (NO and prostanoids, particularly PGE_2) contribute to longer lasting sensitization of the spinal components of the pain pathway. Furthermore, PGE_2 may also evoke disinhibition of spinal neurons by reducing the effects of presynaptic inhibitory inputs regulating spinal neuron excitability. Central neural plasticity can be accompanied by glial cell influx, proliferation, and activation, and the resultant central inflammatory events further enhance neuronal hyperexcitability. Consistent with all of these observations, inhibition of neurokinin receptors, NMDA receptors, PKC, NOS, COX2, and/or glial cells (or glial cell-derived mediators) can prevent and/or reverse the central sensitizing effects of C-fibers.

Processes similar to central sensitization may play a role in heightened airway defensive reflexes, although the mechanisms contributing to this in the vagal afferent system have not

been studied in any great detail. Anatomical and functional studies suggest some degree of afferent convergence in the brain stem (220, 283). Thus vagal afferent C- and A-fiber activation may synaptically drive common neurons in the nucleus of the solitary tract, although as discussed above it has also been debated as to whether any common second-order neurons are in direct convergent inputs (298). It is clear, however, that substance P increases the excitability of nucleus of the solitary tract neurons, thereby facilitating lung afferent transmission in guinea pigs and rabbits (283, 324, 325). Consistent with this, the coincidental activation of bronchopulmonary chemically sensitive afferents lowers the activation threshold for reflexes evoked by pulmonary mechanoreceptor stimulants. Thus, in anesthetized guinea pigs, capsaicin or bradykinin delivery to the airways (to activate chemically sensitive afferents) enhances both coughing and airway parasympathetic outflow evoked by subsequent mechanoreceptor stimulation, and this occurs by facilitating synaptic transmission at brain stem relay neurons (283, 293). Pharmacological evidence suggests that neurokinins play a role in central facilitation, since the central synergistic interactions are prevented by neurokinin receptor antagonists administered intracerebroventricularly and the sensitizing effect of chemically sensitive afferent stimulation can be mimicked by administering substance P to the brain stem. However, this has to be reconciled with the observation that most neurokinin containing (jugular ganglia neurons) have central terminations in the paratrigeminal nucleus, whereas mechanically sensitive nodose neurons terminate in the nucleus of the solitary tract (302). It may be that a nonafferent source of neuropeptides is involved or that the minor population of peptidergic nodose neurons that shares overlapping central termination territories with mechanically sensitive afferent terminals in the nucleus of the solitary tract can provide the source of neurokinins for enhancing synaptic efficacy. In this sense it is also interesting to note that the availability of neurokinins for driving central sensitization likely increases with disease states (discussed above) as nodose neurons greatly upregulate their expression of substance P during pulmonary inflammation (65, 79, 250).

VIII. SUMMARY

Among the some 30,000 vagal afferent nerves in the cat vagus nerves, nearly 20% innervate the trachea and lungs (182). In addition to the greater number of fibers, the complexity of the vagal afferent innervation of the respiratory tract seems richer than in most other visceral organs. For example, subdiaphragmatic vagal afferent nerves are nearly uniformly unmyelinated, whereas in the respiratory tract ~10% of the vagal afferents are myelinated A-fibers. The myelinated fibers are low-threshold mechanosensitive stretch receptors of the RAR and SAR types (and likely subtypes therein) that provide the brain information on a second by second basis that pertains to the mechanical process of breathing, and then feeds back to inform the depth and breadth of respiration. These nerves can also subconsciously regulate an autonomic (mainly parasympathetic) nervous system that has powerful control over airway caliber as well as secretions and vascular tone.

As with the somatosensory system, the majority of afferent nerves innervating the respiratory tract would appear to be devoted to deciphering what is potentially harmful to the lungs. When activated, these nociceptors (mainly C-fibers and some Aδ-fibers) provide an alarm of sorts and initiate defensive reflexes including apnea, cough, bronchospasm, and secretions in an effort to rid the perceived danger. In airway disease this process can become dysregulated such that the response to the perceived danger is out of balance such that at times the proverbial squeak of a mouse evokes a lion's roar.

Impressive progress has been made over the past 50 years in our understanding of vagal afferent innervation of the respiratory tract. Yet, in most instances, our understanding remains vague and lacking in clarity. There is a need for more granular insights into the ionic mechanisms and morphological basis of mechanostransduction in low-threshold mechanosensing Aδ and Aβ fibers of the respiratory tract. Likewise, our understanding of the ionic basis of how the nociceptive stimuli activate C- and Aδ-fibers remains in relatively rudimentary stages. In addition, scientists are only scratching the surface of how the vagal inputs are received and integrated within the brain stem and then in some cases transmitted to higher cortical CNS centers leading to sensations such as dyspnea and urge to cough. Our knowledge is also incomplete when it comes to the cellular and transmitter mechanisms by which vagal afferent nerves regulate autonomic neuronal outflow, and influence the centers of respiration within the CNS.

Progressing knowledge in the areas listed above will continue to depend on thoughtful experimental models which traditionally have been conducted in a wide variety of species. One could therefore reasonably wonder which species is best? However, this seemingly simple question is remarkably difficult to answer. Highlighted throughout this review is the fact that different species offer different advantages depending on the endpoints of the investigation. For example, mice have a poorly developed cough reflex and are therefore not a particularly useful species for studying this as an endpoint. However, mouse sensory neurons themselves display relatively comparable neurophysiology to those in coughing species, such as guinea pigs, and therefore the mouse can be a very powerful model for studying sensory neuron activation in light of the vast array of transgenics available. Compounding the issue is that very little is known about vagal sensory processing in humans to allow for a valid comparison between animal and human systems. Thus, rather than looking for the perfect species to model human airway sensory neurobiology, it is important that scientists understand the limitations of their models when designing and interpreting their experiments.

We can eagerly anticipate future investigations into the vagal sensory innervation of the respiratory tract that should provide fundamental insights specifically related to lung function, but also to visceral sensations in general. A derangement of afferent neuronal control leads to symptoms of many respiratory diseases. Therefore, future investigations into vagal neuroscience should also provide for novel therapeutic targets and strategies aimed at reducing the suffering of those inflicted with airway-related pathology.

ACKNOWLEDGMENTS

S. B. Mazzone andc B. J. Undem contributed equally in the preparation of this manuscript.

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GRANTS

S. B. Mazzone holds National Health and Medical Research Council of Australia Grants 1042528, 1025589, and 1078943. B. J. Undem was supported by National Heart, Lung, and Blood Institute Grant HL112919.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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