Anatomy and Neurophysiology of the Cough Reflex

ACCP Evidence-Based Clinical Practice Guidelines

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Objectives: To describe the anatomy and neurophysiology of the cough reflex.

Methods: A review of the literature was carried out using PubMed and the ISI Web of Knowledge from 1951 to 2004. Most of the referenced studies were carried out in animals.

Conclusions: Studies carried out in animals provide suggestive but inconclusive evidence that C-fibers and rapidly adapting receptors (RARs) arising from the vagus nerves mediate coughing. Recent studies also have suggested that a vagal afferent nerve subtype that is not readily classified as a RAR or a C-fiber may play an important role in regulating cough. Afferent nerves innervating other viscera, as well as somatosensory nerves innervating the chest wall, diaphragm, and abdominal musculature also likely play a less essential but important accessory role in regulating cough. The responsiveness and morphology of the airway vagal afferent nerve subtypes and the extrapulmonary afferent nerves that regulate coughing are described.

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Key words: bronchospasm; capsaicin; C-fiber, convergence; rapidly adapting; vagal

Abbreviations: 5-HT = 5-hydroxytryptamine; ATP = adenosine triphosphate; GERD = gastroesophageal reflux disease; nTS = nucleus of the solitary tract; RAR = rapidly adapting receptor; SAR = slowly adapting stretch receptor; TRPV = transient receptor potential vanilloid

Coughing occurs as a consequence of aspiration, the inhalation of particulate matter, pathogens, accumulated secretions, postnasal drip, inflammation, and mediators associated with inflammation. The elderly, newborns, lung transplant recipients, and patients with paralysis or neuromuscular disorders have a poorly developed and/or compromised cough reflex, and are rendered highly susceptible to lung infections and aspiration pneumonia.1–4 Under normal conditions, therefore, cough serves an important protective role in the airways and lungs. In diseases such as asthma, COPD, gastroesophageal reflux disease (GERD), and upper airway cough syndrome due to a variety of rhinosinus conditions (previously referred to as postnasal drip syndrome), however, cough may become excessive and nonproductive, and is potentially harmful to the airway mucosa.4 These contrasting consequences of coughing can be attributed to the parallel afferent pathways regulating this important defensive reflex of the airways.

Our current understanding of the afferent neuronal pathways regulating the cough reflex is derived almost entirely from studies in animals. In these studies,5–10 conclusive evidence that vagal afferent nerves are responsible for initiating the cough reflex has been provided. The terminations of these vagal afferents are found in abundance in the airway mucosa and in the airway wall from the upper airways to the terminal bronchioles and lung parenchyma.

Afferent neuronal subtypes can be identified based on their physicochemical sensitivity, adaptation to sustained lung inflation, neurochemistry, origin, myelination, conduction velocity (A-fiber, > 3 m/s; C-fiber, < 2 m/s), and sites of termination in the airways (Fig 1). The utility of each of these
approaches for defining airway afferent nerve subtypes is limited in large part by the lack of specificity of the various characteristics studied.5–21 When evaluated in combination, however, these attributes can be used to identify at least three broad classes of airway afferent nerves (Fig 2, Table 1).

In this article, the classes of vagal afferent nerves innervating the airways and their role in regulating the cough reflex will be defined. The mechanisms by which extrapulmonary stimuli (eg, refluxate, upper airway irritation, or postnasal drip) initiate coughing will also be discussed. The information presented in this article was gathered by a literature search from 1951 to 2004 using both PubMed and the ISI Web of Knowledge.

Properties of Airway Afferent Nerve Subtypes and Their Potential Role in Regulating Cough

Rapidly Adapting Receptors

The anatomy of rapidly adapting receptor (RAR) terminations in the airway wall is unknown. Functional studies11–17 of RARs suggest that they terminate within or beneath the epithelium of both intrapulmonary and extrapulmonary airways, but primarily the intrapulmonary airways. RARs are differentiated from other airway afferents by their rapid adaptation (ie, 1 to 2 s) to sustained lung inflations (Fig 2).13–19 Other distinguishing properties of RARs include their sensitivity to lung collapse and/or lung deflation, their responsiveness to alterations in dynamic lung compliance (and thus their sensitivity to bronchospasm), and their conduction velocity (4 to 18 m/s), which is suggestive of myelinated axons.11–21

The sustained activation of RARs produced by dynamic lung inflation, bronchospasm, or lung collapse indicates that the adaptation of RARs is not attributable to an electrophysiologic adaptation.11,13,20,21 Perhaps RARs are thus better defined as dynamic receptors that respond to changes in airway mechanical properties (eg, diameter, length, and interstitial pressures).

RARs are sporadically active throughout the respiratory cycle (Fig 2), are activated by the dynamic mechanical forces accompanying lung inflation and deflation, and become more active as the rate and volume of lung inflation increase.13,20,21 It follows, therefore, that RAR activity during respiration correlates to respiratory rate, is higher in guinea pigs, rats, and newborns of all species (16 to 27 impulses per second), and is almost immeasurable in larger animals such as dogs (< 1 to 5 impulses per second). It also follows that, at least in smaller animals, RAR-dependent reflexes require a heightened activity in the already active RARs.

RARs are insensitive to many “direct” chemical stimuli (Fig 2). RARs are, however, activated by stimuli that evoke bronchospasm or obstruction resulting from mucus secretion or edema.11,14,15,19,22–28 Substances such as histamine, capsaicin, substance P, and bradykinin activate RARs in a way that can be markedly inhibited or abolished by preventing the local end-organ effects that these stimuli produce (eg, bronchospasm and mucus secretion). This sensitivity of RARs to bronchospasm becomes critical when interpreting the ability of stimuli such as capsaicin or bradykinin to evoke coughing in animals and in human subjects.

RAR activation initiates reflex bronchospasm and mucus secretion through parasympathetic pathways. RARs can also respond to stimuli that evoke cough and fulfill many criteria for mediating cough.5,8,14,17,29–31 Further evidence for their role in coughing comes from studies of vagal cooling, which blocks cough at temperatures that selectively abolish activity in myelinated fibers (including RARs) while preserving C-fiber activity.14,30,31 Surprisingly, how-

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Figure 1. Wholemounts of (left, A) rat trachea, (middle, B) guinea pig trachea, and (right, C) human bronchus stained immunohistochemically with antisera to the nonspecific neuronal marker protein gene product 9.5. A dense neuronal plexus is found beneath and within the airway epithelium of all species studied occupies this region of the airway mucosa. The afferent nerves regulating cough likely reside in this plexus. Provided by Dr. S.B. Mazzone (unpublished observations).
ever, many stimuli that are extremely effective at activating RARs (eg, thromboxane, leukotriene C4, histamine, tachykinins, methacholine, and inspiratory or expiratory efforts against a closed glottis) are ineffective or only modestly effective at evoking cough.14,28,32–35 Moreover, it is difficult to reconcile the observation that RARs are spontaneously activate throughout the respiratory cycle in many species, and yet that cough is only induced under special circumstances and in response to very specific stimuli. This indicates that if RARs are indeed responsible for regulating cough, their pattern of activation must be changed or a specific subset of RARs must be recruited in response to a stimulus that results in coughing. Alternatively, RARs may act synergistically with other afferent nerve subtypes to induce coughing.

**Slowly Adapting Stretch Receptors**

Slowly adapting stretch receptors (SARs) are highly sensitive to the mechanical forces that are put on the lung during breathing. SAR activity increases during inspiration and peaks just prior to the initiation of expiration (Fig 2).13,36 SARs are thus thought to be the afferent fibers involved in the Hering-Breuer reflex, which terminates inspiration and initiates expiration when the lungs are adequately inflated.36 SARs can be differentiated from RARs in some species based on action potential conduction velocity, and in most species by their lack of adaptation to sustained lung inflation. SARs may also be differentially distributed throughout the airways.36 In cats, guinea pigs, and rats, few if any SARs, but many RAR-like receptors and/or C-fibers, can be found in the extrapulmonary airways. Rather, SARs appear to terminate primarily in the intrapulmonary airways (in dogs, SARs may also be localized to the extrapulmonary airways). SARs also differ from RARs with respect to the reflexes they precipitate. SAR activation results in the central inhibition of respiration and the inhibition of the cholinergic drive...
to the airways, leading to decreased phrenic nerve activity and decreased airway smooth muscle tone (due to a withdrawal of cholinergic nerve activity).25,36,37

Yu and colleagues38 have morphologically defined the structure of electrophysiologically identified SARs innervating rabbit intrapulmonary airways and lungs. The sensory terminals of SARs assume a complex and varying position within the airway wall. Most of these SARs were found in the peripheral airways (associated with alveoli or bronchioles). Occasionally, but not uniformly, SAR dendritic arbors were associated with the bronchiolar smooth muscle. This contrasts with the SARs innervating the dog trachea, which are intimately associated with the smooth muscle and are activated during bronchospasm.39 As mentioned above, however, cats, guinea pigs, rabbits, and rats appear to have few if any SARs in their extrapulmonary airways.36

Single-unit recordings from the vagus nerve in rabbits have suggested that SAR activity does not increase prior to or during ammonia-induced coughing.9 Although this suggests that SARs are unlikely to play a primary role in the cough reflex, their profound influence over the respiratory pattern makes it likely that they influence coughing and other airway defensive reflexes. It has been proposed, for example, that enhancing baseline SAR activity with the loop diuretic furosemide may account for the reported antitussive effects of this agent in animals and in human subjects.40 In contrast, preloading, which would likely increase baseline SAR activity, has been reported to increase expiratory efforts during cough.41,42 Experiments performed on rabbits8,43 in which inhaled SO2 has been used in an attempt to selectively block SAR activity show that the cough reflex is coincidentally attenuated. However, the selectivity of SO2 for airway SARs is questionable because several reports44,45 have indicated an excitatory action of SO2 on airway C-fibers. C-fiber activation may be inhibitory to cough.

Studies6 of CNS processing have suggested that SARs may facilitate coughing. A central cough network in which SARs facilitate cough via activation of brainstem second-order neurons (termed pump cells) of the SAR reflex pathway has been proposed. In this model, SARs, through the activation of pump cells, open an as-yet-unidentified “gate” in the brainstem that is thought to promote cough. But an excitatory role of pump cells in cough is difficult to reconcile with studies25,46 showing that SARs (via pump cells) inhibit other RAR-mediated reflex pathways. Clearly, much about the role of SARs in coughing remains poorly defined.

<table>
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<th>Properties</th>
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<th>C-Fibers</th>
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*Typical attributes of the afferent nerve subtypes are listed. Species differences and subtypes of each class with distinct physiologic properties and responsiveness have been reported.
†The activation of RARs by capsaicin and bradykinin is prevented by bronchodilator pretreatment, suggesting that activation occurs secondary to obstruction in the lung.
‡C-fiber activation by bradykinin and capsaicin is enhanced by bronchodilators such as adrenaline, adenosine, and prostaglandin E, suggesting that agents directly stimulate C-fibers in the airways. See text for further details and references.
C-Fibers

The majority of afferent nerves innervating the airways and lungs are unmyelinated C-fibers. They are similar in many ways to the unmyelinated somatic sensory nerves innervating the skin, skeletal muscle, joints, and bones that respond to noxious chemical and mechanical stimuli (called nociceptors). In addition to their conduction velocity (< 2 m/s), airway vagal afferent C-fibers are distinguished from RARs and SARs by their relative insensitivity to mechanical stimulation and lung inflation (Fig 2). C-fibers are further distinguished from RARs by the observation that they are directly activated by bradykinin and capsaicin, not indirectly through effects on smooth muscle or the airway vasculature. Moreover, prostaglandin E2, adrenaline, and adenosine, which by bronchodilating the airways might inhibit RAR activation by bradykinin and capsaicin, actually sensitize C-fibers to capsaicin and bradykinin through direct effects on their peripheral nerve terminals.

Morphologic studies in rats (Fig 3) and in guinea pigs have revealed that afferent C-fibers innervate the airway epithelium as well as other effector structures within the airway wall. C-fibers may synthesize neuropeptides that are subsequently transported to their central and peripheral nerve terminals. This unique neurochemical property of bronchopulmonary C-fibers has been exploited to describe the distribution and peripheral nerve terminals of these unmyelinated airway afferent nerve endings. Although the expression of neuropeptides may be species dependent, C-fibers innervating the airways of other species are likely to be morphologically (if not neurochemically) similar to those that have been well characterized in guinea pigs and rats.

In dogs, airway afferent C-fibers may be further subdivided into bronchial and pulmonary C-fibers, a distinction that is based both on sites of termination but also on responsiveness to chemical and mechanical stimuli. Notably, pulmonary C-fibers in dogs may be unresponsive to histamine, while bronchial C-fibers are activated by histamine. Whether similar physiologic distinctions between bronchial and pulmonary afferent C-fibers can be defined in other species, and what if any differences in reflexes are produced on their activation, is unknown. Kollarik et al and Undem et al have described C-fiber subtypes innervating the intrapulmonary airways and lungs of mice and guinea pigs. In guinea pigs, C-fiber subtypes may be distinguished based on their ganglionic origin and on the sites of termination in the airways. C-fibers arising from the jugular (superior vagal) ganglia in guinea pigs innervate both intrapulmonary and extrapulmonary airways, and almost uniformly express the neuropeptides substance P and calcitonin gene-related peptide. These C-fibers

Figure 3. Tachykinin-containing C-fibers innervate the rat tracheal mucosa. Double-labeling immunohistochemistry with antisera for (left top, A) the nonspecific neuronal marker protein gene product 9.5 and (left bottom, B) substance P (SP) in wholemounts of rat trachea. Substance P-containing nerve fibers occupy a dense neuronal plexus beneath and within the airway epithelium. The majority of the nerves in this epithelial plexus in rats are C-fibers. (Middle, C) Retrograde neuronal tracing with fast blue indicates that the perikarya of the tracheal afferent nerves are located in the vagal sensory ganglia. Right, D: Many of the retrogradely labeled neurons stain for TRPV1, the capsaicin receptor. Provided by Dr. S.B. Mazzone (unpublished observations).
are not activated by adenosine triphosphate (ATP), 5-hydroxytryptamine (5-HT), or adenosine, but are readily activated by capsaicin, bradykinin, and acid. By contrast, C-fibers with cell bodies in the nodose (inferior vagal) ganglia terminate almost exclusively in the intrapulmonary airways. These C-fibers rarely express substance P, and are activated by capsaicin, bradykinin, ATP, adenosine, and 5-HT. In mice, ATP activates all C-fibers, whereas capsaicin and bradykinin activated only a subset of the identified bronchopulmonary C-fibers. Unlike the situation reported in dogs, histamine appears to be without effect on bronchopulmonary C-fibers in other species.

C-fibers regulate airway defensive reflexes. Although C-fiber endings are polymodal, responding to both chemical and mechanical stimulation, their threshold for mechanical activation is much higher than that of RARs and SARs.12,13,18,28 Accordingly, C-fibers are generally quiescent throughout the respiratory cycle but are activated by chemical stimuli such as capsaicin, bradykinin, citric acid, hypertonic saline solution, and SO2.12,13,16,18,23,45,47 Reflex responses evoked by C-fiber activation include increased airway parasympathetic nerve activity, and the chemoreflex, characterized by apnea (followed by rapid shallow breathing), bradycardia, and hypotension.18,25,47 In some species (particularly guinea pigs and rats) activated C-fibers release neuropeptides from their peripheral nerve terminals without involvement of the CNS. This process is called an axon reflex. The axon reflex in guinea pigs and rats results in bronchospasm, mucus secretion, and neurogenic inflammation.47,50

Several lines of evidence support the hypothesis that the activation of airway C-fibers precipitates cough. Stimulants (including several putatively selective stimulants) of C-fibers such as capsaicin, bradykinin, SO2, and citric acid evoke cough in conscious animals and in humans.7,18,60–63 Capsaicin desensitization abolishes citric acid-induced coughing in guinea pigs, but has no effect on cough evoked by mechanical probing of the airway mucosa in these same animals.7,60 Finally, pharmacologic studies that take advantage of the unique expression of tachykinins by airway C-fibers have shown that cough induced by bradykinin, citric acid, and capsaicin in cats and guinea pigs is attenuated or abolished by neurokinin receptor antagonists.62,63

Although the evidence summarized above supports a role for C-fibers in the cough reflex, there is also considerable evidence to indicate that airway C-fibers do not evoke cough but may inhibit cough evoked by RAR stimulation. In anesthetized animals, for example, C-fiber stimulation has consistently failed to evoke coughing, even though cough can be induced in these animals by mechanically probing the airway mucosa.5,10,28–31 The systemic administration of C-fiber stimulants can even inhibit cough evoked by RAR stimulation in various species.5,28–31

The fact that vagal cooling to temperatures that can preserve C-fiber-dependent reflexes yet abolish cough is further evidence against a role for C-fibers in cough.5,30,31

It is unclear why so much conflicting evidence about C-fibers in cough has been reported. Perhaps general anesthesia selectively disrupts the ability of C-fibers to evoke cough in animals without adversely affecting cough that is induced by mechanoreceptor stimulation. General anesthesia has a profound influence over the cough reflex.64 Alternatively, because coughing in anesthetized animals is typically studied following tracheotomy with stimuli delivered to lower airways, larynx, and trachea, perhaps C-fiber-dependent coughing is evoked from or requires airflow through the bypassed airways (eg, the pharynx). It is unlikely, however, that anesthesia prevents C-fiber activation and C-fiber-mediated reflex effects entirely. C-fibers are readily activated in anesthetized animals and can precipitate profound cardiopulmonary reflexes.18,23,25,28,65–68 Rather, anesthesia must selectively inhibit cough-related neural pathways or may act by accentuating the inhibitory effects of C-fiber activation on cough. Alternatively, general anesthesia may interfere with the conscious perception of airway irritation and the resulting urge to cough. In this context, it is interesting that capsaicin-evoked cough can be consciously suppressed in human subjects.69

Evidence for a “Cough Receptor”

The conflicting evidence that either C-fibers or RARs regulate coughing makes it reasonable to hypothesize that a previously unrecognized subtype of airway afferent nerve (ie, cough receptors) plays the primary role in regulating this defensive reflex. Subtypes of the recognized classes of airway vagal afferents have been described in several species,70–72 and recent studies in guinea pigs support the notion of a “cough receptor.” Selective nerve cuts and a comprehensive analysis of the stimuli that evoke coughing and afferent nerve activation in guinea pigs reveal that the tracheal, laryngeal, and bronchial afferents that are primarily responsible for regulating cough arise from the nodose ganglia.28 These afferent nerves are polymodal, being activated by punctuated mechanical stimuli, acid, water, and the potassium channel blocker 4-aminopyridine.12,73,74 However, they are unresponsive to capsaicin, bradykinin, or hypertonic saline solution, and do not express transient receptor potential vanilloid
(TRPV)-1, the capsaicin receptor. These putative cough receptors are myelinated and do not synthesize neuropeptides under normal conditions. Their myelination and insensitivity to capsaicin clearly differentiate the putative cough receptors from bronchopulmonary C-fibers.

Because they are myelinated and adapt rapidly to punctuate mechanical stimulation, it is tempting to conclude that the cough receptors are merely the RARs of the extrapulmonary airways. However, there are a number of attributes that differentiate the cough receptors from the classically defined RARs. Unlike RARs, which primarily innervate the intrapulmonary airways, the putative cough receptors are found primarily in the extrapulmonary airways (ie, larynx, trachea, and mainstem bronchi) where cough is most readily initiated. Also unlike RARs, the cough receptors are utterly unresponsive to a wide variety of spasmogens and autacoids that induce airway smooth muscle contraction, including methacholine, histamine, leukotriene C4, substance P, neurokinin A, 5-HT, ATP, and adenosine. All of these stimuli have been shown to activate RARs, and yet none of them are effective, or are only modestly effective, at inducing cough. Maximal inspiratory or expiratory efforts against a closed glottis, like bronchospasm, are also very effective at evoking cough in healthy individuals. Cough receptors are unresponsive to changes in luminal pressure, even pressures changes exceeding –100 to +100 cm H2O. Finally, the cough receptors may also be distinguished from RARs based on conduction velocity. Laryngeal, tracheal, and bronchial cough receptors in guinea pigs conduct action potentials at approximately 5 m/s, whereas intrapulmonary RARs in guinea pigs conduct action potentials at a much faster rate (upward of 15 m/s).

Using the styryl dye FM2–10, the receptive fields of putative cough receptors innervating the guinea pig tracheal and bronchial mucosa have been identified (Fig 4). Their terminals are found in abundance and in a stereotypical position of the airway mucosa, with complex dendritic arbors that are invariably arranged along the circumferential axis of the airways. Terminations are confined to the space between the smooth muscle and the epithelial cell layers. As might be expected, then, neither smooth muscle contraction nor epithelial removal activates or alters the excitability of these receptors and does not inhibit cough that is evoked electrically or by citric acid. The left and right vagus nerves contribute approximately equal numbers of these receptors, with terminals located primarily ipsilateral to their vagal origin. Comparable structures have been identified in the airways of fixed tissue using the osmium tetroxide-staining method. The terminal adhesions of the cough receptors and their orientation within the tissue indicate that they have attached to components in the extracellular matrix comprising the basement membrane, which undergoes extensive remodeling in disease conditions.

FM2–10 labeling of the putative cough receptors has facilitated the identification of a key regulatory mechanism for the excitability of these afferent nerves. Labeling with FM2–10 is absolutely dependent on Na+/K+/ATPase activity. Subsequent immunohistochemical analyses revealed that the putative cough receptors, but not C-fibers, express an isoform of the sodium pump containing the α3 subunit. The unique expression of this isoform is of interest, given its association with mechanoreceptors and not with the C-fibers in the somatic nervous system. The intense and brilliant labeling of the cough receptors with FM2–10 suggests that sodium pump activity is particularly high in the putative cough receptor endings, which may play an essential role in regulating their excitability. Indeed, ouabain potently and selectively inhibited coughing evoked by citric acid, mechanical stimulation, or electrical stimulation of the tracheal and laryngeal mucosa in anesthetized guinea pigs while having no effect on C-fiber-dependent reflexes evoked from the trachea. In vitro electrophysiologic recordings confirm the potent and selective inhibitory effects of ouabain on the putative cough receptor. This inhibitory effect of ouabain on the cough receptors contrasts with the ability of this compound to greatly enhance the excitability of SARs in the lung, aortic baroreceptors, and renal arterial mechanoreceptors.

**INTERACTIONS BETWEEN AFFERENT NERVES SUBTYPES EVOKING COUGH**

**Peripheral Interactions**

Activated C-fibers can release the neuropeptides substance P, neurokinin A, and calcitonin gene-related peptide from their peripheral nerve terminals without involvement of the CNS, and even without action potential formation. This process is called the axon reflex, and has been well-described in human skin and in the viscera of many species. Axon reflexes in the airways and lungs of guinea pigs result in bronchospasm, mucus secretion, vasodilatation, edema, leukocyte recruitment, altered parasympathetic nerve activity, and the stimulation of endothelial and epithelial cells. The neuropeptide-evoked RAR activation occurs indirectly, secondary to actions on structural cells in the airway wall that in turn activate...
RARs. As might be predicted, then, preventing the consequences of the axon reflex with \(\beta\)-agonists, inhaled neurokinin receptor antagonists, or inhaled neutral endopeptidase (which enzymatically inactivates tachykinins and bradykinin) is effective at preventing RAR activation and cough evoked by capsaicin, cigarette smoke, bronchospasm, or the neutral endopeptidase inhibitor phosphoramidon. The receptive fields of the cough receptors assume a characteristic position in the mucosa, between the smooth muscle and the epithelium (note the intact epithelium in the living wholemount in the bottom left corner of the photomicrograph). Approximately four to six receptive fields per cartilage ring are labeled using the styryl dye (200 to 300 receptors over the entire trachea and mainstem bronchi of guinea pigs). Comparable staining of the rat airways reveals that this rodent species has few if any cough receptors (<10 in the entire trachea) in the tracheal or bronchial mucosa. Not surprisingly, then, neither electrical (\(n = 5\)) nor mechanical (\(n = 5\)) stimulation of the tracheal or laryngeal mucosa of rats evokes coughing, while both of these stimuli readily initiate coughing in guinea pigs. See the text for further details.

It is unclear whether axon reflexes or any peripheral interactions between C-fibers and RARs play a role in defensive reflex responses in the airways of humans or in any species other than guinea pigs. In cats and dogs, bradykinin and capsaicin evoke bronchospasm, bronchial vasodilatation, and mucus secretion, but these responses can be prevented with the administration of atropine or with vagotomy, indicating a CNS-dependent parasympathetic reflex, not an axon reflex. Similar findings have been reported in humans. Morphologic and functional studies also have indicated that a neuropeptide-dependent axon reflex is unlikely to play a major role in humans (young or adult), as few substance P-containing nerve fibers are found in...
Mechanoreceptors and capsaicin-sensitive C-fibers likely play an important role in regulating cough. C-fibers might initiate cough secondary to the axon reflex-dependent activation of RARs. C-fibers might also mediate cough by acting synergistically with RARs or other afferent nerves (eg, cough receptors) at the level of the brainstem. C-fiber-mediated cough, but apparently not cough mediated by RAR activation, is highly sensitive to general anesthesia. Indeed, C-fiber activation under general anesthesia is inhibitory to cough reflexes initiated by RAR activation. Dashed lines indicate the potential pathways and mechanisms regulating cough for which supporting or opposing evidence is limited. Likely sites of action for antitussive agents are indicated in red. Excitatory pathways regulating cough are highlighted in green. Inhibitory pathways regulating cough are highlighted in red. See text for further details.29 Bo¨ tzinger complex/pre-Bo¨ tzinger complex/rostral ventral respiratory group; C1-C4 = cervical spinal cord; CB = cannabinoid; E = expiratory-related; GABA = γ-aminobutyric acid; I = inspiratory-related; MN = motorneuron; PDE = phosphodiesterase (eg, theophylline or rolipram).
human airways, and electrically or capsaicin-induced responses of human airway preparations in vitro are not dependent on tachykinins. C-fibers may, however, release other transmitters such as purines (eg, adenosine or ATP) during an axon reflex, and these transmitters may initiate responses in human airways that are similar to the effects of neuropeptides that have been reported in guinea pig and rat airways.101

The lack of an axon reflex in the airways of humans (and other species) notwithstanding, there is good evidence that C-fiber activation is extremely effective at evoking cough. This indicates that other mechanisms may underlie C-fiber-dependent coughing. Thus, peripheral interactions between C-fibers and RARs may proceed independently of axon reflexes (Fig 5). C-fiber activation evokes CNS-dependent parasympathetic reflex-induced bronchospasm, vasodilatation, and mucus secretion.18,25,47,65–67,95–97 These end-organ effects are mediated in large part by acetylcholine released from airway parasympathetic nerves and may be sufficient to activate RARs in the airway wall. That inhaled anticholinergic agents have some antitussive properties in animals and in human subjects is consistent with this notion.102,103

Central Interactions

The central integration of airway afferent nerve input is poorly understood. Insights into how C-fibers and RARs might interact in the brainstem may be gained from studies in other systems, particularly the nervous pathways regulating somatic tissues such as the skin, skeletal muscles, joints, and bones. C-fibers and mechanoreceptors arising from somatic tissues interact in the spinal cord in a process known as central sensitization.48,104 The consequence of this central interaction manifests as a heightened reflex responsiveness and exaggerated sensations of pain following cutaneous stimulation. Studies of central sensitization in the spinal cord have revealed two features of the somatosensory system that facilitate this hyperreflexia. First, C-fiber and mechanoreceptor reflex pathways appear to converge through common integrative circuits in the spinal cord. Second, this convergent input can amplify afferent signaling following the coincident activation of both afferent nerve subtypes. The synergy and resulting hyperreflexia is often dependent on tachykinins released from the central terminals of somatosensory C-fibers, producing a long-lasting hyperexcitability of spinal integrative neurons.49,104

Several lines of evidence have suggested that a process similar to central sensitization regulates airway defensive reflexes. The morphologic, electrophysiologic, and pharmacologic properties of airway C-fibers and mechanoreceptors are similar to those of the somatic nervous system.12,13,18,47,48,104 Anatomic and functional studies105–107 have also demonstrated the convergence of vagal afferents at sites of brainstem integration, particularly in the nucleus of the solitary tract (nTS). As mentioned above, lung mechanoreceptors are sporadically active throughout the respiratory cycle, whereas C-fibers are typically quiescent, even during large lung inflations.13–18 The central processing of C-fiber activity must therefore be integrated into a reflex pathway that is continually receiving input from airway mechanoreceptors. C-fiber activation, via central interactions with RARs, may promote coughing by facilitating synaptic transmission at RAR relay neurons in the brainstem (Fig 5). Indeed, substance P can facilitate synaptic transmission between lung afferents and nTS neurons in guinea pigs.106,109

Direct evidence for central interactions between C-fibers and RARs in the regulation of airway parasympathetic tone has been documented.107 Airway C-fiber activation evokes profound increases in cholinergic tone in the airways by facilitating airway mechanoreceptor actions in the brainstem. Without airway mechanoreceptor activity, C-fibers are ineffective at evoking reflex responses. The facilitating effects of C-fibers on the brainstem RAR reflex pathways appear to be mediated by tachykinins, as the central synergistic interactions are prevented entirely by neurokinin receptor antagonists administered intracerebroventricularly. The sensitizing effect of nociceptor stimulation can also be mimicked, in the absence of C-fiber stimulation, by administering substance P to the brainstem.107 Importantly, a comparable interaction between cough receptors and C-fibers has been documented in studies of cough. Thus, in anesthetized guinea pigs, C-fiber activation does not evoke cough but greatly sensitizes the cough reflex evoked by activating the cough receptors. Neurokinin receptor antagonists prevent this sensitizing effect of nociceptor stimulation.110 Similar sensitizing effects of nociceptor stimulation on cough may be evoked from the esophagus, and observation that may explain how GERD initiates and/or exacerbates coughing.111–113

Extrapulmonary Modulation of Cough

Cough Evoked From the Ear (Arnold Reflex)

Afferent nerves carried by the auricular branch of the vagus nerve (ie, the Arnold nerve) innervate the external auditory meatus.114 In a small subset of patients (<5%), several visceral reflexes, including cough, may be evoked by the mechanical stimulation of the ear. Tracing studies115 in cats have shown that afferent neurons carried by the Arnold nerve have their cell bodies in the superior vagal (jugular)
ganglia, and terminate in several areas throughout the brainstem, including the commissural sub-nTS. It is interesting that in guinea pigs the nociceptors that can modulate cough also have their cell bodies in the jugular ganglia and terminate centrally in the commissural subnucleus of the nTS.\textsuperscript{107,110} It seems likely that the cough initiated by the stimulation of the ear involves the integration of both ongoing airway vagal afferent nerve input with the additional afferent input arising from the ear.

**Cough Initiated From the Pharynx**

Pharyngeal stimulation initiates coughing in human subjects.\textsuperscript{116} It is possible but unproven that the pharyngeal afferent nerves regulating cough are vagal in origin, but pharyngeal afferents may also arise from the glossopharyngeal nerves or from nerve branches emanating from the trigeminal ganglion.\textsuperscript{117,118} The physiologic properties of the pharyngeal afferent nerves regulating cough are poorly defined, but they may be similar to the cough receptors innervating the larynx, trachea, and bronchi. Thus, mechanical stimulation, postnasal drip, and a water bolus placed into the pharynx evoke vigorous coughing in human subjects and in animals, while capsaicin may be unable to initiate coughing when applied selectively to the pharynx.\textsuperscript{117,119,120}

**Cough Initiated From the Esophagus**

There have been conflicting reports\textsuperscript{111,112} regarding the ability of esophageal afferent nerve activation to initiate coughing. In animals, neither distension nor luminal challenge of the esophagus with acid or capsaicin evokes coughing.\textsuperscript{121} Comparably negative studies have been reported in human subjects.\textsuperscript{111} Nevertheless, the association between chronic cough and GERD is quite clear, and the ability to markedly reduce coughing in subjects with GERD with effective treatment of the reflux disease argues strongly for the hypothesis that reflux initiates coughing in susceptible patients.\textsuperscript{4,113} A simple explanation for these results is that refluxate is aspirated into the airways and directly activates the airway vagal afferent nerves regulating cough. However, pH monitoring in GERD patients has provided little evidence\textsuperscript{122} that refluxate can or needs to reach the proximal esophagus, much less the airways, to initiate pulmonary symptoms or coughing. Rather, it is well-established that airway reflexes (i.e., mucus secretion, bronchospasm, and cough) can be initiated from the stomach or the esophagus.\textsuperscript{123,124} These reflexes are effectively reduced or abolished by vagotomy. Although untested directly and thus unproven, these results may indicate that esophageal and airway afferent nerves converge centrally to regulate and initiate parallel and overlapping reflex effects in the airways and GI tract.

Studies in GERD patients have provided evidence that refluxate may also sensitize the cough reflex. Acid challenges of the esophagus\textsuperscript{111,112} markedly reduced the concentration of capsaicin required to evoke cough or resulted directly in coughing. Ing et al\textsuperscript{112} reported that this sensitizing effect of the esophageal challenge was prevented by the administration of inhaled atropine, suggesting that a CNS-dependent reflex initiated from the esophagus resulted in bronchospasm and/or mucus secretion in the airways that initiated coughing. Medical treatment of GERD has also been reported\textsuperscript{113} to greatly reduce the sensitivity to tussive stimuli. This sensitizing effect of refluxate or experimental acid challenges likely depends on CNS integration and amplification of the parallel vagal sensory inputs controlling cough.\textsuperscript{123}

**The Role of Consciousness in Cough**

Coughing, like swallowing, belching, urinating, and defeating, is unique in that there is higher cortical control of this visceral reflex.\textsuperscript{125} The cortical control of coughing can manifest both as inhibition (e.g., coughing that is suppressed until a break in a live performance or activity) but also as voluntary cough.\textsuperscript{125,126} The implications of this conscious control of cough are severalfold. It is clear, for example, that placebos can have a profound effect on coughing that has been studied experimentally.\textsuperscript{126} As such, any study of a novel or existing cough treatment should include a blinded (preferably double-blinded) placebo arm. It is also clear, however, that cough may be an affective behavior, perhaps indicating that an underlying physiologic disease (e.g., GERD or asthma) is poorly controlled or that a psychological issue, which is more difficult to detect, needs to be considered (called psychogenic cough).\textsuperscript{127} The motor pathways controlling voluntary and induced cough are identical, so it is not possible to distinguish physiologically a cough that is voluntary from a cough that is a consequence of some visceral disease. The cortical pathways controlling cough are also not well-understood. Addressing the psychological impact of coughing on a patient’s sense of well-being should therefore be a component of any treatment regimen for chronic cough, whether or not an underlying cause has been identified.\textsuperscript{125}

**Concluding Remarks**

Studies carried out in animals have provided clear evidence that vagal afferent nerves regulate coughing. It remains unclear, however, what relative role
the identified afferent nerve subtypes play in mediating cough. Evidence both for and against the role of C-fibers and RARs has been reported. These conflicting data suggest that the activation of both afferent nerve subtypes may be required to induce coughing, or that a previously unrecognized airway afferent nerve subtype subserves a primary role in cough. Recent studies in guinea pigs and circumstantial evidence gathered from the existing literature suggest that a cough receptor quite distinct from either C-fibers or RARs may exist. Moreover, these afferent nerve subtypes may interact to produce cough and heightened sensitivity to tussive stimuli. It seems imperative that future studies should identify the mechanisms of integration of afferent nerve input in the CNS, the role of consciousness and perception in coughing, and the mechanisms by which afferent nerves are activated.

Evidence for the extensive remodeling of airway afferent innervation in diseases associated with cough has been conflicting. Perhaps chronic coughing occurs not because of a hyperinnervation of the airways but because there are more triggers for coughing in the airways or in other organs associated with coughing. Consistent with this notion, the levels of inflammatory mediators are elevated in the lavage fluid of asthmatic and nonasthmatic chronic coughers, and therapeutic agents that target the identified triggers of coughing or inflammation have proven to be effective in treating cough in several patient populations. Future therapies may also target the mechanisms by which afferent nerve excitability is regulated, perhaps by blocking the ion channels associated with nerve activation (eg, TRPV1) or perhaps other enzymes, ion channels, or ion pumps that regulate the repetitive, high-frequency action potential formation that is required to initiate coughing. But it is unknown whether any meaningful changes in afferent nerve excitability or ion channel expression occur in patients with chronic cough. Other rational therapeutic strategies may target the interactions between the afferent nerves regulating cough at the level of the CNS (Fig 5). These central synapses may change with inflammation, with new transmitters being selectively expressed in and released from the afferent nerve terminals regulating cough. This selective expression in disease and in subsets of afferent nerves may limit the unwanted side effects associated with CNS-penetrant therapeutic agents.

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