

Inflammation-Induced Plasticity of the Afferent Innervation of the Airways

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The activation of primary afferent neurons that innervate the airways leads to homeostatic and defensive reflexes. The anatomic and physiologic characteristics of these afferent fibers do not appear to be static properties but rather appear to change rapidly in response to inflammation. The threshold for activation of airway afferent neurons to various stimuli, for example, is not fixed; these fibers can become sensitized during inflammation. A subset of nociceptive-like (C-fibers) airway afferent neurons not only participates in centrally mediated reflexes but is also thought to release neuropeptides at their peripheral terminals, leading to neurogenic inflammation. An increase in the content of tachykinins is commonly seen in inflamed tissues, and there is accumulating evidence that irritation and inflammation of the airways is associated with the induction of tachykinin synthesis in non-nociceptive airway afferent fibers that under normal conditions do not contain neuropeptides. The release of neurokinins from the peripheral terminals in the airways and their central terminals in the brain stem may contribute to the symptoms of inflammatory airway diseases. Elevated release of neurokinins from peripheral terminals may promote local inflammatory responses, and the release of neurokinins in the brainstem, together with inflammation-induced increases in the excitability of afferent fibers, may culminate in altered visceral autonomic reflex activity, changes in breathing pattern, and cough. *Key words:* airway inflammation, airway innervation, nerve growth factor, substance P, tachykinins, vagal afferent. — *Environ Health Perspect* 109(suppl 4):567–571 (2001).

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A variety of stimuli including chemicals, extremes in osmolarity, elevated H⁺ ion concentrations as well as mechanical stimuli are sensed by afferent neurons that innervate the airways. These stimuli evoke action potentials at the peripheral terminals of afferent neurons that are conducted to the central nervous system, therein causing release of neurotransmitters that excite secondary neurons in the brain stem or spinal cord, leading to homeostatic and defensive reflexes. Reflexes initiated in the airways include sneezing, coughing, changes in autonomic drive, and alterations in the depth and pattern of breathing.

In a number of tissues and organs, the various anatomic and physiologic characteristics of afferent fibers do not appear to be static properties, but rather appear to change rapidly in response to inflammation (1–5). In this overview, literature relevant to inflammation-induced anatomic and physiologic plasticity of airway primary afferent neurons is discussed.

Anatomy and Physiology of Airway Afferent Nerves

There are several subtypes of afferent fibers in the airways (6–8), and it is difficult to devise an unambiguous classification scheme that takes all features of these fibers into account (9). One scheme classifies afferent fibers according to the location of their cell bodies. For example, the nasal mucosa is innervated by sensory neurons whose cell bodies reside in the trigeminal ganglion (10,11), whereas those that innervate the trachea and bronchus have cell bodies residing in inferior (nodose) or

superior (jugular) vagal ganglia (12). Although some afferent neurons that innervate the lung have cell bodies that reside in dorsal root ganglia (12,13) few studies have concentrated on the spinal innervation of the airways.

Afferent fibers innervating the airways may also be broadly classified into two physiologic types: those that monitor normal physiologic activity and those activated by potentially damaging noxious stimuli. Afferent fibers that can be activated by mechanical forces occurring during normal respiration include slowly adapting stretch receptors (SARs) and rapidly adapting receptors (RARs). Activation of SARs leads to inhibition of inspiration, relaxation of airway smooth muscle, peripheral vasodilation, and tachycardia (7,8). Activation of RARs leads to increased inspiratory effort, contraction of airway smooth muscle, and secretion by airway submucosal glands (7,8). Afferent fibers activated by noxious stimuli and by mediators associated with tissue damage and inflammation include some thinly myelinated A δ -fibers and unmyelinated C fibers. Reflexes resulting from activation of these fibers include inhibition of inspiration and expiration (causing an initial apnea followed by rapid shallow breathing), bronchoconstriction, an increase in bronchial blood flow, and bradycardia (8).

In the guinea pig, airway afferent fibers whose cell bodies reside in the nodose ganglia appear to correspond to myelinated fibers that monitor normal physiologic activity. They are exquisitely sensitive to mechanical stimuli but do not respond to a variety of

chemical stimuli, including capsaicin and bradykinin (Figure 1) (14). By contrast, the jugular ganglia contain the cell bodies of myelinated A δ fibers and unmyelinated C fibers. Jugular afferent fibers innervating guinea pig airways resemble the nociceptive fibers of the somatosensory system in that they have relatively high thresholds to mechanical stimuli, but respond vigorously to classic nociceptive fiber-selective stimuli such as capsaicin and bradykinin (Figure 1) (14).

A subset of airway afferent neurons not only participate in centrally mediated reflexes, but are also thought to release neuropeptides at their peripheral terminals. The locally released peptides can activate neurokinin (NK) receptors in a variety of airway tissue and cell types, leading to alterations in bronchomotor tone, mucus secretion, and neurogenic inflammation. The predominant neuropeptides found in this subset of airway afferent fibers are calcitonin gene-related peptide (CGRP) and the tachykinins substance P and NK-A (15). Under normal conditions the tachykinergic innervation to the airways is thought to be derived exclusively from neurons that project nociceptive-like C fibers. In the guinea pig and rat airways, the cell bodies of the vagal afferent fibers that contain tachykinins are C fibers whose cell bodies reside in the jugular ganglia (12,14,16,17).

Inflammation and the Excitability of Afferent Neurons

The threshold of primary afferent neurons to various stimuli is not fixed, but rather can become sensitized during inflammation (4). Perhaps the most dramatic examples of this sensitization occur when an inflammatory mediator fails to overtly stimulate action potentials, but influences the nerve ending in such a way that the nerve responds to stimuli that were previously subthreshold. This is known to occur following experimental

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arthritis in rats, where afferent neurons that normally fail to respond even to noxious rotation of a rat joint respond to simple flexion following arthritis (18,19). Likewise, afferent fibers insensitive to mechanical distension of the bladder respond to modest distension following inflammation (20). Allergic rhinitis is associated with increased nasal neural responsiveness to stimuli that evoke sneezing, the nasosensory reflex, and axon reflex-mediated plasma extravasation (Figure 2) (21). In the human upper airway sub-threshold concentrations of a sensory nerve irritant reach threshold and evoke autonomic and sneezing reflexes when applied during the time of allergic inflammation (22).

Increases in afferent nerve excitability may contribute to the responsiveness of the airway

to various bronchoconstrictor stimuli. Airway hyperresponsiveness is a key feature of asthma and is defined as that phenomenon reflected in a substantial increase in the resistance to airflow in response to a bronchoconstrictive stimulant at doses that have little or no effect in the nonasthmatic population. Many mediators found in the inflamed airways of asthmatics may modulate the sensitivity of afferent neurons. This has led to speculation that analogies may be drawn between asthma-associated airway hyperresponsiveness and hyperalgesia (heightened sensitivity to painful stimuli) that often accompanies inflammation in the somatosensory system (23). Most stimuli administered to assess airway hyperresponsiveness, including histamine (24,25), capsaicin (26), bradykinin (27), distilled water (28), and

sulfur dioxide (29), cause bronchoconstriction by stimulating primary afferent nerve fibers and initiating central parasympathetic cholinergic reflexes. Experiments in animal models suggest that even the response to a so-called direct smooth muscle spasmogen like methacholine is likely to be mediated in part by a centrally mediated cholinergic reflex (30). It follows, therefore, that an increase in the excitability of the afferent endings associated with inflammation in the airway wall will lead to a heightened reflex bronchoconstriction at a given dose of stimulant and thereby contribute to airway hyperreactivity.

Studies of respiratory viral infections support the hypothesis that the afferent innervation of the lung contributes to certain types of hyperresponsiveness. Respiratory tract viral infections are often associated with exacerbations of asthma and the induction of bronchial hyperresponsiveness in otherwise healthy subjects (31–35). The reasons for this association are yet to be resolved and appear to involve multiple mechanisms (36) including increases in the excitability of airway afferent neurons. Empey and co-workers (35) reported that respiratory viral infections cause subjects to develop airway hyperresponsiveness to inhaled histamine. The observation that muscarinic cholinergic antagonist atropine prevented this response suggests the exaggerated bronchoconstriction was due to an increase in the excitability of afferent neurons that innervate the airways, leading to enhanced parasympathetic, cholinergic reflex-mediated bronchoconstriction. This is supported by their observation that the threshold concentration of citric acid that produced cough in subjects with viral infections was significantly lower than in control subjects or in subjects after recovery from infection. These observations suggest that respiratory tract viral infections can sensitize the airway afferent fibers that initiate reflex bronchoconstriction and cough.

The mechanisms underlying increases in excitability of nerve endings at the sites of airway inflammation are not known. In an *in vitro* study on airway nerve endings, the mechanical sensitivity of A δ -fibers innervating guinea pig isolated trachea from immunologically sensitized animals increased following exposure to the relevant antigen (37). In this model, exposure of the trachea/bronchus to the relevant antigen results in inflammatory mediator release, but does not overtly activate the afferent nerve endings. Rather, antigen challenge increases the excitability of the nerve endings, resulting in a 4-fold decrease in the amount of mechanical force required to activate the nerve fibers (37). In general terms the data available on this subject are compatible with the hypothesis that various

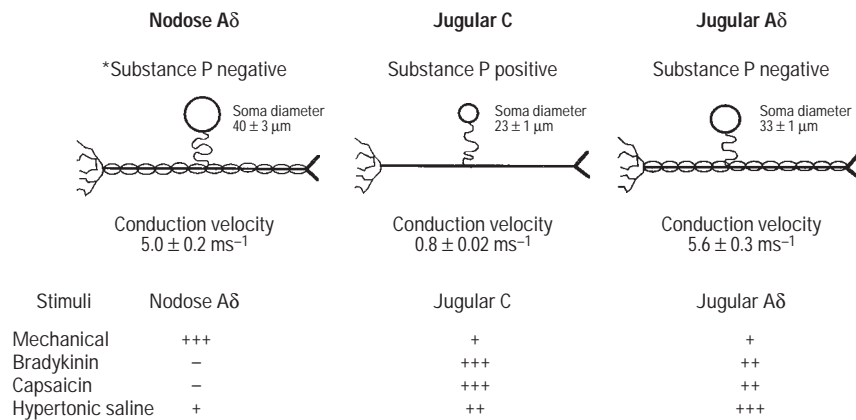


Figure 1. In the guinea pig, airways afferent fibers whose cell bodies reside in the nodose ganglia have large cell bodies, are myelinated, conduct action potentials in the A δ range and are exquisitely sensitive to mechanical stimuli but do not respond to a variety of chemical stimuli including capsaicin and bradykinin. These fibers appear to correspond to those fibers that monitor normal activity during breathing. In contrast, the jugular ganglia contain cell bodies of myelinated A δ and unmyelinated C fibers. These jugular fibers resemble the nociceptive fibers of the somatosensory system in that they have relatively high thresholds to mechanical stimuli and respond to classic nociceptive fiber-selective stimuli such as capsaicin and bradykinin. *Under normal conditions the tachykinergic innervation to the guinea pig bronchus is derived almost exclusively from C fibers whose cell bodies reside in the jugular ganglia. However, following allergen-induced airway inflammation or intratracheal instillation of NGF, the cell bodies of myelinated A-fibers were also found to contain immunoreactive substance P. Thus it appears that after inflammation, non-nociceptive (nodose fibers) as well as nociceptive-like (jugular fibers) airway afferent fibers contain tachykinins. Based on data from Riccio et al. (74), Hunter et al. (16), and Fischer et al. (17).

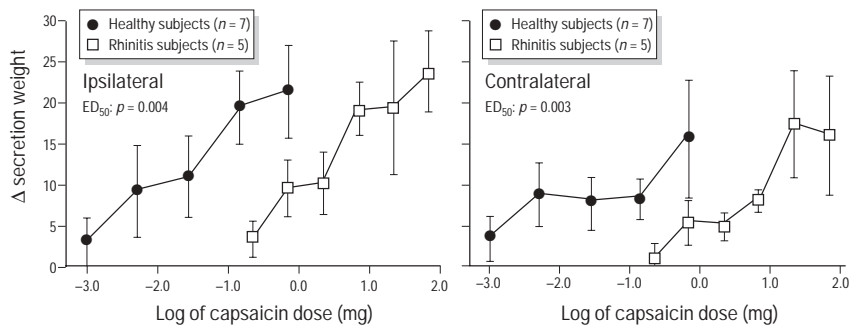


Figure 2. Central reflex induced by increasing doses of capsaicin applied, using filter paper discs, onto the right nasal septum. The changes between diluent- and capsaicin-induced secretions collected at the right (ipsilateral) and left (contralateral) septal mucosae are shown, depicting a 2-log greater responsiveness in the rhinitis group. The dose of capsaicin that induced at least 50% of the maximum response (ED_{50}) was significantly lower in subjects with allergic rhinitis compared with the healthy volunteers. Figure reprinted from Sanico et al. (27) with permission of S. Karger AG.

inflammatory mediators bind to receptors on the nerve endings and modulate current flow through various types of ion channels (38). Inflammation-induced changes in the viscoelastic properties of the airway wall may also contribute to increased excitability of afferent nerves (39).

Inflammation, Tachykinin Levels, and the Density of Afferent Innervation

An increase in the content of tachykinins is commonly seen in inflamed tissues (1,40–43). With respect to airways the baseline amount of substance P in human nasal lavage was greater in atopic subjects with grass pollen allergy than in healthy nonallergic subjects, and allergen challenge elevated these levels further (44). The concentration of substance P in the sputum of patients with chronic obstructive pulmonary disease or asthma was also greater than found in the sputum of healthy volunteers (45). Heaney and co-workers, however, found no immunoreactive substance P or CGRP in bronchoalveolar lavage fluid (BALF) of asthmatics or control subjects. However, they did find NK-A, and the levels of this tachykinin were similar in BALF from asthmatics and healthy subjects (46). Lilly and co-workers found significantly less substance P in lung tissue from asthmatics compared with nonasthmatics (47). One possible explanation for these conflicting data may be variable proteolytic breakdown of tachykinins within the airways. An alternative method of assessing the expression of tachykinins may be to quantitate the levels of tachykinin immunoreactivity or mRNAs encoding tachykinin precursor peptides in the cell bodies of afferent neurons. This approach has been useful in studies of rodents (see below).

The influence of inflammation on the density of the neuropeptide-containing innervation to the human airways is controversial. Nasal turbinate blood vessels from rhinitic children were more richly innervated with CGRP immunoreactive fibers than those from nonrhinitic children (48). The density of substance P immunoreactive nerves in the airway mucosa of asthmatics has also been reported to be increased (49), although this is not consistently observed (50,51). More recently, Chu and co-workers (52) also found that substance P was present in greater levels in the subepithelial airway mucosa of asthmatics compared with healthy control subjects; however, they did find a significantly greater level of immunoreactive substance P in the airway epithelium of asthmatics.

Induction of Tachykinin Synthesis in Afferent Neurons

Although nonneuronal sources of tachykinins may contribute to elevated levels of

NKs in inflamed airways [for example some inflammatory cells can synthesize tachykinins (53)], there is accumulating evidence that irritation and inflammation of the airways can induce the synthesis of tachykinins in afferent neurons.

An example of this induction was demonstrated following exposure of the rat nasal mucosa to toluene diisocyanate (TDI) (54), a chemical used in the production of polyurethane polymers, plastics, foams, and adhesives. Exposure to TDI is associated with nasal irritation, rhinitis, and occupational asthma, and there is some evidence from animal models that activation of afferent C fibers plays a role in these responses (55). Acute exposure to inhaled TDI is associated with increased levels of mRNA encoding tachykinin precursor peptides in cell bodies of afferent fibers that innervate nasal mucosa as well as an increased density of substance P immunoreactive nerve fibers in rat nasal epithelium (54). These data suggest that exposure of airway epithelium to inhaled irritants can lead to increases in neuropeptide gene transcription and translation within the neuronal cell bodies located in sensory ganglia.

Allergen-induced inflammation of the airways may also stimulate the neuronal synthesis of tachykinins in sensory ganglia. In sensitized guinea pigs, challenge of the nasal mucosa with the sensitizing allergen enhanced the synthesis of substance P and CGRP in the cell bodies of trigeminal ganglion neurons and the axonal transportation of these peptides to their terminals in the nasal mucosa (56). Similarly, within 24 hr of allergen inhalation, there is a 3- to 5-fold elevation in the amount of substance P, NK-A and CGRP immunoreactivity in the lungs of guinea pigs (17). Although decreased enzymatic breakdown of tachykinins within the airways may contribute to this elevation (57), at least a component of the increased content of tachykinins stems from an increase in tachykinin synthesis (17). A somewhat surprising observation was that, following allergen challenge, nodose neurons whose axons projected to the airways were shown to express tachykinins. This was surprising, as nodose fibers that innervate guinea pig airways do not normally contain immunoreactive tachykinins. In sensitized guinea pigs not challenged with antigen, only 1% of cell bodies of airways innervating nodose fibers were immunoreactive for substance P. In challenged guinea pigs, approximately 10% of cell bodies of nodose neurons retrogradely labeled from the airways were immunoreactive for substance P (17). Physiologic studies support the hypothesis that allergic inflammation causes a substantive qualitative change in the type of airway afferent fibers that contained tachykinins, such that non-nociceptive (capsaicin-insensitive,

low-threshold mechanosensors) as well as nociceptive airway afferent fibers contribute to the neuropeptide innervation (Figure 1) (58). The implications of this qualitative difference are discussed below in the section on enhanced release of tachykinins.

The mechanism by which airway exposure to allergens or inhaled irritants leads to increases in neuropeptide synthesis within cell bodies of remotely located ganglia is unknown. A family of molecules known to act on nerve endings to send growth signals to their cell bodies is the neurotrophins. Neurotrophins [e.g., nerve growth factor (NGF), brain derived neurotrophic factor, neurotrophin 3 and 4] support the survival, differentiation, and function of neurons of the central and peripheral nervous system (59). Inflammation of the airways is associated with increased levels of neurotrophic factors that may stimulate plasticity in afferent innervation. Potential cellular sources for this increase in airway neurotrophins include the respiratory epithelium, T lymphocytes, alveolar macrophages, and mast cells (60–62). Neurotrophins initiate their effects in vagal afferent neurons, in part, by binding to high-affinity receptors of the tyrosine kinase family, followed by uptake and retrograde transport to the cell body in the vagal ganglia (63). Among the family of neurotrophins most attention has been given to NGF as a potential mediator in afferent plasticity in inflamed airways. NGF is found in human airways, and the NGF content of airway lavage fluid is increased following allergen challenge (61,64–66). NGF is capable of regulating the expression of mRNAs encoding the precursors of substance P and CGRP in mature afferent neurons (67). Moreover, instillation of NGF into the trachea of guinea pigs was associated with a greater percentage of airway-specific afferent neurons that express immunoreactive substance P and also lead to a qualitative switch in the nature of the nodose afferent neurons (16), results similar to those following allergen challenge. That is, after NGF exposure, non-nociceptive-like (nodose fibers) as well as nociceptive-like (jugular fibers) airway afferent fibers contained tachykinins.

Enhanced Release of Tachykinins

The release of tachykinins from the peripheral terminals of C fibers in the airways is thought to occur via an axon reflex. An axon reflex occurs when stimulation of a C fiber ending results in action potentials which, in addition to traveling orthodromically toward the central nervous system, also travel antidromically via collateral branches to effect the release of tachykinins. The release of tachykinins from the peripheral terminals of afferent nerves can be induced by a variety of stimuli including

capsaicin (68), bradykinin (69), electrical nerve stimulation (70), cigarette smoke (71), low pH (72), and hypertonic saline (73). The release of tachykinins from the peripheral terminals of C fibers in the airways may result in increased blood flow through the microvascular bed of the airways, increased microvascular permeability, inflammatory cell recruitment, stimulation of airways secretions, and airway smooth muscle contraction (74).

There is some evidence to suggest inflammation of the airways can enhance the release of tachykinins from the peripheral terminals of vagal afferent neurons. In guinea pig isolated airway preparations, specific allergen, at a concentration that caused only a threshold level of mediator release, profoundly elevated tachykinergic nerve-mediated contraction of airways smooth muscle (70). This was independent of tachykinin synthesis (the cell bodies were removed); however, it was mimicked by histamine. Moreover, the histamine H₁ receptor-selective antagonist pyrilamine could prevent and reverse the antigen-induced potentiation. Other mast cell mediators such as the cysteinyl leukotrienes (Cys-LTs) may also contribute to allergen-induced potentiation of tachykinergic nerve-mediated responses

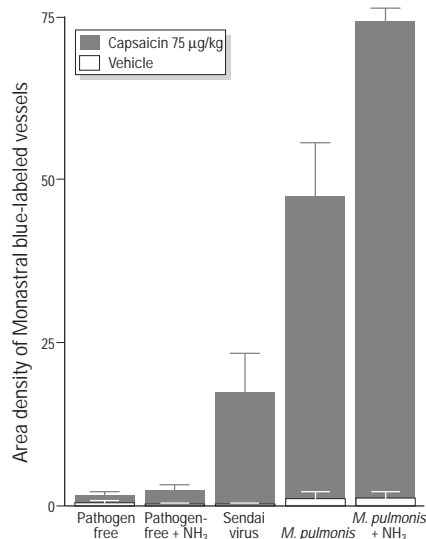


Figure 3. Histogram showing the effect of a 75 µg/kg dose of capsaicin (closed columns) or vehicle (open columns) on vascular permeability in the tracheas of 5 groups of rats: pathogen free, pathogen free with 4 weeks of NH₃ exposure, 6 days after inoculation with *M. pulmonis*, and 4 weeks after inoculation with *M. pulmonis* with continuous NH₃ exposure. Vascular permeability was assessed using Monastral blue as a tracer. Values are mean ± SE ($n = 3-7$) of the amounts of Monastral blue pigment, expressed as area densities (percentage of mucosal surface area in which labeled blood vessels are present). Figure reprinted from McDonald (77) with permission of American Lung Association.

in the airways via the activation of Cys-LT₁ receptors on afferent nerve endings (70,75,76).

Respiratory tract infections may also intensify the tachykinergic nerve-mediated responses in the airways. Infections with *Mycoplasma pulmonis* can produce a 30-fold increase in the magnitude of neurogenic plasma extravasation in rat airways (Figure 3) (77). Similarly, respiratory infections of rodents with Sendai virus (parainfluenza type 1) can potentiate substance P-induced increases in airways blood flow (78) and enhance tachykinergic nerve-mediated contraction of airway smooth muscle (79). These effects appear to be at least partially due to decreased activity of neutral endopeptidase, a protease normally present in the airways, which degrades tachykinins (80-82).

Although much less studied, neuropeptide release from the central terminals of afferent fibers in the brain stem may have more influence on airway physiology than neuropeptide release from their peripheral terminals in the airway wall. The primary neurotransmitter released from the central terminals of vagal airway afferent neurons appears to be glutamate (83). In the somatosensory system, tachykinins act as neuromodulators that enhance the excitability of secondary neurons to primary neurotransmitters, thereby enhancing the perception of pain and/or reflex responses to noxious stimuli (84). Relatively little work has been published on the mechanisms by which NKs modulate synaptic neurotransmission in the brain stem. Nevertheless, pharmacologic studies have demonstrated that the NK receptors NK₁, NK₂, and NK₃ are present in secondary neurons within the nucleus of the solitary tract, and stimulation of these receptors can profoundly affect vagal reflex physiology (85). Also consistent with a role for tachykinins in modulating airway reflexes, the NK₁ receptor-selective antagonist CP-99,994 and SR-48968, an NK₂ receptor-selective antagonist, were able to inhibit mechanically induced cough in the guinea pig and cat by an action in the central nervous system (86).

Conclusions

Various anatomic and physiologic properties of airway afferent neurons change in response to irritation and inflammation in the airways. Perhaps one of the more dramatic changes that occurs is the induction of NK synthesis in airways afferent fibers that under normal conditions do not normally contain neurokinins. As neurokinin release in the brain stem may enhance airway reflexes, stimuli leading to the qualitative change in the type of airway afferent producing and releasing tachykinins like that seen following allergen challenge may contribute to the symptoms of airway inflammation. Thus it may be that in

inflamed airways, it is not only that there is more neurokinin in the nerves innervating the airways, but also that the nerves that are more easily activated (non-nociceptive fibers) now contain and release neurokinins. Inflammation is known to induce a similar qualitative switch in "touch fibers" of the somato-sensory system so they, like nociceptive C fibers, express substance P. Substance P released from the central terminals of these touch fibers appears to contribute to inflammatory hyperalgesia by enhancing transmission in the spinal cord and exaggerating the central response to normally innocuous stimuli (87). It is reasonable to speculate that the release of tachykinins from non-nociceptive afferent neurons that innervate the airways will influence the excitability of synaptic transmission in the central nervous system, such that the stimulation of these mechanically sensitive neurons may lead to exaggerated reflex responses to innocuous stimuli.

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