Cough Sensors. V. Pharmacological Modulation of Cough Sensors

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Contents

Abstract Several airway afferent nerve subtypes have been implicated in coughing. These include bronchopulmonary C-fibers, rapidly adapting airway mechanoreceptors and touch-sensitive tracheal Aδ-fibers (also called cough receptors). Although the last two afferent nerve subtypes are primarily sensitive to mechanical stimuli, all can be acted upon by one or more different chemical stimuli. In this review we catalogue the chemical agents that stimulate and/or modulate the activity of the airway afferent nerves involved in cough, and describe the specific mechanisms involved in these effects. In addition, we describe the mechanisms of action of a number of chemical inhibitors of these afferent nerve subtypes, and attempt to relate this information to the regulation of coughing in health and disease.

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1 Introduction

The mammalian airways are innervated by a variety of afferent nerve subtypes that play differing roles in the regulation of cough. This topic has been recently reviewed elsewhere (Mazzone 2004a, Mazzone 2005) and is also covered in detail by Canning and Chou in this volume. Sensory innervation to the airways and lungs is derived from vagal and spinal sources, although there are only data to support the role of vagal afferents in the cough reflex. There are two broad classes of airway vagal sensory nerve types: one class is predominately mechanically sensitive, and includes rapidly and slowly adapting airway mechanosensors, while the other class is predominately chemosensitive, and includes bronchopulmonary C-fibers and chemosensitive Aδ-fibers (Canning et al. 2001; Ho et al. 2001). Accordingly, airway sensory nerve subtypes are differentiated by their physiological, anatomical, and neurochemical characteristics and the reflexes that they initiate. Although there is debate over which nerve subtypes are involved in initiating and/or regulating coughing, a general consensus suggests that rapidly adapting receptors (RARs) and bronchopulmonary C-fibers, are directly responsible for the initiation of cough, whereas slowly adapting receptors (SARs) play a more modulatory role in shaping the reflex response (Mazzone 2005). More recently, tracheal touch-sensitive Aδ-fibers (also known as cough receptors) have been shown to be involved in coughing in guinea pigs (Canning et al. 2004a). These fibers display characteristics that are distinct from those of other classically described pulmonary mechanoreceptors.

In this chapter we review the current knowledge relating to the chemical activation, modulation and inhibition of airway afferent nerves, focusing particularly on touch-sensitive Aδ-fibers, RARs, and bronchopulmonary C-fibers as these are the likely afferents nerves that are primarily involved in coughing. Although the most up-to-date information is presented, it is readily apparent from reading the proceeding chapters that a great many more substances are known to modulate the activity of bronchopulmonary C-fibers compared with the other types of airway afferent nerves. This regrettably necessitates that a larger portion of the chapter be devoted to C-fiber pharmacology. It is also worth noting that distinct subtypes of bronchopulmonary C-fibers have been described (e.g., pulmonary and bronchial C-fibers in dogs, placodal and neural crest C-fibers in guinea pigs) and there is some debate about the role of each of these afferent subclasses in the cough reflex. This matter has not been addressed in the current chapter, but is dealt with in detail in the chapter by Canning and Chou. Rather, the discussion has been limited to the pharmacological modulation of each afferent nerve type.

2 Increasing Activity

2.1 Touch-Sensitive Aδ-Fibers (Cough Receptors)

Studies in guinea pigs have identified a distinct airway afferent nerve subtype, termed the "cough receptor," which appears to play an essential role in regulating

the cough reflex in this species (Canning et al. 2004a; reviewed in Mazzone 2005). Cough receptors are characterized as extrapulmonary, low-threshold Aδ-fiber mechanosensors that are highly sensitive to touch. The functional and structural properties of cough receptors are considered quite distinct from those of the classic intrapulmonary RARs and SARs. Their conduction velocity is about 3–5 times slower than that of the intrapulmonary RARs and SARs, but about 3–5 times faster than that of C-fibers in this species (Canning et al. 2004a). Although this sensory nerve subtype adapts rapidly to mechanical stimuli (McAlexander et al. 1999), touch-sensitive Aδ-fibers are not sensitive to airway stretch or bronchospasm (stimuli that activate classic RARs). Rather touch-sensitive Aδ-fibers respond to punctate mechanical stimuli (touch) and rapid changes in pH (Canning et al. 2004a; .Kollarik and Undem 2002). These are two stimuli that readily evoke cough in conscious and anesthetized animals and humans. Their location within the airways and functional and structural profiles makes cough receptors the likely sensory nerve subtype involved in defensive coughing in guinea pigs (Canning et al. 2004a; Mazzone 2004a, Mazzone 2005). Circumstantial evidence also suggests that similar nerve fibers may regulate defensive coughing in other species, including humans.

2.1.1 Acid

Touch-sensitive Aδ-fibers in the guinea pig extrapulmonary airways (cough receptors) are largely insensitive to many chemical stimuli. However, they are activated in response to sudden reductions in pH via a mechanism that does not involve stimulation of the capsaicin-sensitive channel, TRPV1 (involved in the acid sensitivity of other airway afferent nerve subtypes; see later) (Kollarik and Undem 2002). Indeed, cough evoked by citric acid activation of touch-sensitive Aδ-fibers in anesthetized guinea pigs is actually potentiated in the presence of the TRPV1 antagonist capsazepine (Canning et al. 2006), albeit via an unknown mechanism since this afferent nerve subtype does not express TRPV1 channels (Myers et al. 2002) and capsazepine has no effect on acid-evoked firing of individual cough receptors in vitro (Kollarik and Undem 2002).

The mechanism by which acid activates tracheal touch-sensitive Aδ-fibers is presently unclear, although possible insights may be gained by assessing the unique kinetics of the acid-evoked responses. For example, acid only activates touch-sensitive Aδ-fibers in the guinea pig extrapulmonary airways when the reduction in pH occurs very rapidly (from pH 7.4 to 5.0 in 1–2 s) (Kollarik and Undem 2002). A comparable reduction in pH over a much slower time scale (e.g., 60 s) does not activate touch-sensitive Aδ-fibers (Kollarik and Undem 2002) and does not cause cough in anesthetized guinea pigs (Mazzone, unpublished observations). Furthermore, following an initial activation, there is a rapid inactivation of the response to acid application. This time course resembles that of certain acid-sensing ion channels (ASICs). The ASICs are a family of channels that are related to the epithelial/sodium (degenerin) channels (ENaCs). There are several subtypes and splice variants, including ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4. Of these subtypes,

ASIC3 has the characteristics that best reflect the acid-evoked responses in touch-sensitive Aδ-fibers. Consistent with this hypothesis, using a single-cell PCR strategy, we have identified ASIC3 messenger RNA (mRNA) within individual nodose ganglion neurons (Undem, unpublished observations). It is also of note that expression of the ASICs in sensory neurons is increased by inflammatory mediators (Mamet et al. 2002). In theory, this may translate into enhanced acid responsiveness of the sensory nerves. Nevertheless, amiloride and other inhibitors of ASICs are only modestly effective, at best, at reducing acid-evoked coughing mediated via touch-sensitive Aδ-fibers in guinea pigs (Canning et al. 2006). Given that there are many other types of ion channels sensitive to decreases in pH, it remains unknown whether an ASIC or some other ionic mechanism is involved in acid-induced activation of touch-sensitive Aδ-fibers.

2.2 Rapidly Adapting Receptors

RARs are sporadically active throughout the respiratory cycle, excited by the dynamic mechanical forces accompanying lung inflation and deflation and becoming more active as the rate and volume of lung inflation increase (Ho et al. 2001; Pack and DeLaney 1983). Indeed a wide range of mechanically related stimuli can modify the activity of RARs. Conversely, reports of chemical stimuli that can directly activate RAR nerve fibers are limited. RARs are insensitive to the direct effects of serotonin (5-hydroxytryptamine, 5-HT), capsaicin, bradykinin, and adenosine, stimuli that readily excite bronchopulmonary C-fibers and other chemosensitive nerve subtypes (see later) (Canning et al. 2001; Ho et al. 2001). Nevertheless, some chemical stimuli may activate RARs indirectly, secondary to producing mechanical-like perturbations in the local lung tissue environment surrounding individual RAR nerve terminals.

2.2.1 Adenosine Triphosphate

In guinea pigs, RARs may be activated directly by some purinergic receptor agonists (Canning et al. 2004a). For example, intrapulmonary RARs in vitro are robustly excited by adenosine triphosphate (ATP) and the selective P2X receptor agonist α,β-methylene ATP. Responses to both ATP and α,β-methylene ATP are abolished by the P2X receptor antagonist pyridoxal phosphate 6-azophenyl-2′,4′-disulfonic acid (Canning et al. 2004a). It is not known if purinergic mechanisms are involved in the endogenous excitation of RARs in vivo, nor is it known which specific subtype of P2X receptor is expressed by RARs. With respect to the latter, given the pharmacological profile of α,β-methylene ATP and on the basis of studies assessing the expression of purinergic receptors in the vagal (nodose) sensory ganglia, it is reasonable to speculate that receptors incorporating $P2X_2$ and $P2X_3$ subunits may be involved.

Inhaled ATP causes dyspnea and cough in asthmatic subjects. Whether this is secondary to activation of RAR or C-fibers (see later) is not known (Basoglu et al. 2005). However, inhaled α,β-methylene ATP does not evoke cough in conscious guinea pigs (at concentrations up to 1 mM; Canning et al. 2004a) or anaesthetized guinea pigs (at concentrations up to 10 mM; Mazzone, unpublished observations) despite these same animals responding readily to citric acid. Purinergic agonists may, however, potentiate cough evoked by other stimuli (Kamei et al. 2005), and endogenous ATP may be involved in the sensitizing effects of histamine on the cough reflex (Kamei and Takahashi 2006).

2.2.2 Agents That Evoke Smooth-Muscle Contraction and Vascular Congestion

Although RARs are insensitive to the direct actions of many chemicals, the sensitivity of RARs to stretch and other mechanical perturbations means that their activity can be increased by any chemical stimulus that evokes bronchospasm or obstruction resulting from mucus secretion or edema in the airways (Bergren and Sampson 1982; Bonham et al. 1996; Canning et al. 2001; Jonzon et al. 1986; Pack and DeLaney 1983; Mohammed et al. 1993). Bronchospastic agents such as histamine, methacholine, and leukotirene C4, and agents that evoke plasma extravasation, including substance P (or capsaicin, via neurogenic release of substance P), produce robust activation of RARs, not necessarily because RARs express receptors for these agents, but rather owing to the secondary pulmonary end organ effects (Canning et al. 2001). Not surprisingly then, RAR activation by chemicals such as histamine, capsaicin and substance P is markedly inhibited or abolished by preventing the local end organ effects that these stimuli evoke. This is perhaps best highlighted by the fact that pretreatment with the bronchodilator isoproteronol prevents bronchospasm-evoked activation of RARs, whereas blocking endogenous β-adrenoceptors with propranolol potentiates bronchospasm-evoked activation of RARs (Bergren 1997; Canning et al. 2001).

Indirect activation of RARs by chemical stimuli may confound attempts to ascribe a sensory nerve subtype to a particular reflex response. For example, the well-known cough-evoking effects of capsaicin could be presumed to be mediated via airway capsaicin-sensitive C-fibers. However, peripherally acting neurokinin receptor antagonists effectively reduce capsaicin-evoked cough in several models, suggesting that axon reflex mediated end organ effects, and the subsequent activation of RARs, may also be involved (reviewed in Mazzone 2004b). Nevertheless, although lung inflation may be associated with cough under certain circumstances, many stimuli that produce robust activation of RARs (e.g., thromboxane, leukotriene C_4, histamine, neurokinins, methacholine) are ineffective or only modestly effective at evoking cough (Barnes et al. 1984; Canning et al. 2004a; Joos et al. 1987). Moreover, in some coughing species (e.g., guinea pigs) many RARs are spontaneously active throughout the respiratory cycle and yet cough is only induced in response to very specific stimuli (Bergren and Sampson 1982; Canning et al. 2001; Mazzone and Canning 2002).

2.3 Bronchopulmonary C-Fibers

Afferent C-fibers are thin unmyelinated nerves that conduct action potentials at a velocity typically less than 1 m/s. It should be kept in mind, however, that there is more than one subtype of afferent C-fibers. The respiratory tract is innervated mainly by afferent C-fibers arising from the vagus nerve, but also by a substantial number of spinal afferent C-fibers with cell bodies situated in the thoracic dorsal root ganglia (DRG). In addition to vagal and spinal C-fiber subtypes, the Coleridges and their colleagues noted in a series of elegant studies that there are subtypes of C-fibers arising from the vagus nerve (Coleridge and Coleridge 1984). The vagal C-fibers innervating the large airways (referred to as bronchial C-fibers) may be pharmacologically distinguishable from those situated in more peripheral lung tissue (pulmonary C-fibers). Part of the phenotypic distinction between different vagal C-fiber subtypes may be based on whether their cell bodies are located in the nodose or jugular vagal ganglia. Neurons in the nodose ganglia are derived embryonically from the epibranchial placodes, whereas the neurons in the jugular ganglia are derived from the neural crest (Baker 2005). In this sense, jugular ganglia neurons are similar to spinal DRG neurons. Several studies have demonstrated pharmacological and neurochemical differences between the neural crest and placodal C-fibers innervating the respiratory tract (Kollarik and Undem 2006).

C-fibers respond to a variety of chemical irritants and endogenous inflammatory mediators, and inhalation of chemicals known to stimulate bronchopulmonary C-fibers is effective at evoking cough in conscious animals, including guinea pigs, rabbits, cats, dogs, and humans. Unlike mechanical stimulation of the extrapulmonary airways, selective C-fiber stimulants are less effective or ineffective at evoking cough in anesthetized animals (Canning et al. 2004a). Whether the placodal C-fibers, neural crest C-fibers or both subtypes evoke cough when activated is not yet understood.

Chemicals activate (evoke action potential discharge) in the C-fiber terminals via one of two general mechanisms: they either act directly on ligand-gated ion channels that result in an inward current upon stimulation, or they act on metabotropic receptors (e.g., G-protein-coupled receptors) that evoke inward currents in the nerve terminal secondary to various signal transduction schemes that ultimately alter ion channel kinetics.

2.3.1 Activation via Ligand-Gated Ion Channels

Nicotinic Receptor Agonists

The nicotinic cholinergic receptor is perhaps the most thoroughly studied ligand-gated ion channel. The functional receptor takes the form of a pentomere comprising various combinations of α and β subunits (Lukas et al. 1999). There are ten identified α subunits (α1–α10) and four β subunits (β1–4). Nine (α2–α10) of the α subunits have been identified in the vertebrate nervous system, although α8 is limited to avians.

Inhalation of cigarette smoke, in naïve smokers, causes intense airway irritation and cough. A series of studies revealed that this is largely accounted for by nicotinic receptor activation (Lee et al. 1993). The most parsimonious explanation of how inhaled nicotine could lead to sensations of irritation and urge to cough is that nicotine directly activates C-fibers near the airway epithelium. This hypothesis has been addressed using extracellular recording of C-fibers in vivo (Lee et al. 2007). Nicotine and cigarette smoke both lead to action potential discharge in a population of airway C-fibers, and this can be blocked by the nicotininc receptor antagonist hexamethonium. A population of RAR fibers is also activated upon inhalation of nicotine (Lee et al. 2007). From these in vivo studies one cannot be certain whether the nerve activation is due to a direct effect of nicotine on the nerve terminals, or secondary to other effects in the lungs (vascular effects, bronchoconstriction, etc.; see earlier).

That nicotine directly interacts with receptors on the afferent nerves is supported by studies showing that nodose neurons express nicotinic receptors (Mao et al. 2006). Furthermore, in functional studies that assess increases in cytosolic calcium as a measure of neuronal activation, nicotine and other nicotinic receptor agonists cause activation of a subset of capsaicin-sensitive neurons retrogradely labeled from the lungs. Nicotine also activated a subset of capsaicin-insensitive lung afferent nodose neurons (perhaps RAR neurons) (Xu et al. 2007).

The nicotinic receptor subtype responsible for stimulation of pulmonary afferent nerves is not known. Immunoprecipitation and immunhistochemical studies provide evidence of $\alpha 2$, $\alpha 3$, $\alpha 4$, and $\alpha 5$ subunits along with $\beta 2$ and $\beta 4$ in the nodose ganglion neurons (Mao et al. 2006). The $\alpha 3$ and $\alpha 5$ subunits may be particularly prominent in the capsaicin-sensitive (i.e., C-fiber) population (Spies et al. 2006). It is also not known whether the nicotinic receptors on afferent nerve terminals in the lungs are ever stimulated by endogenous agonists. There is little evidence that cholinergic parasympathetic nerves synapse with afferent C-fibers. There, are however, potential nonneuronal sources of acetylcholine in the airways that may be juxtaposed to afferent nerve terminals, including the airway epithelial cells (Klapproth et al. 1997). Moreover, choline, a rather ubiquitous chemical, is an effective agonist at certain subtypes of nicotinic receptors (Lukas et al. 1999).

5-Hydroxytryptamine

Among the dozen or so 5-HT receptor subtypes only 5-HT3 is an ionotropic receptor. The functional 5-HT3 receptor, like the nicotinic receptor, takes the form of a pentomer (Hoyer et al. 1994). So far three 5-HT3 subunits have been described (5-HT3A, 5-HT3B, and 5HT-3C). In the 1970s and 1980s several laboratories were using phenyldiguanide to stimulate pulmonary C-fibers (or J-receptors) in classical studies of the pulmonary chemoreflex (Coleridge and Coleridge 1984). These studies predated the discovery that phenyldiguanide is a very selective 5-HT3 receptor agonist; nevertheless, in retrospect, they provided the first evidence that 5-HT3 receptor activation can lead to stimulation of pulmonary C-fibers. Incidentally,

phenyldiguanide is still a useful 5-HT3-selective agonist, but owing to slight 5HT3 receptor sequence differences among species, it fails to stimulate 5-HT3 receptors in some mammals, including guinea pigs (Lankiewicz et al. 1998). 2-Methyl-5-hydroxytryptamine is a selective 5-HT3 receptor agonist that is more universally effective among species.

5-HT effectively activates C-fibers (but not RARs or SARs) in rabbit lungs and this is blocked by a selective 5-HT3 antagonist. Interestingly, the same 5-HT3 receptor antagonist prevents C-fiber activation caused by experimentally induced pulmonary embolism (Kay and Armstrong 1991). In guinea pigs, 5-HT was not effective at stimulating all C-fiber subtypes. The nodose (placodal) C-fibers in the lungs were effectively stimulated by 5-HT (and 5-HT3-selective agonists), whereas the jugular (neural-crest)-derived C-fibers were not stimulated by 5-HT (Chuaychoo et al. 2005). Consistent with these finding, 5-HT evoked an inward current in patch-clamped pulmonary nodose neurons, but failed to evoke such a current in pulmonary jugular neurons. Like the jugular neurons, the dorsal root ganglion C-fiber neurons labeled from the lungs are also unresponsive to 5-HT. In dogs, 5-HT was found to evoke a larger response in bronchial than in pulmonary C-fibers.

Adenosine Triphosphate

ATP binds to P2Y and P2X purinergic receptors, but only P2X receptors are ionotropic. There are seven subtypes of P2X receptors (P2X1–P2X7) (Chizh and Illes 2001). The functional receptors are trimeric and can be either homopolymers or heteropolymers. As described earlier, the P2X receptors in nodose neurons are generally thought to be P2X2, P2X3, and heteromeric P2X2,3 receptors (Cockayne et al. 2005).

Pelleg and Hurt (1996) were first to show ATP and P2X-selective agonists cause action potential discharge in dog lungs studied in vivo. The P2X receptors seem to define the placodal population of afferent neurons innervating the respiratory tract (Undem et al. 2004; Canning et al. 2004a). In patch-clamp studies, P2X currents can be seen on virtually all nodose neurons (Cockayne et al. 2005). By contrast, jugular ganglion neurons and lung-specific dorsal root ganglion neurons (neural crest in origin) do not respond to selective P2X agonists. In extracellular recording studies, ATP, or the P2X3-selective agonist $\alpha s\beta$-methyl ATP, evokes action potential discharge in nodose C-fiber terminals, but not jugular C-fibers (Undem et al. 2004). Given that inhaled α,β-methyl ATP (up to 1 mM) does not evoke cough in conscious guinea pigs, a question could be raised as to the specific role in cough played by nodose-derived C-fiber afferent nerves (Canning et al. 2004a).

Stimuli of TRPV1 Channels

Capsaicin is commonly used as a pharmacological tool to activate visceral and somatosensory C-fibers. It had long been suspected that capsaicin may act via a

specific receptor, but it was not until 1997 that the capsaicin receptor was identified (Caterina et al. 1997). It was found to be a ligand (capsaicin)-gated ion channel originally termed vanilloid receptor-1, but later renamed TRPV1. TRPV1 is a member of a large family of transient receptor potential (TRP) channels that is thoroughly reviewed in Nilius et al. (2007).

The relevance of TRPV1 in C-fiber biology was amplified by the findings that this ion channel can be gated by mechanisms independently of chemicals found in hot peppers (Tominaga et al. 2008). Temperature itself can open TRPV1 and lead to action potential discharge, but this typically requires temperature in excess of what would be found in the lower respiratory tract. A decrease in pH can also activate C-fibers in the airways via TRPV1 gating (see later). This is almost certainly relevant to acid-provoked cough, and may be relevant in inflammatory diseases in general inasmuch as the pH of inflamed tissues is typically acidic. TRPV1 can also be gated by signaling mechanisms downstream of stimulation of metabotropic receptors. Chuang et al. (2001) reported that TRPV1 is tonically inhibited by the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2). Activation of phospholipase C results in the conversion of PIP_2 to diacylglycerol and inositol 1,4,5-trisphosphate, thereby removing the tonic inhibition. Another way in which phospholipase C activation can lead to activation is through the downstream production of lipoxygenase products of arachidonic acid (Shin et al. 2002). Lipoxygenase metabolites such as 12-hydroxyeicosatetraenoic acid or 15-hydroxyeicosatetraenoic acid are structurally similar to capsaicin and are effective at intracellularly gating the TRPV1 channel (Hwang et al. 2000) and evoking TRPV1-dependent reflex bronchospasm in guinea pigs (Mazzone and Canning 2002). These findings explain why bradykinin-induced action potential discharge in airway C-fibers is inhibited in a nonredundant manner by TRPV1 antagonists (or in TRPV1 knockout mice) and 12-lipoxygenase inhibitors (Carr et al. 2003; Kollarik and Undem 2003).

Stimuli of TRPA1 Channels

TRPA1 is another member of the TRP family of ion channels and is named for its prominent ankyrin repeats in the N-terminal domain (Nilius et al. 2007). In preliminary studies we found that all lung-specific C-fibers in the mouse express both TRPV1 and TRPA1 mRNA, and ligands known to activate either TRPV1 or TRPA1 lead to action potential discharge in mouse and guinea pig bronchopulmonary C-fibers. TRPA1 agonists also evoke neurogenic (tachykinin-mediated) bronchospasm in anesthetized guinea pigs (Mazzone, unpublished observations). TRPA1 is not activated by capsaicin, but is stimulated by cinnamaldahyde and allyl isothiocyante (active ingredient in mustard oil, wasabi, horseradish, etc.) (Jordt et al. 2004; Bandell et al. 2004). Like TRPV1, TRPA1 may be gated by autacoids that act through certain G-protein-coupled receptors, e.g., bradykinin acting via B2 receptors. The mechanism(s) underlying G-protein-coupled-receptor-dependent activation of TRPA1, however, appears to be different from those involved in TRPV1 activation. TRPA1 has an N-terminal EF-hand calcium-binding domain making it

sensitive to any stimulus that increases intracellular calcium. These mechanisms provide a pathway through which inflammatory mediators acting via metabotropic receptors may increase the activation rate of TRPA1 containing nerves (Doerner et al. 2007). Stimuli that lead to calcium influx through other ligand-gated ion channels, including TRPV1, may also indirectly activate TRPA1 via this mechanism. Decreases in temperature can activate TRPA1 and this may have relevance in cold-air-induced reflex activity in the airways. In addition, certain relevant environmental irritants such as isothiocyantes and acrolein may directly activate TRPA1, providing a mechanism by which air-pollutants can lead to nasal and bronchial C-fiber activation (Bautista et al. 2006).

Acid

Acid is an effective stimulator of bronchopulmonary C-fibers (Fox et al. 1995a; Hong et al. 1997; Kollarik and Undem 2002). The mechanism by which C-fibers are activated by acid depends on both the acid concentration and the rate of change in pH. Sustained acidification of the airway mucosal surface in the guinea pig evokes sustained action potential discharge in vagal C-fiber nerves. The pH threshold for activation of these nerves is about 6.0 (Fox et al. 1995a; Kollarik and Undem 2002). Pharmacological and TRPV1 knockout studies revealed that this activation is in part mediated by TRPV1; however, up to 50% of this activation is TRPV1-independent (Kollarik and Undem 2004).

An analysis of the characteristics of the TRPV1-independent acid-induced response in the nerve terminals of the touch-sensitive Aδ-fibers and C-fibers predicts an acid transducer with low pH threshold (pH $>$ 6.7) that rapidly (within seconds) inactivates in the presence of acid (Kollarik and Undem 2002). It also predicts that inactivation may occur in the absence of activation if the rate of pH change is slow. As described earlier for tracheal touch-sensitive Aδ-fibers, ASIC3 most closely matches the properties of the TRPV1-independent acid-induced response in vagal afferent C-fibers.

2.3.2 Activation via G-Protein-Coupled "Metabotropic" Receptors

There are a large number of G-protein-coupled-receptor agonists that through various second messenger systems can affect the afferent function of airway sensory nerves. With a few notable exceptions, the ligands acting via G-protein-coupled receptors do not overtly activate the C-fiber terminals. Rather, they alter the excitability of the nerve such that the net response of any given stimulus is enhanced or inhibited. This change in excitability, or "neuromodulation," typically involves phosphorylation of various ion channels or pumps in the nerve terminal. In some cases, ligands acting via G-protein-coupled receptors have been found to directly activate the C-fibers, causing action potential discharge (Table 1).

Table 1 Overview of the known chemicals that activate, modulate, or inhibit the airway sensory nerves involved in cough

Agent	Pharmacological Mechanism of action	Touch sensitive Aδ-fibers	Nodose C-fibers	Jugular C-fibers and Aδ-fibers	RARs[a]
		Activators			
Adenosine	A1/A2A receptors	No	Yes	No	No
Acid	TRPV1 channels	No	Yes	Yes	No
	ASICs[b]	Yes	Yes	Yes	Unknown
Acetylcholine/ nicotine	nAChR[c] receptors	No	Yes[d]	Yes[d]	Some[d]
ATP	P2X[e] receptors	No	Yes	No	Yes
Bradykinin	B2 receptors	No	Yes	Yes	No
Capsaicin, heat, lipoxygenase products	TRPV1 channels	No	Yes	Yes	No
Thrombin	PAR1 receptors	No	Yes	Yes	No
5-HT	5-HT3 receptors	No	Yes	No	No
Cinnamaldehyde, mustard oil, extreme cold	TRPA1 channels	No	Yes[f]	Yes[f]	No
		Modulators			
Histamine	H1 receptors	No	Yes	Unknown	No
Cysteinyl leukotrienes	cys-LT1 receptors	No	Yes	Yes	No
Neurokinins	NK1/NK2 receptors	No	Yes	Unknown	No
Thrombin	PAR2[g] receptors	No	Yes	Yes	No
PGE2	EP3 receptors	No	Yes	Yes	No
		Inhibitors			
Cl^- channel blockers	Cl^- channels	*Possibly*[h]	Yes	Yes	Unknown
Loop diuretics	NKCC1	*Possibly*[h]	Yes	Yes	*Possibly*[h]
Local anesthetics	Na^+ channels	Yes	Yes	Yes	Yes
Menthol[i]	TRPM8/TRPA1 channels	Unknown	Unknown	Unknown	Unknown
Nociceptin	NOP1 receptors	No	Yes	Yes	Unknown
Ouabain	α3-ATPase pumps	Yes	Unknown	No	Unknown
Opioids	Mu receptors	No	Yes	Yes	Unknown
	Kappa receptors	Unknown	Unknown	Unknown	Unknown
	Delta receptors	Unknown	Unknown	Unknown	Unknown

Activators are agents that evoke action potential formation; modulators are agents that alter the membrane potential thereby altering neuronal excitability, and inhibitors are only those compounds that reduce nerve excitability (i.e., although specific antagonists of the known activators will effectively inhibit that activator from evoking afferent responses, such compounds do not reduce the overall excitability of the nerve and are therefore not included in the list). The mechanism of action is the pharmacological receptor, ion channel or ion transporter that the listed compound directly interacts with. All afferent nerves can be activated to punctuate mechanical stimuli and large changes in extracellular osmolarity, albeit with varying sensitivities. The mechanisms of these activations are presently unknown

Table 1 Continued

RARs rapidly adapting receptors, *ASICs*, acid-sensing ion channels, *nAChR* nicotinic acetylcholine receptor, *5-HT* 5- hydroxytryptamine, *PGE2* prostaglandin E2, *PAR2* protease-activated receptor-2, *NKCC1* furosemide-sensitive $Na^+/K^+/2Cl^-$ cotransporter

[a]Only agents that directly activate RARs are listed

[b]Acid activates airway afferent nerves in part via a TRPV1-independent mechanism. Although it is not clear yet, this likely involves activation of one or more ASICs.

[c]The subunits that comprise the nAChR are unknown

[d]The evidence for C-fiber and RAR fiber activation comes from studies in dogs. The precise fibers activated are unclear

[e]The subunits that comprise the P2X receptor are not known, although they are likely to be P2X2 and P2X3

[f]Endogenous activators of TRPA1 are unknown at present. Also, the exact fibers activated by TRPA1 stimulants (cinnamaldehyde, mustard oil, etc.) have not been fully defined, although TRPV1-expressing nociceptors are the likely candidates

[g]Sensitization of airway nociceptors via PAR2 mechanisms is probably secondary to PGE2 release from the epithelium

[h]Functional studies provide some evidence for a role of NKCC1 and/or Cl^-channels in regulating cough receptor excitability

[i]Menthol inhibits cough; however, the mechanism via which it does so has not been investigated

Bradykinin

Bradykinin B2 receptors have been localized on neurons in human nodose ganglia, and bradykinin causes sneezing and coughing when applied to appropriate sites in human airways (Choudry et al. 1989; Riccio and Proud 1996). Bradykinin stimulates action potential discharge in airway C-fiber afferent nerves in several species studied in vivo (Coleridge et al. 1989; Hargreaves et al. 1993). In the guinea pig isolated airway preparation, bradykinin is one of the few G-protein-coupled-receptor agonists that consistently leads to action potential discharge when applied directly to the receptive fields of afferent nerves in the airway wall (Fox et al. 1993; Kajekar et al. 1999). This effect of bradykinin is blocked by B2 receptor antagonists, and is selective for C-fibers and Aδ nociceptive fibers (Kajekar et al. 1999).

The question remains as to the ionic mechanism that leads to the depolarizing generator potential following bradykinin B2 receptor activation. At least two mechanisms appear to be involved. As mentioned above, it is known that bradykinin B2 receptor stimulation can lead to the opening of TRPV1. Supporting this idea are the findings that the bradykinin-induced action potential discharge in airway C-fibers is inhibited by TRPV1 antagonist, and reduced in fibres from TRPV1 knockout mice (Carr et al. 2003; Kollarik and Undem 2004). Blocking TRPV1 does not, however, abolish the bradykinin response. The balance of the response may be due to the opening of certain calcium-activated chloride channels. The efflux of chloride down its concentration gradient leads to the membrane depolarization and action potential discharge. This hypothesis is supported by the observations that the bradykinin-

induced inward current in nodose and jugular neurons is dependent on chloride channel activity (Oh and Weinreich 2004; Lee et al. 2005), and chloride channel blockers inhibit the action potential discharge in guinea pig tracheal C-fiber terminals (Lee et al. 2005). Another potential ion channel that may be involved in the response to bradykinin is TRPA1. In this case, as mentioned earlier, TRPA1 may be gated secondary to an elevation in intracellular calcium.

Adenosine

Adenosine can evoke sensations of dyspnea and cough in humans subjects (Burki et al. 2005; Basoglu et al. 2005). Adenosine increases action potential discharge in rat pulmonary C-fibers (Hong et al. 1998) studied in vivo. This effect is unaffected by adenosine A2 antagonists, but is inhibited by A1 antagonists. In the isolated innervated guinea pig lung preparation, adenosine effectively evokes action potential discharge in C-fiber terminals. The effect of adenosine can be mimicked by adenosine A1 or adenosine A2A receptor selective agonists, and is blocked entirely only with a combination of A2A and A1 receptor antagonists. Single-cell Reverse transcriptase-PCR analysis of guinea pig lung-specific C-fiber neurons revealed that both A1 and A2A receptors are expressed. These data suggest that signaling mechanisms downstream of either A1 or A2A receptor are capable of causing inward currents that activate guinea pig lung C-fiber terminals (Chuaychoo et al. 2006). The precise signaling mechanisms have not yet been studied. The effect of adenosine on guinea pig lung C-fibers is relatively selective for nodose (placodal) C-fibers with the response of jugular C-fibers being either trivial or absent.

Protease-Activated Receptor-1

Among the four known protease-activated receptors (PARs) (PAR1–PAR4), PAR1 and PAR2 appear to the types most often linked to C-fiber activity. In the vagally innervated, isolated mouse lung preparation, thrombin and selective PAR1 activators robustly evoke action potential discharge in bronchopulmonary C-fibers. The PAR1-selective activating peptide also causes a rapid and large inward current in lung-specific C-fiber neurons. In addition, single neuron Reverse transcriptase-PCR analysis of mouse lung-specific C-fiber neurons revealed that the neurons routinely express PAR1 and PAR3 with little evidence of either PAR2 or PAR4 expression (Undem, unpublished observations).

2.3.3 Modulation of Bronchopulmonary C-Fiber Excitability

As mentioned already, most inflammatory mediators fail to directly evoke action potential discharge when applied to C-fibers ex vivo. Within our narrow definition of the term, we may say that these mediators fail to *activate* the nerve. Many of

these mediators, however, increase, or in some cases decrease, the excitability of the nerve.

It is often difficult to determine if a given chemical should be classified as a sensory nerve activator or modulator (or both). Confusing the issue are experiments where a nerve modulator is associated with action potential discharge. Bronchopulmonary C-fibers often reveal little background activity when studied in vivo, but this should not be interpreted as the complete absence of a potential stimulus. In the complex environment of the tissue, one can envisage that bronchopulmonary C-fibers are constantly exposed to potentially activating stimuli (e.g., changes in osmolarity, mechanical forces, acid concentration, ATP, adenosine, choline, bradykinin), but the stimuli are subthreshold for action potential discharge. In the presence of a neuromodulating autacoid that increases excitability, one may observe action potential discharge when a complex system is studied where these subliminal stimuli are present (e.g., in vivo). A modulator would not be expected to evoke action potentials in nerves studied in a more controlled or reduced environment. At the level of the cell soma, studied using patch-clamp techniques, a neuromodulator may influence active and passive electrophysiological properties of nerves, but generally speaking will not cause a large inward current (i.e., it is not sufficient to evoke an action potential).

Histamine

Histamine H1 receptor activation depolarizes a subpopulation of vagal sensory neurons in several species. Unlike the ligand-gated ion channels, and bradykinin, the membrane depolarization evoked by histamine is small and typically associated with a decrease in ion conductance (Undem and Weinreich 1993; Jafri et al. 1997). Histamine inhibits the resting or so-called leak potassium current in nodose ganglion neurons, and in some species inhibits voltage-gated calcium currents and the calcium-activated potassium current that subserves afterspike hyperpolarizations (Jafri et al. 1997). These effects on sensory neurons predict that histamine alone will not directly evoke action potential discharge in airway afferent endings, but will increase the excitability of the nerve. Consistent with this prediction, histamine does not evoke action potential discharge in the guinea pig isolated airway preparation (Fox et al. 1993; Riccio et al. 1996), but it has been shown to increase the mechanical and chemical (capsaicin) sensitivity of afferent C-fibers in dog airways (Lee and Morton 1993).

Eicosanoids

Prostaglandins have long been known to increase the excitability of sensory nerves. Electrophysiological studies on vagal sensory ganglion neurons have demonstrated excitatory affects of several prostaglandins, including prostaglandin E2 (PGE2), prostaglandin D2 (PGD2), and prostaglandin I2 (PGI2; prostacyclin) (Fowler et al. 1985; Undem and Weinreich 1993). PGE2 increases the sodium current through

voltage-gated tetrodotoxin (TTX) resistant sodium channels (likely NaV1.8) in lung-specific nodose neurons (Kwong and Lee 2005). This effect appears to be dependent on EP3 receptors. In addition to enhancing sodium currents, certain prostaglandins (e.g., PGE2, PGI2, and PGD2) have been found to inhibit calcium-activated potassium currents involved in the afterspike hyperpolarization (Weinreich and Wonderlin 1987; Undem and Weinreich 1993), and can lead to an increase in the hyperpolarization-activated cation current (Ingram and Williams 1996). If these effects occur at the nerve terminals they would likely lead to an increase in the peak frequency of action potential discharge. Consistent with this prediction, in studies on vagal ganglion neurons Ho et al. (2000) found that low concentrations of PGE2 do not cause action potential discharge in airway afferent nerves, but effectively increased action potential discharge in pulmonary C-fiber afferents induced mechanically by lung inflation, or chemically with capsaicin. Others have noted that thromboxane, PGE2, PGI2, and prostaglandin F2a (PGF2a) increased the rate of baseline discharge in airway C-fibers (Coleridge et al. 1976; Roberts et al. 1985; Bergren 2006; Karla et al. 1992). In support of a role for prostaglandins in increasing the excitability of human airway afferent C-fibers are the findings that PGE2 and PGF2a inhalation increased the sensitivity of the cough response in human volunteers (Stone et al. 1992).

There has been little investigation into the potential role of cysteinyl leukotrienes (cys-LTs) on airway vagal afferent nerve activity. Cyst-LTs inhibit the afterspike hyperpolarization of vagal sensory ganglion neurons, and cause membrane depolarization of identified airway C-fiber neurons in sensory ganglia (McAlexander et al. 1998; Undem and Weinreich 1993). This latter effect is due to an inhibition of a resting potassium current, and is blocked by cys-LT1 receptor antagonists. Other lipoxygenase products, as discussed earlier, may interact directly with the TRPV1 channel on nociceptive nerve terminals in the airways. Inhibitors of leukotreine cys-LT1 receptors inhibit the heightened cough response in animal models and human subjects (Nishitsuji et al. 2007; Brozmanova et al. 2005; Kopriva et al. 2004; Dicpinigaitis et al. 2002).

Bradykinin

Bradykinin is a mediator that may be considered as both an activator (as discussed earlier) and a modulator. One mechanism by which bradykinin modulates C-fiber excitability is blockade of calcium-dependent potassium currents responsible for an afterspike hyperpolarization (Weinreich et al. 1995). This effect is mediated by bradykinin B2 receptors, although the latter effect on the afterspike hyperpolarization appears to be secondary to prostacyclin production by the neuron.

Neurokinins

Neurokinin 1 receptor agonists have been found to depolarize (Oh et al. 2000) or hyperpolarize (Jafri and Weinreich 1998) the membrane potential of nodose

ganglion neurons depending on the species. Membrane hyperpolarization would be expected to inhibit the excitability of the C-fibers. The hyperpolarization of ferret nodose ganglion neurons is secondary to activation of a calcium-gated potassium current. Neurokinin 2 receptor agonists depolarize guinea pig nodose ganglion neurons secondary to an increase in nonselective cation current (Moore et al. 2000). Interestingly, this effect is "unmasked" by inflammatory mediators. Thus, whereas the membrane potential of none out of 156 guinea pig isolated nodose ganglion neurons depolarized in response to neurokinin 2 receptor activation normally, within a day of allergen challenge of the airways more than 80% of the nodose ganglion neurons responded to neurokinin 2 agonists with membrane depolarization (Moore et al. 2000). Substance P has been associated with an increase in C-fiber activity in guinea pig lungs. This effect was inhibited by indomethacin, suggesting a role for prostaglandins in the response.

Bombesin-Like Peptides

Bombesin and gastrin-releasing peptides have been shown to sensitize C-fiber neurons to subsequent activation by capsaicin and ATP. In addition, these peptides enhance the pulmonary chemoreflex induced by C-fiber stimulants. The relevance of these observations may be heightened in small-cell lung cancers, as the cancer cells are known to secrete various peptides, including bombesin-like peptides (Gu and Lee 2005).

Protease-Activated Receptor-2

PAR2 activating peptides failed to stimulate rat C-fiber neurons, but were effective in enhancing the C-fiber responses to subsequent exposure to capsaicin (Gu and Lee 2006). Inhalation of PAR2-selective activating peptides increases C-fiber mediated reflexes in rats (Gu and Lee 2006) and cough sensitivity in guinea pigs (Gatti et al. 2006). The effect of PAR2 activators on cough is mainly secondary to prostaglandin production (Gatti et al. 2006), likely owing to PAR2-mediated prostaglandin E release from the airway epithelium (Cocks et al. 1999).

3 Decreasing Activity

Relatively few pharmacological inhibitors of airway sensory nerve activity have been described, and even fewer have been investigated carefully to assess their specific modes of action. Whereas some inhibitors exert actions primarily on a single afferent nerve subtype (e.g., bronchopulmonary C-fibers), others, such as the local anesthetics, may act less specifically. With the relatively recent discovery of tracheal touch-sensitive Aδ-fibers, there is a paucity of knowledge about selective pharmacological inhibition of this afferent nerve subtype. Accordingly, the follow-

ing section highlights only the inhibitors that have been studied in some detail or where the mechanism of action can be predicted from work on other systems. Specific references to the afferent nerve subtypes targeted by these agents are made where appropriate.

3.1 Opioids

Often considered the "gold standard" antitussive therapy, opioids acting at mu-, kappa- or delta-opioid receptors have all been shown to be antitussive, although the mechanisms via which opioids inhibit cough are debatable (Takahama and Shirasaki 2007). Given that effective doses of opioids for cough suppression also produce sedation and can lead to addiction, as well as the reported limited antitussive actions of inhaled or peripherally acting compounds in humans, it seems that the site of action is likely primarily somewhere within the central nervous system. Nevertheless, opioid receptors are expressed on the peripheral terminals of sensory nerves and there is evidence that activation of peripheral opioid receptors can suppress sensory nerve activity. Mu-opioid agonists inhibit calcium currents in cultured rat nodose neurons, albeit this inhibitory effect is more frequently observed in neurons from neonatal animals than in those from adults (Hamra et al. 1999). Morphine and mu-opioid receptor agonists inhibit neurogenic bronchospasm and airway plasma extravasation evoked by antidromic electrical stimulation of the vagus nerves in guinea pigs, an effect that is likely due to prejunctional inhibition of tachykinin release from vagal C-fibers in the airways (Belvisi et al. 1988, 1989; Shankley et al. 1992). Consistent with this, mu-opioid receptor transport in rat vagus nerve is greatly reduced by capsaicin pretreatment (Laduron 1984) and immunohistochemical studies have shown that mu-opioid receptors are expressed in substance P-containing vagal ganglia neurons (Ding et al. 1998).

Despite the effect of morphine and related compounds on neurogenic effects in the airways, there have been relatively few studies that have looked specifically at whether opioids inhibit orthodromic activity in pulmonary vagal sensory nerves. In guinea pigs, cough evoked by inhaled citric acid has been shown to be inhibited by inhaled morphine and selective mu-opioid receptor agonists, perhaps indicating a potential inhibitory effect of opioids on centrally directed activity in airway afferent nerves (Adcock et al. 1988; Callaway et al. 1991; Karlsson et al. 1990). Furthermore, in bullfrogs, morphine and dihydrocodeine have been shown to reduce the number of spontaneous action potential discharges in vagal pulmonary afferents (Kontani and Koshiura 1985). There are also no data confirming the presence of either delta-opioid or kappa-opioid receptors on the peripheral terminals of airway vagal afferent nerves, although vagal afferents innervating the stomach are inhibited by selective kappa-opioid receptor agonists, suggesting vagal afferent terminals can express other opioid receptors (Ozaki et al. 2000).

3.2 Nociceptin

Nociceptin (also known as orphanin FQ) is an endogenous peptide that was originally shown to activate the "orphan" opioid receptor-like receptor (ORL1, now known as NOP1). Both nociceptin and NOP1 receptors are expressed by airway vagal sensory neurons (Fischer et al. 1998) and exogenously administered nociceptin, and related agonists, inhibit tachykinergic-mediated airway neurogenic bronchospasm and plasma extravasation in guinea pigs and rabbits (Corboz et al. 2000; D'Agostino et al. 2005; Fischer et al. 1998). Furthermore, intravenously administered nociceptin inhibits cough in guinea pigs and cats, perhaps in part via an action at peripheral NOP1 receptors in the airways (Bolser et al. 2001; Lee et al. 2006; McLeod et al. 2001, 2004).

Recent studies have revealed insights into how activation of NOP1 receptor activation may inhibit vagal sensory nerve activity. Inhibition of capsaicin- and acid-evoked coughing in conscious guinea pigs is associated with an inhibition of TRPV1-dependent increases in intracellular calcium in dissociated guinea pig vagal ganglia sensory neurons (Jia et al. 2002; Lee et al. 2006). Jia et al. (2002) showed that prior treatment with an inhibitor of inward-rectifier potassium channels, but not with an inhibitor of voltage-gated calcium channels, prevented capsaicin-evoked calcium response in dissociated neurons and prevented nociceptin inhibition of capsaicin-evoked contractions of the guinea pig bronchus. These data would suggest that neuronal hyperpolarization mediated via inward-rectifier potassium channels may underlie how nociceptin regulates cystolic calcium responses (and hence peripheral neurotransmitter release) in vagal afferent nerves. In contrast, however, Lee et al. (2006) suggest that this mechanism may not be involved in how nociceptin regulates all TRPV1-dependent responses in vagal sensory neurons. In patch-clamped airway-projecting jugular ganglia neurons, under voltage-clamp conditions that limit the contribution of inward-rectifier potassium channel activity, Lee et al. (2006) showed that inward-rectifier potassium channels contribute little, if any, to nociceptin inhibition of acid-evoked TRPV1-mediated inward currents. Rather they speculate that a secondary signaling event (perhaps a reduction in cytosolic cyclic AMP formation) likely mediates the effect. They also showed that TRPV1-independent acid responses (presumably ASIC-mediated currents) are not affected by nociceptin. Identifying the exact mechanisms via which nociceptin selectively inhibits TRPV1 channel activity may provide novel approaches for sensory nerve activity suppression.

3.3 Sodium Channel Blockers

Local anesthetics such as lignocaine and mexiletine, which block sensory neuron voltage-gated Na^+ channels, are effective antitussive agents in humans and animals. Blockade of neuronal voltage-gated Na^+ channels reduces action potential formation in sensory neurons evoked by a variety of stimuli (Adcock et al. 2003;

Carr 2006). However the effects of local anesthetic drugs are typically transient and are limited by acute tachyphylaxis with repeated administration. Insights into the specific sites of action of local anesthetics have been made in the hope of discovering novel anesthetic-like molecules which more effectively inhibit sensory nerve activity. For example, Carr (2006) showed that mexilitine can inhibit action potential formation in guinea pig tracheal touch-sensitive Aδ-fibers (cough receptors) at concentrations that do not block action potential conduction along the sensory nerve axon. This observation would suggest that cough receptor nerve terminals express Na^+ channel characteristics that are different from those along the axons. The observation that the novel local anesthetic RSD931 inhibits RAR-evoked cough in guinea pigs and rabbits via a mechanism that is distinct from that of lidocaine (Adcock et al. 2003) also suggests heterogeneity in Na^+ channels on sensory nerves. Understanding the nature of this heterogeneity could lead to the development of more selective Na^+ channel blockers (perhaps modified local anesthetic-based drugs) for inhibiting sensory-neuron-mediated events, including cough.

In addition to playing a role in the basic generation of action potentials, Na^+ channels are also likely involved in altered sensory neuron excitability in disease. For example, the TTX-resistant Na^+ channel is expressed by specific subsets of sensory nerve subtypes, specifically those displaying the characteristics of capsaicin-sensitive nociceptors (Kwong and Lee 2005). In guinea pigs, neurons that originate in the jugular ganglia and project to the trachea (but not those from the nodose ganglia) specifically express a TTX-resistant Na^+ current (Carr, personal communication). As discussed, this population of nerves consists exclusively of capsaicin-sensitive C-fibers and Aδ-fibers, and is distinct from the nodose derived touch-sensitive Aδ-fibers in this species (Canning et al. 2004a). Capsaicin-sensitive neurons are robustly sensitized by inflammatory mediators such as PGE2, a response that is likely mediated in part by an increase in TTX-resistant Na^+ currents (Kwong and Lee 2005). Physiological studies in guinea pigs have suggested that capsaicin-sensitive nociceptors are likely quiescent in the normal airways, but are recruited during airway inflammation (Mazzone and Canning 2002; Mazzone et al. 2005). Given that nociceptor recruitment is likely to contribute to cough hyperreflexia, selective inhibitors of TTX-resistant Na^+ channels may prove useful cough suppressants in hypertussive states.

3.4 NKCC1 and Chloride Channel Blockers

Furosemide and other loop diuretics reduce cough evoked by citric acid, low-chloride solutions and angiotensin-converting enzyme inhibitors in guinea pigs and humans (Franova 2001; Karlsson et al. 1992; Mazzone and McGovern 2006; Stone et al. 1993; Ventresca et al. 1990), and prevent the activation of laryngeal and tracheobronchial sensory nerves by solutions that are deficient in chloride ions (Fox et al. 1995b; Sant'Ambrogio et al. 1993). This reduction in sensory nerve activity is likely due to inhibition of a neuronally expressed chloride transporter in the

airways (Mazzone and McGovern 2006). The furosemide-sensitive $Na^+/K^+/2Cl^-$ cotransporter (NKCC1) is expressed by the majority of neurons in the vagal sensory ganglia and by the peripheral terminals of touch-sensitive Aδ-fibers in the guinea pig trachea (Mazzone and McGovern 2006). NKCC1 functions to accumulate intracellular chloride ions above the electrochemical equilibrium. Opening of membrane chloride channels results in a depolarizing chloride current that contributes to the activation of sensory fibers by some stimuli. The identity of the chloride channels involved in carrying this depolarizing current has received some attention. Lee et al. (2005) recently showed that bradykinin-evoked depolarization of bronchopulmonary C-fibers is inhibited with niflumic acid, a selective inhibitor of calcium-activated chloride channels. Consistent with this, niflumic acid reduces citric acid evoked cough in guinea pigs, although other chloride channel subtypes may also be involved (Mazzone and McGovern 2006). These findings encourage additional studies to further assess whether compounds selective for either NKCC1 or neuronally expressed chloride channels are likely to be useful inhibitors of specific sensory neuron subtypes in the airways.

3.5 α3 Na^+/K^+ ATPase Inhibitors

Immunohistochemical analyses have revealed that a population of somatic and visceral myelinated mechanosensitive afferent nerve fibers are characterized by the expression of an isozyme of the sodium pump containing the alpha3 subunit (Brouns et al. 2006; Canning et al. 2004b; Dobretsov et al. 2003; Mazzone and McGovern 2006; Yu et al. 2003). In the airways, α3 Na^+/K^+ ATPase is expressed by SARs and RARs in rabbits and rats (Yu et al. 2003), myelinated afferent fibers associated with neuroepithelial bodies in rats (Brouns et al. 2006) and touch-sensitive Aδ-fibers (cough receptors) in guinea pigs (Canning et al. 2004b; Mazzone and McGovern 2006), and is distinctly absent from capsaicin-sensitive C-fibers (Canning et al. 2004b; Mazzone and McGovern 2006). Although the exact role of α3 Na^+/K^+ ATPase is unclear, pharmacological studies suggest an essential role for this isozyme in regulating the excitability of mechanosensory nerves. The Na^+/K^+ ATPase inhibitor ouabain, at doses that are reportedly selective for inhibiting the α3 subunit, selectively inhibits cough receptor activation and coughing evoked by citric acid, mechanical stimulation or electrical stimulation of the tracheal mucosa in anesthetized guinea pigs, but has little effect on C-fiber-dependent reflexes evoked from the trachea (Canning et al. 2004b). This inhibitory effect of ouabain on the cough receptors contrasts with the findings of other studies showing that ouabain enhances the excitability of baroreceptors, SARs in the lung and mechanoreceptors innervating the renal artery (Chapleau et al. 1993; Kopp et al. 1994; Winner et al. 2005). Preliminary electrophysiological studies suggest that unique kinetic properties of the α3 Na^+/K^+ ATPase isozyme may facilitate high-frequency action potential conduction along the cough receptor nerve terminal (Mazzone and Canning, unpublished results). Indeed, functional studies have confirmed that cough

can only be evoked when cough receptors are activated at high firing frequencies (Canning et al. 2004b; Mazzone et al. 2005). Although these data are promising, it is too early at present to speculate whether selective inhibition of $\alpha 3$ Na^+/K^+ ATPase is a viable option for cough suppression.

3.6 Menthol

Menthol, a natural compound extracted from peppermint, eucalyptus and citronella oils, has been used for many years to alleviate itch and pain and cough. However, despite its long therapeutic history, insights into the specific pharmacological actions of menthol have only been described in recent years. Menthol, when applied topically to the skin or inhaled, produces a characteristic cooling sensation owing to an agonist activity at the TRPM8 ion channel (reviewed in Patel et al. 2007; Reid 2005). TRPM8 is a distant relative of the TRPV1 channel (capsaicin receptor), and like TRPV1, TRPM8 is expressed by subsets of sensory nerves that are involved in thermosensation. However, unlike TRPV1, which has a temperature threshold for activation of 43°C and therefore senses moderate heat, TRPM8 is activated by cool temperatures in the 8–28°C range (McKemy et al. 2002). Although TRPM8 is expressed by subsets of sensory neurons in the mouse nodose ganglia (Zhang et al. 2004), very little is known about the specific characteristics of these visceral sensory neurons. Therefore, only inferences can be made at present about how menthol inhibits cough on the basis of data from somatosensory neurons in the DRG.

TRPM8 is generally considered to be expressed in a population of small-diameter DRG neurons that do not express classic nociceptive markers, like TRPV1 (Kobayashi et al. 2005; McKemy et al. 2002, Peier et al. 2002); however, there is controversy in relation to this point. Several studies have provided evidence for TRPM8 expression in, and/or menthol activation of, TRPV1-expressing neurons (Babes et al. 2004; Reid et al. 2002; Xing et al. 2006). This discrepancy warrants some discussion as understanding the sensory neuron populations that express TRPM8 may provide important insights into the mechanisms that underlie how menthol exerts its antipruritic, analgesic, and antitussive effects (see later). Many experiments that have investigated the expression of TRPM8 and TRPV1 in DRG neurons have used neurons that were cultured in the presence of nerve growth factor, which has been shown to induce TRPV1 expression in neurons that do not normally express the capsaicin receptor (Story et al. 2003). In addition, functional studies that show menthol sensitivity in TRPV1-expressing neurons (that have been cultured without nerve growth factor) must take into account that menthol is not selective for TRPM8 and can activate other TRP channels, including TRPV3 (Macpherson et al. 2006). This is of course not conclusive evidence against the coexpression of TRPM8 and TRPV1 in DRG neurons, but rather highlights some of the issues that confound these studies.

Menthol inhibits cough in both animals and humans (Morice et al. 1994; Laude et al. 1994), although how it does so is not readily apparent. Prolonged or repeated

exposure to menthol desensitizes nociceptive responses, including those evoked by subsequent exposure to capsaicin (Cliff and Green 1994; Green and McAuliffe 2000), which may provide a potential peripheral antitussive mechanism if TRPM8 is expressed in neurons responsible for cough (i.e., TRPV1-expressing bronchopulmonary C-fibers). Recent studies have shown that, in addition to activating TRPM8, menthol also inhibits TRPA1 channels (Macpherson et al. 2006). Given that TRPA1 is likely coexpressed with TRPV1 (Story et al. 2003), blocking TRPA1 channels expressed by airway sensory neurons could conceivably reduce the activity of capsaicin-sensitive neurons, in particular the increased activity that occurs during airway inflammation. One study in guinea pigs has showed that menthol can directly relax airway smooth muscle (Wright et al. 1997). This bronchodilator effect could have antitussive effects by reducing RAR activity in response to bronchospastic agents. Indeed, inhaled β-agonists have antitussive effects in humans and animals (Bolser et al. 1995; Horiuchi et al. 1995). Alternatively, menthol may inhibit cough via central mechanisms that do not involve the suppression of primary afferent nerve activity. Consistent with this, menthol-induced suppression of capsaicin evoked pain (when both agents are applied to the skin) has been shown to be mediated via specific glutamatergic mechanisms in the spinal cord, rather than via a peripheral mechanism (Proudfoot et al. 2006). In this scenario, input from TRPM8-expressing neurons reduces spinal synaptic transmission of noxious sensory input from TRPV1-expressing neurons. This is of course consistent with the data showing that different primary afferent nerve subtypes express TRPM8 and TRPV1. Finally, the pleasant cooling sensation of inhaled menthol may simply soothe the airways, reducing the urge to cough via psychotropic mechanisms.

4 Concluding Remarks

There is an obvious utility in identifying chemical mediators (and their mode of action) that activate, sensitize or inhibit the airway sensory nerves involved in coughing as understanding these processes will undoubtedly pave the way forward for the design of new therapeutic strategies for the treatment of cough disorders. Although our current knowledge in this area is rapidly progressing, there is still much work to be done. In reality we presently know relatively little about the transduction processes involved in converting cough stimuli into sensory nerve action potentials and therefore, at this stage, suggesting viable targets for cough suppression is difficult. Nevertheless the progress made thus far is promising and ongoing research in this exciting field should lead to new therapeutic discoveries in the future.

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