Respiratory Physiology & Neurobiology 226 (2016) 115–120

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Multiple neural circuits mediating airway sensations: Recent advances in the neurobiology of the urge-to-cough

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ARTICLE INFO

Article history:
Received 10 August 2015
Received in revised form
30 September 2015
Accepted 30 September 2015
Available online 9 October 2015

Keywords:
Somatosensory
Viscerosensory
Anterograde
Herpes virus
fMRI

ABSTRACT

The respiratory system is densely innervated by sensory neurons arising from the jugular (superior) and nodose (inferior) vagal ganglia. However, a distinction exists between jugular and nodose neurons as these ganglia developmentally originate from the neural crest and the epibranchial placodes, respectively. This different embryological origin underpins an important source of heterogeneity in vagal afferent biology, and may extend to include fundamentally different central neural circuits that are in receipt of jugular versus nodose afferent inputs. Indeed, recent studies using viral tract tracing and human brain imaging support the notion that airway sensors contribute inputs to multiple central circuits. Understanding the neural pathways arising from the airways and lungs may provide novel insights into aberrant sensations, such as the urge-to-cough, characteristic of respiratory disease.

1. Introduction

The respiratory tree and lung parenchyma are densely innervated by heterogeneous populations of sensory receptors that respond to a wide variety of chemical and/or mechanical stimuli. When activated, these sensory receptors initiate autonomic reflexes and complex behavioral responses that are critical for the ongoing physiological control of respiratory function as well as for protecting against potentially damaging stimuli that could adversely affect ventilation. It is widely acknowledged that the majority of these sensory receptors are vagal in origin, and accordingly their cell bodies are located in either the jugular (superior) or nodose (inferior) vagal ganglia (Canning et al., 2004; Undem et al., 2004; McGovern et al., 2012a). However, it is often unappreciated that these two ganglia are derived from distinct embryological origins, with neural crest and epibranchial placodal derived cells forming the jugular and nodose ganglia during development, respectively (D'Autréaux et al., 2011). In essence, these distinct embryologically-derived neuron populations are a fundamental source of airway afferent heterogeneity inasmuch as they represent both the somatic (jugular) and visceral (nodose) divisions of the nervous system. This is reflected in the molecular expression profiles and physiological attributes of the constituent sensory neurons (Chuaychoo et al., 2006; Kwong et al., 2008; D'Autréaux et al., 2011; McGovern et al., 2015a). Surprisingly, there has been little recognition that such a fundamental difference in airway afferent phenotype might have important implications for airway sensory biology. Furthermore, the visceral/somatic split of airway afferents could be a factor influencing aberrant airway sensations, and constitutes a possible explanation for the expression of morbidity in respiratory disease. In this mini review of our presentation at the *2014 Oxford Breathing Meeting* we summarize some of the recent animal and human studies that our group has conducted investigating vagal sensory processing pathways and their relationships to the ganglionic distinctions in airway afferents, observations that we believe are providing new and exciting insights into the complex biology of airway sensations.

2. Characterization of airway afferents—updating an old paradigm

Several detailed reviews have described the characteristics of airway afferents (Mazzone, 2005; Canning and Spina, 2009; Mazzone and Undem, 2009), and this section highlights the critical points relevant for this treatise. Vagal airway sensory neurons have classically been discriminated based on their functional characteristics, and as such two classes of sensory receptors can be

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http://dx.doi.org/10.1016/j.resp.2015.09.017
1569-9048/

differentiated on the basis of their physiological and pharmacological properties (Mazzone, 2005; Canning and Spina, 2009; Mazzone et al., 2009). The first of these are the mechanoreceptors, which consist of neurons that display limited sensitivity to a wide variety of chemical mediators but are exquisitely responsive to stretch, touch or other mechanical forces that can occur within airway wall or lung parenchyma. As a class of airway afferents, mechanoreceptors can be further subdivided into three functionally unique subgroups; rapidly adapting receptors (RARs), slowly adapting receptors (SARs) and touch sensitive receptors, depending on their functional activation profile (Yu, 2000; Canning et al., 2004). Regardless of the specific functional subtype, mechanoreceptive afferents are all myelinated, non-peptidergic neurons that conduct action potentials in the Aδ or Aβ range (Ricco et al., 1996; Chuaychoo et al., 2005). By contrast, airway chemoreceptors (also frequently termed nociceptors) are typically considered to be small diameter, unmyelinated (C-fiber) peptidergic neurons that are characteristically sensitive to the Transient Receptor Potential Vanilloid 1 (TRPV1) receptor agonist capsaicin (Undem et al., 2004). In many species there are additional populations of chemoreceptors, some of which are C-fibers that are not capsaicin sensitive (Kollarik et al., 2003) and others that are C- or Aδ fibers that respond to capsaicin but are non-peptidergic (Ricco et al., 1996; Undem et al., 2004).

This classical approach to categorizing airway afferents provides meaningful insights into afferent physiology. However, this purely functional classification scheme ignores the ganglionic origin of the afferents (Fig. 1). Indeed, although airway mechanoreceptors are thought to be derived exclusively from the nodose ganglia (thus representing a class of visceral afferents in the airways) (Ricco et al., 1996), both visceral and somatic chemoreceptors (i.e., originating from both the nodose and jugular ganglia) innervate the airways and lungs (Undem et al., 2004; McGovern et al., 2015a). Recognizing this distinction is not simply a matter of semantics. Nodose and jugular afferents have quite distinct termination patterns within the airways as well as displaying functional response profiles and molecular phenotypes that relate to their embryological origin (Fig. 1). For example, nodose afferents heavily innervate the intrapulmonary airways and lungs, whereas jugular afferents are more restricted to the larger extrapulmonary airway tree (McGovern et al., 2015a). Furthermore, nodose afferents respond to purinergic receptor P2X ligands and generally lack neuropeptide expression (even in the chemoreceptors), whereas jugular afferents do not demonstrate functional P2X ligand responses but express significant amounts of substance P and CGRP (Undem et al., 2004; Kwong et al., 2008). Importantly, in vivo functional studies support the notion that the reflexes and behaviors initiated by nodose and jugular chemoreceptors are also distinct (Chou et al., 2008), further highlighting the value of incorporating this additional level of discrimination into the broadly accepted classification based on functional characteristics.

3. Novel neurotropic viruses shed light on central circuit organisation

The notion that the distinct subsets of airway afferents underpin different functional behaviors points to the existence of multiple neural processing circuits within the CNS. Our group has been particularly interested in understanding the central representation of airway sensation, and we have used novel viral tools for in vivo circuit mapping in animal models as one approach to investigate these questions. In 2012 we reported the development and application of a modified recombinant of the Herpes Simplex Virus 1 Strain H129 (HSV-1H129), a neurotropic virus that had previously been reported to migrate transynaptically in the anterograde direction through the nervous system (McGovern et al., 2012b). Our addition of a green fluorescent protein reporter to the virus, did not alter replication, nor influence transynaptic movement or anterograde motility, but greatly facilitated the visualization of virally-infected neurons in the brain. Insights into the synaptic organization of neural circuits were possible by collecting tissues at multiple time points after viral inoculation to follow the spread of viral infection and accordingly we made use of this tool to report the central neural circuits labeled following injection of the virus into the tracheal lumen (McGovern et al., 2012a; McGovern et al., 2012b). Our findings from these initial studies confirmed that tracheal afferent neurons synapse with second order neurons in the caudal (predominately dorsolateral subnucleus) of the nucleus of the solitary tract (Sol) but additionally highlighted a second termination site within the paratrigeminal nucleus (Pa5) (Fig. 2). The Sol has been extensively studied with respect to vagal afferent biology and has well described roles in the integration of airway afferent inputs (Mazzone and Canning, 2002; Zoccal et al., 2014). By contrast, although vagal inputs to the Pa5 have been previously reported to be involved in baroreceptor control of cardiac function (Junior et al., 2004), a role for the Pa5 in respiratory afferent processing has not been investigated in any detail. Rather, the Pa5 is better known as a key relay nucleus for somatic nociception and pain sensation (Lapa and Watanabe, 2005). Our study further showed that the higher order projections from tracheal afferents (presumably relayed via the Sol and Pa5) terminate broadly throughout the medulla (including the rostral ventrolateral medulla and medullary reticular formation), pons (Kölliker fuse, locus coeruleus and parabrachial nuclei), hypothalamus (paraventricular and lateral nuclei), subthalamus (principally in the zona incerta), thalamus (notably in the ventrobasal, mediodorsal and submedius nuclei), central amygdala and ultimately in the primary and secondary sensory cortices, cingulate, insula and lateral orbital cortices (McGovern et al., 2012a; McGovern et al., 2012b). The pattern of infection over time was suggestive of multiple parallel ascending circuits, although this was difficult to resolve at the time using the techniques employed.

The possibility that the Sol and Pa5 may have different roles in airway sensation is intriguing, especially given their widely acknowledged roles in visceral (Sol) and somatic (Pa5) afferent integration. It seemed reasonable to speculate that these two brainstem integration nuclei may in turn relay primary afferent inputs into distinct ascending circuits. To determine if this was true, our group conducted a neuroanatomical tracing study designed to dissect the Sol higher order projections from those of the Pa5 (McGovern et al., 2015b). This study was made possible by further modifications of the HSV-1H129 viral tracer in order to develop a Cre-conditional version of the green fluorescent virus (HSV1-H129$_{floxed}$) (McGovern et al., 2015b). Thus, in the absence of Cre recombinase, the viral recombinant drives the expression of a green fluorescent protein in infected cells but in the presence of Cre the EGFP expression cassette is excised from the virus and an otherwise silent red (tdTomato) cassette becomes functionally active. In other words, the virus switches from expressing green to a red fluorescence in Cre expressing cells (McGovern et al., 2015b). By first injecting an AAV-Cre expression vector into either the Sol or Pa5, we were able to restrict Cre expression to one or other of the brainstem primary afferent integration nuclei, such that the subsequent delivery of HSV1-H129$_{floxed}$ to the tracheal lumen would enable the circuitry arising from the Cre expressing neurons in the Sol or Pa5 to be distinguished (McGovern et al., 2015b). The study showed clear evidence for anatomical divergence of airway afferent pathways in the CNS, relating to the origin of the primary afferent relays in the Sol or Pa5 (Fig. 2). For example, the Sol specific circuitry included connectivity with the lateral parabrachial nucleus (LPB), locus coeruleus, paraventricular nucleus, lateral hypothalamus, amyg-