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REVIEW

The nervous system of airways and its remodeling in inflammatory lung diseases

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Abstract Inflammatory lung diseases are associated with bronchospasm, cough, dyspnea and airway hyperreactivity. The majority of these symptoms cannot be primarily explained by immune cell infiltration. Evidence has been provided that vagal efferent and afferent neurons play a pivotal role in this regard. Their functions can be altered by inflammatory mediators that induce long-lasting changes in vagal nerve activity and gene expression in both peripheral and central neurons, providing new targets for treatment of pulmonary inflammatory diseases.

Keywords Allergic asthma · Chronic obstructive lung disease (COPD) · Peripheral sensitization · Central sensitization · Transient receptor potential ankyrin 1 (TRPA1) · Transient receptor potential vanilloid 1 (TRPV1) · Vagal ganglia · Nucleus tractus solitarii (NTS)

Introduction

Millions of people suffer from acute and chronic inflammatory respiratory diseases, such as viral and bacterial infections, allergic asthma and chronic obstructive pulmonary disease (COPD). These diseases are accompanied, independent of their trigger, by dyspnea, cough and bronchospasm and, at least in asthma, with an increased sensitivity of the bronchi to various environmental stimuli, the so-called airway

hyperreactivity (AHR). The mechanisms that lead to these symptoms are incompletely understood. In the last decades, the detailed encoding of inflammatory responses was the focus of the majority of studies; however, inflammation alone does not explain most relevant clinical aspects of these inflammatory diseases. The aim of this review is to provide an overview of the crucial role of the pulmonary nervourun s system in the development of symptoms in inflammatory airway diseases.

Innervation of the airways

The nervous system of the airways includes afferent (=sensory) neurons as well as efferent parasympathetic and sympathetic nerve fibers. Afferent neurons are functionally connected with efferent neurons via the brainstem. In addition to effector functions of afferent neurons by neuropeptide release, these connections provide the basis for a neuronal-mediated response after stimulation of afferents by central reflex activity, resulting in efferent reactions such as bronchospasm and an enhanced mucus secretion.

Parasympathetic innervation of the airways

Retrograde tracing studies have demonstrated that preganglionic parasympathetic vagal neurons projecting to neurons innervating the tracheal wall are located in the ambiguus nucleus of the medulla (Haxhiu et al. 1993) and those of intrapulmonary airways are mainly located in the ambiguus nuclei but also, to a lesser extent, in the dorsal motor nucleus of the vagus nerve (DMV) (Hadziefendic and Haxhiu 1999). All nerve fibers travel with the vagus nerve to intramural ganglia where they project to secondary (postganglionic) neurons and distribute centrally generated information to effector cells

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within the airways (Fig. 1). Postganglionic cholinergic neurons represent the predominant bronchoconstrictor pathway in the airways by acetylcholine (ACh) release (Barnes 1992). ACh induces smooth muscle contraction via muscarinic receptors located on the surface of smooth muscle cells. In addition, these neurons also increase secretion from exocrine glands and secretory cells of the epithelium. Nitric oxide (NO) and vasoactive intestinal peptide (VIP) have also been described as transmitters of parasympathetic neurons. In contrast to cholinergic postganglionic neurons, the non-cholinergic postganglionic neurons are located in the myenteric plexus of the esophagus or closely associated with the outer striated longitudinal muscle layers (Fischer et al. 1998; Balentova et al. 2013). Neurons containing VIP or NO, or the combination of both, elicit airway smooth muscle relaxation (Bai and Bramley 1993; Matsuzaki et al. 1980; Zhu and Dey 2001).

Sympathetic innervation of the airways

The primary cell bodies of sympathetic neurons are located in the intermediolateral and intercalate nuclei of the thoracic spinal cord. Their function is influenced by the hypothalamus, brainstem and the reticular formation (Fig. 2). Preganglionic sympathetic neurons project to postganglionic neurons located in paravertebral sympathetic chain ganglia. The upper thoracic ganglion is fused with the inferior cervical ganglion and forms the stellate ganglion. Postganglionic sympathetic neurons innervating the lung are derived from the stellate and the thoracic sympathetic chain ganglia T2–T4, whereas those supplying the trachea are derived from the stellate and the superior cervical ganglia (Kummer et al. 1992). The typical mediator of postganglionic sympathetic neurons is the catecholamine noradrenaline. Co-localization of the catecholamine synthesizing enzyme tyrosine hydroxylase (TH) and neuropeptide Y (NPY) has been demonstrated (Kummer et al. 1992) but NPY is also expressed in a non-adrenergic subpopulation of neurons containing VIP and originating in the stellate ganglia (Kummer et al. 1992; Bowden and Gibbins 1992). A small percentage of non-adrenergic neurons also express the enzyme for NO synthesis, the NO synthase (Fischer et al. 1996a, b). Sympathetic nerve fibers are located in the vicinity of arteries, arterioles, submucosal glands and the airway smooth muscle (Uddman et al. 1984; Partanen et al. 1982;

Fig. 1 Parasympathetic innervation of the airways. *AN* ambiguus nucleus, *DMV* dorsal motor nucleus of vagus nerve
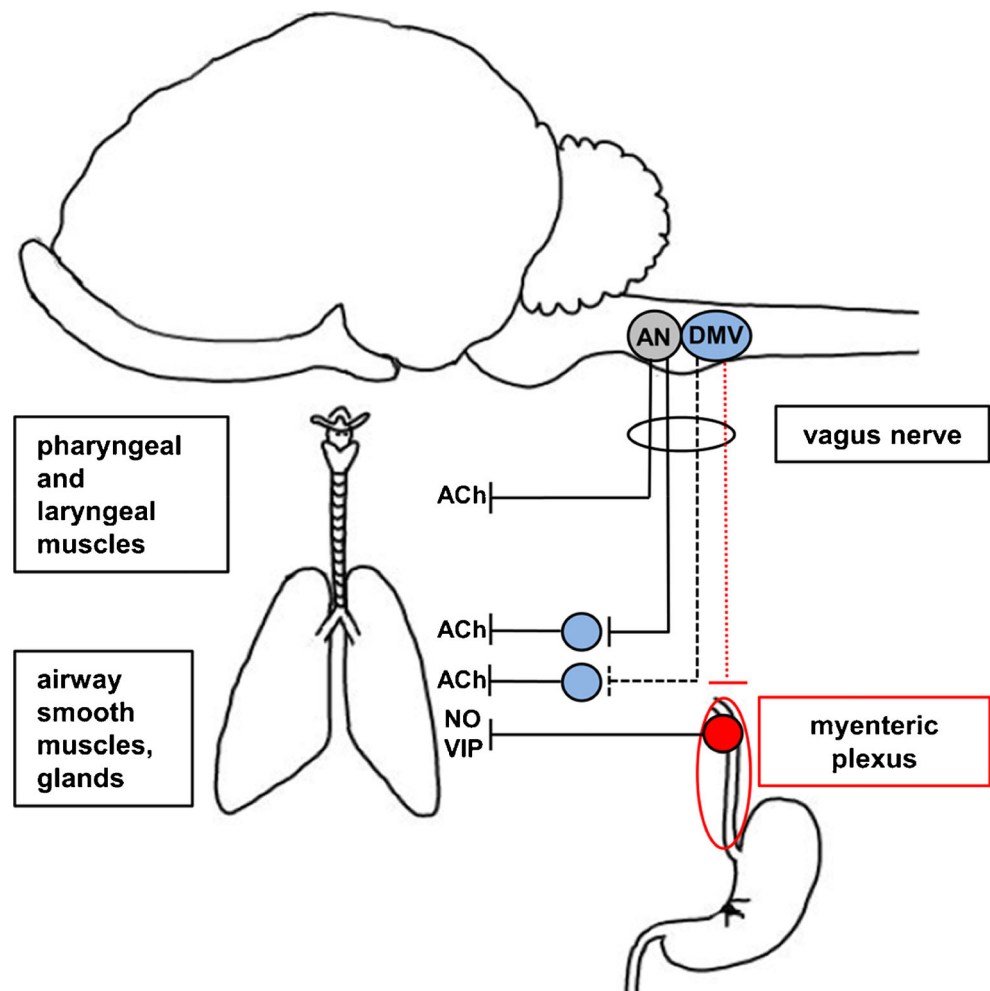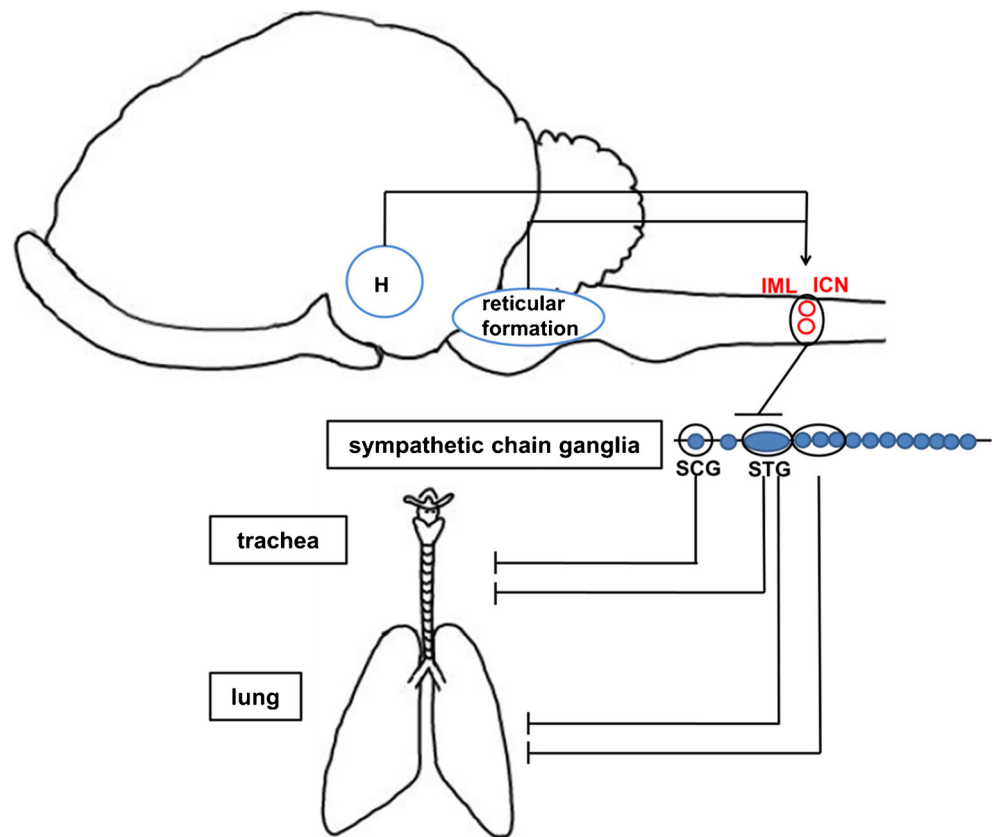
Fig. 2 Sympathetic innervation of the airways. *SCG* superior cervical ganglion, *STG* stellate ganglion, *H* hypothalamus, *IML* intermediolateral nucleus, *IC* intercalate nucleus

Pack and Richardson 1984). Stimulation of the cervical sympathetic trunk elicits relaxation in explanted guinea pig tracheas (Canning and Undem 1993). In vivo, surgical sympathectomy not only negatively affects lung function but is also associated with an enhanced response to methacholine inhalation (Du Kim et al. 2009). Sympathetic neurons are therefore considered to have bronchodilator functions.

Sensory innervation of the airways

The airways are innervated by different subpopulations of sensory neurons. Their cell bodies are located mainly in sensory vagal ganglia as has been shown by comparative studies in different mammals (Düring and Andres 1988) and, to a lesser extent, in dorsal root ganglia (DRG). The vagal sensory ganglion can be differentiated in a jugular and nodose ganglion. The nodose ganglion is derived from epibranchial placodes, whereas the origin of the jugular ganglion and the DRGs is the neural crest. In the last decades, different nomenclatures for different subclasses of neurons have been introduced based on their physiological and pharmacological properties, their transmitters and their conduction velocity. Based on their conduction velocity, afferent neurons can be subdivided into A- and C-fibers and the so-called cough receptors.

A-fibers

The A-fiber population consists of myelinated non-peptidergic mechanoreceptors and conduct action potentials in the Aδ and Aβ range (14–32 m/s). Nerve terminals are associated with airway smooth muscle cells (Krauhs 1984) and neuroepithelial bodies (NEBs) within the airway epithelium. Fibers innervating both targets express similar receptors and transporters, such as the ATP receptor P2X(3), Na(+)/K(+) -ATPase alpha3, vesicular glutamate transporter 1 (VGLUT1) and VGLUT2 (Brouns 2006) and channels that may underlie direct sensing and transduction of mechanical stimuli, e.g., the two-pore domain K(+) channel TRAAK (Lembrechts 2011). A-fibers can be further subdivided into slowly adapting stretch receptors (SARs) and rapidly adapting stretch receptors (RARs) based on their adaption to sustained lung inflation. SARs mediate the Hering–Breuer reflex that terminates inspiration after an adequate inflation of the lung (Luck 1970), whereas the activity of RARs increases with the rate and volume of lung inflation and/or deflation (Pack and DeLaney 1983). In addition, Widdicombe (1954a, b) described a third subtype, the so-called "cough receptor" that has a conduction velocity of 4–6 m/s, is not activated by lung inflation and is located in the extrapulmonary airways (Fig. 3). A-fibers are activated by stretch, touch and other mechanical forces but exhibit a limited sensitivity to a wide variety of
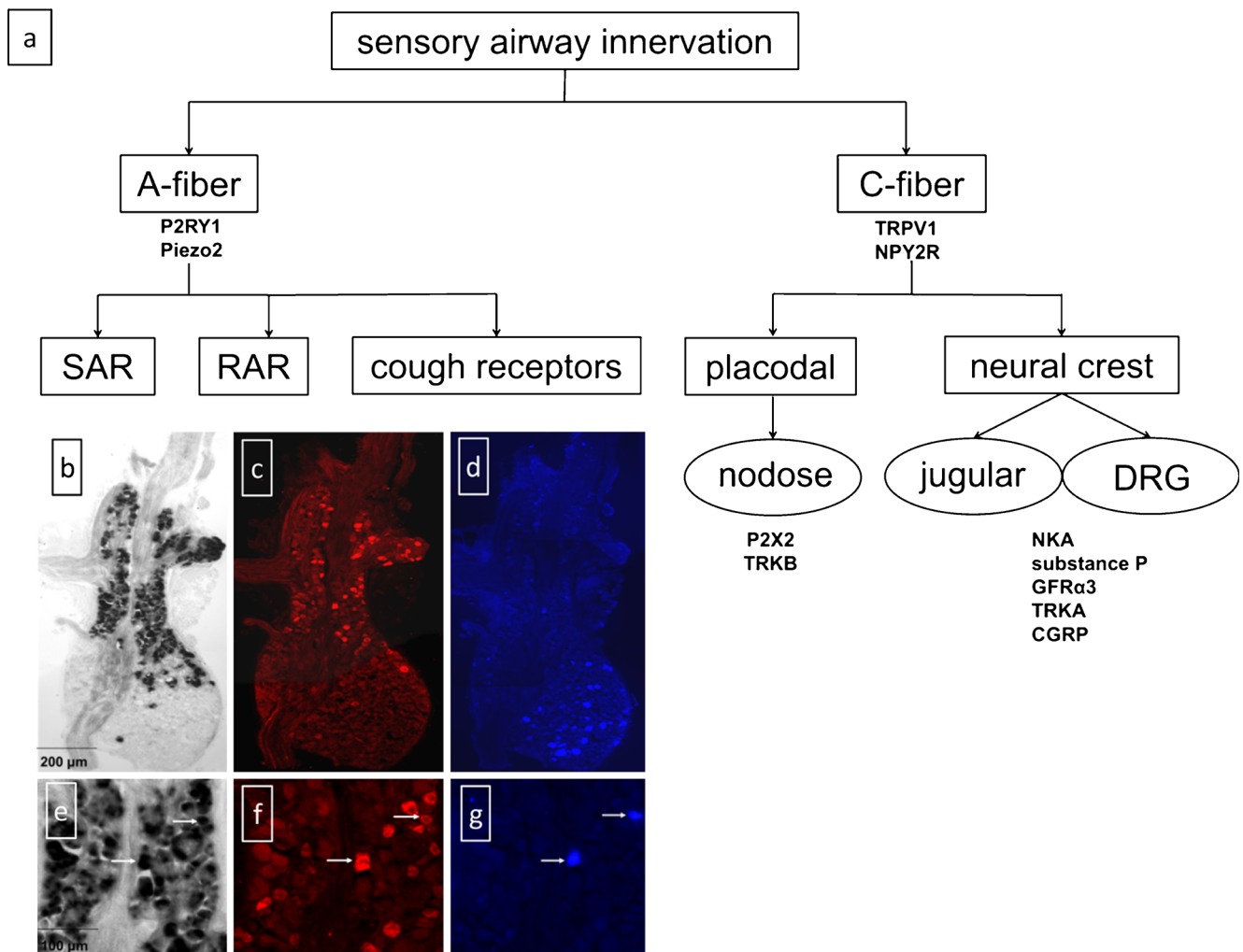
Fig. 3 **a** Schematic overview of the different subpopulations of sensory airway neurons. *SAR* slowly adapting stretch receptors, *RAR* rapidly adapting stretch receptors. **b–g** GFRα3 expression was evaluated by immunohistochemistry in lung-labeled vagal ganglia of Wnt1Cre/R26R mice. **b–d** Overview of a section from a whole jugular nodose ganglion; **e–g** higher magnification from the jugular ganglion. **b**, **e** X-Gal staining; **c**, **f** GFRα3 immunoreactivity; **d**, **g** airway-innervating neurons indicated by aminostilbamidine-labeling. GFRα3 expression was selectively expressed in neural crest-derived (X-Gal+) neurons. *Arrowheads* indicate lung-labeled GFRα3+ neural-crest derived neurons. This image was taken from Nassenstein et al. (2010)

chemical mediators (Ricco et al. 1996). Interestingly, RARs can be activated by several stimuli released during airway inflammation, such as leukotrienes, thromboxane, histamine and neurokinins (Karla et al. 1992; Schelegle et al. 2000; Sano et al. 1992). In addition, methacholine, an agonist for muscarinic ACh receptors, frequently used for bronchial provocation tests to diagnose an AHR in patients with asthma, can also activate RARs. However, it is still under debate if this is a direct effect on RARs, since some responses have instead been described to depend on intact C-fibers (Schelegle et al. 2000) or on the presence of bronchoconstriction (Karla et al. 1992). The precise cellular mechanisms of A-fiber activation by mechanical stimulation have not yet been defined; however, in recent studies, it has been shown that, in comparison to C-fibers, A-fibers express purinergic receptors of the P2RY1

subtype and Piezo-2 (Chang et al. 2015), the latter being a mechanically activated cation channel (Coste et al. 2010).

C-fibers

In comparison to A-fibers, bronchopulmonary C-fibers are slow-conducting unmyelinated neurons that can be activated by physical, chemical and thermal stimuli, such as capsaicin, bradykinin, hyperosmolar fluids, cold air and cigarette smoke. In comparison to nodose C-fibers, neural crest-derived jugular and DRG neurons express neuropeptides, such as the tachykinin substance P (SP), which is synthesized from the precursor preprotachykinin A (PPT-A) (Nassenstein et al. 2010) and calcitonin gene-related peptide (CGRP). Release of neuropeptides upon stimulation of peripheral nerve

endings in the airways induces the so-called "neurogenic inflammation" (Barnes 1991). However, their effects on airway smooth muscle contraction, bronchial edema and mucus secretion are controversial and may be species-dependent (Joos et al. 1994; Szarek et al. 1995).

Even though the transient receptor potential vanilloid 1 (TRPV1), the receptor for capsaicin, is expressed in the vast majority of C-fibers, placodal and neural crest-derived C-fibers show marked differences in their expression of, e.g., purinergic and neurotrophic factor receptors, thus indicating that they respond to different endogenous signals (Fig. 3). P2X2, a purinergic receptor, is selectively expressed in C-fibers from the placodes but absent in neural crest-derived C-fibers. TrkA, the high-affinity receptor for nerve growth factor (NGF) and glial cell-derived neurotrophic factor (GDNF) family receptor alpha 3 (GFRα3), a co-receptor for artemin, are expressed in neural crest C-fibers, whereas TrkB, the high-affinity receptor for the brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) are predominantly expressed in placodal C-fibers (Nassenstein et al. 2010). Recently, Chang et al. (2015) identified that NPY2R receptor, a G-protein coupled receptor (GPCR), is also specifically expressed in a subpopulation of C-fibers. In this study, activation of NPY2R-expressing neurons is associated with rapid and shallow breathing; however, the embryological origin of NPY2R-expressing neurons has not yet been investigated.

Lung diseases: definition, symptoms and pathogenesis

Allergic asthma

Allergic asthma is an inflammatory disease of the airways that is associated with many symptoms, including a reversible bronchoconstriction, dyspnea and cough and is generally associated with AHR. In the last decade, many different cell types have been discussed to play a role in the pathogenesis of this disease, especially various inflammatory cells. However, immune cells alone are not able to evoke the symptoms that mostly require central reflex activity. Studies in which children bearing a high risk to develop asthma have been treated before the onset of first symptoms with anti-inflammatory corticosteroids have clearly demonstrated that corticosteroids failed to prevent the development of asthma (Guilbert et al. 2006). In addition, bronchial biopsies from patients with allergic asthma often show no signs of inflammation despite the presence of symptoms.

COPD

COPD is a common preventable and treatable disease characterized by a persistent airflow limitation, which is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Symptoms of COPD include dyspnea, chronic cough and enhanced sputum production [definition according to the Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2016. Available from: http://goldcopd.org/.] The spectrum of symptoms indicates that alterations of the pulmonary nervous system might be involved in the pathogenesis of COPD.

Infections and exacerbations

Most respiratory infections of the lower airways are due to viral and bacterial infections. Symptoms, including cough and dyspnea, occur transiently and resolve either spontaneously or with adequate treatment. In patients suffering from asthma or COPD, however, these infections can lead to exacerbations that require intense treatment and hospitalization. First evidence has been provided that virus infections contribute to long-lasting changes in the pulmonary nervous system (Colasurdo et al. 1998; Piedimonte et al. 2004).

From sensory nerve fiber activation to symptoms

Mechanisms of peripheral nerve fiber activation

Activation of peripheral neurons results in neuropeptide release and, therefore, leads to neurogenic inflammation but also conveys the signals to the central nervous system (CNS) thus leading to enhanced central reflex activity. This dual function of sensory neurons can be associated with the activation of specific receptors located at the nerve terminals, e.g., receptors of the transient receptor potential (TRP) channel family and purinergic receptors. These receptors are constitutively expressed in the terminals of neurons but their expression and activation can be modified by different pathways, e.g., due to respiratory inflammation.

TRPV1

The first receptor of the TRP channel family was described in 1989 in *Drosophila* (Montell and Rubin 1989). Meanwhile, this family has been subdivided into seven subfamilies based on their structural and functional similarities (Pedersen et al. 2005). All TRP channels are ion channels with a high permeability for cations. Several members respond to mechanical, thermal and chemical stimuli (Pedersen et al. 2005; Moran et al. 2004). TRPV1, a member of the vanilloid family of TRP channels, was shown almost 20 years ago to be activated by various stimuli causing pain, including capsaicin, heat and low pH. The

expression of TRPV1 in neurons is almost limited to sensory C-fibers and has been extensively described in vagal C-fibers innervating the airways (Lee and Pisarri 2001). TRPV1 activation is associated with calcium influx and depolarization leading to neurogenic inflammation by release of tachykinins and CGRP, an enhanced action potential discharge and central reflex activity (Caterina et al. 1997; Kollarik and Undem 2006). Meanwhile, it has been shown that TRPV1 can be activated by diverse exogenous and endogenous agonists, such as anandamide, 12-hydroperoxy-eicosatetraenoic acid (12-HPETE), N-arachidonoyl-dopamine (NADA), piperine (ingredient of black pepper), camphor, heat (temperatures higher than 43 °C), voltage, NO and sweeteners (Riera et al. 2007; Jara-Oseguera et al. 2008). Interestingly, TRPV1 sensitization is often associated with GPCRs, which can be activated by pro-inflammatory agents, e.g., histamine, prostaglandins and ATP (Tominaga et al. 2001), leading to phospholipase C (PLC)- or protein kinase A (PKA)-dependent phosphorylation and consecutively to a sensitization of TRPV1. Another mechanism causing an enhanced response to TRPV1 activaton is the translocation of preformed TRPV1 to the cell membrane as has been demonstrated after NGF treatment (Stein et al. 2006).

TRPA1

The non-selective cation channel transient receptor potential ankyrin 1 (TRPA1) is a chemosensitive receptor that is co-expressed with TRPV1 on airway-innervating C-fibers (Nassenstein et al. 2008). TRPA1 is activated by covalent modifications of the cysteine residues within the N-terminal (Hinman et al. 2006) by a number of noxious exogenous chemical stimuli including nicotine (Talavera et al. 2009), acrolein (Bautista et al. 2006), ozon, menthol, PF-4840154 (Ryckmans et al. 2011), cinnamaldehyde (Bandell et al. 2004), formalin, tear gases, allyl isothiocyanate (mustard oil), gingerol and eugenol. In addition, TRPA1 serves as a sensor for cold temperatures and environmental irritants (Karashima et al. 2009). TRPA1 is also regarded as a receptor for the early detection of bacterial lipopolysaccharide (LPS) independent from Toll-like receptor (TLR4), even before the onset of an immune response (Meseguer et al. 2014). In addition, TRPA1 is activated by several endogenous stimuli, such as reactive oxygen species and oxidized lipids, indicating that TRPA1 could also be a sensor for oxidative stress. Its activation has been associated with enhanced respiratory reflex activity (Nassenstein et al. 2008), pain, neurogenic inflammation and hyperalgesia (Nassini et al. 2014). Similar to TRPV1, TRPA1 function can be modulated via PLC and/or PKA (Dai et al. 2007; Wang et al. 2008).

Purinergic receptors

Sensory nerve terminals express purinergic receptors of the P2 receptor subfamily. P2 receptors can be subdivided into metabotropic GPCRs, including P2Y, P2U, P2T and P2D receptors and ionotropic receptors, such as P2X and P2Z receptors, which form a non-selective ligand-gated ion channel (Fredholm et al. 1994). Several members of the P2X family, such as P2X3, P2X2/3, P2X4 and P2X7 and members of the P2Y subfamily, e.g., P2Y12, have been suggested to play an important role in neuropathic and inflammatory pain (Burnstock and Kennedy 2011). P2X receptors possess two transmembrane domains with a large extracellular loop and intracellularly located N- and C-termini (North 2002). The structure of the C-terminus is responsible for the specific properties of the different P2X subunits. P2X receptors are activated by binding of ATP but also of α,β-methylene ATP and $2',3'$-O-(benzoyl-4-benzoyl)-ATP (BzATP), to the extracellular loop. This causes a structural change and, consecutively, cation influx and depolarization of the cell (Kawate et al. 2011). Purinergic receptors are located in nociceptive neurons located in DRG and vagal sensory neurons (Gerevich and Illes 2004; Ford 2012). A functional interaction between purinergic receptors and TRPV1 as well as purinergic receptors and neuropeptides released after TRPV1 activation has been suggested (Park et al. 2010; Gerevich and Illes 2004). Interestingly, P2X2, forming a heterodimer with P2X3, is restricted to neurons derived from the placodes, whereas P2X3 is expressed in both placodal neurons and neural crest-derived neurons (Nassenstein et al. 2010; Kwong et al. 2008). Besides ATP, purinergic receptors can be stimulated by reactive oxygen species, e.g., H_2O_2 and OH (Ruan et al. 2005; Gerevich and Illes 2004).

Muscarinic receptors

ACh is the typical agonist of all muscarinic receptors and is released by, e.g., pre- and postganglionic parasympathetic neurons and preganglionic sympathetic neurons. An increased cholinergic activity, known to be associated with COPD and asthma, results in activation of muscarinic receptors and plays a key role in increased airway resistance (Zaagsma et al. 1997; Fryer and Jacoby 1998; Barnes 1993). Muscarinic receptors can be subdivided into 5 subtypes (M1R–M5R), all of which possess 7 transmembrane domains and belong to GPCRs. They differ markedly in their downstream signal cascade. M1R, M3R and M5R are Gq/11 protein-coupled and lead to calcium increase in a phospholipase C-dependent pathway. In contrast, M2R and M4R are Gi protein-coupled and can open potassium channels. M2R activation can alternatively lead to inhibition of adenylate cyclase and thus to a reduction of cAMP levels (Caulfield 1993). The M1R, M2R and M3R subtypes have been detected within the airways of different

species (Fryer and El-Fakahany 1990; Garssen et al. 1993; Barnes 1993). Cholinergic constriction of murine peripheral airways is mediated by the concerted action of the M2 and M3 receptor subtypes (Struckmann et al. 2003). M2R is expressed in the airway smooth muscle and in parasympathetic postganglionic neurons. M3R is preferentially expressed in the airway smooth muscle. However, there has so far been no evidence that M3R function is altered in asthmatic patients exhibiting an AHR to cholinergic agonists. Thus, airway smooth muscle taken from patients with asthma and stimulated in vitro with cholinergic agonists showed a similar contraction compared to that of healthy people (Whicker et al. 1988; Black 1991).

Neurotrophic factor receptors

Expression of receptors for different neurotrophic factors have been demonstrated preferentially in vagal C-fibers. In particular, the effects of neurotrophins and the GDNF family ligands on neuronal functions in inflammatory airway diseases have been extensively studied. The family of neurotrophins consists in NGF, BDNF, neurotrophin-3 (NT-3) and NT-4. Their signals are mediated via the low-affinity receptor p75NTR, which can form heterodimers with the high-affinity TRK receptors. Neurotrophins are expressed, e.g., in the airway epithelium and infiltrating immune cells and are secreted in enhanced levels after induction of an allergic airway inflammation (Virchow et al. 1998; Braun et al. 1998, 1999). The GDNF family comprises GDNF, neurturin, artemin and persephin. They are ligands of the GDNF co-receptors GFRα1-4, which then can bind to RET, a receptor tyrosine kinase. In previous studies, it has been shown that GDNF is expressed in airway epithelial cells and is upregulated in a mouse model of allergic airway inflammation (Lieu et al. 2012), whereas neurturin is predominantly expressed in human immune cells (Vargas-Leal et al. 2005) and seems to suppress both airway inflammation and AHR (Michel et al. 2011). Receptors for neurotrophins and the GDNF family are expressed in vagal sensory neurons. Nodose as well as jugular C-fibers express p75NTR and RET. However, depending on the phenotype, the distribution of Trk and GFRα receptors is heterogeneously distributed. Thus, TrkB is predominantly expressed in nodose C-fibers, whereas TrkA and GFRα3 are expressed preferentially in jugular C-fibers (Nassenstein et al. 2010).

Symptoms of inflammatory airway diseases and their neuronal circuitry

AHR

Allergic asthma is the only inflammatory disease of the airways that is generally accompanied by AHR, even though the diagnosis of AHR is not absolutely specific for asthma

(Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2016. Available from: www. ginasthma.org). Thus, AHR is also present in, e.g., some patients suffering from COPD, allergic rhinitis and non-inflammatory pulmonary diseases such as cystic fibrosis. Symptoms occurring after inhalation of cigarette smoke, cold air, ozone, chlorine and several other irritants including epithelial, microbial and inflammatory triggers are attributed to AHR. AHR is commonly diagnosed based on bronchial provocation tests to methacholine, histamine and exercise but also to eucapnic voluntary hyperventilation or inhaled mannitol. The different irritants evoking symptoms in daily life and the different provocation tests in the clinical assessment of AHR point toward the unsolved question about the pathogenesis of AHR. Thus, there is still discussion concerning which cell types are primarily activated by this wide variety of stimuli and which cell population represents the main effector cell type. Since nerve endings of sensory neurons possess specific receptors for irritants and inflammatory mediators that were associated with AHR and are able to elicit all relevant symptoms experienced by patients, such as bronchospasm, dyspnea and cough via peripheral and central mechanisms, the aim is to first describe the pathogenesis of these symptoms followed by a description of related alterations that might be associated with AHR and pulmonary inflammation by itself.

Bronchospasm

Airway caliber and thus resistance to airflow is primarily mediated via the parasympathetic nervous system (Widdicombe and Nadel 1963; Coleridge et al. 1989; Kesler and Canning 1999). In all mammalian species, the baseline tone of the airway smooth muscle is primarily mediated by cholinergic neurons as indicated by a marked dilatation after treatment with muscarinic receptor antagonists, ganglionic blockade and vagotomy (Coleridge et al. 1989; Mills and Widdicombe 1970; Holtzman et al. 1980; Jammes and Mei 1979). However, cholinergic activity is closely associated with afferent nerve activity in intrapulmonary airways, mainly the activity of RARs (Kesler and Canning 1999). This observation raises the question whether bronchospasm is mechanistically "just" an enhanced baseline tone of the airways. Indeed, evidence has been provided that activation of RARs contributes to bronchospasm. Thus, bronchospasm still occurs in response to inhaled histamine after capsaicin-induced neuropeptide depletion (Forsberg et al. 1988). However, since capsaicin can also induce bronchospasm (Forsberg et al. 1988) and TRPV1, its receptor, is mainly expressed in sensory C-fibers, it has been proposed that neuronal cell populations other than RARs are involved in the onset of bronchospasm (Fig. 4). C-fibers may contribute via two distinct mechanisms First, neuropeptides, such as SP, neurokinins and CGRP are released from peripheral nerve endings located in the airway wall. Neuropeptides

Fig. 4 Neural mechanisms leading to bronchospasm. *AN* ambiguus nucleus, *DMV* dorsal motor nucleus of vagus nerve, *NTS* nucleus tractus solitarii, *RAR* rapidly adapting stretch receptors
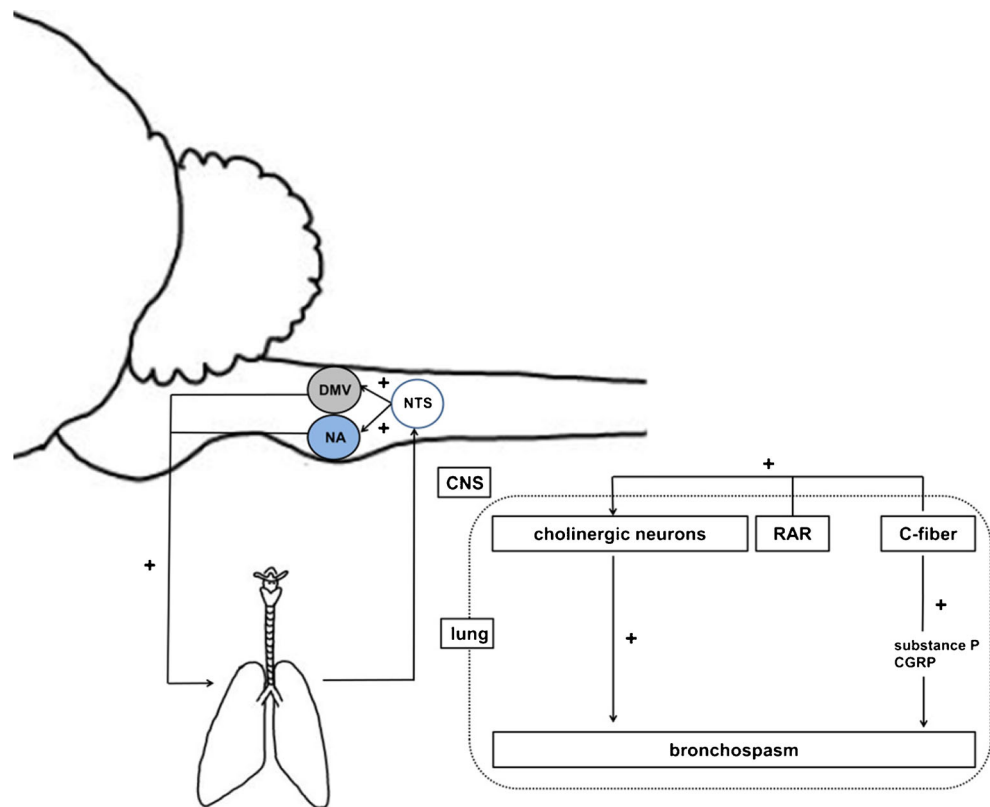
can induce airway smooth muscle contraction via neurokinin receptors 1–3 in different species (Corboz et al. 2010; Schelfhout et al. 2009) and in addition contribute to airway obstruction via plasma extravasation, an increase in local blood flow in the airway mucosa and stimulation of mucus secretion (Khansaheb et al. 2011; Martling et al. 1988; Lundberg et al. 1991). Second, they can initiate bronchospasm by enhancing central reflex activity that consecutively increases cholinergic outflow. Bronchoconstrictive signals detected by airway afferents are transmitted predominantly to cell bodies of airway-related vagal preganglionic neurons (AVPNs) located in the most rostral part of the DMV and the rostral nucleus ambiguus region via the nucleus of the solitary tract (NTS) (Hadziefendic and Haxhiu 1999; Kc et al. 2004). AVPN neurons, which are cholinergic and co-express VIP (Kc et al. 2004), convey the central information through descending fibers, which in airway intramural ganglia synapse to cholinergic postganglionic parasympathetic neurons. These neurons finally end at the neuromuscular junction at the airway smooth muscle or, alternatively, at submucosal glands and local blood vessels. It is not yet clear whether individual AVPNs provide parallel innervation to those different target tissues within the airways. Airway smooth muscle contraction and an increased mucus production occur after release of ACh. However, parasympathetic non-cholinergic neurons containing VIP and NO have also been shown to innervate the airways. These neurons exhibit contrary functions

compared to cholinergic neurons and relax airway smooth muscle cells (Matsuzaki et al. 1980; Zhu and Dey 2001). Neuronal cell bodies are located within the myenteric plexus of the esophagus or are closely associated with the outer striated longitudinal muscle layers of the esophagus (Canning et al. 1996; Fischer et al. 1998). However, the origin of their inputs is not yet clear. Chemical stimulation of the rostral nucleus ambiguus had no significant effect on precontracted airway smooth muscle tone after muscarinic receptor blockade (Kc et al. 2004) and stimulation of the DMV also remained uneffective on airway smooth muscle tone (Haselton et al. 1992). Thus, it is still being discussed whether the major influence is provided by local sensory excitatory factors or intrinsic communication pathways (Canning et al. 2002; Dey 2003).

Cough induction/cough reflex

Cough is a defensive reflex that is initiated to preserve the air conductance in the extra- and intrathoracic airways and the gas exchange in the alveoli while cleaning the airways from diverse irritants, secretions and inhaled particulate matter. Acute cough, often associated with acute viral or bacterial airway infection, is typically transient (<3 weeks duration) and generally self-limited (Dicpinigaitis 2015). In contrast, the etiology of chronic cough (>8 weeks duration) often remains unidentified. Thus, an underlying pulmonary or nonpulmonary disease, such as

asthma, cystic fibrosis and bronchiectasis or gastroesophageal reflux disease, was involved in about half the patients exhibiting chronic cough (Chamberlain et al. 2015). However, almost all patients had in common that treatment effects with over-the-counter drugs were very limited or completely absent (Chamberlain et al. 2015).

Cough can be initiated in humans by distinct irritants, such as cigarette smoke, bradykinin, capsaicin, prostanoids, acids, particulate matter, hyper- and hypotonic solutions and mechanical probing of the laryngeal, tracheal and large bronchial mucosa (Widdicombe 1954b; Gravenstein et al. 1954; Lalloo et al. 1995; Choudry et al. 1989; Nishino et al. 1996; Karlsson and Fuller 1999). Recently, the existence of different "disease-specific neurophenotypes" has been discussed because of the observation that patients suffering from different respiratory conditions exhibit a divergent profile of tussive responses to cigarette smoke, capsaicin and prostaglandin E2 (Belvisi et al. 2016) indicating different underlying mechanisms. However, the nature of these different phenotypes has not yet been defined. Coughing can be completely abolished by vagotomy or vagal cooling, respectively, abolishing activity of myelinated neurons while preserving C-fiber activity (Widdicombe 1954b; Klassen et al. 1951; Tatar et al. 1988; Canning et al. 2004) and is diminished by anesthesia, abolishing predominantly C-fiber activity while preserving activity of myelinated neurons (Canning et al. 2004). This indicates that different nerve fiber subtypes are involved in coughing, each of which possesses an individual spectrum of mediators or conditions initiating cough. In comparison to vagal C-fiber subtypes and cough fibers that are suggested to contribute to initiate cough, pulmonary stretch receptors are not directly activated by tussive components but may indirectly contribute to cough. They can alter autonomic neuronal activity of airways and lungs (Canning et al. 2001; Richardson et al. 1984), influence respiratory rate and inspiratory volume, the latter being associated with the peak expiratory flow and expired volume during cough (Smith et al. 2012). Nerve fibers involved in coughing travel via the superior laryngeal nerve to cell bodies located in the jugular ganglia, or the recurrent nerve to cell bodies in the nodose ganglia, respectively and project to second-order neurons located in the NTS of the medulla oblongata (Canning et al. 2004; Hewitt et al. 2016; Muroi et al. 2013; Mazzone and Canning 2002; McGovern et al. 2015b; Driessen et al. 2015) (Fig. 5). Myelinated and unmyelinated lung afferents project bilaterally but with an ipsilateral predominance, to second-order neurons located in the ventrolateral subnucleus (probably receiving input from SARs; Kalia and Richter 1985) and in the rostral, medial and the dorsal commissural subnucleus of the NTS as has been shown in cats (Kubin et al. 1991), dogs, ferrets, rats (Haxhiu and Loewy 1996) and guinea pigs (Mazzone and Canning 2002). Interestingly, the possibility has been suggested that a single nerve fiber may give several terminal branches that terminate in different NTS subnuclei (Bellingham and Lipski 1992). Efferents from NTS neurons are connected to the lateral parabrachial nucleus, locus coeruleus, paraventricular nucleus, lateral hypothalamus, amygdala, zona incerta and the mediodorsal thalamus (McGovern et al. 2015b). Many of these are components involved in autonomic neural pathways (Saper 2002).

In addition to the NTS, a second termination site was demonstrated by retrograde tracing techniques. Thus, a small number of airway innervating neurons projects to the dorsolateral portion of the spinal trigeminal nucleus and the cervical dorsal horn in dogs, ferrets and rats (Haxhiu and Loewy 1996). Recent studies using a refined viral tracing strategy in guinea pigs defined a nucleus lateral to the spinal trigeminal tract of the medulla and medial to the spinocerebellar tracts at the level of the spinal trigeminal nucleus as a second termination site of afferent tracheal neurons, the paratrigeminal nucleus (Pa5) (McGovern et al. 2015b). Pa5 receives primary afferent sensory fibers of vagal neurons, especially from peptidergic C-fibers whose cell bodies are located in the jugular ganglion (McGovern et al. 2015a; Driessen et al. 2015), as well as trigeminal and glossopharyngeal neurons. It has been suggested to play a differential modulatory role in the central processing of mechanical and chemical nociceptive information (Koepp et al. 2006). Efferents from Pa5 neurons labeled by tracheal viral application projected not only to the ventral posteromedial thalamic nucleus but furthermore to the submedius thalamic nuclei and the medullary as well as the pontine trigeminal nuclei, which are considered to be important for nociceptive sensation (Zhao et al. 2006). The precise roles of NTS and Pa5 neurons in cough remain to be investigated. After sensing irritants in the airways and subsequent activation of NTS and Pa5 neurons, respectively, urge-to-cough is induced and a complex processing is initiated to coordinate cough motor mechanisms. First evidence from guinea pigs argues against a major role of Pa5 at least in mediating cough motor mechanisms, since electrical laryngeal stimulation led to respiratory slowing and eventual apnea but not to coughing (Driessen et al. 2015). However, since the Pa5 shows a strong connectivity to the somatosensory thalamus, it might play a major role in higher brain regulations.

Urge-to-cough The term urge-to-cough describes the desire to cough after sensing airway irritation and involves higher brain regulation (Fig. 5). In humans, inhalation of capsaicin in doses below threshold levels to evoke cough induced signal changes in the anterior midcingulate cortex, the supplementary motor cortex (probably both involved in actively suppressing sensory-driven events like cough; Kase et al. 1984; Haggard and Whitford 2004) and the primary somatosensory cortex as observed in fMRI scans that correlated with the experienced urge-to-cough. A moderate correlation was also
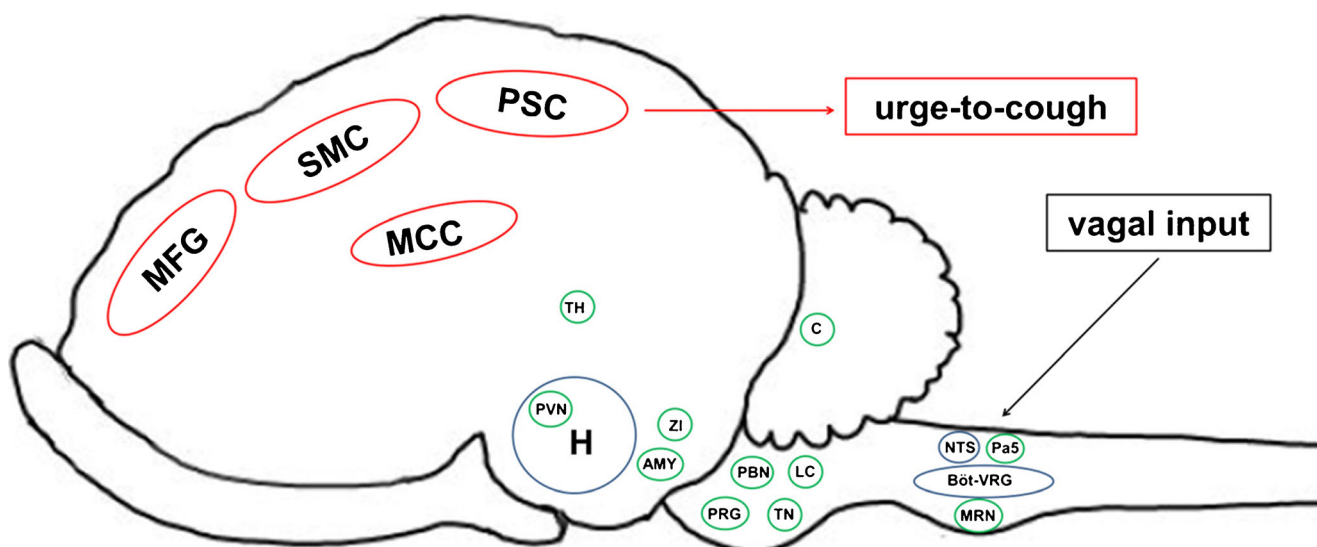
Fig. 5 Schematic overview of the localization of centers involved in mediating cough and urge-to-cough. *MFG* middle frontal gyrus, *PSC* primary somatosensory cortex, *SMC* supplementary motor cortex, *MCC* midcingulate gyrus, *NTS* nucleus tractus solitarii, *Pa5* paratrigeminal nucleus, *Th* thalamus, *ZI* zona incerta, *AMY* amygdala, *HYP* hypothalamus, *PVN* paraventricular nucleus, *TN* trigeminal nucleus, *LC* locus coeruleus, *PBN*, parabrachial nucleus, *C* cerebellum, *MRN*, medullary raphe nuclei, *PRG* Pontine Respiratory Group

found between urge ratings and signal changes in the middle frontal gyrus and left cerebellum (Mazzone et al. 2007). Even though the precise roles of some of these activated regions remain unclear, a similar cortical activation pattern was reported in association with control of continence and micturition, which involve visceral and/or somatosensory inputs (Lotze et al. 2001; Kavia et al. 2005), suggesting that these centers are involved in sensing irritation resulting in urge and a subsequent modification in the behavioral responses.

Cough motor mechanism Cough motor mechanisms include primary and secondary motor mechanisms. Primary cough motor mechanisms are responsible for the "four-phase cough motor pattern", which consists of an inspiratory phase characterized by enhanced contraction of the diaphragm and the abductor muscles of the larynx, a compressive phase, which is defined by closure of the glottis due to activation of the laryngeal adductor muscles and contraction of expiratory muscles, the expulsive phase resulting from a sudden opening of the glottis while the expiratory muscle contraction is continued and, finally, the cessation phase characterized by activation of laryngeal adductor muscles. Secondary motor mechanisms include all mechanisms that enhance the defensive function of cough (such as bronchoconstriction and mucus secretion), mechanisms that oppose the mechanical stress of cough (e.g., the esophageal sphincter and the perineal muscles), changes in breathing pattern and cardiovascular changes (e.g., changes in cardiac output, blood pressure and heart rate) (Fontana and Lavorini 2006). Cough motor mechanisms have been attributed to sensory neurons projecting via the NTS to neurons in the ventrolateral medulla (Bötzinger, Pre-Bötzinger and rostral Ventral Respiratory Group) (Fig. 5). Ventrolateral

medullary neurons are essential for generating the basic respiratory rhythm neurons (Smith et al. 2007; Rybak et al. 2004) and, in addition, they play a major role in regulation of motor activity of the diaphragm and the intercostal, abdominal and laryngeal muscles (Shannon et al. 1998, 2000). Ventrolateral medullary neurons are connected to neural networks located in the medulla, pons and cerebellum. In the medulla, the caudal medullary raphe nuclei transmit and modulate sensory information that consecutively influences breathing, respiratory drive and respiratory pattern (Shannon et al. 2004b; Poliacek et al. 2014; Segers et al. 1985). In the pons, neurons of the rostral dorsal lateral pons and pons/mescencephalic junction, summarized as the Pontine Respiratory Group, are reciprocally connected to the ventrolateral medullary neurons (Ezure and Tanaka 2006; Smith et al. 1989). These neurons display a characteristic tonic discharge pattern during eupneic respiration that is strongly altered during cough (Shannon et al. 2004a). The precise mechanism that the cerebellum participates in cough is still unknown. First evidence demonstrating that cough frequency was modulated by the rostral interposed nucleus of the cerebellum was published almost 20 years ago by Xu et al. (1997) in cats. Capsaicin aerosol challenge also shows an activation in the cerebellar region in humans (Mazzone et al. 2007; Farrell et al. 2012).

Dyspnea

Many diseases, including inflammatory airway diseases such as asthma, COPD and airways infection, are associated with dyspnea. According to the current definition provided by the American Thoracic Society (1999), dyspnea is "a subjective experience of breathing discomfort that consists of

qualitatively distinct sensations that vary in intensity". From the patient's view, dyspnea comprises chest tightness, enhanced breathing effort, an unrewarded inspiration, rapid breathing and, finally, urge-to-breath ("air hunger") (Scano et al. 2005). Studying the cellular and molecular mechanisms underlying dyspnea is limited by the fact that an accurate measurement of dyspnea in animal experiments is impossible, as is the precise assessment of neuronal airway activity in humans. Nevertheless, multiple pathways in concert seem to be necessary to explain the different descriptors of dyspnea, which is associated with enhanced activation of the muscle spindles in muscles of the chest wall (kinesthetic information about inspiratory muscle placement), Golgi tendon organs (tension development) and changes in airway flow due to altered respired volume and flow. The latter leads to an enhanced vagal activity (Kelsen et al. 1981), mainly characterized by indirect activation of SARs and direct activation of RARs but less by C-fiber activation (Taguchi et al. 1991). This triggers a reflex that increases respiratory motor activity (Shannon and Zechman 1972) mediated by the motor cortex, and simultaneously induces a central corollary discharge of cortical interneurons projecting to the sensory cortex thereby conveying the information of an enhanced voluntary effort to increase ventilation (Scano et al. 2005) and possibly leading to awareness of dyspnea by sensing the increased breathing effort (Fig. 6a). However, breathing effort and air hunger seem to be two qualitatively different sensations. The perceived breathing effort is dependent on changes in ventilation and involves corollary discharge from the cortical motor center, whereas urge-to-breath is independent from respiratory muscle activity (Banzett et al. 1990) but dependent on hypercapnia-associated central (e.g., within the medullary raphe, Richerson 2004; NTS, Dean et al. 1990; retrotrapezoid nucleus, Li and Nattie 1997; and locus coeruleus, Oyamada et al. 1998) or peripheral (mainly the carotid body; Smith et al. 2006), chemoreceptor activation (Demediuk et al. 1992) or medullary corollary discharge. Activation of medullary neurons may subsequently affect the cortico-limbic circuitry, namely the amygdala, anterior cingulate and insular cortices and therefore might contribute to discomfort and anxiety experienced during dyspnea (Binks et al. 2014; Chen et al. 1991; Evans et al. 2002; Peiffer et al. 2001) (Fig. 6b).

Inflammation and neuroplasticity

Inflammation causes plasticity and sensitization in peripheral nerve fibers innervating the airways

Peripheral sensitization in autonomic neurons is characterized by an enhanced transmitter release from postganglionic neurons, thus increasing their effector functions. In sensory neurons, inflammation causes structural changes characterized by an increase in the density of tachykinin-containing nerve fibers, e.g., by enhanced NGF release (Hoyle et al. 1998; Braun et al. 1998). The increased density of tachykinin-containing nerve fibers is associated with an enhanced release of tachykinins during airway inflammation (Nieber et al. 1992) and might be, at least partly, due to a de novo synthesis in sensory nerve fibers, which under non-inflammatory conditions lack neuropeptide expression (Fischer et al. 1996a, b). However, the pathophysiological relevance of tachykinin release is still inconclusive in humans as seen by non-satisfactory outcomes in clinical studies employing NK1 and NK2 receptor antagonists (Fahy et al. 1995; Boot et al. 2007). The impact of CGRP-dependent signaling has not yet been investigated in clinical studies.

Airway inflammation also leads to an overt activation of nerve terminals in the airways. This so-called peripheral sensitization is associated with an enhanced action potential discharge in their respective ganglia and/or supraordinate brain regions. In the following, we aim to give examples of how peripheral neurons can be sensitized by inflammation.

TRPV1 Inhalation of capsaicin in mice suffering from an allergic airway inflammation leads to marked changes in the respiratory breathing pattern compared to healthy controls that are considered to be equivalent with an urge-to-cough in other species. Considerable evidence has been provided that sensitization of sensory nerve terminals is induced by neurotrophin-dependent pathways in allergic asthma. Thus, overexpression of NGF in the airways is associated with an enhanced sensitivity to inhaled capsaicin in vivo (Hoyle et al. 1998; Path et al. 2002) and inhibition of p75NTR or signaling via their high-affinity neurotrophin receptors TRK is able to abrogate these effects (Kerzel et al. 2003; Nassenstein et al. 2006, 2007). Neurotrophin-induced sensitization can be explained by increases in TRPV1 membrane insertion mediated by a phosphatidylinositol 3-kinase/Src kinase-dependent pathway (Zhang et al. 2005). This pathway is similarly also involved in interleukin (IL)-6 mediated TRPV1 upregulation (Fang et al. 2015). In addition, inflammatory mediators like tumor necrosis factor-α and prostaglandin metabolites have been shown to cause TRPV1 translocation to the cell membrane (Shibata et al. 2016; Meng et al. 2016) or are able to regulate the excitability of TRPV1, as demonstrated for serotonin (5HT) (Salzer et al. 2016), artemin, neurturin and GDNF (Malin et al. 2006). A recent study indicated that human rhinovirus infection, which is often associated with asthma exacerbations, has a direct effect on TRPV1 expression in a human neural cell line (Abdullah et al. 2014). This effect is mimicked by application of soluble factors released within 2–4 h after neuronal infection and contains enhanced NGF, IL-6 and IL-8 levels. These data indicate that TRPV1 can also be regulated by auto- and/or paracrine mechanisms and does not necessarily involve the immune system. Similarly,
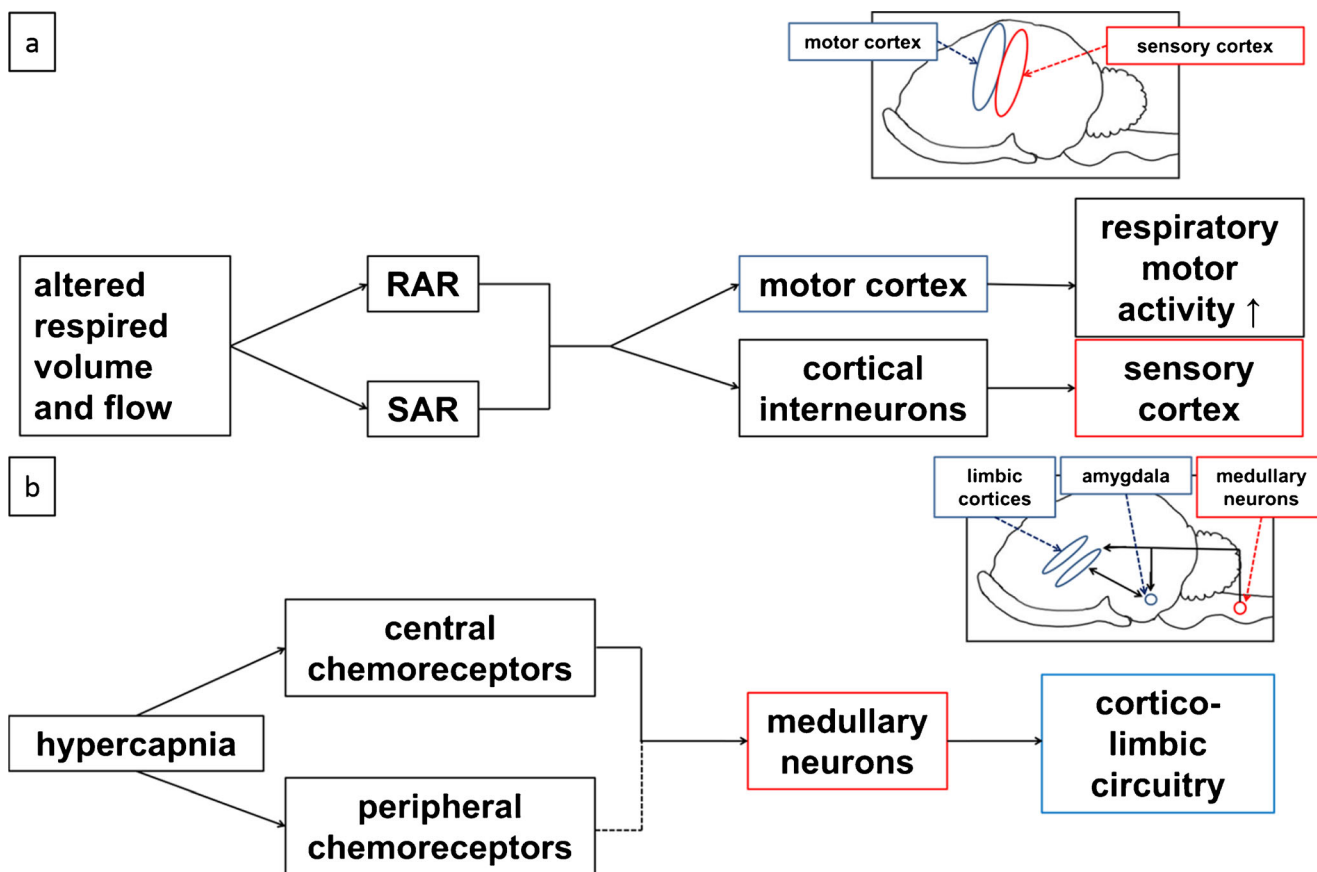
Fig. 6 **a** Concept about neuronal mechanisms involved in an enhanced breathing effort and **b** urge-to-cough

parainfluenza-3 infection associated with a hypertussive state leads to a marked upregulation of TRPV1 in jugular C-fiber neurons as well as a de novo synthesis of this channel in Aδ cough receptors that were associated with an enhanced BDNF and artemin expression in the tracheal wall (Zaccone et al. 2016). Inhibition of TRPV1 expression therefore seems to be an interesting target for pharmaceutical interventions in asthma- and infection-related symptoms, even though a study in mice claimed that it is not TRPV1 receptors by themselves but sensory C-fibers expressing TRPV1 that are important for the pathogenesis of asthma (Trankner et al. 2014). However, in humans, it has been shown that different TRPV1 single nucleotide polymorphisms may enhance susceptibility to cough in current smokers and in subjects with a history of workplace exposures (Smit et al. 2012), whereas other polymorphisms lower the risk of current wheezing and cough (Cantero-Recasens et al. 2010). A first series of clinical studies targeting TRPV1 was performed about a decade ago but did not result in an approval from the FDA. TRPV1 antagonists had severe side effects resulting in hyperthermia (Gavva et al. 2008), which is most likely due to the central and peripheral expression of TRPV1. TRPV1 antagonists that are currently investigated are well tolerated, mostly applied topically and now their efficiency is being tested in clinical studies

for a variety of diseases that are associated with C-fiber activation, such as pruritus (Gibson et al. 2014), nasal hyperreactivity (Holland et al. 2014), refractory chronic cough (Khalid et al. 2014) and pain perception (Chizh et al. 2007).

TRPA1 It is still under discussion whether peripheral sensitization is associated with an altered expression and/or sensitization of TRPA1. Due to its activation xprofile, it is reasonable to predict the latter, since TRPA1 activation can explain a wide variety of symptoms in patients suffering from asthma or COPD and might also play a crucial role in infection-induced exacerbations. TRPA1 expression had been identified in bronchopulmonary vagal C-fibers (Nassenstein et al. 2008) and first evidence for a role in asthma was provided shortly afterwards by Caceres et al. (2009). In this study, it was demonstrated that AHR as well as airway inflammation were markedly reduced both after genetic ablation of TRPA1 or after treatment with a TRPA1 antagonist. Even though TRPA1 represents, in our opinion, one of the most promising targets for pharmaceutical interventions in inflammatory airway diseases, there has not to our knowledge been any proof so far for a dysregulation of TRPA1 in peripheral nerve fibers innervating the airways. Indirect evidence that TRPA1 might be upregulated in neuronal cells during airway

inflammation was provided by Abullah et al. (2014) who demonstrated that TRPA1 expression is upregulated in a neuroblastoma cell line that has been infected with the human rhinovirus HRV-16. Since this effect can be mimicked by incubation of the cell line with a supernatant from infected cells that contain high levels of NGF, IL-1β, IL-6 and IL-8 (Abdullah et al. 2014), it has been hypothesized that this effect is indirectly mediated due to virus-induced release of inflammatory mediators similar to those released in inflamed airways in vivo. That inflammatory mediators are generally able to potentiate TRPA1 currents in primary vagal C-fibers has been demonstrated in studies investigating murine esophagus innervating vagal afferents and DRG neurons. Specific adenosine A2A receptor agonists potentiated TRPA1 currents evoked by TRPA1 agonists (Brozmanova et al. 2016), whereas artemin contributes to an enhanced TRPA1 expression and concomitant enhanced calcium influx in DRG neurons in vitro and an enhanced mechanical and heat hyperalgesia in vivo, by an unidentified intracellular pathway (Ikeda-Miyagawa et al. 2015; Elitt et al. 2006). A combination of NGF, GDNF and NT-3 was also able to upregulate TRPA1 in cultured human DRG neurons (Anand et al. 2008). In addition, in a model of eosinophilic esophagitis, a prolonged antigen challenge also induced an enhanced calcium influx and increased current densities in response to TRPA1 agonists in nodose and jugular neurons (Liu et al. 2015). In an IL-13-transgenic mouse model of atopic dermatitis, TRPA1 was upregulated in skin-innervating afferents, a finding that could also be demonstrated in afferents taken from lesional skin biopsies from patients with atopic dermatitis (Oh et al. 2013).

Purinergic receptors Activation of purinergic receptors, especially that of the ionotropic P2X receptors in lung-innervating sensory afferents, is associated with an action potential discharge in the vagal ganglia complex (Pelleg and Hurt 1996; Undem et al. 2004). This effect has gained widespread attention since in vivo studies have clearly demonstrated that this response is associated with cough. Thus, exposure to ATP can increase the number of coughs elicited by citric acid (Kamei et al. 2005), indicating that ATP has a modulatory function by enhancing cough sensitivity. The effects of ATP are transduced preferentially by activation of P2X2/3 receptors. Nebulized ATP induced more dyspnea and cough in patients with COPD, who also showed enhanced ATP levels in the exhaled breath condensate compared with healthy subjects (Basoglu et al. 2015). In addition, ATP levels are also increased in patients with asthma, probably due to a reduced extracellular ectonucleotidase activity that inactivates ATP (Burnstock 2012; Ford 2012). Interestingly, evidence has been provided that ATP is released due to bronchoconstriction and thus may lead to a mechanical activation of nodose C-fibers (Weigand et al. 2012). Antagonization of P2X3 receptors is currently being tested in clinical studies about chronic cough.

First evidence demonstrates that this approach significantly reduces cough (Abdulqawi et al. 2015). However, it still remains unclear whether purinergic receptors are altered during peripheral sensitization in airway neurons or whether changes in receptor activities are mainly due to enhanced extracellular ATP levels. P2X3 receptor function is facilitated by CGRP, NGF and BDNF due to phosphorylation of N- or C-terminal P2X3 domains (Fabbretti and Nistri 2012).

Muscarinic receptors Within the airways, ACh released from parasympathetic postganglionic neurons is thought to be a major bronchoconstrictor via binding to muscarinic receptors in the airway smooth muscle. Release of ACh is predominantly mediated by an enhanced activity of preganglionic parasympathetic neurons; however, evidence has also been provided that ACh release can be modulated at the level of the postganglionic parasympathetic neuron. Thus, an in vitro antigen challenge in ovalbumin-sensitized guinea pigs leads to degranulation of mast cells surrounding airway parasympathetic ganglia and consecutively to an enhanced depolarization (Myers et al. 1991). Similarly, NGF, CGRP and prostaglandins, which are released after allergen exposure, also induce an enhanced excitability of parasympathetic ganglia neurons (Hazari et al. 2007; Kajekar et al. 2003; Kajekar and Myers 2008). This contributes to increases in ACh release and consecutively in an increased airway smooth muscle tone in the lower airways. Another mechanism that might contribute to an enhanced ACh release from parasympathetic neurons is M2R dysfunction. M2R activation exerts an inhibitory effect on ACh release, thereby limiting M3R-mediated contraction of the airway smooth muscle. Eosinophils, which are increased during allergic airway inflammation, can lead to a dysfunction of M2R and therefore to a loss of its inhibitory function, which is interestingly associated with an exaggerated vagal reflex activity and AHR (Adamko et al. 1999). Interestingly, AHR has turned out to be mediated by the parasympathetic neurons and not by a change in the responsiveness of airway smooth muscle, since bronchoconstriction induced by intravenous ACh was not different among the groups (Adamko et al. 1999). The main question that arises from these data and has yet not been solved, is why patients with asthma show an enhanced bronchoconstriction and central reflex activity upon inhalation of muscarinic receptor agonists, a test that is routinely performed to assess AHR in patients with asthma. From the study published by Adamko et al. (1999), the conclusion can be drawn that airway smooth muscle responsiveness is unaltered in allergic asthma, a finding that is supported by studies employing human airway smooth muscle (Whicker et al. 1988; Black 1991). Interestingly, Tränkner et al. (2014) clearly showed that AHR in response to muscarinic agonists could not any longer be demonstrated in the absence of vagal C-fibers. These findings together raise the question if activation of muscarinic

receptors expressed in C-fibers might explain a positive clinical AHR test that is routinely performed by lung function measurement after inhalation of increasing doses of methacholine. Data on muscarinic receptor expression in airway innervating vagal neurons are very limited. Thus, in our experiments, only a small percentage of airway-innervating nodose C-fibers express M2R, M3R or M4R (Fig. 7). The presence of a low percentage of M3R positive C-fibers is in line with the data published by Weigand et al. (2012). However, in this latter study, methacholine-induced action potential discharge in vagal ganglia neurons was attributed to bronchoconstriction-dependent mechanical stimulation of C-fibers, whereas our study provides clear evidence that C-fibers can be directly activated by methacholine. This is indicated by calcium imaging analysis in dissociated vagal neurons. Thus, about 12 % of nodose C-fibers and about 6 % of jugular C-fibers show an increase in intracellular calcium after muscarin stimulation (Fig. 7). Also, a synergistic action of direct muscarinic receptor activation and an indirect mechanism due to bronchoconstriction-mediated mechanical activation of sensory C-fibers has to be discussed.

Inflammation causes central sensitization

Despite the fact that central mechanisms have been extensively studied in the somatosensory system, e.g., explaining allodynia (Treede et al. 1992; Woolf 2007), the understanding about central alterations in the viscerosensory system leading to cough, dyspnea and an enhanced bronchospasm is still incomplete. First electrophysiological evidence that central sensitization contributes to visceral hypersensitivity in humans was described by Sarkar et al. (2001) in the esophagus. If and how sensitization of second-order neurons of airway innervating neurons occur during inflammation is relatively unexplored. Thus, it has been shown that NTS neurons in a non-human primate model of allergic asthma undergo intrinsic increases in excitability (Chen et al. 2001). In rats, allergen exposure increased the neural firing response to intratracheal capsaicin in NTS neurons and induced endocannabinoid expression (Spaziano et al. 2015). In addition, exposure to antigen aerosol after sensitization increased c-fos in NTS neurons and glial fibrillary acidic protein (GFAP) expression in their surrounding glia cells (Chen et al. 2008). These effects are reversed by corticosteroid treatment, indicating that they are induced by inflammation (Chen et al. 2008). How peripheral inflammation alters NTS neurons in the brainstem is still unclear. Different mechanisms have been suggested, including crossing of proinflammatory cytokines across the blood–brain barrier by specific transporters or via circumventricular organs. In addition, indirect mechanisms by which proinflammatory mediators stimulate endothelial cells of the blood–brain barrier that give rise to secondary paracrine signals may contribute to inflammation-induced alterations, or, last but not least, that inflammatory mediators activate peripheral neurons that then project to second-order neurons and may release altered levels of transmitters or neuropeptides (Hale et al. 2012). However, the exact mechanisms have to be elucidated in further studies.
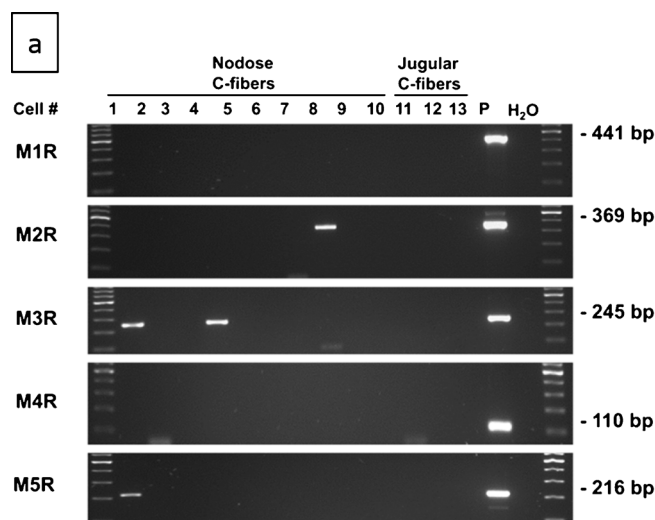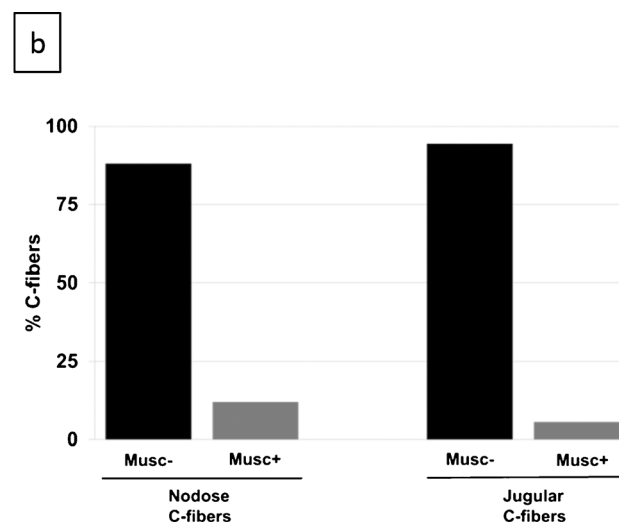

Fig. 7 **a** Single cell RT-PCR was performed to detect mRNA transcripts for M1-5R in individual cells using intron-spanning primers. Individual murine lung-labeled TRPV1+ vagal neurons (C-fibers) were divided into two groups according to their P2X2 expression. All P2X2+ neurons were defined as nodose neurons and all P2X2- cells were defined as jugular neurons. *P* positive control (cDNA from murine jugular nodose complex), H_2O water control. **b** Calcium imaging experiments indicate that small percentages of capsaicin + vagal neurons (C-fibers; 300 nM capsaicin) respond to muscarine stimulation (10 μM) with an increase in $(Ca^{2+})_i$. 23/192 (12 %) nodose (α,β-methylene ATP+) C-fibers and 6/112 (5.6 %) jugular (α,β-methylene ATP-) C-fibers responded to muscarine stimulation

Conclusion

Both efferent and afferent vagal innervation of the airways play a pivotal role in the onset of symptoms experienced by patients during inflammatory airway diseases. Different mediators released during inflammation can sensitize both peripheral and central neurons. However, suppression of inflammation may not be sufficient as a therapeutic approach since the neuronal sensitization might be long-lasting and become independent. Suppressing or reversing peripheral and/or central sensitization of neuronal activity might therefore represent new pathways for the treatment of inflammatory airway diseases.

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