



# WAO-ARIA consensus on chronic cough - Part 1: Role of TRP channels in neurogenic inflammation of cough neuronal pathways

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## ABSTRACT

**Background:** Cough features a complex peripheral and central neuronal network. The function of the chemosensitive and stretch (afferent) cough receptors is well described but partly understood. It is speculated that chronic cough reflects a neurogenic inflammation of the cough reflex, which becomes hypersensitive. This is mediated by neuromediators, cytokines, inflammatory cells, and a differential expression of neuronal (chemo/stretch) receptors, such as transient receptor potential (TRP) and purinergic P2X ion channels; yet the overall interaction of these mediators in neurogenic inflammation of cough pathways remains unclear.

**Objectives:** The World Allergy Organization/Allergic Rhinitis and its Impact on Asthma (WAO/ARIA) Joint Committee on Chronic Cough reviewed the current literature on neuroanatomy and pathophysiology of chronic cough. The role of TRP ion channels in pathogenic mechanisms of the hypersensitive cough reflex was also examined.

**Outcomes:** Chemoreceptors are better studied in cough neuronal pathways compared to stretch receptors, likely due to their anatomical overabundance in the respiratory tract, but also their distinctive functional properties. Central pathways are important in suppressive mechanisms and behavioral/affective aspects of chronic cough. Current evidence strongly suggests neurogenic

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inflammation induces a hypersensitive cough reflex marked by increased expression of neuro-mediators, mast cells, and eosinophils, among others. TRP ion channels, mainly TRP V1/A1, are important in the pathogenesis of chronic cough due to their role in mediating chemosensitivity to various endogenous and exogenous triggers, as well as a crosstalk between neurogenic and inflammatory pathways in cough-associated airways diseases.

**Keywords:** TRP channel, P2X3, Pathogenesis, Chronic cough, Chemoreceptors, Mechanoreceptors

## INTRODUCTION

Persistent coughing lasting for  $\geq 8$  weeks in adults<sup>1</sup> or  $4 \geq$  weeks in children<sup>2-4</sup> can be defined as chronic cough, although prolonged acute cough can persist for 3 to 8 weeks in children.<sup>3</sup> The physiologic cough reflex has well-defined vagus nerve-mediated afferent and efferent neuro-anatomical and signal pathways.<sup>5-11</sup> The peripheral afferent cough receptors, namely the chemosensitive and stretch receptors, are selectively distributed along the airways. They also manifest different triggers and functions (Table 1). Current research suggests that stimulation of these hyperreactive afferent vagus nerve endings can result in chronic cough, the so-called hypersensitive cough reflex (HCR).<sup>12</sup> HCR manifests as variable lower than normal trigger thresholds to cough in various inflammatory and non-inflammatory airways pathologies and gastroesophageal reflux-related diseases. These trigger thresholds can be assessed by topical and inhalation tussigen challenges. HCR, however, can also occur in the absence of known triggers. Based on clinical models, it is speculated the underlying mechanism of HCR is a concomitant neurogenic and inflammatory pathological alteration of the cough reflex which becomes hypersensitive.<sup>13</sup> Moreover, HCR in different anatomical regions is associated with a plethora of biomarkers which can be expressed individually or act collectively. These encompass neuronal receptors such as transient receptor potential (TRP) and purinergic P2X ion channels,<sup>14,15</sup> exogenous and endogenous neuromediators,<sup>16,17</sup> and cytokines and inflammatory cells.<sup>18</sup> The current part I of the consensus (see Appendix) examines the neuroanatomical pathways, pathogenic mechanisms, and the role of TRP ion

channels in modulation of chronic cough. The different phenotypes of abnormal cough presentation and their management will be published subsequently in part II and III of the consensus, respectively.

## APPLIED ANATOMY AND PHYSIOLOGY OF COUGH STRETCH AND CHEMORECEPTORS

Cough reflex entails a physiological halt of the central “respiratory generator pattern” of normal respiration, in parallel with activation of the medullary premotor and motor neuronal pathways which initiate cough, collectively called the “cough generator pattern”.<sup>19</sup>

### Peripheral afferent cough pathway

Two parallel vagal afferent pathways of cough reflex originate in the nodose and the jugular ganglia. These have different embryological origins<sup>20</sup> and distinct peripheral anatomical distributions (see Table 1) at which therapeutic trials are directed when investigating chronic cough. This approach is collectively called the “anatomic-diagnostic protocol” for cough management.<sup>5</sup>

### Chemoreceptors

Both jugular and nodose ganglia have chemosensitive (nociceptive) neurons which are unmyelinated C fibers, mainly sensitive to chemical stimuli and lung inflation (Table 1). These chemoreceptors are associated with TRP ion channels, constitute the majority of airway afferent neurons in the lung and nose<sup>6</sup> and innervate the lower respiratory tract from the

larynx to alveoli. Their activation causes airway constriction and mucus secretion, cough, and sneezing. The serious signs and symptoms of apnea, bradycardia, and hypotension can also be activated via these ganglia.<sup>6</sup> Data suggest that chemoreceptors (eg, TRP-Vanilloid-1) are stretch-insensitive;<sup>21</sup> however, they can also act as high threshold mechanoreceptors<sup>8</sup> when activated by severe mechanical stimuli such as lung hyperinflation and aggressive touch stimuli. Despite lack of solid evidence, a "two pathways model" of cough has been suggested, whereby jugular chemo-sensitive receptors initiate cough in the proximal airways, whereas nodose chemosensory activation in the distal airways can inhibit cough.<sup>22,23</sup> This is based on the fact that some mediators such as the cellular breakdown byproduct adenosine triphosphate (ATP) and serotonin, both of which are selective activators of nodose-chemosensitive neurons, are not potent tussigens. In summary, chemoreceptors can have a dual tussive role in view of their high sensitivity (low threshold) to inhaled chemicals, but also low activation (high threshold) by severe mechanical stimuli.

### Stretch receptors

Nodose ganglia, but not jugular ganglia, incorporate stretch (mechanical) receptors which can be rapidly- (RARs) or slowly- (SARs) adapting stretch receptors and are present mainly in the distal airways<sup>6</sup> (Table 1). Their main function is protecting the airways from generally aspirated or inhaled matter.<sup>8</sup> Stretch receptors are relatively insensitive to a wide range of chemical stimuli,<sup>8,24</sup> although data to the contrary exist.<sup>25</sup> RARs are mainly intrapulmonary dynamic receptors triggered by change in intrapulmonary airway mechanical properties, namely diameter, length, and interstitial pressures.<sup>6</sup> Their activation triggers parasympathetically-mediated reflex bronchospasm and mucus secretion. The act of coughing and subsequent gasping inhalation can provoke RAR-stretch receptors and a pathogenic amplification loop. Contrary to their role in promoting bronchospasm, RARs can also attenuate cough. This is effected by counteraction of molecules which mediate bronchospasm in end organs, such as substance P and bradykinin, histamine, and capsaicin.<sup>6</sup> This adds to the complexity of

chronic cough. Similarly, bronchodilating agents such as prostaglandin (PG) E<sub>2</sub>, adrenaline, and adenosine can have a dual role. They can inhibit bronchospasm secondary to activation of RAR-stretch receptors by capsaicin and bradykinin. However, bronchodilators can also sensitize C fiber-chemoreceptors to capsaicin and bradykinin thereby promoting cough and bronchospasm.<sup>26,27</sup> SARs are incorporated within the bronchioles and alveoli<sup>6</sup> and have peak activity at end-inspiration when they initiate normal expiration through a centrally mediated inhibition of muscarinic respiratory drive.<sup>5</sup> It is questionable whether SARs, RARs, or both contribute to the bronchoprotective (bronchodilating) effect of deep inspiration.<sup>28</sup> The latter normally becomes suppressed in healthy subjects undergoing methacholine bronchoprovocation challenge.<sup>29</sup> Nevertheless, stretch receptors can promote coughing through central activity at the level of the brainstem. In summary, stretch receptors initiate physiologic expiration and additionally can either trigger or attenuate cough by different pathogenic mechanisms.

### Anatomical overlap of stretch and chemoreceptors

Mechano-receptors originating from nodose ganglia and carried by the vagus nerve can overlap with jugular chemo-sensory terminals in the proximal airways (Table 1). Thus, the larynx, trachea, and main bronchi can be triggered by both mechanical and chemical stimuli.<sup>6</sup> However, it is generally agreed stretch receptors are not activated by chemical stimuli except when mechanical distortion of nerve terminal occurs. This can be secondary to mucosal inflammation, mucus secretion, or alteration in smooth muscle tone.<sup>24,30</sup> Based on animal studies, this overlapping subpopulation of nodose-stretch receptors and jugular-chemoreceptors is sensitive to rapid changes of pH and touch-like stimuli, hence their partial role in clearing the airways specifically from aspiration of food or gastric contents.<sup>22,31</sup>

Taken all together, these data indicate chemoreceptors are overabundant in the respiratory tract and overlap anatomically with stretch receptors. They normally trigger the cough reflex as opposed to stretch receptors which can promote or attenuate coughing. Chemoreceptors can have variable

Vagus nerve	Peripheral afferent distribution <sup>6</sup>	Neuronal type	Triggers <sup>8</sup>	Role <sup>22,25,31,87</sup>	Chemical predilection/ Muscarinic activity <sup>25,88,89</sup>	Capsaicin challenge <sup>25,89</sup>	Receptor agonist <sup>6,90</sup>	Overlap of receptors in proximal airway <sup>8</sup>
<b>Chemoreceptors</b>								
Jugular ganglia	- Main bronchus - Trachea - Lungs	C fibers (unmyelinated)	Chemicals and lung inflation*	Airway clearance of inhaled chemicals and inflammatory products	Chemical predilection to: - Nicotine - Hypertonic solution Muscarinic stimulatory role●	Cough hyper-responsiveness	Bradykinin	Overlap with stretch receptors
Nodose ganglia	- Bronchioles - Alveoli	C fibers (unmyelinated)	Chemicals and lung inflation*	Airway clearance of inhaled chemicals and inflammatory products	Chemical predilection to: - Adenosine triphosphate - Serotonin (exclusive) Muscarinic stimulatory role●	Cough hyper-responsiveness	Bradykinin	No overlap with stretch receptors
<b>Stretch receptors (nodose ganglia)</b>								
RARs	Intrapulmonary (majority)	A-δ fibers	Change in airway mechanical properties	Airway clearance of inhaled matter and aspirated products	Muscarinic stimulatory role●	No response	pH alteration	Overlap with chemoreceptors
SARs	- Bronchioles - Alveoli	A-δ fibers	End-inspiratory cycle	Airway clearance of inhaled matter and aspirated products	Muscarinic inhibitory role o	No response	pH alteration	Overlap with chemoreceptors

**Table 1.** Neuro-anatomical properties and functional role of cough chemosensitive and stretch receptors in vagal afferent neuronal pathways from human and animal clinical models. SARs (slow adapting receptors), RARs (rapid adapting receptors), \*chemoreceptors can act as high threshold mechano (stretch) receptors, ● bronchoconstriction, cough and mucus secretion, o Respiratory drive blockage and initiation of expiration

thresholds to a range of chemical agents including those which trigger stretch receptors.

### Central cough pathways

Centrally the afferent pathways of the nodose and jugular ganglia feature different brainstem projections, namely the nucleus solitarius and paratrigeminal nucleus, respectively<sup>32-34</sup> (Fig. 1). Central cough mechanisms and specific regions involved are unknown. Moreover, ascending pathways from the brainstem to higher cortical areas can be studied with functional brain imaging.<sup>35,36</sup> This imaging helps us understand the behavioral, sensory-discriminative, and affective-motivational aspects of airway irritation.<sup>37</sup> Central descending pathways of the prefrontal cortical region are involved with urge-to-cough and the initiation of a voluntary cough or its suppression; this occurs in the presence or absence of a peripheral activation stimulus.<sup>38</sup> For example, chronic cough patients were less able to suppress cough in response to capsaicin inhalation, when compared to healthy controls, reportedly due to failure of inhibitory descending pathways.<sup>39</sup> In accordance, patients with chronic cough challenged with sufficient inhaled capsaicin to induce urge-to-cough demonstrated lower activation of cough inhibitory descending pathways by functional magnetic resonance imaging, compared to healthy subjects.<sup>40</sup> Upregulation or downregulation of cough can occur centrally or peripherally. Central

modulation of cough can be manifested by voluntary suppression of cough.<sup>37</sup> Both central and peripheral modulation of cough are likely independent of a systemic airway inflammatory disease. This can partly explain the controversial role of atopy as a risk factor for chronic cough in the lower airways.<sup>41</sup> In summary, central cough pathways account for behavioral/affective aspects of cough in addition to modulation of tussive effect as manifested by inhibition or suppression of voluntary cough and urge-to-cough. This is likely independent of a systemic airway inflammation.

### PATHOPHYSIOLOGY OF HYPERSENSITIVE COUGH REFLEX

HCR is best described as a hyperexcitable neuroinflammation of the cough-triggering vagal neuronal pathway. This can occur centrally,<sup>42</sup> peripherally or both,<sup>37,43</sup> and is mediated by increased neuronal expression of TRP ion channels.<sup>44</sup> Triggers, which in otherwise normal individuals do not necessarily exacerbate cough, can cause prolonged coughing in patients with HCR (Fig. 1). These triggers include normal human activity, changes in ambient atmospheric conditions, exposure to airborne irritants and pollutants, an acute infectious or atopic respiratory event, and a chronic inflammatory disease as well as with an experimental inhalation challenge with tussigens.

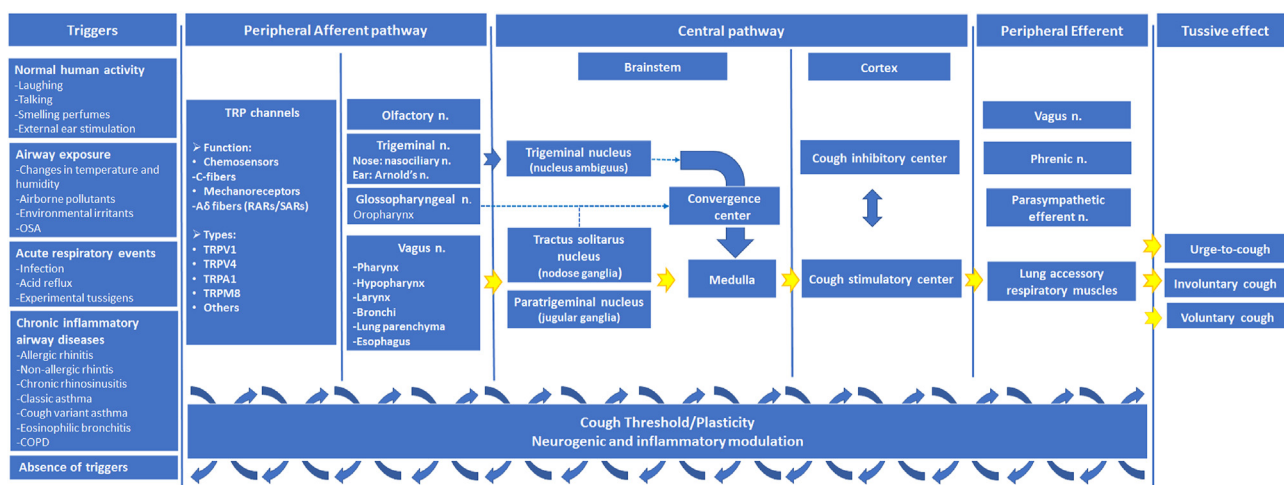


Fig. 1 Neuro-anatomical aspects and mechanistic concepts in modulation of chronic cough from animal and human models, COPD (chronic obstructive pulmonary disease), n. (nerve), OSA (obstructive sleep apnea), TRP channels (Transient receptor potential channels)

## Neurogenic dysfunction

Various triggers can induce acute, subacute, or chronic modification of excitability pattern in the cough central and likely afferent neuronal pathway along the vagus nerve. This can result in exaggeration or suppression of cough reflex, a term called cough plasticity. Cough plasticity can be modified by the following factors: 1) the nature of triggering factors, 2) the ensuing modification in intrinsic neuronal excitability, 3) changes in neuronal synaptic transmission (pre- or postsynaptic), and 4) the subsequent effect of neurotransmitters and neuromodulators, such as the calcitonin gene-related peptide (CGRP),<sup>45</sup> an important neuropeptide with pro-inflammatory potential. Evidence suggests that cough plasticity involves primarily the (vagal) nucleus tractus solitarius,<sup>19</sup> the associated higher central sensory processing circuit<sup>46</sup> and the vagal afferent nerves.<sup>47</sup> Also, it is speculated this abnormal excitability of central sensory neuronal pathways can exist even in the absence of a prior acute or chronic peripheral sensitization; this is very similar to chronic neuropathic pain which results in the "uncoupling of central cough hypersensitivity state to the injury zone".<sup>48</sup> In summary, cough plasticity refers to a peripheral/central variable cough threshold to nonspecific triggers, or their absence, and is driven partly by neurogenic mechanisms involving neuropeptides and neuromodulators, among others.

## Immune alterations

In HCR, the term "neurogenic inflammation" implies cough is primarily a neurogenic response monitored by the immune system. Dysfunction of one or both systems can result in HCR.<sup>49</sup> Direct or indirect release of inflammatory mediators has been reported into the nucleus tractus solitarius and adjacent medullary neuronal complex area.<sup>19</sup> The intricate regulatory role of the immune system in HCR is deciphered by experimental in-vivo and in-vitro models comparing different cough phenotypes to each other or to healthy individuals. Based on specimen findings from broncho-alveolar lavage fluid, induced sputum and lung biopsies of patients with different cough phenotypes,<sup>43</sup> studies suggest inflammatory cells such as eosinophils, neutrophils and mast cells are involved in the

pathogenesis of cough. In fact, there is a considerable similarity in biochemical profile among patients with HCR (see below). A detailed description of immune cascade pathways in HCR is comprehensively described elsewhere.<sup>43,49,50</sup> Importantly, *in vivo* and *in vitro* data from these reviews address the immune modulatory role of cough in a variety of lower airway clinical phenotypes of chronic cough but unfortunately most patient populations included in these studies are small in size. Neurokinins such as substance P, CGRP, and interleukins (IL)-4 and IL-5 stimulate eosinophils to release effector molecules such as major basic proteins, cysteinyl leukotrienes, and eosinophil granule-derived cationic proteins with resultant stimulation of cough sensory and muscarinic nerves.<sup>12,43</sup> Nerve growth factor (NGF), whose main source in humans is eosinophils, increases expression of TRP channels (TRPV1, TRPA1) on sensory nerves with inflammation, thus highlighting the role of allergic airway inflammation in chronic cough.<sup>21,51,52</sup> Similar modulation of cough can also be induced by IL-17-mediated infiltration of neutrophils whose probable role in neurogenic inflammation is generation of reactive oxygen species and sensitization of cough neuronal pathway. The same effect can result from bradykinin-induced secretion of PGs<sup>43</sup> which are potent tussigens.<sup>12</sup> Data also suggest an important role of mast cells in chronic cough patients. Patients with non-asthmatic cough have marked submucosal mast cell infiltration as opposed to patients with asthmatic cough with submucosal infiltration by neutrophils and eosinophils.<sup>53</sup> CGRP, a byproduct of neurogenic inflammation of sensory nerves, induces mast cell hyperplasia.<sup>54,55</sup> These cells, which are in close association with airway mucosal sensory nerves, highlight the role of the innate immune system in regulating neurogenic inflammation in respiratory epithelium of chronic cough patients.<sup>54</sup> For example, increase in histamine and PGD<sub>2</sub> in sputum specimens of patients with non-asthmatic eosinophilic bronchitis compared to asthmatics suggests that mast cells are particularly activated in the former cough phenotype.<sup>56</sup> Meanwhile, data from clinical trials using biological therapy undermine the role of eosinophils in HCR. For example, anti-IL-5 treatment improved cough scores in asthmatics, but not markers of sputum eosinophilia. This speculates a more important role

of mast cells in asthmatic patients with cough.<sup>57</sup> Along the same line, inhaled and nasal topical steroids improved markers of eosinophilic inflammation in cough-associated asthma/eosinophilic bronchitis, and allergic rhinitis/chronic rhinosinusitis with nasal polyps, respectively.<sup>50</sup> Taken together, these data and others suggest airway eosinophilia can be an epiphenomenon in chronic cough, yet maintain evidence that eosinophilic airway inflammation identifies steroid-responsive patients.<sup>58</sup> Other neuro-inflammatory mediators implicated in HCR<sup>59</sup> such as IL-1 $\beta$ , tumor necrosis factor- $\alpha$  and NGF have an integral role in TRPV1 and TRPA1 gene production and channel expression.<sup>60-62</sup> In conclusion, despite ample evidence of inflammatory and biochemical mediators of neurogenic inflammation being involved in different cough phenotypes, their exact role remains obscure. There seems to be an important role of mast cells and innate immune system in HCR. Yet, it is difficult to clearly delineate the signal and cascade pathways involved in different etiologies or phenotypes of chronic cough.

## TRP CHANNELS IN COUGH NEURONAL PATHWAYS

### Neuro-physiological properties

TRP channels are a collective family of ion channel receptors located at afferent airway cough neurons of the vagus and trigeminal nerves and mediate airway protective mechanisms to noxious stimuli<sup>21</sup> (Fig. 1). They are present in the entire respiratory tract from the nose through to the alveoli and are expressed on neuronal and non-neuronal cells such as epithelium, fibroblasts, smooth muscle cells, and inflammatory cells. TRP expression on smooth muscle cells suggests their role also in mediating structural and functional changes in airway mucosal barrier as occurs, for example, in airway remodeling.<sup>21</sup> Broadly speaking, TRPV1 (aka capsaicin receptor) and TRPA1 dominate the intra- and extra-pulmonary cough airway afferents, are carried on unmyelinated C fibers, and are chemosensitive, that is triggered by locally produced or inhaled chemicals and inflammatory mediators. On the other hand, airway sensitivity to mechanical, thermal, and pH-related stimuli is generally featured on a minority of airway cough afferents which are almost

exclusively extrapulmonary (proximal airways), are carried on myelinated A- $\delta$  fibers, and are not mediated by TRPV1 channels.

### Chemosensitivity

A thorough anatomical and functional description of TRP channels in different cough phenotypes is well described in the literature<sup>14,21,63</sup> and summarized in Table 2. Chemoreceptors are the main TRP channels featured in the airways and are, in the majority, TRPV1 ion channels. These co-localize with neuropeptides, such as substance P, CGRP, and receptors for NGF. They also have low threshold for various inflammatory mediators such as bradykinins, histamines and eicosanoids.<sup>11</sup> Simultaneous stimulation by multiple TRP channel agonists, such as occurs with capsaicin in the presence of gastric acid<sup>11</sup> or fever,<sup>64</sup> can further lower the activation threshold. In accordance, the simultaneous activations of TRPA1 and TRPV1 by their respective selective agonists on pulmonary sensory nerves are far more effective than single agonists taken separately.<sup>65</sup> Chemosensitivity of TRPV1 and TRPA1 to endogenously generated chemicals such as bradykinin, arachidonic acid, and its effector, PGE2, coupled to G protein receptor activation<sup>21,66</sup> occurs in inflammatory diseases, such as asthma. Oxidative stress mediators following exposure to air pollutants, such as cigarette smoke (acrolein), diesel exhaust particles, aldehydes, chlorines, and scents, can also trigger chemosensitive TRPA1 channels. Both TRPV1 and TRPA1 activate the mucin-producing MUC5 gene.<sup>67</sup> In mammal models, a calcium mediated activation of TRP channels triggers local release of neuropeptides from afferent terminals peripherally or centrally. Ultimately, a signal is sent through efferent cholinergic pathways to cause bronchoconstriction in parallel with a vagal motor neuron-mediated signal to the larynx, accessory respiratory muscles and diaphragm to initiate cough and induction of various cellular responses.<sup>14</sup> In summary, current data suggest airway chemosensitivity to endogenously produced and/or exogenously inhaled chemicals and inflammatory products is mediated by TRPA1/V1 channels. Central and peripheral mechanisms subsequently induce bronchoconstriction and cough.

	TRPV1	TRPA1	TRPV4	TRPM8	P2X3
<b>Trigger</b>	<p><b>Exogenous:</b> Environmental: - High T (<math>\geq 43</math> °C), - Painful stimuli, <i>Dietary:</i> - Chili pepper (capsaicin), - Mustard, wasabi (allyl isothiocyanate), - Vanilloids, <i>Infectious:</i> - HRV,</p> <p><b>Endogenous:</b> - Lipid and AA metabolites (e.g., PGE<sub>2</sub>), - Bradykinin, - Nerve growth factors, - Low pH (&lt;5.9), - Serotonin, - Histamine, - CGRP, - Cannabinoid and dopamine derivatives, - ATP,</p> <p><b>Others:</b> - Proteases, - TRPA1.<sup>91-99</sup></p>	<p><b>Exogenous:</b> <i>Environmental:</i> - Low T (&lt;17 °C), - Painful stimulus, - TBS, herbicides, antiseptic, etc. (acrolein and formalin), <i>Dietary:</i> - Mustard oil/ Cinnamon/Garlic/ Clove bud oil compounds, <i>Infectious:</i> - HRV, <i>Others:</i> - TRPM8 activators,</p> <p><b>Endogenous:</b> - Lipid and AA metabolites (e.g., PG), - Bradykinin - Nerve growth factors, - NO, - Reactive oxygen species, - Histamine, - Cytokines (e.g., TSLP),</p> <p><b>Others:</b> - Proteases, - TRPV1.<sup>100-109</sup></p>	<p><b>Exogenous:</b> <i>Environmental:</i> - Moderate T (24-38 °C) - Painful stimuli - Citric acid, - Synthetic phorbol ester 4<math>\alpha</math>- PDD, - Shear stress,</p> <p><b>Endogenous:</b> - Arachidonic acid metabolites, - Phospholipase A2 activation, - Mechanical-osmotic changes (hypo-osmolarity).<sup>110-113</sup></p>	<p><b>Exogenous:</b> - Low to warm T (10-25 °C), - Cooling compound (menthol, icilin and eucalyptol), <i>Infectious:</i> - HRV.<sup>21,110</sup></p>	<p><b>Endogenous:</b> - ATP (extracellular), - Thermal stimuli,</p> <p><b>Others:</b> - TRPV4.<sup>114-117</sup></p>
<b>Role in cough reflex</b>	- Increase sensitivity to capsaicin, citric acid, histamine. <sup>118,119</sup>	- Increase sensitivity to capsaicin. <sup>120</sup>	- Insensitive to capsaicin. <sup>121</sup>	- Increase sensitivity to capsaicin, citric acid. <sup>122</sup>	- Increase sensitivity to capsaicin, citric acid, histamine. <sup>123-125</sup>



<p><b>Airway Function</b></p>	<p>- Chemoreceptors (major), <b>Role in:</b> - Increased cytokine gene expression mediating bronchoconstriction - Transition of early defensive immune and inflammatory responses to chronic responses and disease pathology.<sup>51,126</sup></p>	<p>- Chemoreceptor, <b>Role in:</b> Bronchoconstriction secondary to irritant exposure.<sup>21,127</sup></p>	<p>- Polymodal ion channel, <b>Role in:</b> - Neurogenic inflammation - Ciliary beat frequency - Epithelial barrier function - macrophage activation.<sup>128-130</sup></p>	<p>- Cold thermal receptors (major), <b>Role in:</b> - Macrophage and T cell function, <b>Conflicting role in:</b> - Bronchoconstriction and cough in response to cold T, - Cough inhibition in lower airways by menthol effect on nasal neurons, - Airway epithelium production of mucus and inflammatory mediators.<sup>122,131-133</sup></p>	<p>- ATP- gated ion channel, <b>Role in:</b> - Nociception.<sup>116</sup></p>
<p><b>Non-neuronal cellular mapping*</b></p>	<p>- Airway epithelial and smooth muscle cells,<sup>134, 135</sup> - CD4+ T cells.<sup>136</sup></p>	<p>- Airway epithelial and smooth muscle cells, - Lung fibroblasts.<sup>21</sup></p>	<p>- Airway epithelial and smooth muscle cells, - Lung fibroblasts, - Alveolar macrophages and mononuclear cells.<sup>121, 129, 137</sup></p>	<p>- Epithelial nasal mucosa and cilia, - Epithelial bronchial cells.<sup>51,138</sup></p>	<p>- Epithelial cells, - Taste buds.<sup>139,140</sup></p>
<p><b>Cough phenotypes</b></p>	<p>- Allergic rhinitis, - Asthma, - COPD.</p>	<p>- Asthma, - COPD.<sup>14</sup></p>	<p>- Asthma, - COPD.<sup>141</sup></p>	<p>- Cold induced-nasal hyperreactivity, - Cough and bronchoconstriction, - COPD.<sup>51, 133, 142</sup></p>	<p>- Asthma, - COPD.<sup>38</sup></p>

**Table 2.** Functional and tussive properties of P2X3 and important TRP channels in cough-associated airway diseases from in-vivo and in-vitro models. \*cough pertinent non-neuronal tissues, AA (Arachidonic acid), ATP (Adenosine triphosphate), CGRP (Calcitonin gene-related peptide), COPD (Chronic obstructive pulmonary disease), HRV (Human rhinovirus), NO (Nitric oxide), PG (Prostaglandins), T (Temperature), TBS (Tobacco smoke), TSLP (Thymic stromal lymphopoietin), TRP (transient potential receptor)

## Role in neurogenic inflammation, polymorphism, and cough suppression

As described earlier, TRP channels mediate neurogenic inflammation. They can respond differentially to inflammatory mediators and, through Ca-dependent mechanisms, can produce additional mediators. For example, stimulation of TRPV1 and TRPA1 can locally release neurotrophins important for chemotaxis and activation of eosinophils. Once activated, eosinophils secrete NGF resulting in recycling of TRP channels to surface membrane<sup>60,68</sup> and increased expression of TRP channels resulting in an amplification loop.<sup>11</sup> Ultimately, depolarization or hyperpolarization of TRP channels at nerve terminals can respectively increase or decrease excitability of cough afferent neuronal pathway. This crosstalk between inflammatory and neurogenic signal pathways highlights the role of TRP channels in several airway diseases. These can include post-viral-cough, idiopathic cough, asthma, and chronic obstructive pulmonary disease, and other less frequent respiratory diseases such as idiopathic pulmonary fibrosis and airway Pseudomonas infection in cystic fibrosis.<sup>21</sup> This same cross talk likely also occurs in COVID-19 cough.<sup>69,70</sup> Due to their variable expression patterns in neurogenic inflammation, it is also suggested that TRP channels may be partly responsible for a transition from early (acute) defensive immune and inflammatory responses to chronic responses and chronic cough pathology.<sup>21</sup> Unlike other TRP channels, TRPM8 can mediate a decrease in capsaicin-mediated cough sensitivity in the presence of thymol and camphor, which explains the antitussive effect of these agents,<sup>71,72</sup> probably secondary to stimulation of cool receptors in the nasal cavity.<sup>73,74</sup> TRP channel variants have been linked to altered cough presentation: TRPV1 polymorphisms have been associated with decreased wheezing in a group of asthmatics<sup>75</sup> but a higher risk of chronic cough in both smokers and patients with occupational exposure.<sup>76</sup> Other data suggest that TRPA1 polymorphism is associated with poor control of asthma secondary to air pollutant (particulate matter) exposure.<sup>77</sup> Moreover, cough suppression can be mediated by TRP channels. Oral capsaicin was found to reduce cough by desensitization of TRPV1.<sup>78</sup> Also, TRPA1

antagonist suppressed cough in guinea pigs yet TRPV1<sup>79</sup> or TRPA1 antagonists<sup>80</sup> failed to suppress chronic cough in humans. This argues for involvement of central sensitization in HCR. In fact, data from P2X3 antagonists (see below) suggest central rather than peripheral neuronal pathways of vagus nerve are likely important target areas for new antitussive drug development.<sup>81</sup> In conclusion, the anatomical and physiological mapping of TRP channels along cough neuronal pathways and their upregulation in neurogenic inflammation of cough reflex suggest their important role in pathogenesis of chronic cough. This is likely manifested by a crosstalk between neurogenic and inflammatory signal pathways in cough-associated airway diseases.

## P2X3 CHANNELS IN COUGH NEURONAL PATHWAYS

P2X3 purinergic channels are receptors belonging to the P2X family of ion channels, with high predilection to the cellular breakdown product ATP.<sup>82</sup> P2X3 channels are almost exclusively expressed on sensory neurons.<sup>83</sup> Specifically, they are present, in their majority, on both C (chemoreceptors) and A- $\delta$  fibers (mechanoreceptors) of vagal afferent cough pathways with some expression in nucleus tractus solitarius.<sup>84</sup> P2X3 receptor activation by adenosine triphosphate (ATP) depolarizes the peripheral cough neuronal pathway and transmits a central signal interpreted as urge-to-cough.<sup>85</sup> P2X3 channels can also modulate the cough neuronal pathway activity at central synapses in both upper and lower airways, hence the effect of P2X3 antagonists.<sup>15</sup> Mounting evidence suggests that P2X3 receptors can mediate HCR leading to chronic cough.<sup>81</sup> However, it remains unclear if the role of P2X3 in HCR is secondary to increased ATP production in the airways or decreased ATP degradation, an enhanced sensitivity to ATP or a combination of these factors.<sup>86</sup>

## SUMMARY

This paper represents a comprehensive and updated review on neuroanatomy and pathophysiology of HCR, including novel findings on the role of TRP receptors and P2X channels in

modulating chronic cough. Knowledge of immune pathways involved in chronic cough is vital to understand how neurogenic inflammation of HCR, in different anatomical regions of the aero-digestive tract, can express different symptomatology, and thus results in different cough-associated clinical phenotypes. In chronic cough, there is a differential expression of chemo/stretch-sensitive afferent receptors (TRP channels) as well as P2X ion channels. Also neuromediators and inflammatory cells, such as mast cells, eosinophils, and neutrophils, are involved in HCR. It is likely that mast cells and innate immune system have a pivotal role in HCR, whereas current data undermine the role of eosinophils in chronic cough. An important function of TRP channels in chronic cough stems from data on TRP polymorphism and cough suppression by TRP antagonists. TRP channels can also mediate cross talk between neurogenic and inflammatory signal pathways involved in chronic cough. The nature of cough triggers, spectrum of expressed TRP channels and their co-localization with distinct neuro-inflammatory mediators, in addition to tussigen challenge data, contribute significantly to a proper diagnosis and treatment strategies of chronic cough. Currently, we lack a better functional understanding of cough central pathways and how they modulate chronic cough. Tussigen challenge protocols which can better discriminate healthy subjects from patients with HCR need to be standardized. This is more evident considering the value and limitations of animal cough models.

### Abbreviations

ATP (Adenosine triphosphate), CGRP (Calcitonin gene-related peptide), HCR (Hypersensitive cough reflex), IL (Interleukin), NGF (Nerve growth factor), P2X (Purinergic receptors), PG (Prostaglandin), RAR (Rapidly adapting receptors), SAR (Slowly adapting receptors), TRP (Transient receptor potential).

### Authors contributions

PR designed the plan of the article, contributed to the data collection. He wrote the manuscript draft, conceived, and designed the tables/figures, and reviewed all parts of the article.

SI contributed to data collection, tables/figures, and manuscript draft.

JB participated to the manuscript draft and reviewed the whole article.

TL, CA, MAA, AY, MAN, TN, SB, EAJ, FZ, UH, GJ and MR contributed the manuscript draft each according to his/her specialty and domain of interest.

The rest of authors reviewed closely the whole article and added their valuable remarks.

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### Appendix A. Supplementary data

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