Published in final edited form as:

Curr Opin Pharmacol. 2011 June ; 11(3): 238–247. doi:10.1016/j.coph.2011.04.005.

Airway irritation and cough evoked by acid: from human to ion channel

Qihai Gu¹ and Lu-Yuan Lee²

¹ Division of Basic Medical Sciences, Mercer University School of Medicine, Macon, Georgia 31207, USA

² Department of Physiology, University of Kentucky, Lexington, Kentucky 40536, USA

Abstract

Inhalation or aspiration of acid solution evokes airway defense responses such as cough and reflex bronchoconstriction, resulting from activation of vagal bronchopulmonary C-fibers and A δ afferents. The stimulatory effect of hydrogen ion on these sensory nerves is generated by activation of two major types of ion channels expressed in these neurons: a rapidly activating and inactivating current mediated through ASICs, and a slow sustaining current via activation of TRPV1. Recent studies have shown that these acid-evoked responses are elevated during airway inflammatory reaction, revealing the potential convergence of a wide array of inflammatory signaling on these ion channels. Since pH in the airway fluid drops substantially in patients with inflammatory airway diseases, these heightened stimulatory effects of acid on airway sensory nerves may play a part in the manifestation of airway irritation and excessive cough under those pathophysiological conditions.

1. Airway irritation and cough responses evoked by acid

Systemic acidosis can occur as a consequence of respiratory or/and metabolic disorders (e.g., emphysema, diabetes mellitus, etc.). Acidosis can also occur transiently in healthy individuals; for example, after strenuous exercise, arterial blood pH can reach 7.06 in trained athletes resulting from the production of lactic acid [1]. Further, the concentration of hydrogen ion increases locally in the extracellular fluid of inflamed and ischemic tissues. In patients during acute asthma exacerbation, the pH of the airway vapor condensate of exhaled gas was reduced to 5.23, as compared to 7.65 in healthy individuals [2,3]. This abnormally low airway pH returned to normal after anti-inflammatory therapy [2], suggesting the tissue inflammation as the origin of airway acidosis.

Inhalation or aspiration of acid solution triggers immediate airway responses such as cough, airway irritation and reflex bronchoconstriction in both humans and animals, resulting from stimulation of airway sensory nerves [4–6]. In fact, citric acid aerosol is one of the most commonly used inhaled tussive agents in testing cough responsiveness in man [5,7–9] (Figure 1a). Low pH and lack of chloride ion, and not the citrate ion, are mainly responsible for the stimulatory effect of citric acid on the “cough receptors” [7,10*]. In addition, other

© 2011 Elsevier Ltd. All rights reserved.

Corresponding Author: Lu-Yuan Lee, Department of Physiology, University of Kentucky Medical Center, 800 Rose Street, Lexington, KY 40536-0298, USA, Tel: 001-859-323-6339, FAX: 001-859-323-1070, lylee@uky.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

forms of acid solution (e.g., acetic acid, phosphoric acid, etc.) in the same range of pH (approximately 1.5–2.5) are similarly effective in eliciting cough reflex in humans, further indicating a critical role of hydrogen ion in the stimulatory effect on airway sensory nerves [7,8] (Figure 1a). In animal studies, the cough reflex elicited by inhalation of acid aerosol and the bronchoconstriction produced by right heart injection of acid solution can be mostly abolished by blocking the vagal afferent pathways [10*,11]. In guinea pigs, the bronchoconstrictive response is mediated in a large part through the release of sensory neuropeptides such as tachykinins that are released from these sensory endings upon activation because the airway responses are blocked by pretreatment with specific antagonists of neurokinin (NK) receptors [6,12].

Microaspiration of gastric acid has been shown to induce reflex bronchoconstriction in both animal and human studies [13,14]. Acid activation of the sensory nerves innervating larynx and trachea is believed to be the primary cause of chronic cough and airway irritation in gastroesophageal and laryngopharyngeal reflux diseases [15,16*]. Acid can also stimulate the vagal afferents innervating the esophagus in a similar pattern as in the airway afferents [16*]. It has been suggested that activation of the afferents innervating the distal segment of esophagus is responsible for eliciting reflex bronchoconstriction in asthmatics in response to gastroesophageal reflux [17].

2. Effect of acid on sensory nerves innervating the airways and lung

It has been known for decades that extracellular acidification can stimulate nociceptors, the counterpart of bronchopulmonary C-fiber and A δ afferents in other organ systems, and evoke the pain sensation in various somatic and visceral tissues [18]. Indirect evidence of acid stimulation of lung afferents showed that continuous perfusion of isolated guinea pig lungs with acidic buffer at a pH of 5.0 evokes a release of various sensory neuropeptides, such as tachykinins and calcitonin gene-related peptides, from pulmonary C-fiber endings [19]. The direct evidence was first reported by Fox and co-workers [20], who demonstrated that capsaicin-sensitive C-fiber afferents, but not A δ fibers, innervating the guinea pig trachea are stimulated when the isolated guinea pig airway was perfused with acidic buffer at pH of 5.0. The C-fiber response to acid challenge is abrogated by capsazepine [20], a selective antagonist of the transient receptor potential vanilloid receptor (TRPV1) channel. However, using a similar isolated guinea pig airway-nerve preparation, Kollarik and Undem found that both C-fiber and A δ afferents can be activated by acid challenge, dependent upon the magnitude, duration and rate of acid application [21]. Furthermore, their study showed that acid also evokes a rapid-inactivating capsazepine-insensitive current, and postulated a possible involvement of activation of acid-sensing ion channels (ASICs). In awake guinea pigs, the cough reflex triggered by inhalation of aerosolized citric acid can be abolished by pretreatment with capsazepine [22], and specific antagonists of NK-1, NK-2 and NK-3 receptors [23,24]. These findings convincingly suggest that the acid stimulation of C-fibers contributes, directly or indirectly, to the acid-evoked airway irritation and cough response in awake animals. Interestingly, the cough reflex elicited by chemical stimulation (such as acid) of airway C-fibers is completely absent during general anesthesia [25**].

In a series of studies, Canning and coworkers further identified a separate set of airway sensory nerves involved in eliciting the acid-evoked cough reflex, which persists even during anesthesia [10*,25**–27] (Figure 1b–1d). These thin myelinated (A δ) vagal afferent fibers innervating the extrapulmonary airways (trachea and larynx) exhibit exquisite sensitivity to acid challenge and punctuate mechanical stimulation, and their activation evokes coughing in a dose-dependent manner in anesthetized guinea pigs [26]. These “cough receptors” are insensitive to capsaicin [25**]. Furthermore, unlike the traditionally-defined rapidly adapting receptors (RARs) in the lung, they are not activated by lung

inflation, bronchoconstriction, or other autacoids known to stimulate RARs [25**]. The cell bodies of these cough receptors reside in the nodose ganglia; their sensory terminals can be identified by intravital labeling with the styryl dye FM2-10, and are distributed in the airway mucosa, between the smooth muscle and the epithelium [10*,26,27]. In view of their distinct sensitivity to light punctuate stimulation, it seems conceivable that the activation of these receptors is also responsible for the airway irritation and coughs generated by the presence and/or movement of the mucus on the airway mucosa. Another unique feature of these nerve endings is the expression of an isozyme of the sodium pump containing the $\alpha 3$ subunit [10*, 27]. Indeed, the cough response evoked by topical application of citric acid in anesthetized guinea pigs is abolished by ouabain, a sodium pump inhibitor [10*,27].

Lactic acid is produced by anaerobic metabolism such as during acute and chronic hypoxia or ischemia, and locally during tissue inflammation [3]. Lactic acid injected into the right atrium of anesthetized rats produces a transient decrease in arterial blood pH (range: 7.09–7.29), which evokes a short but intense burst of afferent activity in pulmonary C-fibers in a dose-dependent manner [28]. The same nerve endings can be also activated by injection of other acid solutions (e.g., formic acid) that produce the same decrease in pH, suggesting that hydrogen ion is primarily responsible for the action. The dyspneic sensation is generated by cortical convergence of multiple sensory signals and central respiratory motor commands [29], and one of these potential sensory inputs is from the vagal bronchopulmonary C-fiber endings [30,31]. During exertional exercise, a large quantity of lactic acid can be produced by exercising muscles and carried in the venous blood. Considering the pronounced stimulatory effect of lactic acid on these afferents, it seems reasonable to suggest that C-fiber activation by lactic acid may act as one of the contributing factors to the genesis of dyspnea during severe exercise. In addition, hyperthermia, elevated pulmonary arterial pressure, and pulmonary congestion that occur during exercise are also known to stimulate pulmonary C-fibers [32,33], which may further enhance the stimulatory effect of lactic acid on these afferents.

It is also possible that the irritant effects of acid challenge are elicited by indirect effects of the C-fiber stimulation. Stimulation of C-fibers in the airways is known to trigger the release of substance P and other tachykinins [19], which can in turn lead to the production of nitric oxide from endothelial cells [34]; nitric oxide, an endogenous free radical gas, can increase the microvascular permeability and fluid infiltration into the perivascular interstitial space in the airways, and activate RARs [35]. Indeed, it has been shown that right atrial injection of lactic acid stimulates a small percentage of RARs in the rat lung [36]. Furthermore, the possibility that hydrogen ions may act on the surrounding tissue, trigger the release of certain autacoids (e.g., cyclooxygenase metabolites, etc.) [37], and subsequently cause a secondary stimulatory effect on these sensory terminals should also be considered [30].

3. Ion channels activated by hydrogen ion

To determine the direct effect of hydrogen ion, the responses of isolated rat vagal pulmonary sensory neurons to physiological/pathophysiological-relevant low pH (7.0–5.5) were investigated using whole-cell perforated patch-clamp recordings [38**]. In voltage-clamp mode, these neurons exhibit distinct sensitivities to hydrogen ion and different phenotypes of inward current in response to acid challenge (Fig 2a). A mild drop of extracellular pH (pH_o) to 7.0, 6.5, 6.0 and 5.5 activates 45.2%, 83.1%, 91.5% and 92.5% of these neurons, respectively. The current evoked by pH 7.0 consists of mainly a transient, rapidly inactivating component with small amplitude. The amplitude of this transient current increases when the proton concentration is elevated, and apparently reaches the maximum amplitude even at pH 6.5 in some neurons. A slow, sustaining inward current begins to emerge when pH is reduced to below 6.5. This sustaining current increases its amplitude in a

larger percentage of neurons when a lower pH_o is applied. Similarly, in current-clamp mode, the temporal pattern of membrane depolarization also exhibits the transient and sustaining components, coinciding with those displayed in the voltage-clamp mode [38**].

When an I-V curve of the peak amplitude of the transient current evoked by a reduction of pH_o from 7.4 to 5.5 is constructed in the pulmonary sensory neurons, it has a linear I-V relationship with a reversal potential at ~ 65.4 mV, which is close to the theoretical Na^+ equilibrium potential, indicating that the transient current evoked by acid stimulation is selective for Na^+ ions. Pretreatment with amiloride, a general inhibitor of ASICs, dose-dependently attenuates the transient current evoked by acid with EC_{50} at $32.6 \mu\text{M}$. On the other hand, pretreatment with capsazepine significantly attenuates the sustaining, but not transient component of acid-evoked current (Figure 2b, 2c). These results demonstrate that acid stimulates rat vagal pulmonary sensory neurons through the activation of both ASICs and TRPV1 [38**].

The expression and function of TRPV1 in rat pulmonary sensory neurons have been well documented [39]. ASICs are members of the amiloride-sensitive degenerin/epithelial sodium channel family of sodium channels that are gated by protons [40]. To date, four genes encoding six ASIC subunits have been cloned in mammals, namely ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3 and ASIC4. However, ASIC4 has not been shown to produce or modulate proton-evoked current and remains the least understood subunit [41]. Four ASIC subunits (ASIC1a, ASIC1b, ASIC2a and ASIC3) can form functional homomultimers, whereas ASIC2b cannot form functional channels by itself but may co-assemble with other ASIC subunits to form heteromultimers with new biophysical and pharmacological properties [42]. Different homomeric and heteromeric ASICs are known to have distinct pH sensitivity, ion selectivity, and channel kinetics [42]. A recent study showed that all four functional ASIC subunits (1a, 1b, 2a, and 3) are expressed in rat pulmonary sensory neurons [43*]. It is assumed that the expression of ASICs in these neurons, like that in dorsal root ganglion (DRG) neurons [44], are most likely heteromultimeric channels. However, the exact expression of ASIC subtypes/subunit combinations in these pulmonary sensory neurons is not known. In addition, whether ASICs are the sole candidates of the TRPV1-independent acid activation in pulmonary sensory neurons of animal species other than rats remains to be determined.

4. Response to hydrogen ion altered by physiological stresses and disease conditions

The airway irritation and cough responses to acid challenge are altered in many physiological and disease conditions. The modulation of the acid-induced airway responses can be attributed at least in part to the altered sensitivity/expression of acid-sensitive ion channels and therefore, the altered excitability of bronchopulmonary sensory nerves under these conditions.

Protease-activated receptor-2 (PAR_2)

PAR_2 belongs to a family of G-protein coupled receptors that are activated by specific proteases. PAR_2 activation occurs after proteolytic cleavage of its extracellular N-terminal domain by proteases, which reveals a tethered ligand domain that binds to and activates the cleaved receptor [45]. Trypsin, mast cell tryptase, and coagulation factors VIIa and Xa are considered as the endogenous agonists of PAR_2 [46]. PAR_2 can also be activated by airborne allergens such as house dust mite allergens Der p1, p3 and p9 under certain experimental conditions [47]. In the respiratory system, PAR_2 is distributed in various cells in the lung and airways [46]. The elevated levels of both endogenous agonists and

expression of PAR₂ have been reported from patients and animals under airway inflammatory conditions [48]. Although PAR₂ may also be anti-inflammatory under certain conditions [49], the proinflammatory role of PAR₂ has been consistently observed in the airways of mouse, guinea pig and human, and confirmed by studies with PAR₂-deficient mice [48].

Stimulation of PAR₂, obtained with two diverse PAR₂ agonists, PAR₂-AP and trypsin, does not activate the cough reflex but can significantly potentiate the cough responses induced by citric acid in a guinea pig model [50*]. Involvement of PAR₂ in the potentiation is further indicated by the observation that the cough response induced by citric acid stimulation is not affected by the PAR₂-RP, which is unable to stimulate the receptor, or the inactivated trypsin (by soy bean trypsin inhibitor) [50*]. In isolated rat vagal pulmonary sensory neurons, pre-incubation of PAR₂-AP markedly enhances the Ca²⁺ transient evoked by extracellular acidification [43*]. In perforated patch-clamp recordings, pretreatment with PAR₂-AP significantly potentiates the acid-evoked both ASIC- and TRPV1-like whole-cell inward currents (Figure 3a–3c). In current clamp mode, activation of PAR₂ also potentiates the excitability of these neurons to acid (Figure 3d–3f), but not to electrical stimulation [43*]. In addition, the potentiation of acid-evoked responses is not prevented by inhibiting either phospholipase C or protein kinase C, nor is it mimicked by activation of protein kinase C. The short-lasting and PKC-independent feature of the potentiating effect may indicate a direct interaction between PAR₂ agonist and the two acid-sensitive ion channels, ASIC and TRPV1 [43*]. It is reasonable to assume that the potentiation of acid signaling by PAR₂ activation is relevant to airway inflammation and other pathophysiological conditions in which acidification and PAR₂ activation may occur simultaneously.

Hyperthermia

Hyperthermia can occur in the lung and airways under either physiological (e.g., exercise) or pathophysiological (e.g., fever or airway inflammation) conditions. Indeed, a significant increase in the exhaled air temperature ($\Delta = 2.7^{\circ}\text{C}$) has been reported in asthmatic patients [51], indicating an increase in airway tissue temperature during inflammatory reaction. A recent study demonstrated that increasing temperature within the normal physiological range (36–40.6 °C) exerts a paradoxical effect on the two types of the acid-evoked current in pulmonary sensory neurons (Figure 4a); although the slow, sustaining component mediated by the TRPV1 receptor is markedly potentiated, the rapid, transient component mediated by ASICs is consistently inhibited [52*] (Figure 4b–4e). The potentiating effect on the TRPV1-mediated current is consistent with the finding of a positive interaction at the TRPV1 channel between hydrogen ion and heat [53]. In addition, more rapid activation and inactivation of the TRPV1-mediated current are noticeable during hyperthermia compared with that at room temperature, which is probably due to the faster opening and closing of the TRPV1 channel at a higher temperature [52*]. The inhibitory effect of hyperthermia on the ASICs in pulmonary sensory neurons is in general agreement with previous observations that the proton-gated channel activation is inhibited by increasing temperature in both transfected cells and DRG neurons [54]. Hyperthermia appears to exert an effect on ASIC channels via the same site as proton does, because mutation of a conserved residue that regulates the ASIC channel gating also abolishes the desensitizing effect of increasing temperature on acid-induced current [54]. These findings further suggest different roles of these two types of channels in sensing the acidity under different conditions in pulmonary sensory neurons. In the physiological range of body core (intrathoracic) temperature, TRPV1 plays a dominant role, whereas ASICs contribute very little or no response to acid challenge. However, when one breathes through the mouth, the mucosal temperature in the extrathoracic airways (larynx and trachea) is lowered by the inspired air (ambient temperature), acidification of airway fluid (e.g., during asthma exacerbation) or acid

aspiration is then expected to evoke the ASIC-mediated current in the airway sensory nerves [52*].

Eosinophil-derived cationic proteins

Airway infiltration of eosinophils and the release of their granule proteins are the hallmark of a variety of airway inflammatory diseases including asthma [55]. These eosinophil granule-derived, low molecular weight and highly cationic proteins, including major basic protein, eosinophil cationic protein, eosinophil peroxidase and eosinophil-derived neurotoxin, are believed to play a pathogenic role in airway inflammatory reactions [56]. It has been reported recently that pretreatment with eosinophil major basic protein significantly enhances the excitability of rat vagal pulmonary sensory neurons to acid stimulation (an increase in the numbers of action potentials evoked by acid in current-clamp recordings); surprisingly this potentiating effect is absent on the acid-induced inward current when tested in voltage-clamp recordings [57]. The same study further reveals that the hyperexcitability of pulmonary sensory neurons to acid challenge results, at least partially, from the inhibition of both fast inactivating and sustaining voltage-gated K^+ currents by the eosinophil-derived cationic protein.

Nerve growth factor (NGF)

NGF is a member of neurotrophin family, and known for its important role as an inflammatory mediator during acute tissue injury. NGF is synthesized and released from inflammatory cells such as eosinophils, mast cells and macrophages. In patients with asthma or other types of airway inflammatory reactions, there is a pronounced increase in the NGF level in the serum and bronchoalveolar lavage fluid [58,59]. NGF has been shown to induce airway hyperresponsiveness in anesthetized guinea pigs [60] and in vitro in human bronchus [61]. Exposure of guinea pigs to NGF immediately before citric acid inhalation results in a significant increase in the citric acid-induced cough and airway obstruction [62]. The latter is significantly inhibited by the pretreatment with K252a, a NGF TrkA receptor antagonist, or by iodo-resiniferatoxin, a TRPV1 antagonist. These results indicate that although NGF does not directly induce cough or airway obstruction, it can significantly enhance the citric acid-induced both cough and airway obstruction. The effect is likely mediated through the TrkA receptor and TRPV1, but not the p38 MAPK-dependent pathway [62].

Tumor necrosis factor-alpha (TNF α)

An important role of TNF α , a pro-inflammatory cytokine, in the pathogenesis of allergic asthma has been extensively documented [63,64]. TNF α can be released from a variety of cell types in the airways, particularly macrophages, monocytes and mast cells. Increased levels of TNF α have been detected in bronchoalveolar lavage fluid, sputum and serum of asthmatic patients during asthmatic attack or following antigen inhalation challenge [65,66]. A recent study demonstrated that prolonged treatment with TNF α induces a pronounced potentiating effect on the acid-evoked Ca^{2+} transient in pulmonary sensory neurons, but the underlying mechanism is not known [67*]. The biological effects of TNF α are mediated by activation of two distinct types of TNF receptors (TNFR) on the cell surface: TNFR1 and TNFR2 [68]; the former is believed to be primarily responsible for the pro-inflammatory effects induced by TNF α in the pathogenesis of asthma [64]. Coincidentally, an important role of TNFR1 in the upregulation of TRPV1 expression in DRG neurons has been recently reported, and the potentiating effect of TNF α is absent in these neurons isolated from mice lacking TNFR1 [69]. Whether the TNFR1-mediated signaling pathways are also involved in the potentiating effect of TNF α on pulmonary sensory neurons remains to be determined [67*].

5. Conclusion

Tissue acidification in the airways can occur under both normal and pathophysiological conditions. The stimulatory effect of hydrogen ion on vagal bronchopulmonary sensory nerves is primarily responsible for the acid-evoked airway irritation, cough and reflex bronchoconstriction, and is generated by activation of two major types of ion channels: a rapid transient current mediated through the ASIC channels, and a slow sustaining current mediated through the TRPV1. Recent studies have further revealed that the responses of isolated pulmonary sensory neurons to acid are up-regulated by certain inflammatory mediators (e.g., tryptase, NGF, TNF α , etc.). These findings are particularly important for the reason that tissue acidification and endogenous release of these chemical mediators often occur concurrently during airway inflammatory reaction.

There are several important and interesting questions that require further investigation on this subject. For example, studies are required to elucidate the mechanisms by which the ASIC and TRPV1 current responses to hydrogen ion are amplified under the pathophysiological conditions described above (e.g., PAR₂ activation, hyperthermia, etc.). Further, it is known that pH in the airway fluid drops substantially in patients during asthma exacerbation, but to what extent that the acidification of airway fluid or tissue is responsible for the manifestation of airway irritation and excessive cough in these patients is not known. Answers to these questions should lead to a better understanding of the pathogenic role of tissue acidification in the airway dysfunction occurring in the various inflammatory pulmonary diseases.

Acknowledgments

The authors thank Marcus Geer for technical assistances. The work was supported in part by USPHS grants from the National Institutes of Health (HL58686 & HL96914 to L.Y.L.; AI76714 to Q.G.).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

1. Medbo JJ, Sejersted OM. Acid-base and electrolyte balance after exhausting exercise in endurance-trained and sprint-trained subjects. *Acta Physiol Scand.* 1985; 125:97–109. [PubMed: 4050490]
2. Hunt JF, Fang K, Malik R, Snyder A, Malhotra N, Platts-Mills TA, Gaston B. Endogenous airway acidification. Implications for asthma pathophysiology. *Am J Respir Crit Care Med.* 2000; 161:694–699. [PubMed: 10712309]
3. Kostikas K, Papatheodorou G, Ganas K, Psathakis K, Panagou P, Loukides S. pH in expired breath condensate of patients with inflammatory airway diseases. *Am J Respir Crit Care Med.* 2002; 165:1364–1370. [PubMed: 12016097]
4. Simonsson BG, Jacobs FM, Nadel JA. Role of autonomic nervous system and the cough reflex in the increased responsiveness of airways in patients with obstructive airway disease. *J Clin Invest.* 1967; 46:1812–1818. [PubMed: 6070326]
5. Karlsson JA, Fuller RW. Pharmacological regulation of the cough reflex--from experimental models to antitussive effects in Man. *Pulm Pharmacol Ther.* 1999; 12:215–228. [PubMed: 10500001]
6. Ricciardolo FLM, Rado V, Fabbri LM, Sterk PJ, Di Maria GU, Geppetti P. Bronchoconstriction induced by citric acid inhalation in guinea pigs - Role of tachykinins, bradykinin, and nitric oxide. *Am J Respir Crit Care Med.* 1999; 159:557–562. [PubMed: 9927373]

7. Lowry RH, Wood AM, Higenbottam TW. Effects of pH and osmolarity on aerosol-induced cough in normal volunteers. *Clin Sci*. 1988; 74:373–376. [PubMed: 3356109]
8. Wong CH, Matai R, Morice AH. Cough induced by low pH. *Respir Med*. 1999; 93:58–61. [PubMed: 10464850]
9. Morice AH, Kastelik JA, Thompson R. Cough challenge in the assessment of cough reflex. *Br J Clin Pharmacol*. 2001; 52:365–375. [PubMed: 11678780]
- *10. Canning BJ, Farmer DG, Mori N. Mechanistic studies of acid-evoked coughing in anesthetized guinea pigs. *Am J Physiol Regul Integr Comp Physiol*. 2006; 291:R454–R463. This is a comprehensive study of the mechanisms possibly involved in the acid-evoked cough reflex in anesthetized guinea pigs. [PubMed: 16914432]
11. Nault MA, Vincent SG, Fisher JT. Mechanisms of capsaicin- and lactic acid-induced bronchoconstriction in the newborn dog. *J Physiol*. 1999; 515:567–578. [PubMed: 10050022]
12. Tsuchida H, Takahashi S, Nosaka E, Mukaiyama O, Yamashita M, Morimoto K. Novel triple neurokinin receptor antagonist CS-003 strongly inhibits neurokinin related responses. *Eur J Pharmacol*. 2008; 586:306–312. [PubMed: 18353309]
13. Tuchman DN, Boyle JT, Pack AI, Schwartz J, Kokonos M, Spitzer AR, Cohen S. Comparison of Airway Responses Following Tracheal or Esophageal Acidification in the Cat. *Gastroenterology*. 1984; 87:872–881. [PubMed: 6468875]
14. Jack CIA, Calverley PMA, Donnelly RJ, Tran J, Russell G, Hind CRK, Evans CC. Simultaneous tracheal and esophageal pH measurements in asthmatic-patients with gastroesophageal reflux. *Thorax*. 1995; 50:201–204. [PubMed: 7701464]
15. Canning BJ, Mazzone SB. Reflex mechanisms in gastroesophageal reflux disease and asthma. *Am J Med*. 2003; 115:45S–48S. [PubMed: 12928074]
- *16. Kollarik M, Ru F, Udem BJ. Acid-sensitive vagal sensory pathways and cough. *Pulm Pharmacol Ther*. 2007; 20:402–411. This paper provides an overview of the acid-sensitive sensory pathways involved in the cough reflex, and discuss the mechanisms of acid-evoked stimulation of airway afferents. [PubMed: 17289409]
17. Harding SM. Gastroesophageal reflux, asthma, and mechanisms of interaction. *Am J Med*. 2001; 111:8–12.
18. Bevan S, Geppetti P. Protons: small stimulants of capsaicin-sensitive sensory nerves. *Trends Neurosci*. 1994; 17:509–512. [PubMed: 7532332]
19. Lou YP, Lundberg JM. Inhibition of low pH evoked activation of airway sensory nerves by capsazepine, a novel capsaicin-receptor antagonist. *Biochem Biophys Res Commun*. 1992; 189:537–544. [PubMed: 1280428]
20. Fox AJ, Urban L, Barnes PJ, Dray A. Effects of capsazepine against capsaicin-evoked and proton-evoked excitation of single airway c-fibers and vagus nerve from the guinea-pig. *Neuroscience*. 1995; 67:741–752. [PubMed: 7675200]
21. Kollarik M, Udem BJ. Mechanisms of acid-induced activation of airway afferent nerve fibres in guinea-pig. *J Physiol*. 2002; 543:591–600. [PubMed: 12205192]
22. Laloo UG, Fox AJ, Belvisi MG, Chung KF, Barnes PJ. Capsazepine inhibits cough induced by capsaicin and citric acid but not by hypertonic saline in guinea pigs. *J Appl Physiol*. 1995; 79:1082–1087. [PubMed: 8567546]
23. Bolser DC, DeGennaro FC, Oreilly S, McLeod RL, Hey JA. Central antitussive activity of the NK1 and NK2 tachykinin receptor antagonists, CP-99,994 and SR 48968, in the guinea-pig and cat. *Br J Pharmacol*. 1997; 121:165–170. [PubMed: 9154323]
24. Daoui S, Cognon C, Naline E, Emonds-Alt X, Advenier C. Involvement of tachykinin NK3 receptors in citric acid-induced cough and bronchial responses in guinea pigs. *Am J Respir Crit Care Med*. 1998; 158:42–48. [PubMed: 9655705]
- **25. Canning BJ, Mazzone SB, Meeker SN, Mori N, Reynolds SM, Udem BJ. Identification of the tracheal and laryngeal afferent neurones mediating cough in anaesthetized guinea-pigs. *J Physiol*. 2004; 557:543–558. This paper provided an in-depth description of the cough receptors located in the extrapulmonary airways, and their distinct physiological properties and function in regulating cough reflex elicited by topical acid application and punctuate stimulation. [PubMed: 15004208]

26. Canning BJ, Mori N, Mazzone SB. Vagal afferent nerves regulating the cough reflex. *Respir Physiol Neurobiol.* 2006; 152:223–242. [PubMed: 16740418]
27. Mazzone SB, Reynolds SM, Mori N, Kollarik M, Farmer DG, Myers AC, Canning BJ. Selective expression of a sodium pump isozyme by cough receptors and evidence for its essential role in regulating cough. *J Neurosci.* 2009; 29:13662–13671. [PubMed: 19864578]
28. Hong JL, Kwong K, Lee LY. Stimulation of pulmonary C fibres by lactic acid in rats: contributions of H⁺ and lactate ions. *J Physiol.* 1997; 500:319–329. [PubMed: 9147320]
29. Lansing RW, Gracely RH, Banzett RB. The multiple dimensions of dyspnea: Review and hypotheses. *Respir Physiol Neurobiol.* 2009; 167:53–60.
30. Lee LY. Respiratory sensations evoked by activation of bronchopulmonary C-fibers. *Respir Physiol Neurobiol.* 2009; 167:26–35.
31. Burki NK, Lee LY. Mechanisms of dyspnea. *Chest.* 2010; 138:1196–1201. [PubMed: 21051395]
32. Paintal AS. Sensations From J-receptors. *News Physiol Sci.* 1995; 10:238–243.
33. Ruan T, Gu Q, Kou YR, Lee LY. Hyperthermia increases sensitivity of pulmonary C-fibre afferents in rats. *J Physiol.* 2005; 565:295–308. [PubMed: 15760937]
34. Nguyen LS, Villablanca AC, Rutledge JC. Substance P increases microvascular permeability via nitric oxide-mediated convective pathways. *Am J Physiol.* 1995; 268:R1060–R1068. [PubMed: 7537470]
35. Hargreaves M, Ravi K, Kappagoda CT. Responses of slowly and rapidly adapting receptors in the airways of rabbits to changes in the Starling forces. *J Physiol.* 1991; 432:81–97. [PubMed: 1886073]
36. Ho CY, Gu Q, Lin YS, Lee LY. Sensitivity of vagal afferent endings to chemical irritants in the rat lung. *Respir Physiol.* 2001; 127:113–124. [PubMed: 11504584]
37. Franco-cereceda A, Kallner G, Lundberg JM. Cyclooxygenase products released by low pH have capsaicin-like actions on sensory nerves in the isolated guinea-pig heart. *Cardiovasc Res.* 1994; 28:365–369. [PubMed: 8174157]
- **38. Gu Q, Lee LY. Characterization of acid signaling in rat vagal pulmonary sensory neurons. *Am J Physiol Lung Cell Mol Physiol.* 2006; 291:L58–L65. Using perforated patch-clamp recordings, this study demonstrated that physiological/pathophysiological-relevant low pH stimulates rat vagal pulmonary sensory neurons via the activation of both ASIC and TRPV1 channels. [PubMed: 16443641]
39. Lee LY, Gu Q. Role of TRPV1 in inflammation-induced airway hypersensitivity. *Curr Opin Pharmacol.* 2009; 9:243–249. [PubMed: 19269247]
40. Waldmann, R.; Champigny, G.; Lingueglia, E.; De Weille, JR.; Heurteaux, C.; Lazdunski, M. H⁺-gated cation channels. In: Rudy, B.; Seeburg, P., editors. *Molecular and Functional Diversity of Ion Channels and Receptors.* Vol. 868. *Annals of the New York Academy of Sciences*; 1999. p. 67-76.
41. Wemmie JA, Price MP, Welsh MJ. Acid-sensing ion channels: advances, questions and therapeutic opportunities. *Trends Neurosci.* 2006; 29:578–586. [PubMed: 16891000]
42. Hesselager M, Timmermann DB, Ahring PK. pH dependency and desensitization kinetics of heterologously expressed combinations of acid-sensing ion channel subunits. *J Biol Chem.* 2004; 279:11006–11015. [PubMed: 14701823]
- *43. Gu Q, Lee LY. Regulation of acid signaling in rat pulmonary sensory neurons by protease-activated receptor-2. *Am J Physiol Lung Cell Mol Physiol.* 2010; 298:L454–L461. This study showed that PAR₂ agonist significantly potentiates acid-evoked both ASIC- and TRPV1-like inward currents in rat pulmonary sensory neurons, and enhances the excitability of these neurons to acid stimulation. [PubMed: 20044436]
44. Alvarez de la Rosa D, Zhang P, Shao D, White F, Canessa CM. Functional implications of the localization and activity of acid-sensitive channels in rat peripheral nervous system. *Proc Natl Acad Sci U S A.* 2002; 99:2326–2331. [PubMed: 11842212]
45. Ossovskaya VS, Bunnett NW. Protease-activated receptors: Contribution to physiology and disease. *Physiol Rev.* 2004; 84:579–621. [PubMed: 15044683]
46. Reed CE, Kita H. The role of protease activation of inflammation in allergic respiratory diseases. *J Allergy Clin Immunol.* 2004; 114:997–1008. [PubMed: 15536399]

47. Ramachandran R, Hollenberg MD. Proteinases and signalling: pathophysiological and therapeutic implications via PARs and more. *Br J Pharmacol*. 2008; 153:S263–S282. [PubMed: 18059329]
48. Sokolova E, Reiser G. A novel therapeutic target in various lung diseases: Airway proteases and protease-activated receptors. *Pharmacol Ther*. 2007; 115:70–83. [PubMed: 17532472]
49. Cocks TM, Fong B, Chow JM, Anderson GP, Frauman AG, Goldie RG, Henry PJ, Carr MJ, Hamilton JR, Moffatt JD. A protective role for protease-activated receptors in the airways. *Nature*. 1999; 398:156–160. [PubMed: 10086357]
- *50. Gatti R, Andre E, Amadesi S, Dinh TQ, Fischer A, Bunnett NW, Harrison S, Geppetti P, Trevisani M. Protease-activated receptor-2 activation exaggerates TRPV1-mediated cough in guinea pigs. *J Appl Physiol*. 2006; 101:506–511. This study showed that PAR₂ stimulation exaggerates TRPV1-dependent cough by activation of diverse mechanism(s), including PKC, PKA, and prostanoid release. [PubMed: 16627674]
51. Piacentini GL, Peroni D, Crestani E, Zardini F, Bodini A, Costella S, Boner AL. Exhaled air temperature in asthma: methods and relationship with markers of disease. *Clin Exp Allergy*. 2007; 37:415–419. [PubMed: 17359391]
- *52. Ni D, Lee LY. Effect of increasing temperature on TRPV1-mediated responses in isolated rat pulmonary sensory neurons. *Am J Physiol Lung Cell Mol Physiol*. 2008; 294:L563–L571. This study demonstrated that increasing temperature within the normal physiological range (36–40.6 °C) exerts opposite effects on the two types of the acid-induced current in pulmonary sensory neurons: the TRPV1-mediated current being potentiated, but the ASICs-mediated current inhibited. [PubMed: 18178674]
53. Vyklíček L, Vlachova V, Vitabskova Z, Dittert I, Kabat M, Orkand RK. Temperature coefficient of membrane currents induced by noxious heat in sensory neurones in the rat. *J Physiol*. 1999; 517:181–192. [PubMed: 10226158]
54. Askwith CC, Benson CJ, Welsh MJ, Snyder PM. DEG/ENaC ion channels involved in sensory transduction are modulated by cold temperature. *Proc Natl Acad Sci U S A*. 2001; 98:6459–6463. [PubMed: 11353858]
55. Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma - From bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med*. 2000; 161:1720–1745. [PubMed: 10806180]
56. Gleich GJ. Mechanisms of eosinophil-associated inflammation. *J Allergy Clin Immunol*. 2000; 105:651–663. [PubMed: 10756213]
57. Gu Q, Lim ME, Gleich GJ, Lee LY. Mechanisms of eosinophil major basic protein-induced hyperexcitability of vagal pulmonary chemosensitive neurons. *Am J Physiol Lung Cell Mol Physiol*. 2009; 296:L453–L461. [PubMed: 19136577]
58. Bonini S, Lambiase A, Bonini S, Angelucci F, Magrini L, Manni L, Aloe L. Circulating nerve growth factor levels are increased in humans with allergic diseases and asthma. *Proc Natl Acad Sci U S A*. 1996; 93:10955–10960. [PubMed: 8855290]
59. Hoglund CO, de Blay F, Oster JP, Duvernelle C, Kassel O, Pauli G, Frossard N. Nerve growth factor levels and localisation in human asthmatic bronchi. *Eur Respir J*. 2002; 20:1110–1116. [PubMed: 12449162]
60. de Vries A, Dessing MC, Engels F, Henricks PAJ, Nijkamp FP. Nerve growth factor induces a neurokinin-1 receptor-mediated airway hyperresponsiveness in guinea pigs. *Am J Respir Crit Care Med*. 1999; 159:1541–1544. [PubMed: 10228123]
61. Frossard N, Naline E, Hoglund CO, Georges O, Advenier C. Nerve growth factor is released by IL-1 beta and induces hyperresponsiveness of the human isolated bronchus. *Eur Respir J*. 2005; 26:15–20. [PubMed: 15994384]
62. El-Hashim AZ, Jaffal SM. Nerve growth factor enhances cough and airway obstruction via TrkA receptor- and TRPV1-dependent mechanisms. *Thorax*. 2009; 64:791–797. [PubMed: 19497920]
63. Thomas PS. Tumour necrosis factor-alpha: The role of this multifunctional cytokine in asthma. *Immunol Cell Biol*. 2001; 79:132–140. [PubMed: 11264706]
64. Berry MA, Hargadon B, Shelley M, Parker D, Shaw DE, Green RH, Bradding P, Brightling CE, Wardlaw AJ, Pavord ID. Evidence of a role of tumor necrosis factor alpha in refractory asthma. *N Engl J Med*. 2006; 354:697–708. [PubMed: 16481637]

65. Cembrzynskanowak M, Szklarz E, Inglot AD, Teodorczykkinjeyan JA. Elevated release of tumor-necrosis-factor-alpha and interferon-gamma by bronchoalveolar leukocytes from patients with bronchial-asthma. *Am Rev Respir Dis.* 1993; 147:291–295. [PubMed: 8430950]
66. Keatings VM, Oconnor BJ, Wright LG, Huston DP, Corrigan CJ, Barnes PJ. Late response to allergen is associated with increased concentrations of tumor necrosis factor-alpha and IL-5 in induced sputum. *J Allergy Clin Immunol.* 1997; 99:693–698. [PubMed: 9155837]
- *67. Hu YM, Gu Q, Lin RL, Kryscio R, Lee LY. Calcium transient evoked by TRPV1 activators is enhanced by tumor necrosis factor-alpha in rat pulmonary sensory neurons. *Am J Physiol Lung Cell Mol Physiol.* 2010; 299:L483–L492. This study showed that a prolonged treatment with TNF α induces a marked potentiation of the calcium influx in isolated pulmonary sensory neurons evoked by TRPV1 activators including hydrogen ion. [PubMed: 20639352]
68. Baud V, Karin M. Signal transduction by tumor necrosis factor and its relatives. *Trends Cell Biol.* 2001; 11:372–377. [PubMed: 11514191]
69. Hensellek S, Brell P, Schaible HG, Brauer R, von Banchet GS. The cytokine TNF alpha increases the proportion of DRG neurones expressing the TRPV1 receptor via the TNFR1 receptor and ERK activation. *Mol Cell Neurosci.* 2007; 36:381–391. [PubMed: 17851089]

Figure 1.

Cough response to acid challenge. **(a)** Mean dose responses of cough to inhalation challenges of aerosolized capsaicin ($n = 18$), citric acid ($n = 48$), phosphoric acid ($n = 22$) and acetic acid ($n = 26$) in healthy subjects (age 18–60 years; each subject was tested in at least two study series). **(b)** Cumulative coughs evoked by citric acid ($n = 83$), sodium citrate ($n = 3$), and HCl ($n = 3$) solutions applied topically in 100 μ l aliquots directly to the tracheal mucosa of anesthetized guinea pigs; concentration response curves were created cumulatively, with increasing concentrations added in 100 μ l aliquots at 1-min intervals. **(c)** The number of coughs evoked by each dose of citric acid, applied in 100 μ l aliquots in anesthetized guinea pigs ($n = 33$). **(d)** The number of coughs evoked by a single, suprathreshold (0.1 M) concentration of citric acid and by water (the vehicle for citric acid), applied in increasing volumes at 1-min intervals ($n = 4$) are shown (adapted from Ref. [8,10*]).

Figure 2. Acid-evoked whole cell inward currents and inhibition by amiloride and capsazepine in rat vagal pulmonary sensory neurons. **(a)** Low pH with increasing proton concentrations are applied for 6 s (as indicated by the horizontal bars) to four different neurons. Note distinct pH sensitivities and different phenotypes of inward currents in response to acidic challenges. **(b)** Effect of 2-min pretreatments with amiloride (100 μ M) and capsazepine (CPZ; 10 μ M) on acid (pH 5.5)-evoked inward currents. **(c)** Acid (pH 6.5–5.5)-evoked both transient and sustaining components are inhibited by amiloride and CPZ. * $P < 0.05$ as compared with the corresponding control (adapted from Ref. [38**]).

Figure 3.

Potentiation of acid-induced responses by PAR₂-AP in rat pulmonary sensory neurons. **(a)** Acid (pH 5.5, 18 s)-evoked ASIC-like (1st peak) and TRPV1-like (2nd peak) inward currents at control, after pretreatment with amiloride (300 μ M, 2 min), amiloride + PAR₂-AP (300 μ M and 100 μ M, respectively; 2 min) and PAR₂-AP (100 μ M, 2 min) alone, and after 10-min washout. **(b)** and **(c)** Group data showing the peak amplitudes of pH 5.5-evoked ASIC-like and TRPV1-like inward currents, respectively, after different pretreatments as shown in **(a)**. * Significantly different from the control response. † Significant difference between pretreatments with amiloride + PAR₂-AP and amiloride alone. **(d)** Acid (pH 5.5, 6 s)-evoked changes in membrane potential (V_m) at control, after pretreatment with PAR₂-AP, and after 5-min washout. V_m-1 and V_m-2 refer to the values of membrane potential at the 1st peak and at the termination of acid challenge, respectively. **(e)** Effect of PAR₂-AP on the pH 5.5-evoked membrane potential changes. **(f)** Effect of PAR₂-AP on the number of pH 5.5-evoked action potentials. * Significantly different from the corresponding control (adapted from Ref. [43*]).

Figure 4.

Effect of increasing temperature on the response of vagal pulmonary sensory neurons to acid. **(a)** Experimental records illustrating the acid-induced current exhibiting both rapid, transient and slow, sustained components in a jugular pulmonary neuron (22.5 pF) at 24.4°C. Transient component of the acid (pH 5.5)-induced current was almost completely inhibited whereas the sustained component was potentiated when the temperature was raised; the responses recovered when the temperature was returned. Pretreatments with amiloride (1 mM, 1 min) and capsazepine (CPZ; 10 μ M, 3 min) blocked the transient and sustained components, respectively. **(b)** Acid (pH 6.5)-evoked rapid transient current was completely inhibited when the temperature was increased in a nodose neuron (27.9 pF). **(c)** Group data for the acid (pH 6 or 6.5)-evoked transient current tested at the three temperatures in the order shown in (a). RT: room temperature ($22.8 \pm 0.58^\circ\text{C}$); BT, body temperature ($35.8 \pm 0.10^\circ\text{C}$); HT, hyperthermic temperature ($40.6 \pm 0.12^\circ\text{C}$). $**P < 0.01$, $*P < 0.05$ compared with RT. **(d)** Acid (pH 6.0)-evoked slow, sustained current was increased when the temperature was increased from 35.7 to 40.3°C in a nodose neuron (24.6 pF). **(e)** Group data for the acid (pH 5.5 or 6.0)-evoked sustained current response at the two temperatures: BT, $36.0 \pm 0.07^\circ\text{C}$; HT, $40.5 \pm 0.08^\circ\text{C}$. $*P < 0.05$ compared with BT (adapted from Ref. [52*]).