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Cough and gastroesophageal reflux: Insights from animal models

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Abstract

Chronic cough in gastroesophageal reflux disease (GERD) has been attributed to irritation of the esophagus and/or upper airways by reflux of gastric content. Animal models have provided insight into both of these putative mechanisms. In patients with chronic cough and GERD, stimuli associated with reflux in the esophagus sensitize the cough reflex. This sensitization can be reproduced in the guinea pig and is most likely mediated by the esophageal afferent nerve fibers carried by the vagus nerves. Studies in animals have identified several subtypes of vagal esophageal C-fibers that may subserve this function. The putative nociceptive vagal C-fibers in the guinea pig esophagus are stimulated by acid and express the TRPV1 and TRPA1 receptors that confer responsiveness to disparate noxious stimuli. Acute and/or chronic irritation of the upper airways by reflux may contribute to cough by stimulation and/or sensitization of the airway afferent nerves. Studies in animals have identified airway nerves that likely initiate cough due to aspirated reflux; have characterized their pharmacology; and have provided insight into changes of their sensitivity. Studies in animal models have also described the neurophysiology of reflexes that protect the airways from reflux. In conclusion, animal models provide mechanistic insight into the modulation of cough from the esophagus and the pharmacology of neural pathways mediating cough in GERD.

Modeling cough in animals has inherent advantages and limitations (reviewed in [1]). The major advantage of animal models is the amenability to mechanistic manipulations. Thus animal models are the source of important mechanistic information that cannot be readily obtained in humans. The major disadvantage of studying cough in animals is that animal models rarely completely mimic all aspects of a human disorder. Nonetheless, useful extrapolations regarding the pathogenesis of cough can be made from animal models with appropriate caution. In this review we focus on the insights made from animal models regarding the neurophysiology and pharmacology of neural pathways mediating cough associated with GERD. Animal models have also provided invaluable information about the pathogenesis of GERD, however, the discussion of this extensive research area is beyond the scope of the current review.

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Putative mechanisms of cough in gastroesophageal reflux disease

Based on data from human studies, two major common mechanisms of cough in GERD have been proposed: cough mediated by the activation of the afferent nerves in the esophagus and cough as a result of acute or chronic (micro-) aspiration of the refluxed content into the airways [2,3]. Studies in animal models have identified and characterized the neural pathways for each of these two putative mechanisms.

It has been suggested that activation of the vagal sensory nerves in the esophagus triggers cough in patients with GERD and chronic cough [4]. A strong temporal association between the reflux episodes and cough would argue that cough is directly triggered from the esophagus. Unfortunately, the reported association between reflux and cough is variable among the studies (depending also on patient selection and methods for detection of cough and reflux, see for example [5–9]). Cough is occasionally encountered during prolonged infusion of acid into the esophagus [4], but this finding is inconsistent across the studies [10,11]. Thus, based on the available evidence it is difficult to unequivocally conclude that cough is directly initiated from the esophagus in patients with chronic cough and GERD.

More circumstantial evidence supports the hypothesis that activation of afferent nerves in the esophagus leads to sensitization of the cough reflex (cough reflex hypersensitivity) in patients with chronic cough and GERD. Increased cough reflex sensitivity is a regular finding in patients with gastroesophageal reflux [12,13]. Increased cough reflex sensitivity is thought to contribute to chronic coughing because the endogenous and environmental stimuli are more effective in triggering cough in these patients. Additional support for the role of the cough reflex hypersensitivity in the pathogenesis of cough comes from reports that the cough reflex sensitivity decreases with effective treatment of gastroesophageal reflux [13,14,15,16].

While the mechanisms of cough reflex hypersensitivity in humans are incompletely understood, the data from clinical studies indicate that the hypersensitivity is at least in part caused by the stimulation of distal esophagus. Infusion of acid into the distal esophagus of patients with GERD and chronic cough induced the cough reflex hypersensitivity (documented as lowered cough threshold to inhaled capsaicin) [11]. The increase in the cough reflex sensitivity developed within 15 min (the earliest time point evaluated). Similar rapid (within 10 minutes) sensitization of cough reflex by infusion of acid into the esophagus was also demonstrated in patients with asthma [17]. The relatively rapid change in cough sensitivity is best explained by assuming a neurally-mediated process. Indeed, the effects of acid infusion on cough are inhibited by esophageal instillation of the local anesthetic lignocaine [4].

Regardless of whether the cough reflex is only sensitized or also directly triggered from the esophagus, the data from human studies predict an afferent neural pathway mediating the effects of esophageal stimuli on the cough reflex. Since the afferent pathways of the cough reflex have been so far convincingly demonstrated only in the vagus nerves, the simplest hypothesis predicts a vagal pathway from the esophagus that sensitizes the cough reflex. Although it cannot be excluded that the esophageal inputs sensitizing cough are also carried by other than vagal afferent pathways (i.e. spinal or other cranial nerves), this alternative remains speculative in the absence of supporting evidence. It should be also noted that a subgroup of patients with chronic cough due to gastroesophageal reflux do not present with heartburn and chest pain. A likely interpretation of this finding is that the pathways mediating the effects on cough (presumably vagal pathways) are different from the pathways mediating heartburn and pain (spinal pathways).

Vagal afferent pathways from the esophagus and cough reflex

The afferent nerves in the esophagus responsible for modulation of the cough reflex are predicted to respond to stimuli associated with reflux. A number of clinical studies have shown that acid is an important mediator of cough in GERD (reviewed in [2,3]). In addition to acid, other chemicals in the refluxed fluid such as components of bile and pepsin have been implicated in the activation of esophageal nerves in GERD [2,18]. Another stimuli likely relevant for gastroesophageal reflux are the mechanical stimuli [18]. While mechanical stimuli are present in the esophagus during its normal activity, the abnormal mechanical pattern associated with reflux or sustained esophageal contractions [19] may provide an adequate stimulus for modulation of cough. The effects of weakly acidic (pH>4) reflux have been, at least in part, attributed to its volume (i.e. mechanical effects) and the association of cough with weakly acidic reflux has been reported in several studies [8,20–22], reviewed in [23]. Thus the afferent pathways modulating the cough reflex are likely responsive to chemical stimuli including acid and to mechanical stimulation.

A complex picture of the vagal afferent innervation of the esophagus has emerged from anatomical and neurophysiological studies in various species [24]. A number of thorough anatomical studies have characterized neural structures in the esophagus; however, with few exceptions it is still difficult to assign a morphological correlate to a functionally characterized afferent nerve. We will therefore base our discussion on the functional neurophysiological studies and refer to histological data whenever possible. In general the afferent nerves in the esophagus fit into two broad categories: (1) afferent nerves sensitive to low intensity mechanical stimuli - low threshold mechanosensors and (2) afferent nerves discriminately responsive to noxious stimuli - nociceptors. In this discussion, we use the term nociceptive to describe the capacity of an afferent nerve to respond to noxious stimuli.

Vagal nociceptive C-fiber subtypes in the esophagus

Vagal nociceptive afferent nerves in the esophagus have been defined based on their discriminative responsiveness to noxious mechanical stimuli and their sensitivity to noxious chemical stimuli [25,26]. Vast majority of the vagal nociceptive nerves in the guinea pig esophagus conduct action potentials in the C-fiber range (<1m/s). The vagal nociceptive afferent nerves described in the guinea pig [25,26] are likely analogous to mucosal-tension receptors described in the ferret [27] and a subset of vagal afferent nerves described in the rat [28]. Vagal nociceptive C-fibers express the TRPV1 receptor, which is activated by acid and certain lipid mediators [25,26]. Vagal C-fibers are also activated and/or sensitized by a wide range of the endogenous mediators associated with inflammation such as bradykinin (B₂ receptor) [29], adenosine (A₁ and possibly A_{2B} receptor, unpublished data), purines (P2X receptors for ATP) [30], histamine (H₁ receptor) [31] and serotonin (5-HT₃ receptor) [25]. We have recently investigated the activation of the vagal esophageal C-fibers via the TRPA1 receptor (Fig. 1). As discussed elsewhere in this issue, TRPA1 confers the afferent nerve sensitivity to a number of endogenous molecules associated with inflammation and tissue damage (Taylor-Clark & Undem, this issue). We found that TRPA1 agonists induce robust activation of the vagal esophageal C-fibers (Fig. 1), an observation consistent with the findings of a recently-published study [29]

Given their capacity to detect noxious stimuli and their vagal origin, vagal nociceptive C-fibers appear to be the most likely candidates for mediating the effects of reflux on cough. The sensitization of the cough reflex by esophageal acid demonstrated in patients with GERD and cough can be reproduced in the guinea pig model (BJ Canning, personal communication). Similar observations were made in the guinea pig after infusion of the TRPV1 receptor agonist capsaicin into the esophagus. The cough sensitization is mimicked by the activation of central terminals of vagal nerves evoked by injection of capsaicin into

the appropriate areas of the brainstem [32]. These data also support the notion that sensitization of the cough reflex from the esophagus is mediated by the vagal afferent capsaicin-sensitive C-fibers innervating the esophagus.

Some studies in the lung and esophagus have demonstrated that there are at least two subtypes of vagal nociceptive C-fibers. Vagal C-fiber subtypes are determined by their embryonic origin and distinguished by certain activation properties and anatomical distribution [25,26,30,33,34]. In most species the neurons of the neural crest-derived nociceptive subtype reside in the vagal jugular ganglion, while the neurons of the placodes-derived subtype reside in the vagal nodose ganglion[35]. In the guinea pig both vagal afferent subtypes project into the esophagus[25,26]. It is currently unknown which vagal C-fiber subtype in the esophagus mediates the cough reflex sensitization, but available evidence suggests a role for the jugular nociceptive subtype. First, the jugular C-fiber subtype is more likely to express neurokinins implicated in the central sensitization of the cough reflex[26,36]. Second, the circumstantial afferent nerve terminals of the jugular nerve fibers are likely located in the esophageal mucosa and submucosa close to esophageal lumen[28,36]. Finally, in the respiratory system the activation of the jugular nociceptive subtype has been shown to sensitize the cough reflex [32]. Interestingly, vagal jugular and nodose nociceptive subtypes have the opposite effects on respiration [37]. Given the differences in the pharmacology of the nodose and jugular subtypes, the identification of the subtype involved in the cough sensitization is important for pharmacological targeting of the cough sensitization.

It should be also noted that animal studies have revealed a unique pattern of vagal afferent innervation in the uppermost portion of the esophagus [28,36,38]. In this region the extremely high density of vagal afferent nerves located in the mucosa and their responsiveness to acid suggests a role in protection against proximal reflux.

The cough reflex can be sensitized from the esophagus, but direct activation of cough from the esophagus in animal models has not been reported to our knowledge. Even administration of large concentrations of acid into the intact or damaged esophagus has not been reported to initiate cough in anesthetized animals. Similarly, we found that intraesophageal administration of capsaicin failed to trigger cough in the conscious guinea pigs with intact or damaged esophageal mucosa [39]. While the regulation of cough in patients with cough and GERD can be, in theory, altered in a way that the stimulation of esophageal afferent nerves triggers cough, the available data from animal studies do not support this phenomenon.

Vagal low threshold mechanosensors in the esophagus

A proportion of vagal esophageal afferent nerves are exquisitely sensitive to mechanical stimuli [2,27,40,41]. The best characterized in this group are tension mechanosensors (also termed tension mechanoreceptors) activated by esophageal distention. The nerve terminals of tension mechanosensors have been anatomically identified as the intraganglionic laminar endings located in the muscular layers of the esophagus[41]. Tension mechanosensors regulate esophageal motor reflexes[42]. Another subset of the low threshold mechanosensors is most effectively activated by low intensity mechanical stimuli applied to the esophageal mucosa and therefore often termed mucosal receptors [27,43]. While it is reasonable to assume that the nerve terminals of mucosal receptors are located in the mucosa, this assumption still awaits experimental confirmation.

The vagal low threshold mechanosensitive afferent nerves in the esophagus probably do not directly contribute to sensitization of the cough reflex. Because of their low mechanical threshold the mechanosensors are strongly activated by physiological events in the

esophagus. For example, the esophageal distention-induced activity of tension mechanosensors reaches its maximum within the physiological levels of esophageal distention (intraesophageal pressure ≤ 60 mmHg). Therefore, the central nervous system receives strong afferent input from the mechanosensors even during normal activity of the esophagus. Furthermore, acid and other potentially noxious mediators are not effective activators of the esophageal mechanosensors (tension mechanosensors also lack TRPV1 and TRPA1) [25,26,29]. Thus, although the participation of mechanosensors in the direct modulation of cough in GERD cannot be excluded (for example due to inflammation-induced alteration in neurotransmitter expression), this possibility seems unlikely. However, the altered function of vagal mechanosensors may contribute to cough indirectly. Since vagal tension mechanosensors regulate esophageal motor reflexes, their normal function is required to limit and remove the reflux. In particular, some of the reflexes that protect the upper airways from aspiration such as the upper esophageal sphincter constricting reflex and secondary peristalsis are mediated by vagal tension mechanosensors [42,44,45]. We have recently reported that acid directly inhibits the vagal tension mechanosensors [46]. Thus, acid acting on the vagal low threshold mechanosensors may create conditions favorable for reflux and aspiration.

In addition to its effects on cough, stimulation of the esophagus in animals has been shown to modulate airway physiology in a manner that may influence cough. Esophageal acidification can cause a variety of effects in the lungs and airways such as increased airway resistance and increased microvascular leakage [47,48]. A recent elegant study in cats demonstrated that the esophageal acidification increased the output of mucus from the tracheobronchial tree (by $\sim 150\%$) and decreased tracheal mucociliary transport rate (by $\sim 50\%$) [49]. While the physiological relevance of such effects may be uncertain, it can be speculated that their perturbation in disease results in potentiation of cough.

Cough due to irritation of the larynx and trachea

Clinical evidence indicates that the irritation of the large airways by reflux contributes to the cough in some patients with cough due to GERD. The proposed mechanisms of this cough include irritation of the airways leading to inflammation, and micro- and macro-aspiration [2,3]. Animal models have provided information about the neural pathways that trigger cough from the airways and their sensitivity to stimuli associated with reflux.

Larynx and trachea are innervated by two major types of afferent nerves known to regulate the cough reflex: the C-fibers and the low threshold mechanosensitive (“touch sensitive”) A δ -fibers. The physiology and pharmacology of airway afferent nerves have been reviewed in detail elsewhere [46,50–52]. Here we focus on the selected aspects of airway nerves that are potentially important for cough in GERD.

The touch sensitive A δ -fibers have been conclusively shown to initiate cough in response to mechanical stimuli and acid [53,54]. The typical cough attributed to activation of the touch sensitive A δ -fibers is the immediate violent cough triggered by aspiration. The capacity of C-fibers in the large airways to initiate cough is implicated from the ability of selective C-fiber activators (such as capsaicin) to trigger cough [50]. Unlike the immediate A δ -fibers-mediated cough, capsaicin inhalation in humans evokes an increasing urge to cough that is eventually relieved by coughing [55].

Both the touch-sensitive A δ -fibers and C-fibers in the large airways are activated by acid, but the receptors mediating this activation are different (reviewed in [46]). The response to acid in C-fibers is mediated by the capsaicin TRPV1 receptor and a TRPV1-independent mechanism [56,57]. In contrast the touch-sensitive A δ -fibers lack the TRPV1 receptor and the kinetics of their response to acid resembles the Acid sensing ion channels (ASICs) [56].

The threshold for acid-induced activation is lower in the touch-sensitive A δ -fibers (pH₅₀=6.5 compared to pH=5–5.5 required for an effective activation of C-fibers).

Reflux-induced airway inflammation may lead to sensitization of the airway afferent pathways mediating cough. Although it has not been studied in reflux models, the sensitization of airway nerves has been demonstrated in several models of airway inflammation. Inflammation has been shown to acutely sensitize the touch-sensitive A δ -fibers [58]. Airway inflammation also triggers changes in the neurotransmitter expression in the touch-sensitive A δ -fibers, which would be predicted to amplify signaling in cough pathways[59,60]. The TRPV1 and other receptors expressed in the tracheal C-fibers confer these afferent nerves sensitivity to a number of mediators associated with inflammation[50]. Changes in the excitability induced by inflammation have not been studied in detail in the tracheal C-fibers yet. However, inasmuch as these nerves are similar to polymodal nociceptors in somatosensory system, it is likely that inflammation enhances their excitability.

Because of their acid-sensitive properties, the touch-sensitive A δ -fibers are predicted to be more sensitive than C-fibers to stimuli associated with reflux in the intact airways. However, the central and peripheral sensitization (discussed above) may result in enhanced sensitivity of the C-fiber mediated cough to the stimuli associated with reflux. Indeed the pattern of cough in GERD is rarely consistent with the typical aspiration-induced cough[61], but rather resembles the pattern of the capsaicin-induced cough mediated by C-fibers. Thus, it seems more likely that in those patients with GERD in which the cough is initiated from the airways, the cough is triggered by the C-fibers.

Conclusion

Multiple neural pathways innervating the esophagus and airways are likely involved in the pathogenesis of chronic cough in GERD. The nature of the pathways involved depends on whether the irritation of the airways, modulation of the cough by sensory inputs from the esophagus or their combination is the predominant cough mechanism. Animal models have provided mechanistic insight into the modulation of cough from the esophagus and the pharmacology of neural pathways mediating cough in GERD.

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References

1. Canning BJ. The cough reflex in animals: relevance to human cough research. *Lung* 2008;186(Suppl 1):S23–8. 10.1007/s00408-007-9054-6 [PubMed: 18080711]
2. Irwin RS. Chronic cough due to gastroesophageal reflux disease: ACCP evidence-based clinical practice guidelines. *Chest* 2006;129:80S–94S. 129/1_suppl/80S [pii]. 10.1378/chest.129.1_suppl.80S [PubMed: 16428697]
3. Ing AJ. Cough and gastro-oesophageal reflux disease. *Pulm Pharmacol Ther* 2004;17:403–13. [PubMed: 15564084]
4. Ing AJ, Ngu MC, Breslin AB. Pathogenesis of chronic persistent cough associated with gastroesophageal reflux. *Am J Respir Crit Care Med* 1994;149:160–7. [PubMed: 8111576]
5. Ing AJ, Ngu MC, Breslin AB. Chronic persistent cough and gastro-oesophageal reflux. *Thorax* 1991;46:479–83. [PubMed: 1877035]
6. Ours TM, Kavuru MS, Schilz RJ, Richter JE. A prospective evaluation of esophageal testing and a double-blind, randomized study of omeprazole in a diagnostic and therapeutic algorithm for chronic cough. *Am J Gastroenterol* 1999;94:3131–8. [PubMed: 10566703]

7. Paterson WG, Murat BW. Combined ambulatory esophageal manometry and dual-probe pH-metry in evaluation of patients with chronic unexplained cough. *Dig Dis Sci* 1994;39:1117–25. [PubMed: 8174426]
8. Blondeau K, Dupont LJ, Mertens V, Tack J, Sifrim D. Improved diagnosis of gastro-oesophageal reflux in patients with unexplained chronic cough. *Aliment Pharmacol Ther* 2007;25:723–32. APT3255 [pii]. 10.1111/j.1365-2036.2007.03255.x [PubMed: 17311606]
9. Sifrim D, Dupont L, Blondeau K, Zhang X, Tack J, Janssens J. Weakly acidic reflux in patients with chronic unexplained cough during 24 hour pressure, pH, and impedance monitoring. *Gut* 2005;54:449–54. 54/4/449 [pii]. 10.1136/gut.2004.055418 [PubMed: 15753524]
10. Irwin RS, French CL, Curley FJ, Zawacki JK, Bennett FM. Chronic cough due to gastroesophageal reflux. Clinical, diagnostic, and pathogenetic aspects. *Chest* 1993;104:1511–7. [PubMed: 8222816]
11. Javorkova N, Varechova S, Pecova R, Tatar M, Balaz D, Demeter M, Hyrdel R, Kollarik M. Acidification of the oesophagus acutely increases the cough sensitivity in patients with gastro-oesophageal reflux and chronic cough. *Neurogastroenterol Motil* 2008;20:119–24. NMO1020 [pii]. 10.1111/j.1365-2982.2007.01020.x [PubMed: 17999650]
12. Ferrari M, Olivieri M, Sembenini C, Benini L, Zuccali V, Bardelli E, Bovo P, Cavallini G, Vantini I, Lo Cascio V. Tussive effect of capsaicin in patients with gastroesophageal reflux without cough. *Am J Respir Crit Care Med* 1995;151:557–61. [PubMed: 7842220]
13. O'Connell F, Thomas VE, Pride NB, Fuller RW. Capsaicin cough sensitivity decreases with successful treatment of chronic cough. *Am J Respir Crit Care Med* 1994;150:374–80. [PubMed: 8049818]
14. McGarvey LP, Heaney LG, Lawson JT, Johnston BT, Scally CM, Ennis M, Shepherd DR, MacMahon J. Evaluation and outcome of patients with chronic non-productive cough using a comprehensive diagnostic protocol. *Thorax* 1998;53:738–43. [PubMed: 10319055]
15. Benini L, Ferrari M, Sembenini C, Olivieri M, Micciolo R, Zuccali V, Bulighin GM, Fiorino F, Ederle A, Cascio VL, Vantini I. Cough threshold in reflux oesophagitis: influence of acid and of laryngeal and oesophageal damage. *Gut* 2000;46:762–7. [PubMed: 10807885]
16. Ziora D, Jarosz W, Dzielicki J, Ciekalski J, Krzywiecki A, Dworniczak S, Kozielski J. Citric acid cough threshold in patients with gastroesophageal reflux disease rises after laparoscopic fundoplication. *Chest* 2005;128:2458–64. [PubMed: 16236909]
17. Wu DN, Yamauchi K, Kobayashi H, Tanifuji Y, Kato C, Suzuki K, Inoue H. Effects of esophageal acid perfusion on cough responsiveness in patients with bronchial asthma. *Chest* 2002;122:505–9. [PubMed: 12171823]
18. Ang D, Sifrim D, Tack J. Mechanisms of heartburn. *Nat Clin Pract Gastroenterol Hepatol* 2008;5:383–92. ncpgasthep1160 [pii]. 10.1038/ncpgasthep1160 [PubMed: 18542113]
19. Balaban DH, Yamamoto Y, Liu J, Pehlivanov N, Wisniewski R, DeSilvey D, Mittal RK. Sustained esophageal contraction: a marker of esophageal chest pain identified by intraluminal ultrasonography. *Gastroenterology* 1999;116:29–37. S0016508599000165 [pii]. [PubMed: 9869599]
20. Zerbib F, Roman S, Ropert A, des Varannes SB, Poudroux P, Chaput U, Mion F, Verin E, Galmiche JP, Sifrim D. Esophageal pH-impedance monitoring and symptom analysis in GERD: a study in patients off and on therapy. *Am J Gastroenterol* 2006;101:1956–63. AJG711 [pii]. 10.1111/j.1572-0241.2006.00711.x [PubMed: 16848801]
21. Mainie I, Tutuian R, Shay S, Vela M, Zhang X, Sifrim D, Castell DO. Acid and non-acid reflux in patients with persistent symptoms despite acid suppressive therapy: a multicentre study using combined ambulatory impedance-pH monitoring. *Gut* 2006;55:1398–402. gut.2005.087668 [pii]. 10.1136/gut.2005.087668 [PubMed: 16556669]
22. Tutuian R, Mainie I, Agrawal A, Adams D, Castell DO. Nonacid reflux in patients with chronic cough on acid-suppressive therapy. *Chest* 2006;130:386–91. 130/2/386 [pii]. 10.1378/chest.130.2.386 [PubMed: 16899836]
23. Blondeau K, Sifrim D, Dupont L, Tack J. Reflux cough. *Curr Gastroenterol Rep* 2008;10:235–9. [PubMed: 18625132]

24. Neuhuber WL, Raab M, Berthoud HR, Worl J. Innervation of the mammalian esophagus. *Adv Anat Embryol Cell Biol* 2006;185:1–73. back cover. [PubMed: 16573241]
25. Yu S, Ru F, Ouyang A, Kollarik M. 5-Hydroxytryptamine selectively activates the vagal nodose C-fibre subtype in the guinea-pig oesophagus. *Neurogastroenterol Motil* 2008;20:1042–50. NMO1136 [pii]. 10.1111/j.1365-2982.2008.01136.x [PubMed: 18482251]
26. Yu S, Undem BJ, Kollarik M. Vagal afferent nerves with nociceptive properties in guinea-pig oesophagus. *J Physiol* 2005;563:831–42. jphysiol.2004.079574 [pii]. 10.1113/jphysiol.2004.079574 [PubMed: 15649987]
27. Page AJ, Blackshaw LA. An in vitro study of the properties of vagal afferent fibres innervating the ferret oesophagus and stomach. *J Physiol* 1998;512(Pt 3):907–16. [PubMed: 9769431]
28. Lennerz JK, Dentsch C, Bernardini N, Hummel T, Neuhuber WL, Reeh PW. Electrophysiological characterization of vagal afferents relevant to mucosal nociception in the rat upper oesophagus. *J Physiol* 2007;582:229–42. jphysiol.2007.130823 [pii]. 10.1113/jphysiol.2007.130823 [PubMed: 17478536]
29. Yu S, Ouyang A. TRPA1 in Bradykinin-induced Mechano-hypersensitivity of Vagal C Fibers in Guinea Pig Esophagus. *Am J Physiol Gastrointest Liver Physiol*. 2008 90530.2008 [pii]. 10.1152/ajpgi.90530.2008
30. Kwong K, Kollarik M, Nassenstein C, Ru F, Undem BJ. P2X2 receptors differentiate placodal vs. neural crest C-fiber phenotypes innervating guinea pig lungs and esophagus. *Am J Physiol Lung Cell Mol Physiol* 2008;295:L858–65. 90360.2008 [pii]. 10.1152/ajplung.90360.2008 [PubMed: 18689601]
31. Yu S, Kollarik M, Ouyang A, Myers AC, Undem BJ. Mast cell mediated long-lasting increases in excitability of vagal C fibers in guinea pig esophagus. *Am J Physiol Gastrointest Liver Physiol* 2007;293:G850–6. 00277.2007 [pii]. 10.1152/ajpgi.00277.2007 [PubMed: 17702952]
32. Mazzone SB, Mori N, Canning BJ. Synergistic interactions between airway afferent nerve subtypes regulating the cough reflex in guinea-pigs. *J Physiol* 2005;569:559–73. jphysiol.2005.093153 [pii]. 10.1113/jphysiol.2005.093153 [PubMed: 16051625]
33. Undem BJ, Chuaychoo B, Lee MG, Weinreich D, Myers AC, Kollarik M. Subtypes of vagal afferent C-fibres in guinea-pig lungs. *J Physiol* 2004;556:905–17. jphysiol.2003.060079 [pii]. 10.1113/jphysiol.2003.060079 [PubMed: 14978204]
34. Chuaychoo B, Lee MG, Kollarik M, Pullmann R Jr, Undem BJ. Evidence for both adenosine A1 and A2A receptors activating single vagal sensory C-fibres in guinea pig lungs. *J Physiol* 2006;575:481–90. jphysiol.2006.109371 [pii]. 10.1113/jphysiol.2006.109371 [PubMed: 16793905]
35. Baker CV, Bronner-Fraser M. Vertebrate cranial placodes I. Embryonic induction. *Dev Biol* 2001;232:1–61. [PubMed: 11254347]
36. Wank M, Neuhuber WL. Local differences in vagal afferent innervation of the rat esophagus are reflected by neurochemical differences at the level of the sensory ganglia and by different brainstem projections. *J Comp Neurol* 2001;435:41–59. [PubMed: 11370010]
37. Chou YL, Scarupa MD, Mori N, Canning BJ. Differential effects of airway afferent nerve subtypes on cough and respiration in anesthetized guinea pigs. *Am J Physiol Regul Integr Comp Physiol* 2008;295:R1572–84. 90382.2008 [pii]. 10.1152/ajpregu.90382.2008 [PubMed: 18768768]
38. Dutsch M, Eichhorn U, Worl J, Wank M, Berthoud HR, Neuhuber WL. Vagal and spinal afferent innervation of the rat esophagus: a combined retrograde tracing and immunocytochemical study with special emphasis on calcium-binding proteins. *J Comp Neurol* 1998;398:289–307. [pii]. 10.1002/(SICI)1096-9861(19980824)398:2<289::AID-CNE9>3.0.CO;2-X [PubMed: 9700572]
39. Kollarik M, Plevkova J, Brozmanova M, Revalló M, Varechova S, Bartos V, Plank L, Tatar M. The effect of chemical stimulation of esophageal mucosa on citric acid induced cough and specific airway resistance in guinea pig. *Bratisl Lek Listy* 2005;106:101–6. [PubMed: 16026143]
40. Sengupta JN, Kauvar D, Goyal RK. Characteristics of vagal esophageal tension-sensitive afferent fibers in the opossum. *J Neurophysiol* 1989;61:1001–10. [PubMed: 2723726]
41. Zagorodnyuk VP, Brookes SJ. Transduction sites of vagal mechanoreceptors in the guinea pig esophagus. *J Neurosci* 2000;20:6249–55. 20/16/6249 [pii]. [PubMed: 10934275]
- 42.

43. Page AJ, Martin CM, Blackshaw LA. Vagal mechanoreceptors and chemoreceptors in mouse stomach and esophagus. *J Neurophysiol* 2002;87:2095–103.10.1152/jn.00785.2001 [PubMed: 11929927]
44. Shaker R, Hogan WJ. Normal physiology of the aerodigestive tract and its effect on the upper gut. *Am J Med* 2003;115(Suppl 3A):2S–9S. S0002934303001864 [pii]. [PubMed: 12928068]
45. Lang IM, Medda BK, Shaker R. Mechanisms of reflexes induced by esophageal distension. *Am J Physiol Gastrointest Liver Physiol* 2001;281:G1246–63. [PubMed: 11668034]
46. Kollarik M, Ru F, Undem BJ. Acid-sensitive vagal sensory pathways and cough. *Pulm Pharmacol Ther* 2007;20:402–11. S1094-5539(06)00146-5 [pii]. 10.1016/j.pupt.2006.11.010 [PubMed: 17289409]
47. Hamamoto J, Kohrogi H, Kawano O, Iwagoe H, Fujii K, Hirata N, Ando M. Esophageal stimulation by hydrochloric acid causes neurogenic inflammation in the airways in guinea pigs. *J Appl Physiol* 1997;82:738–45. [PubMed: 9074957]
48. Barthelemy P, Sabeur G, Jammes Y. Reflex relationships between the cervical esophagus and the respiratory system in cats. *Neurosci Lett* 1996;217:81–4. [PubMed: 8916077]
49. Lang IM, Haworth ST, Medda BK, Roerig DL, Forster HV, Shaker R. Airway responses to esophageal acidification. *Am J Physiol Regul Integr Comp Physiol* 2008;294:R211-9. 00394.2007 [pii]. 10.1152/ajpregu.00394.2007 [PubMed: 17928508]
50. Canning BJ, Mori N, Mazzone SB. Vagal afferent nerves regulating the cough reflex. *Respir Physiol Neurobiol* 2006;152:223–42. S1569-9048(06)00103-0 [pii]. 10.1016/j.resp.2006.03.001 [PubMed: 16740418]
51. Lee LY, Pisarri TE. Afferent properties and reflex functions of bronchopulmonary C-fibers. *Respir Physiol* 2001;125:47–65. S0034568700002048 [pii]. [PubMed: 11240152]
52. Widdicombe JG. Afferent receptors in the airways and cough. *Respir Physiol* 1998;114:5–15. S0034568798000760 [pii]. [PubMed: 9858046]
53. Mazzone SB, McGovern AE. Na⁺-K⁺-2Cl⁻ cotransporters and Cl⁻ channels regulate citric acid cough in guinea pigs. *J Appl Physiol* 2006;101:635–43. 00106.2006 [pii]. 10.1152/japplphysiol.00106.2006 [PubMed: 16627683]
54. Canning BJ, Mazzone SB, Meeker SN, Mori N, Reynolds SM, Undem BJ. Identification of the tracheal and laryngeal afferent neurones mediating cough in anaesthetized guinea-pigs. *J Physiol* 2004;557:543–58. jphysiol.2003.057885 [pii]. 10.1113/jphysiol.2003.057885 [PubMed: 15004208]
55. Lee PC, Cotterill-Jones C, Eccles R. Voluntary control of cough. *Pulm Pharmacol Ther* 2002;15:317–20. S1094-5539(02)90365-2 [pii]. 10.1006/pupt.2002.0365 [PubMed: 12099785]
56. Kollarik M, Undem BJ. Mechanisms of acid-induced activation of airway afferent nerve fibres in guinea-pig. *J Physiol* 2002;543:591–600. PHY_022848 [pii]. [PubMed: 12205192]
57. Kollarik M, Undem BJ. Activation of bronchopulmonary vagal afferent nerves with bradykinin, acid and vanilloid receptor agonists in wild-type and TRPV1^{-/-} mice. *J Physiol* 2004;555:115–23. jphysiol.2003.054890 [pii]. 10.1113/jphysiol.2003.054890 [PubMed: 14634201]
58. Riccio MM, Myers AC, Undem BJ. Immunomodulation of afferent neurons in guinea-pig isolated airway. *J Physiol* 1996;491(Pt 2):499–509. [PubMed: 8866873]
59. Myers AC, Kajekar R, Undem BJ. Allergic inflammation-induced neuropeptide production in rapidly adapting afferent nerves in guinea pig airways. *Am J Physiol Lung Cell Mol Physiol* 2002;282:L775–81.10.1152/ajplung.00353.2001 [PubMed: 11880304]
60. Carr MJ, Hunter DD, Jacoby DB, Undem BJ. Expression of tachykinins in nonnociceptive vagal afferent neurons during respiratory viral infection in guinea pigs. *Am J Respir Crit Care Med* 2002;165:1071–5. [PubMed: 11956047]
61. Morice AH. Is reflux cough due to gastroesophageal reflux disease or laryngopharyngeal reflux? *Lung* 2008;186(Suppl 1):S103–6.10.1007/s00408-007-9038-6 [PubMed: 17909892]

Fig 1. TRPA1 agonists stimulate vagal nociceptive afferent nerves in the esophagus

(A) The activation of a vagal nodose C-fiber in the esophagus by the TRPA1 agonist allyl isothiocyanate (AITC). Shown is the extracellular single unit recording trace (top) and histogram (bottom). *Inset*: action potential shape. (B) The activation induced by AITC was comparable to activation evoked by a noxious esophageal distention with intraesophageal pressure 100mmHg (n=25). AITC activated 7/8 nodose C-fibers. *p<0.05