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Acid-sensing by airway afferent nerves

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Abstract

Inhalation of acid aerosol or aspiration of acid solution evokes a stimulatory effect on airway C-fiber and A δ afferents, which in turn causes airway irritation and triggers an array of defense reflex responses (e.g., cough, reflex bronchoconstriction, etc.). Tissue acidosis can also occur locally in the respiratory tract as a result of ischemia or inflammation, such as in the airways of asthmatic patients during exacerbation. The action of proton on the airway sensory neurons is generated by activation of two different current species: a transient (rapidly activating and inactivating) current mediated through the acid-sensing ion channels, and a slowly activating and sustained current mediated through the transient receptor potential vanilloid type 1 (TRPV1) receptor. In view of the recent findings that the expression and/or sensitivity of TRPV1 are up-regulated in the airway sensory nerves during chronic inflammatory reaction, the proton-evoked irritant effects on these nerves may play an important part in the manifestation of various symptoms associated with airway inflammatory diseases.

Keywords

airway irritation; cough; proton; acid-sensing ion channels (ASICs); transient receptor potential vanilloid type 1 receptor (TRPV1); inflammation

1. Introduction

The concentration of hydrogen ion in body fluids can be elevated substantially during various physiological and pathophysiological conditions. For example, lactic acid is produced in large quantities by the skeletal muscles during anaerobic exercise in healthy individuals [1]. Because lungs are perfused by the total venous return, they are fully exposed to the lactic acid produced by the peripheral tissues. Furthermore, the production of lactic acid is known to be elevated locally in the inflamed and/or ischemic tissues [2,3]. Indeed, in patients during asthmatic attack, the pH of the airway vapor condensate of exhaled gas is reduced to 5.23, as compared to 7.65 in healthy individuals [4,5]. This abnormally low airway pH returns to normal after anti-inflammatory therapy, suggesting the tissue

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inflammation as the origin of airway acidosis [4]. In addition, acidosis resulting from retention of carbon dioxide in the body fluid is one of the most common and debilitating symptoms in patients with severe chronic pulmonary diseases. All these findings indicate that tissue acidosis occurs in the airways and lungs in health as well as in disease conditions. Although the airway responses evoked by an increase in acidity in the respiratory tract has been extensively documented, recent studies began to further identify the specific types of ion channels involved and uncover the underlying mechanisms of the airway irritation caused by tissue acidification.

2. Airway irritation evoked by acid

Inhalation or aspiration of acid solution causes airway irritation and triggers various airway defense reflex responses such as cough and bronchoconstriction. More than four decades ago, Simonsson et al. first reported that inhalation of citric acid aerosol (20% solution) evoked an abrupt increase in airway resistance in patients with asthma, and the response was completely abolished by a pretreatment with atropine [6] (Fig. 1A). Accompanying the immediate bronchoconstriction, citric acid aerosol inhalation challenge also evoked airway irritation and vigorous coughs in these patients, suggesting that cholinergic reflex elicited by acid stimulation of airway sensory nerves was responsible [6]. Similarly, the bronchoconstriction induced by right heart injection of acid solution was mostly abolished by atropine in anesthetized newborn dogs [7]. However, in anesthetized guinea pigs, the bronchoconstrictive response to inhaled citric acid aerosol was mediated in a large part through the action of sensory neuropeptides such as tachykinins and calcitonin gene-related peptide (CGRP) released from these sensory endings upon activation because the airway responses were blocked by pretreatment with specific antagonists of neurokinin receptors [8]. The evidence of a dominant role of tachykinins in regulating the airway responses to inhaled irritants such as acid is well documented in rodents [9], but their relative contribution (as compared to the cholinergic reflex) in the airway responses to these irritants remains to be clearly defined in humans.

In patients with gastroesophageal and laryngopharyngeal reflux diseases, aspiration of gastric acid is known to trigger reflex bronchoconstriction and cough [10]. It has been clearly demonstrated in experimental animals that these reflex responses were elicited by acid stimulation of the sensory nerves innervating larynx and trachea [11–13]. In addition, gastric acid can also stimulate the vagal afferents innervating the distal segment of the esophagus, which may be involved in eliciting the reflex bronchoconstriction triggered by gastroesophageal reflux in asthmatics [10,14].

Association of inhaled acid and asthma symptoms is also frequently reported when asthmatics are exposed to acid fog or aerosol in the environmental air [15–17], and the airway irritation, bronchoconstriction and coughing are also attributed to acid stimulation of airway sensory nerves [18]. These symptoms can be further aggravated during exercise because air (and acid) intake is increased during hyperventilation and the filtering function of the nasal passage is bypassed when breathing via the mouth.

Inhaled citric acid aerosol was first used for experimental production of cough in man about six decades ago [19], and remains as one of the most frequently used agent for testing cough responsiveness in humans [20–23]. The cough response to inhaled citric acid increased in a dose-dependent pattern in healthy subjects (Fig. 1B). Other forms of acid solution (e.g., acetic acid, phosphoric acid, etc.) in the same range of pH (approximately 1.5–2.5) were similarly effective in generating cough, indicating a key role of hydrogen ion in the stimulatory effect on airway sensory nerves [20,21] (Fig. 1B). The cough sensitivity to citric acid was heightened in patients with airway inflammatory diseases such as COPD [24,25].

3. Airway sensory nerves stimulated by acid

It is well documented that increasing acidity in the extracellular fluid activated nociceptive nerve endings in various somatic and visceral tissues, and evoked pain sensation [26–29]. In the respiratory tract, indirect evidence of an activation of C-fiber sensory nerves by proton was first reported in an isolated perfused guinea-pig lung preparation. Constant perfusion of pulmonary arteries with the acid buffer at pH of 5.0 caused the release of tachykinins and CGRP from these sensory nerves, which could be blocked by capsazepine, a selective antagonist of the "capsaicin receptor", suggesting that "capsaicin receptors" were activated during acid stimulation [30,31]. Direct evidence of acid stimulation of C-fiber sensory nerves was established by Fox and coworkers in an isolated airway-nerve preparation [32]. They demonstrated that C-fiber afferents innervating the guinea pig trachea, but not A δ fibers, were stimulated when the pH of the perfusing buffer was reduced to 5.0. Their study further showed that the stimulatory effect of proton is mediated through activation of the capsaicin receptor because it could be abolished by capsazepine [32].

In anesthetized rats, lactic acid injected as a bolus into the right atrium caused a transient decrease in pulmonary venous blood pH (dropped from 7.41 to 7.09–7.29), and a short but intense burst of afferent activities in pulmonary C-fibers [33] (e.g., Fig. 2). This stimulatory effect of lactic acid was dose dependent. Formic acid, with a pK_a value (the negative logarithm of the acid dissociation constant) similar to that of lactic acid (3.79) and thus at the same molar concentration decreasing blood pH to the same degree, evoked a similar response in pulmonary C-fibers, further suggesting that hydrogen ions were primarily responsible for the action [33]. The stimulatory effect of lactic acid was abrogated by capsazepine in 75% (8 out of 12) of the pulmonary C-fibers tested but was not altered in the remaining 25% (Fig. 2), despite that this dose of capsazepine was sufficient to block the stimulatory effect of a large dose of capsaicin (five folds of its threshold dose). This finding suggested that an activation mechanism not mediated through the capsaicin receptor was also involved in the action of hydrogen ion on some of pulmonary C-fiber endings. In addition, right atrial injection of lactic acid also stimulated a small sub-population of the rapidly adapting receptors (RARs) in the rat lung [34] (Fig. 3A), which was somewhat surprising because RARs are recognized as mechanosensitive airway afferents, and generally do not exhibit chemosensitivity [34].

It has been reported that acid solution can act on the airway tissue surrounding the sensory terminals or circulating blood, and trigger the release of certain chemical mediators such as thromboxanes and prostaglandins [31,35], which may in turn cause a secondary stimulatory and/or sensitizing effect on these nerve endings [31,32,36]. However, a possible involvement of cyclooxygenase metabolites in the stimulatory effect of lactic acid can be ruled out because the responses were not affected by pretreatment with indomethacin [37].

In a more recent study, Kollarik and Undem recorded separately from vagal nodose and jugular ganglia in an isolated airway-nerve preparation similar to that used by Fox et al. [32], and found that both jugular C-fiber and nodose A δ afferents could be activated by acidification (Fig. 3B), dependent upon the magnitude, duration and rate of the pH change [38]. A rapid and transient application of acid stimulated both C and A δ afferent fibers, whereas a slow and sustained reduction in pH stimulated only the C fibers. Furthermore, the afferent nerve discharge recorded from jugular C-fibers was greater than those from nodose A δ fibers in response to a given concentration of citric acid (therefore the same pH) (Fig. 3B). Whether these A δ afferents belonged to a sub-group of the RARs was not known.

In search for "cough receptors", Canning and coworkers identified a specific type of A δ vagal afferents innervating the extrapulmonary airways (trachea and larynx) with cell bodies

residing in the nodose ganglia [39]. Their sensory terminals exhibited exquisite sensitivity to acid challenge and punctuate mechanical stimulation, but was insensitive to capsaicin. These receptors are believed to be primarily responsible for eliciting the cough reflex triggered by acid challenge in the upper airways in anesthetized guinea pigs. Their transduction properties appeared to be different from that of the traditionally defined RARs; for example, they were not activated by lung inflation or bronchoconstriction [39].

4. Ion channels involved in acid-evoked airway irritation

Long before the transient receptor potential vanilloid type 1 (TRPV1) channel was cloned in 1997 [40], a number of studies have shown that low-pH solutions evoked a sustained increase of the cation conductance in a sub-population of isolated rat dorsal root ganglion neurons that exhibited capsaicin sensitivity, suggesting a direct effect of hydrogen ion on "capsaicin receptors" on the soma membranes of these neurons [26,27]. This hypothesis was supported by the observations that the stimulatory effect of acid on the vagal and trigeminal C-fiber endings can be abrogated by capsazepine [32,41]. The definitive evidence was established later when Caterina et al. demonstrated that proton can activate and modulate the cloned TRPV1, the "capsaicin receptor", expressed in oocytes [40].

To avoid the possible indirect effects generated by endogenous chemical mediators released from the surrounding tissue or circulating blood, the direct stimulatory effect of hydrogen ion on the airway sensory nerves was studied in isolated rat vagal pulmonary sensory neurons identified by retrograde labeling [42]. When the pH of the perfusing buffer was reduced by steps of 0.5 in the physiological-relevant range (7.0–5.5), different phenotypes of inward current were recorded in these neurons using the whole-cell patch-clamp recording technique (Fig. 4). The percentages of pulmonary sensory neurons responded to step reductions of pH to 7.0, 6.5, 6.0 and 5.5 were 45.2%, 83.1%, 91.5% and 92.5%, respectively. A mild drop of pH to 7.0 evoked only a transient (rapidly activating and inactivating) current with small amplitude in <50% of these neurons. Both the amplitude of this transient current and the percentage of responding neurons began to increase when pH was further lowered (Fig. 4). In addition, a slowly activating and sustained inward current began to emerge when pH was reduced to below 6.5. The current-voltage curve indicated that the transient component of acid-evoked current was carried predominantly by Na^+ , which was dose-dependently inhibited by amiloride, a known blocker of the acid-sensing ion channels (ASICs) (Fig. 5). The amplitude of the slow, sustained current also increased progressively with lowering pH, and was significantly attenuated by capsazepine, indicating that it was mediated primarily through the activation of TRPV1 (Fig. 5). These two components of acid-evoked current also displayed distinct recovery kinetics from desensitization [42]. A similar observation was reported earlier in isolated rat dorsal root ganglion neurons that acid solution (pH < 6.0) evoked two different types of inward currents [27].

Because there was a clear difference in the pH activation threshold between these two current species (with a higher pH threshold for activating the ASIC-mediated transient current than that for TRPV1), their relative roles in response to a given acid challenge depended on the level of pH (Fig. 4). The amplitudes of these two different currents varied between neurons, but the difference did not seem to be related to the cell size. In current-clamp recordings, lowering the pH in the same manner caused membrane depolarization and evoked action potentials in these isolated neurons in a proton-concentration dependent manner [42]. Recent studies have shown that the sensitivity of ASIC channels to acid was attenuated as the temperature was raised to body temperature, whereas that of TRPV1 was enhanced by an increase in temperature [43,44]. Therefore, the local tissue temperature is an important factor in determining the relative contributions of TRPV1 and ASICs to the

irritant effect of acid in the airways [43]. This difference has important clinical relevance and implication because airway temperature is known to be significantly elevated in asthmatic patients during exacerbation [45].

ASICs are proton-gated ion channels that are voltage-insensitive, and belong to the super family of degenerins/epithelial Na⁺ channels [46–48]. They are widely expressed in mammalian sensory neurons. Six different proteins arising from four genes have been cloned to date [49]: ASIC1a and ASIC1b (spliced forms of the ASIC1 gene), ASIC2a and ASIC2b (spliced forms of ASIC2 gene), ASIC3 and ASIC4. Among them, ASIC2b and ASIC4 do not form functional homomeric channels. However, these subunits can be assembled to form functional heteromeric channel complexes or homomeric channels [49,50], and it is believed that the biphasic current responses (a rapid desensitizing component and a late sustained component) to proton shown by the native nociceptive sensory neurons involve heteromeric ASICs channels (e.g., ASIC2a and ASIC3) [50,51].

TRPV1 is a polymodal transducer, and a tetrameric membrane protein with four identical subunits. Each subunit contains six transmembrane-spanning domains, which form a non-selective cation channel with a high permeability to Ca²⁺ [52]. In the respiratory tract, TRPV1 is expressed predominantly in non-myelinated (C-fiber) afferents [34,53], which represent >75% of the afferent fibers in the pulmonary branch of the vagus nerve [54]. These TRPV1-expressing nerve endings are located near the luminal surface of airway mucosa, either between epithelial cells or forming networklike plexus immediately beneath the basement membrane of epithelium [55], suggesting an involvement of these afferents in regulating the airway responses to inhaled irritants. Activation of these TRPV1-expressing C-fiber sensory terminals triggered centrally-mediated reflex responses that include reflex bronchoconstriction and mucus hypersecretion via the cholinergic pathway, accompanied by the sensation of airway irritation and urge to cough [53]. In addition, activation of TRPV1 also triggers Ca²⁺ influx and a subsequent release of tachykinins and CGRP from the nerve endings, eliciting the local "axon reflex" responses including bronchoconstriction, protein extravasation and inflammatory cell chemotaxis [9].

In a recent study, the expression of messenger RNAs (mRNAs) encoding for TRPV1 and four functional ASIC subunits ASIC1a, ASIC1b, ASIC2a and ASIC3 was detected in rat pulmonary sensory neurons using RT-PCR (Fig. 6). This finding has lent additional support to the electrophysiological evidence that has characterized these two current species involved in the neuronal response to acid challenge [56].

In addition to the vagal sensory neurons described above, expressions of ASIC3 and/or TRPV1 have also been identified in the cell bodies of the dorsal root ganglion neurons (between spinal segments C6-T6) innervating the lung and pleura; either one or both of these two proton-sensitive channels are expressed in these sympathetic afferents arising from the pleura (97%) and the lung (74%) [57]. The more abundant expression of these proton sensors in the pleural afferents [57,58] may contribute to the high nociceptive sensitivity of the parietal pleura to infection/inflammation during pleurisy [59].

Conclusion

When tissue acidification occurs in the respiratory tract under pathophysiological conditions such as inflammation, ischemia and carcinogenesis, it evokes airway irritation, cough and bronchoconstriction. These responses are elicited by the stimulatory effect of hydrogen ion on vagal bronchopulmonary C-fiber and A δ afferents, which is mediated through activation of two major channel species: ASICs and TRPV1. The distinct difference in the activation and inactivation pattern between these two current species suggests that the ASIC channels

are primarily responsible for triggering the sharp response to acid assault in the airways (e.g., acid aspiration), whereas TRPV1 is more involved in the development of slow lingering airway irritation associated with airway disease conditions (e.g., airway inflammation). Recent studies have shown that TRPV1 expression is up-regulated in the sensory nerves innervating the lung and airways during chronic allergic airway inflammation [60] and the laryngeal mucosa in patients with laryngopharyngeal reflux [61]. Furthermore, the responses of isolated pulmonary sensory neurons to acid are enhanced by certain inflammatory mediators [62], such as tryptase [56,63], human eosinophil-derived cationic proteins [64], tumor necrosis factor α [65], and nerve growth factor [66]. These positive interactions may have important clinical implications because tissue acidification and endogenous release of these chemical mediators often occur concurrently during airway inflammatory reaction [62]. In view of the important role of the TRPV1 expressed on airway sensory nerve terminals in the regulation of the cardiopulmonary function, the increased TRPV1 expression and/or sensitivity to proton may play a part in the manifestation of various symptoms associated with airway inflammatory reaction [53,67–69].

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Figure 1.

Airway irritation generated by inhaled acid aerosol in humans. *Panel A*, changes in total lung resistance (RL) after inhalation of one breath of citric acid aerosol (20% solution) before (solid lines) and 10 min after injection of atropine sulfate (2 mg, intravenous; dotted lines) in a patient with asthma; the inhalation challenge was repeated in the same patient to test the reproducibility. *Panel B*, 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). (adapted from Ref. 6 and 21).

Figure 2.

Effect of capsazepine (CPZ), a selective TRPV1 antagonist, on pulmonary C-fiber responses to lactic acid (LA) in rats. *Panel A*, LA (0.2 mmol/kg; injectate volume=0.2 ml; pH=2.1) was injected as a bolus into the right atrium at the arrow during control (top), constant intravenous infusion of CPZ (0.3 mg/kg/min for 5 min; middle) and recovery (20 min after termination of CPZ; bottom) in an anesthetized, open-chest and mechanically ventilated rat (weight 348 g). This dose of CPZ administered was sufficient to block the stimulatory effect of a large dose of capsaicin (2.0 μ g/kg; ~5 folds of its threshold dose; data not shown). Note that two C-fibers distinguished by different spike heights were recorded; locations of receptors: one in upper and one in middle lobe of the right lung. AP, action potentials; Ptr, tracheal pressure; ABP, arterial blood pressure. *Panel B*, experimental procedures and doses identical to that in A were administered in a different rat (362 g); receptor locations: both in the right lower lobe. *Panel C*, group data of 12 pulmonary C-fibers recorded from 8 rats. FA, fiber activity; B, baseline FA averaged over 10-s interval; LA, peak response (1-s average) after the injection of LA. *, $P < 0.05$, significantly different from the baseline. †, $P < 0.05$, significantly different from corresponding response obtained during control. (adapted from Ref. 33).

Figure 3.

Stimulatory effects of acid on both C and A δ afferent fibers innervating the lung and airways in anesthetized rats. *Panel A*, relationship between conduction velocities and fiber activity (FA) evoked by right-atrial bolus injection of capsaicin (1 μ g/kg; left) and lactic acid (0.2 mmol/kg; right). ΔFA represents the difference between the peak FA (1-s average) and the baseline FA (averaged over 10-s interval) in each fiber. *Panel B*, concentration-response curve of nodose A δ -fibers and jugular C-fibers to citric acid. Citric acid was administered as 500 μ l volume in 3 s into superfusion over the mechanically sensitive receptive field at 5 min intervals. Each point represents mean $\pm$ SE of at least 5 experiments. * $P < 0.05$, ** $P < 0.01$.

0.001. Inset, representative traces of a nodose A δ fiber (left) and a jugular C-fiber (right) response to citric acid (500 μ l, 1 mM). (adapted from Ref. 34 and 38).

Figure 4.

Representative acid-evoked whole-cell inward currents in isolated rat vagal pulmonary sensory neurons. Acid buffers with increasing proton concentrations were applied for 6 s (horizontal bars) to four different jugular ganglion neurons. The cell capacitances for the neurons in *Panels A, B, C, and D* were 24.2, 23.1, 15.6, and 11.1 pF, respectively. Note distinct sensitivities to low pH and different phenotypes of inward currents in response to acidic challenges. (adapted from Ref. 42).

Figure 5.

Effects of amiloride and capsazepine on the acid-evoked currents in rat vagal pulmonary sensory neurons. *Panel A:* representative records illustrating the effects of amiloride (100 μ M; 2 min) and capsazepine (CPZ; 10 μ M, 2 min) pretreatments on pH 5.5 (6 s)-evoked inward currents in a jugular neuron (23.1 pF). *Panel B:* group data showing the effects of 2-min pretreatment with amiloride (100 μ M) and CPZ (10 μ M) on both transient and sustained components evoked by low pH (6.5–5.5; 6 s) in the same neurons. * $P < 0.05$ as compared with the corresponding control. Data represent means $\pm$ SE. (adapted from Ref. 42).

Figure 6.

Detection of the presence of acid-sensing ion channels (ASICs) and transient receptor potential vanilloid receptor 1 (TRPV1) mRNAs in rat pulmonary sensory neurons using RT-PCR. Cytoplasm of 20 individual pulmonary nodose neurons was collected into single PCR tubes. One-step RT-PCR and the nested PCR were carried out to detect the presence of the transcripts for TRPV1 and ASIC subunits 1a, 1b, 2a, and 3. M, DNA molecular weight marker. (adapted from Ref. 56).