Alveolar hypercapnia augments pulmonary C-fiber responses to chemical stimulants: role of hydrogen ion

QIHAI GU AND LU-YUAN LEE
Department of Physiology, University of Kentucky Medical Center, Lexington, Kentucky 40536

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Gu, Qihai, and Lu-Yuan Lee. Alveolar hypercapnia augments pulmonary C-fiber responses to chemical stimulants: role of hydrogen ion. J Appl Physiol 93: 181–188, 2002. First published March 15, 2002; 10.1152/japplphysiol.00062.2002.—To determine whether the excitabilities of pulmonary C fibers to chemical and mechanical stimuli are altered by CO2-induced acidosis, single-unit pulmonary C-fiber activity was recorded in anesthetized, open-chest rats. Transient alveolar hypercapnia (HPC) was induced by administering CO2-enriched gas mixture (15% CO2, balance air) via the respirator inlet for 30 s, which rapidly lowered the arterial blood pH from a baseline of 7.40 ± 0.01 to 7.17 ± 0.02. Alveolar HPC markedly increased the responses of these C-fiber afferents to several chemical stimulants. For example, the C-fiber response to right atrial injection of the same dose of capsaicin (0.25–1.0 μg/kg) was significantly increased from 3.07 ± 0.70 impulses/s at control to 8.48 ± 1.52 impulses/s during HPC (n = 27; P < 0.05), and this enhanced response returned to control within ~10 min after termination of HPC. Similarly, alveolar HPC also induced significant increases in the C-fiber responses to right atrial injections of phenylbiguanide (4–8 μg/kg) and adenosine (0.2 mg/kg). In contrast, HPC did not change the response of pulmonary C fibers to lung inflation. Furthermore, the peak response of these C fibers to capsaicin during HPC was greatly attenuated when the HPC-induced acidosis was buffered by infusion of bicarbonate (1.36–1.82 mmol·kg−1·min−1 for 35 s). In conclusion, alveolar HPC augments the responses of these afferents to various chemical stimulants, and this potentiating effect of CO2 is mediated through the action of hydrogen ions on the C-fiber sensory terminals.

CO2; capsaicin; hydrogen ion; acidosis

Carbon dioxide (CO2) is a main product of cellular metabolism. Hypercapnia (HPC) develops when the production of CO2 is exceedingly high and/or the elimination of CO2 from the lungs is hindered; such changes can occur in both normal and abnormal physiological conditions. Previous investigators have postulated that an increase in venous CO2 flux in the lungs can stimulate certain pulmonary afferents, which play an important role in regulating ventilatory response to meet the metabolic demand during exercise (36). Although a stimulatory effect of systemic HPC on pulmonary stretch receptors has been reported by previous investigators (12, 29), results obtained from the experiments using differential cooling or anodal blockade of myelinated fibers in the vagus nerves seemed to suggest a possible involvement of bronchopulmonary C fibers in regulating the tachypneic response to HPC (27, 30). However, there is no direct electrophysiological evidence in support of this hypothesis.

In a recent study, Hong et al. (18) demonstrated a distinct sensitivity of vagal pulmonary C-fiber afferents to hydrogen ion (H+). Furthermore, other investigators have shown that an increase in H+ concentration within the physiological range of pH enhances the response to capsaicin in isolated dorsal root ganglion (DRG) nociceptive neurons, the counterpart of bronchopulmonary C-fiber endings in the peripheral tissue (20, 29). CO2 is highly soluble and permeant to all cell membranes. Through the catalytic action of carbonic anhydrase, hydration of CO2 forms carbonic acid, which rapidly dissociates to H+ and bicarbonate ion (HCO3−). We reasoned that an increase in alveolar CO2 concentration and the accompanying higher H+ concentration in pulmonary blood and interstitial fluid may, in turn, modulate the sensitivity of C-fiber endings to capsaicin in the lungs. If this is the case, it would be important to find out whether this potentiating effect of H+ is also present in the C-fiber responses to other chemical stimulants that activate different ligand-gated ion channels on the nerve terminals. Furthermore, although bronchopulmonary C fibers are usually quiescent during eupneic breathing, they can be activated by lung inflation (15, 22), and an increase in inspired volume occurs commonly during hyperventilation (e.g., during exercise). Whether the response of these afferents to lung inflation is enhanced by an increase in CO2 in the pulmonary blood is not known. In light of these unanswered questions, the present study was carried out to determine 1) whether the responses of pulmonary C fibers to chemical stimulants and to lung inflation are enhanced by transient HPC, which produces a rapid but readily reversible increase in CO2 in the alveolar gas, and 2) if so, whether these effects are mediated through an increase in the H+ concentration in the pulmonary venous blood and interstitial fluid.

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Address for reprint requests and other correspondence: L.-Y. Lee, Dept. of Physiology, Univ. of Kentucky Medical Center, 800 Rose St., Lexington, KY 40536-0298 (E-mail: lylee@uky.edu).

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METHODS

The procedures described below were performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals [DHEW Publication No. (NIH) 86-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20892] and were also approved by the University of Kentucky Institutional Animal Care and Use Committee.

Animal preparation. Male Sprague-Dawley rats (330–435 g) were initially anesthetized with intraperitoneal injection of α-chloralose (100 mg/kg) and urethane (500 mg/kg); smaller (<1/10), supplemental doses of the same anesthetics were given intravenously, whenever necessary, to maintain abolition of pain reflex elicited by paw pinch. The right femoral artery and the left jugular vein were cannulated for recording arterial blood pressure (ABP) and for injections, respectively. The jugular venous catheter was advanced until its tip was slightly above the right atrium. In some animals, the right femoral vein and left femoral artery were also cannulated for infusion of pharmacological agents and collection of the blood samples, respectively; the tip of the arterial cannula was placed under 3-cmH2O pressure to maintain a near-normal physiological range (4.5–5.1%), monitored by a CO2 gas analyzer (Novametrix 1260). A midline thoracotomy was performed, and the expiratory outlet of the respirator was placed under 3-cmH2O pressure to maintain a near-normal functional residual capacity. Body temperature was maintained at ~36°C throughout the experiment by a heating pad placed under the animal lying in a supine position. Animals were killed at the end of the experiments by an intravenous injection of overdose of pentobarbital sodium.

Recording of pulmonary C-fiber activity. Single-unit pulmonary C-fiber activity was recorded as previously described (16). Briefly, the right cervical vagus nerve was separated from the carotid artery and sectioned rostrally. The caudal end of the cut vagus nerve was placed on a small dissection platform and desheathed; a thin filament was teased away from the nerve trunk and placed on a platinum-iridium hook electrode. Action potentials were amplified (Grass P511K), monitored by an audio monitor (Grass AM8RS), and displayed on an oscilloscope (Tektronix 2211). The thin filament was further split until the afferent activity from a single unit was electrically isolated. Both vagi were ligated just above the diaphragm to eliminate the electrical signals arising from abdominal viscera. The afferent activity of a single unit was first searched for by hyperinflation (3–4 × Vt) and then identified by the immediate (delay <1 s) response to bolus injection of capsaicin (0.5–1 μg/kg) into the right atrium. The conduction velocity of the afferent fiber was measured as previously described (16). Finally, the general locations of pulmonary C fibers were identified by their responses to the gentle pressing of the lungs with a blunt-ended glass rod.

The signals of the afferent activities, tracheal pressure (Ptr) (Validyne MP 45-28), and ABP (Statham P23AA) were recorded on a Gould Thermal Writer (TW11) and on a video-cassette recorder (Vetter 500H). Fiber activity (FA) was analyzed later by a computer for each 0.5-s interval.

Experimental protocols. Two series of experiments were carried out. In series 1, the effect of HPC on the response of pulmonary C fibers to chemical stimulants and lung inflation was examined. Transient HPC was induced by connecting a balloon containing a CO2-enriched gas mixture (15% CO2, balance air) to the inlet of the respirator for 30 s. The reason for choosing the method of transient HPC was its ability to produce a rapid and substantial increase in alveolar CO2 without any sustained systemic effects of HPC and acidosis; it shortened the length of the protocol and thus enabled us to maintain the single-fiber recording throughout the entire experiment. Three different chemical stimulants [capsaicin, 0.25–1.0 μg/kg; phenylbiguanide (PBG), 4–8 μg/kg; adenosine, 0.2 mg/kg] were applied separately during the last 5–10 s of a transient HPC challenge when alveolar CO2 concentration reached a steady state. Lung inflation (Ptr = 30 cmH2O; 10 s) was applied during the last 10 s of HPC. Two minutes before each injection or inflation challenge, the rat’s lung was hyperinflated (3 × Vt) to maintain a constant volume history (24). The sequence of injection and lung inflation was randomized among fibers. At least 10 min elapsed between two tests. Series 2 was carried out in another group of rats to determine whether the effect of HPC on the C-fiber responses was mediated through the increase in the H+ concentration in the pulmonary venous blood. The experimental protocol identical to that described in series 1 was repeated during a constant infusion of sodium bicarbonate (NaHCO3: 1.36–1.82 mmol·kg−1·min−1; 35 s) via a femoral venous catheter. Arterial blood samples were collected during the last 5–10 s of the HPC challenge for measurements of pH (pHa) and partial pressure of CO2.

Materials. A mixture of 2% α-chloralose (Sigma Chemical) and 10% urethane (Sigma Chemical) was dissolved in a 2% borax (Sigma Chemical) solution. Capsaicin (Sigma Chemical) was dissolved in a stock solution at 200 μg/ml in a vehicle of 10% Tween 80, 10% ethanol, and 80% isotonic saline. A stock solution of PBG (Sigma Chemical) was dissolved in distilled water at 1 mg/ml, and that of the hemisulfate salt of adenosine (Sigma Chemical) (at 10 mg/ml) was prepared in saline. NaHCO3 (Sigma Chemical) was dissolved in a stock solution at 10 mmol/ml in distilled water. A solution of each of these agents at the desired concentration was prepared daily by diluting the stock solution with saline on the basis of the animal’s body weight. The volume of each bolus injection of these agents and the infusion rate of NaHCO3 were kept at 0.2 ml and 1 ml/min, respectively.

Statistical analysis. A one-way or two-way repeated-measures analysis of variance was used for the statistical analysis. For the latter, one factor was the effect of transient HPC and the other factor was HCO3− infusion. When the two-way analysis of variance showed a significant interaction, pairwise comparisons were made with a post hoc analysis (Newman-Keuls test). Data are expressed as means ± SE. P < 0.05 was considered significant.

RESULTS

In total, 64 pulmonary C fibers were studied in 46 rats. The conduction velocities were 0.77–1.60 m/s (1.07 ± 0.06 m/s; n = 42). The distribution of locations of these receptors was as follows: 18 in the upper lobe, 13 in the middle lobe, 14 in the lower lobe, 5 in the accessory lobe, and 3 in the opposite (left) lung. The locations of the remaining 11 fibers were not determined. Baseline activity was detectable in only 5 of the 64 C-fiber afferents; the baseline FA averaged over a 10-s interval was 0.02 ± 0.01 impulses/s (imp/s). During transient HPC (15% CO2, balance air; 30 s), there was no significant change in the baseline activity of
after capsaicin injection and the baseline FA (averaged over a 10-s duration) was 3.07 ± 0.70 imp/s. However, the stimulatory effect of the same dose of capsaicin on the same group of C fibers was markedly enhanced (ΔFA: 8.48 ± 1.52 imp/s; P < 0.05; n = 27) during the transient HPC challenge (Figs. 1 and 2). The C-fiber responses increased in both peak activity and the duration of firing (Fig. 2). Transient HPC clearly augmented the stimulatory effect of capsaicin on all fibers, including two fibers in which the same dose of capsaicin (1 µg/kg) failed to generate a clear stimulatory effect at control. The response to capsaicin returned completely to the control level (∆FA: 2.93 ± 0.68 imp/s) when the same fibers were tested again ~10 min after termination of HPC (e.g., Fig. 1).

The potentiating effect of transient HPC was not limited only to the response to capsaicin. The responses of these pulmonary C fibers to injections of a low dose of PBG (4–8 µg/kg) and adenosine (0.2 mg/kg) were also enhanced during the transient HPC challenge; ΔFA induced by PBG were 1.78 ± 0.29 imp/s at control and 6.31 ± 1.34 imp/s during HPC (P < 0.05; n = 13); ΔFA caused by adenosine was 4.46 ± 0.76 imp/s at control and 7.88 ± 1.33 imp/s during HPC (P < 0.05; n = 12). Similarly, the fiber responses increased in both the peak activity and the duration of firing during HPC, but the degree of potentiation varied considerably among different chemical agents (Figs. 3 and 4).

these C fibers (0.06 ± 0.02 imp/s; P > 0.05; n = 64), despite a slight but distinct increase in four receptors. However, the transient HPC did induce mild but significant decreases in baseline ABP and heart rate (HR) (e.g., Figs. 1 and 2): ABP decreased from 94.1 ± 4.7 mmHg at control to 80.7 ± 5.9 mmHg during HPC (P < 0.05; n = 64), and HR decreased from 263.2 ± 13.4 beats/min at control to 235.2 ± 12.4 beats/min during HPC (P < 0.05; n = 64). Both ABP and HR returned to control shortly (<30 s) after the termination of transient HPC.

The response to capsaicin was always tested at an initial dose of 0.5 µg/kg. In 7 of 27 C fibers, the dose was increased to 1.0 µg/kg when the initial dose failed to produce a detectable and consistent stimulatory effect on these fibers. At control, injection of this low dose of capsaicin immediately evoked a mild and short burst of discharge (e.g., Fig. 1); the difference (∆FA) between the peak FA (averaged over a 2-s duration)
buffered by the HCO$_3^-$ infusion, the peak response of pulmonary C fibers to the same challenge of capsaicin during HPC was significantly reduced (e.g., Figs. 5 and 6); ∆FA were $10.60 \pm 1.91$ and $5.35 \pm 0.99$ imp/s before and during the HCO$_3^-$ infusion, respectively ($P < 0.05$; $n = 10$; Figs. 6 and 7). In fact, there was no significant difference in the FA response to the same challenge of capsaicin between control (air breathing, before HCO$_3^-$ infusion) and HPC during the HCO$_3^-$ infusion ($P > 0.05$; $n = 10$; Fig. 7).

**DISCUSSION**

Our results showed that transient HPC, which decreased pH in the arterial blood by 0.23 units, markedly increased the responses of pulmonary C-fiber afferents to various chemical stimulants. During infusion of HCO$_3^-$, the acidosis generated by HPC was diminished whereas $P_{CO_2}$ in the arterial blood was elevated to an even higher level. However, the HPC-induced increase in pulmonary C-fiber response to chemical stimulation was significantly attenuated. Hence, these results suggest that the elevated H$^+$...
concentration in the pulmonary venous blood (i.e., systemic arterial blood) or lung interstitial fluid, and not the elevated PCO₂, enhances the responses of these afferents to chemical stimulation during transient HPC.

The three chemical stimulants used in this study activate C-fiber endings through different transduction mechanisms. Capsaicin, the pungent ingredient of the red pepper, has been widely used as a chemical tool for selectively studying the function and properties of somatic and visceral C-fiber afferents. Electrophysiological studies (2, 25, 37) have shown that capsaicin excites nociceptive neurons by increasing permeability of cellular membrane to cations. The recent cloning of the vanilloid receptor subtype 1, VR1, has offered new insight into the molecular nature of capsaicin action and its signaling mechanism (5). The cloned VR1 receptor is a ligand-gated, nonselective cation channel. Although H⁺ by itself does not activate VR1, it markedly potentiates the capsaicin-evoked, VR1-mediated response (5), which is consistent with the observations that low pH enhances the capsaicin response in isolated rat DRG neurons reported by other investigators (20, 23, 26). On the other hand, sensory neurons isolated from the DRG of VR1-deficient mice showed profoundly reduced response to acid (pH 5.0), both in culture and in isolated skin-nerve preparation (4). In addition, the experiments measuring the release of neuropeptides from capsaicin-sensitive neurons also illustrated the close similarity between the actions of H⁺ and capsaicin (14, 31), which raised the possibility that H⁺ functions as an endogenous modulator of the ion channels associated with the putative capsaicin receptor. A possible explanation of the interaction between H⁺ and capsaicin is that H⁺ may enter the open VR1 channel and neutralize negative charges, which impedes the movement of Ca²⁺; as a consequence, the passage of monovalent cations is facilitated, which leads to an increase in total conductance (20). A recent study using the oligonucleotide-directed mutagenesis technique further suggested an extracellular Glu residue of the VR1 receptor as the key regulatory site for the proton-induced potentiation of VR1 sensitivity (19).

However, these hypotheses cannot explain the observation that HPC-induced low pH potentiates the excitability of pulmonary C-fiber response not only to capsaicin, but also to PBG and adenosine. These two chemical agents are known to stimulate pulmonary C fibers through completely different mechanisms from that of capsaicin. PBG, which has been used frequently to activate vagal sensory C neurons in different species, is a specific 5-HT₃ receptor agonist (11, 15). Unlike most of other G-protein-coupled 5-HT receptor subtypes, a 5-HT₃ receptor subtype identified in neuronal tissue is coupled to an ion channel exhibiting a...
fast activating and inactivating inward current (21). In contrast, adenosine activates pulmonary C fibers with a relatively longer latency (5–8 s), and the effect usually lingers for a longer period of time (e.g., Fig. 3). This delayed stimulatory effect of adenosine is generated by the activation of adenosine A1 receptor (16), which is known to be coupled to several types of G-proteins that, in turn, initiate different second-messenger transduction cascades (13).

An alternative explanation is that the observed effect of H+ was on the voltage-sensitive ion channels, which became active when membrane depolarization occurred as a result of activation of one of the ligand-gated channels described above. Indeed, changes in intracellular H+ have been shown to modify several types of voltage-gated ion channels, including Ca2+ channels (9), inward rectifier K+ (Kir) channels (34), delayed rectifier K+ channels (35), and Na+ channels (3). Although more than one channel species is probably involved in the regulation of membrane excitability during HPC, K+ channels, especially the Kir channels, may play a potentially significant role (28). Recent studies have shown that several Kir channels are inhibited by high CO2 (28, 34, 38). The effect is probably mediated by intracellular acidification rather than molecular CO2 because selective intracellular acidification to the same level as that during HPC inhibited the channel activation as effectively as HPC (34, 38). In contrast, extracellular acidification failed to do so in the same preparation. The Kir channels play an essential role in the regulation of resting membrane potential; inhibition of these K+ channels leads to depolarization and increase of membrane excitability. The change in intracellular pH during HPC could not be determined in our study, but presumably it was in parallel with that in the extracellular fluid. Whether the sensitizing effect of the H+ on C-fiber terminals was generated by its action taking place in intracellular (34, 38), extracellular (19, 20), or both compartments remains to be determined.

Delpierre et al. (10) have reported an increase in baseline C fiber discharge during HPC in anesthetized cats when alveolar CO2 partial pressure reached the level similar to that in this study. In contrast, we did not find any significant difference in the baseline C-fiber activity between control and transient HPC (Figs. 2 and 3); this discrepancy could be related to the difference in animal species. However, it is somewhat surprising to find that transient HPC has no effect on the C-fiber response to lung inflation in this study, despite a striking potentiating effect on their response to chemical stimuli. In a rat skin nerve preparation in vitro, Steen and co-workers (32, 33) demonstrated that stimulus-related response of a distinct subpopulation of mechano-heat-sensitive (“polymodal”) C-fiber nociceptors increased with extracellular H+ concentration, and the response followed the time course of the pH change. In their study, the threshold pH levels ranged from 6.9 to 6.1, and mean maximum discharge occurred at pH 5.2. Thus a plausible explanation is that the local drop in pH induced by transient HPC in our study did not reach the threshold levels and therefore failed to generate a sensitizing effect on the response to lung inflation in a majority of the pulmonary C fibers studied. The wide-range variation of the effect of CO2 on the response to lung inflation among different C fibers reflects the heterogeneity of physiological properties of these afferents (7, 22). On the other hand, we cannot rule out the possibility that H+ simply did not alter the sensitivity of ion channels that mediate the lung inflation-induced membrane depolarization of the C-fiber sensory terminals. In any event, our results obtained from this study do not provide sufficient evidence to support an involvement of bronchopulmonary C-fiber afferents in “sensing” the increase in Vt during HPC (27, 30).

We realize that the pH of systemic arterial blood (or pulmonary venous blood) may not accurately measure the local pH surrounding the C-fiber endings of which
the afferent activity was measured in this study. However, a direct and precise measurement of the local interstitial fluid pH was not feasible because these C-fiber endings are located either in the walls of intrapulmonary airways or in the lung parenchyma. Alternatively, we measured the changes of pH in mixed pulmonary "venous" blood (i.e., systemic arterial blood) as an estimation of the overall change of interstitial fluid pH in the whole lung; our assumption is based on the fact that H⁺ exchanges freely between the blood and the interstitial fluid through the pores (the interendothelial junctions) existing in the capillary wall as the blood flows through the pulmonary capillary (8). Thus the diffusion of H⁺ between capillary blood and interstitial fluid should reach an “equilibrium” before the blood leaves the capillary.

In the present study, systemic arterial blood pH decreased from a baseline of 7.40 to 7.17 at the end of the 30-s transient HPC (Fig. 7). This change was within the physiological range despite the high concentration of CO₂ (15%) administered, and it rapidly reversed after termination of HPC. The relatively mild and short-lasting reduction in arterial pH is believed to be related to the fact that the amount of CO₂ intake during the transient HPC was limited by the brief duration of inhalation. In comparison, much more severe acidosis occurs naturally in ischemic, damaged, or inflamed tissue and in and around malignant tumors (1, 6). Under those pathophysiological conditions, interstitial fluid pH as low as 5.7 has been reported (6).

Bronchopulmonary C fibers represent >75% of vagal afferents innervating the respiratory tract. Responses evoked by activating these afferents are mediated both by central reflex pathways and by local or axon reflexes involving the release of tachykinins from sensory endings (22). The overall responses to C-fiber stimulation include bronchoconstriction, hypersecretion of mucus, airway mucosa edema, and cough (7, 22). Hence, when the excitability of these afferent endings is enhanced by acidosis, such as during tissue inflammation or ischemia, the centrally mediated and axonal reflex responses elicited by a given level of stimulus are expected to be augmented.

In summary, these results demonstrated that transient alveolar HPC markedly and reversibly enhances the excitability of pulmonary C fibers to chemical stimuli. This sensitizing effect of CO₂ is probably mediated through the action of H⁺ on the terminal membrane of C fibers because the sensitization was significantly attenuated by infusion of HCO₃⁻, which prevented the HPC-induced acidosis in the pulmonary venous blood. However, the site(s) of this action of H⁺ on the C-fiber sensory terminals remains to be determined.

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