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Stimulatory effect of CO₂ on vagal bronchopulmonary C-fiber afferents during airway inflammation

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Abstract

This study investigated: 1) whether pulmonary C-fibers are activated by a transient increase in the CO₂ concentration of alveolar gas; 2) if the CO₂ sensitivity of these afferents is altered during airway inflammation. Single-unit pulmonary C-fiber activity was recorded in anesthetized, open-chest rats. Transient alveolar hypercapnia (HPC) was induced by administering a CO₂-enriched gas mixture (25–30% CO₂, 21% O₂, balance N₂) for 5–8 breaths, which increased alveolar CO₂ concentration progressively to near or above 13% for 3–5 s and lowered the arterial pH transiently to 7.10 ± 0.05 . Our results showed: 1) HPC evoked only a mild stimulation in a small fraction (4/47) of pulmonary C-fibers, and there was no significant change in fiber activity ($\Delta FA = 0.22 \pm 0.16$ impulses/s; $P > 0.1$, $n = 47$); 2) in sharp contrast, after airway exposure to poly-L-lysine (PLL), a cationic protein known to induce mucosal injury, the same challenge of transient HPC activated 87.5% of the pulmonary C-fibers tested and evoked a distinct stimulatory effect on these afferents ($\Delta FA = 6.59 \pm 1.78$ impulses/s; $P < 0.01$, $n = 8$); 3) similar potentiation of the C-fiber response to HPC was also observed after acute exposure to ozone ($n = 6$), and during a constant infusion of inflammatory mediators such as adenosine ($n = 15$) or prostaglandin E₂ ($n = 12$); and 4) The enhanced C-fiber sensitivity to CO₂ after PLL was completely abrogated by infusion of NaHCO₃ (1.82 mmol/kg/min) that prevented the reduction in pH during HPC ($n = 6$). In conclusion, only a small percentage (< 10%) of the bronchopulmonary C-fibers exhibit CO₂ sensitivity under control condition, but alveolar HPC exerts a consistent and pronounced stimulatory effect on the C-fiber endings during airway inflammation. This effect of CO₂ is probably mediated through the action of hydrogen ions.

Keywords

hydrogen ion; airway mucosal injury; airway hyperreactivity; hypercapnia

INTRODUCTION

Previous investigators have suggested the existence of sensory receptors that can detect an increase in CO₂ in the lung (32). However, no direct and definitive evidence has been established in identifying the presence of “CO₂ sensors” in the lung structures. Experiments employing various techniques to block the conduction of myelinated fibers in the vagus have yielded compelling evidence suggesting the involvement of bronchopulmonary C-fibers in the hyperpneic response to CO₂ (26,28). One possibility is that the increase in alveolar CO₂ concentration in those experiments led to a decrease in the pulmonary interstitial pH, which then activated the pulmonary C-fibers. This hypothesis is supported by a recent study demonstrating that pulmonary C-fibers are consistently activated when pH in the arterial blood (pulmonary venous blood) is lowered to ~7.1 by a bolus intravenous injection of acid solution (e.g., lactic acid, formic acid) (18).

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The important role of vagal bronchopulmonary C-fiber afferents in the regulation of airway function is well documented (2,23). Recently, extensive studies have shown that airway mucosal injury/inflammation can cause a pronounced increase in the sensitivity of pulmonary C-fiber afferents to various stimuli including hydrogen ion (23,24). More importantly, both CO₂ retention and airway inflammation are common symptoms encountered in patients who suffer from either acute or chronic obstructive pulmonary diseases. It is, therefore, important to understand the potential influence of airway inflammation on the sensitivity of pulmonary C-fibers to CO₂.

In view of these existing unanswered questions, this study was designed to investigate: 1) whether pulmonary C-fibers are activated by an increase in the CO₂ concentration of alveolar gas; 2) if the CO₂ sensitivity of these afferents is altered during airway inflammation; and 3) if a stimulatory effect on these afferents is generated by CO₂, whether the action is mediated through the production of hydrogen ions. To avoid any lingering systemic effects of hypercapnia (HPC) (e.g., change in bronchomotor tone, blood pressure, etc.) and their potential secondary influence on the C-fiber activity, a transient (< 10 s) alveolar HPC was employed in this study.

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 ($n = 60$; body weight, 345–470 g) were initially anaesthetized with intraperitoneal injection of α -chloralose (100 mg/kg) and urethane (500 mg/kg); smaller (1/10 of the initial dose) 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, the left jugular vein, and the right femoral vein were cannulated for recording arterial blood pressure (ABP), injection and infusion of various chemical agents, respectively. The tip of the femoral arterial catheter was advanced into the abdominal aorta; the jugular venous catheter was advanced until its tip was slightly above the right atrium. The trachea was cannulated just below the larynx, and the lungs were artificially ventilated with a respirator (UGO Basile 7025, Comerio-Varese, Italy). Tidal volume (V_T) and respiratory frequency were set at 8–10 ml/kg and 50 breath/min, respectively, to mimic those of unilaterally vagotomized rats; the end-tidal CO₂ concentration in such a setting was within normal physiological range (4.5–5.1%), monitored by a CO₂ gas analyzer (Novamatrix 1260). Tracheal pressure (P_t) was measured via a side port of the tracheal cannula. A mid-line 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 (the normal body temperature in rats during sleep) 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 sodium pentobarbital.

Recording of Pulmonary C-fiber Activity

Single-unit pulmonary C-fiber activity was recorded as previously described (18). 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 ($3-4 \times V_T$), and then identified by the immediate (delay < 1 s) response to bolus injection of capsaicin ($0.5-1.0 \mu\text{g/kg}$) into the right atrium. 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, P_t (Validyne MP 45-28) and ABP (Statham P23AA) were recorded on a Gould Thermal Writer (TW11) and on a videocassette recorder (Vetter 500H). Fiber activity (FA) was analyzed continuously by a computer for each 0.5-s interval.

Transient Alveolar HPC

To induce an abrupt and substantial increase in alveolar CO_2 concentration (e.g., Fig. 1), transient HPC was induced by connecting a balloon containing a CO_2 -enriched gas mixture (25–30% CO_2 , 21% O_2 , balance N_2) to the inlet of the respirator for 5–8 consecutive breaths (average: 6.04 ± 0.21 breaths); the increase in alveolar CO_2 concentration was accompanied by a rapid and progressive decrease of pH in the arterial (or pulmonary venous) blood that was then followed by a rapid and complete reversal without any detectable lingering systemic effects (Fig. 1A).

Experimental Protocols

Three series of experiments were carried out. *Study 1* was carried out in 44 rats to determine whether bronchopulmonary C-fibers were stimulated by transient HPC under control conditions. To determine the change in pH in the arterial blood (pH_a) during the HPC challenge, single arterial blood samples (0.1 ml) were drawn before and at the end of the HPC challenge when the end-tidal CO_2 concentration reached the peak, and the pH of these samples was measured (i-STAT, Heska Co., Fort Collins, CO) in 7 rats. In another group of rats ($n = 4$), a blunt-tipped catheter (PE-90; 15 cm length) was inserted into the right carotid artery and advanced retrogradely until its tip was positioned in the left ventricle, which was determined by monitoring the pressure and verified later by postmortem examination. The other end of the catheter was connected to the bottom of an open-ended micro reservoir (volume = 80 μl) in which the tip (0.75 mm) of a miniature pH electrode (IC 501, Samuel Agulian, Hamden, CT) (time constant = 1.3 s) was positioned. During the 15-s baseline and throughout the challenge, the well was continuously filled by the blood ejected from the left ventricular catheter at a flow rate of ~ 2.0 ml/min. This method provided a continuous measurement of the rapid and progressive change of pH_a , but the measurement had to be carried out in a separate group of rats to avoid the possibility that the receptor response recorded may be influenced by the blood loss. In *Study 2*, the same protocol as that described in *Study 1* was carried out to determine if the C-fiber response to HPC was altered by airway mucosal inflammation; the latter was induced either by intratracheal instillation of poly-L-lysine (PLL; 0.25 mg/ml; 0.1 ml), a synthetic cationic protein, or by acute exposure of the airways to ozone (O_3 ; 2.5 ppm; 30–45 min). Both these procedures have been demonstrated previously to induce a sustained (> 30 min) potentiation of the sensitivity of bronchopulmonary C-fibers (10,14). In two separate groups of animals, the bronchopulmonary C-fiber responses to the HPC challenge were determined during a constant intravenous infusion of prostaglandin E_2 ($1-2 \mu\text{g/kg/min}$ for 3 min) or adenosine ($40-120 \mu\text{g/kg/min}$ for 2 min); both these inflammatory mediators are known to be released endogenously in the lung during airway inflammation and have been shown previously to enhance the sensitivity of these afferents (11,12). *Study 3* was carried out to determine if the effect of HPC on C-fiber afferents is mediated through the action of hydrogen ions; the effects of intratracheal instillation of PLL and intravenous infusion of adenosine on the response to HPC (*Study 2*) were determined and compared in the same fibers between

before and during a constant infusion of sodium bicarbonate (NaHCO_3) (1.82 mmol/kg/min; 35 s) via a femoral venous catheter in order to prevent the acidosis caused by the HPC challenge. Some of the animals were used in two different studies (e.g., *Study 1* and 2).

Materials

All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). A mixture of 2% α -chloralose and 10% urethane was dissolved in a 2% borax solution. Capsaicin was dissolved in a stock solution at 250 $\mu\text{g/ml}$ in a vehicle of 10% Tween 80, 10% ethanol and 80% isotonic saline. A stock solution of PGE_2 was dissolved in 5% ethanol and 95% isotonic saline at 125 $\mu\text{g/ml}$, and that of the hemisulphate salt of adenosine (at 10 mg/ml) was prepared in saline. NaHCO_3 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 NaHCO_3 were kept at 0.15 ml and 1 ml/min, respectively.

Statistical Analysis

The change in FA (ΔFA) in response to HPC was calculated as the difference between the peak FA (2-s average) during the HPC challenge and the base-line FA (20-s average) in each fiber. Data were then analyzed with either one-way or two-way repeated-measures ANOVA, unless mentioned otherwise. When the ANOVA showed a significant interaction, pair-wise comparisons were made with a *post hoc* analysis (Fisher's least significant difference). A P value < 0.05 was considered significant. Data are reported as means $\pm$ SE.

RESULTS

A total of 67 vagal bronchopulmonary C-fibers were studied in 60 rats. The distribution of locations of these receptors was as follows: 10 in the upper lobe, 19 in the middle lobe, 25 in the lower lobe, and 3 in the accessory lobe; one receptor was found in the left lung. The locations of the remaining 9 fibers were not identified, but all of them were activated by lung inflation and responded to bolus injection of capsaicin with a latency of < 1 s; these receptors are therefore considered as pulmonary C-fibers (13).

Study 1

During the transient HPC challenge, the end-tidal (alveolar) CO_2 concentration increased rapidly and progressively to near or above 13% at the end of the HPC challenge (e.g., Fig. 1A). Continuous measurement of the pH in the blood ejected from left ventricle showed that pH_a decreased very rapidly, reaching a lowest value of ~ 7.1 , and then returned to the base-line within 10 s after resuming air ventilation. The continuous decrease in pH_a coincided closely with the increase in the end-tidal CO_2 concentration (e.g., Fig. 1A), and was reproducible in repeated challenges in the same rats. The average pH_a decreased from a base-line of 7.41 ± 0.03 to the lowest value of 7.10 ± 0.03 ($P < 0.05$, $n = 4$; Fig. 1B). When pH_a was measured in single arterial blood samples drawn separately during base-line and at the end of the HPC challenge, the average change in pH_a produced by the same HPC challenge (base-line: 7.43 ± 0.05 ; at peak HPC: 7.10 ± 0.05 ; $n = 7$, $P < 0.01$) (Fig. 1C) was similar to that obtained from the continuous measurement described above (Fig. 1B). Thus, to minimize the blood loss, measurements of pH_a in this and later study series were made from single arterial blood samples (Fig. 1C).

Transient HPC challenge evoked a mild stimulation ($\Delta\text{FA} > 1$ impulses/s) in only 4 of the 47 (8.5%) bronchopulmonary C-fibers tested; an example is shown in Fig 2. In these 4 fibers, the discharges began to emerge when the end-tidal CO_2 concentration exceeded 10%, and ceased shortly after the termination of HPC. The group data, however, showed no significant

stimulatory effect of HPC on these C-fibers ($\Delta\text{FA} = 0.22 \pm 0.16$ imp/s; $P > 0.1$; $n = 47$; Fig. 1D).

In some rats, the HPC challenge elicited a spontaneous hyperventilatory drive, as indicated by the irregular P_t signals during mechanical ventilation (e.g., Fig. 1A), which subsided several breaths later. In addition, a mild and short-lasting hypotension usually emerged several seconds after termination of the HPC challenge (e.g., Fig. 1A).

Study 2

In sharp contrast to the weak and inconsistent responses shown above, the sensitivity of bronchopulmonary C-fibers to HPC was markedly enhanced by airway mucosal inflammation. For example, after the intratracheal instillation of PLL (0.25 mg/ml; 0.1 ml), transient HPC activated 7 of the 8 (87.5%) pulmonary C-fibers tested. Furthermore, the peak response of these afferents to the same HPC challenge was drastically elevated ($\Delta\text{FA} = 0.06 \pm 0.06$ imp/s at control and 6.59 ± 1.78 imp/s after PLL; $P < 0.01$, $n = 8$; Figs. 3 and 4A). This enhanced response to HPC gradually returned to control at 60–120 min after the PLL treatment (Figs. 3D and 4A). Similarly, after acute exposure to O_3 (2.5 ppm; 30–45 min), the same HPC challenge evoked a distinct stimulatory effect on these afferents ($P < 0.05$, $n = 6$; Fig. 4B). In addition, when individual inflammatory mediators known to enhance the sensitivity of these afferents were administered, the C-fiber responses to HPC were also clearly augmented. For example, infusion of adenosine (40–120 $\mu\text{g/kg/min}$) produced a significantly greater stimulatory effect of HPC on the C-fibers ($\Delta\text{FA} = 1.21 \pm 0.24$ imp/s), compared to that during control ($\Delta\text{FA} = 0.00 \pm 0.00$ imp/s; $P < 0.01$, $n = 15$; e.g., Figs. 4C and 5). Similar increase in the response to HPC was also found during infusion of PGE_2 (1–2 $\mu\text{g/kg/min}$) in a separate group of rats ($P < 0.05$, $n = 12$; Fig. 4D). Within 30 min after the termination of infusion of either adenosine or PGE_2 , the C-fiber response to HPC returned completely to control (Fig. 4, C and D).

Study 3

After intratracheal instillation of PLL, the C-fiber response to HPC was markedly elevated ($\Delta\text{FA} = 4.16 \pm 2.5$ imp/s) in a manner similar to that shown in Study 3. However, during intravenous infusion of NaHCO_3 (1.82 mmol/kg/min), this enhanced response to HPC induced by PLL was completely abolished ($\Delta\text{FA} = 0.56 \pm 0.26$ imp/s; $P < 0.05$, $n = 6$; Fig. 6A). Similar attenuating effect of NaHCO_3 was also found when the C-fiber sensitivity to HPC was augmented by adenosine infusion ($n = 8$; Fig. 6B).

Before the instillation of PLL, the HPC challenge caused the base-line pH_a to decrease from 7.42 ± 0.01 to 7.16 ± 0.03 ($n = 6$); after PLL, HPC decreased pH_a from 7.40 ± 0.02 to 7.13 ± 0.03 . During infusion of NaHCO_3 , the base-line pH_a was elevated to 7.54 ± 0.03 after the instillation of PLL, which was only reduced to 7.31 ± 0.03 during the challenge of HPC.

DISCUSSION

Results of this study indicated that under control condition only ~8.5% of the bronchopulmonary C-fibers exhibited mild sensitivity to transient alveolar HPC that reduced the arterial blood pH to ~7.1. However, after acute airway exposure to either PLL or O_3 , agents known to induce airway inflammation, or when inflammatory mediators such as PGE_2 were administered, the same HPC challenge exerted a consistent and pronounced stimulatory effect on the C-fiber endings. This stimulatory effect of CO_2 was probably mediated through the action of H^+ ions on the terminal membrane of these C-fiber afferents because the enhanced C-fiber sensitivity to CO_2 was significantly attenuated by infusion of HCO_3^- that prevented the HPC-induced acidosis in the pulmonary venous blood. Furthermore, during the HCO_3^-

infusion, the same HPC challenge produced a higher arterial CO₂ partial pressure because of the additional CO₂ generated by the reaction between H⁺ and HCO₃⁻ ions in the blood. Thus, our results further indicated that CO₂ itself was not responsible for the stimulatory effect of HPC on these afferents. After CO₂ enters the pulmonary capillary from alveoli, it reacts with water and releases H⁺ in the blood via the enzymatic action of carbonic anhydrase. Because H⁺ ions can diffuse rapidly into the interstitial fluid through the inter-endothelial junctions/pores in the wall of pulmonary capillary (5), they can have easy access to and act on the endings of pulmonary C-fibers.

Based upon the observations made in the experiments when the myelinated fibers in the vagus nerves were blocked by differential cooling or anodal blockade, previous investigators have suggested an important role of bronchopulmonary C-fibers in the increase in respiratory rate during hypercapnia (26,28). However, direct experimental evidence of a stimulatory effect of CO₂ on these afferents has not been clearly established. Dickinson and Paintal first reported the activation of J receptors (pulmonary C-fiber endings) by injecting CO₂ gas bubbles into the right ventricles of cats (7). On the basis of response latency, they further postulated that these "CO₂ sensors" are located between alveoli and capillaries. A paradoxical stimulatory effect of CO₂ on pulmonary C-fibers was reported in cats by Delpierre *et al.* (6), but the locations of most of the receptors were not identified in the lung in their study. In comparison, our study showed a much smaller percentage of pulmonary C-fibers exhibiting CO₂ sensitivity in rats under normal conditions. This discrepancy is probably not due to solely the species difference because a low percentage of CO₂-sensitive pulmonary C-fibers was also found previously in other animal species in our laboratory: dogs (9.1%, *n* = 11; 25% CO₂) and guinea pigs (12.5%, *n* = 8; 20–25% CO₂) (L.Y. Lee and J.L. Hong, unpublished data). A lack of stimulatory effect of CO₂ on pulmonary C-fibers has also been reported by other investigators in dogs (2).

In sharp contrast, our study demonstrated that pulmonary C-fibers were consistently activated by the same level of alveolar hypercapnia after acute exposure of the lungs to either PLL or O₃. Both of these agents have been shown to induce airway mucosal inflammation and injury, accompanied by airway hyperresponsiveness (AHR) (3,8,15,16,29). The possible involvement of vagal pulmonary C-fiber afferents in the AHR induced by PLL or O₃ has been suggested (4,33). Indeed, intratracheal instillation of PLL, a synthetic cationic protein, at the same dosage as that used in this study markedly enhances the sensitivity of pulmonary C-fibers to both lung inflation and chemical stimuli in anesthetized rats (10). Similar effects on the airways can be produced by airway exposure to endogenous cationic proteins derived from eosinophil granules (22). Acute exposure to O₃, one of the major air pollutants in urban areas, is known to cause AHR in a number of species including humans (8,15,33) and also elevate the sensitivity of pulmonary C-fiber afferents to mechanical and chemical stimulations (14). In both cases of PLL and O₃, the mucosal injury and release of inflammatory mediators are believed to be the primary cause of the hypersensitivity of pulmonary C-fiber afferents (10,14).

Our study showed that administration of individual inflammatory mediators such as PGE₂ and adenosine also significantly enhanced the CO₂ sensitivity in pulmonary C-fibers. PGE₂ is a potent autacoid derived from arachidonic acid metabolism through the enzymatic action of cyclooxygenase and PGE synthase. The airway epithelium, which is the main target of initial assault by the inhaled irritants, is also the primary cell type that releases this autacoid (17). PGE₂ administered at a low dose that did not cause any systemic effects markedly enhanced the excitability of pulmonary C-fibers in anesthetized rats (12). In cultured vagal (nodose and jugular ganglia) pulmonary sensory neurons, PGE₂ (1 μM) markedly increased the neuronal excitability to chemical and electrical stimulations (9,20,21). These studies suggest that the sensitizing effect of PGE₂ is mediated through an activation of the G_s protein-coupled EP prostanoid receptors, which increases the enzyme activity of adenylyl cyclase and activates

the cyclic AMP/protein kinase A (PKA) transduction pathway (9,21). The elevated PKA activity can then enhance the neuronal excitability by increasing the phosphorylation of certain ligand-gated channels and voltage-sensitive channels (9,21), which presumably includes the target channel(s) of H^+ in this study.

Adenosine is a purine nucleoside product of ATP metabolism and is produced by virtually all metabolically active cells, particularly when the energy demand cannot be matched by oxygen supply such as during tissue inflammation. Published evidence has strongly suggested the involvement of adenosine as an inflammatory mediator in the pathogenesis of AHR (25,27). A recent study reported by Gu *et al.* showed that adenosine administered in the same dose and manner as in this study did not produce significant changes in the basal cardiovascular conditions, but induced a pronounced and reversible sensitizing effect on the pulmonary C-fibers in rats (11). Although the mechanisms underlying the sensitizing effect of adenosine is not known, their results suggest that activation of the adenosine A_1 receptor expressed on the neuronal membrane of these sensory terminals is probably involved (11,25). However, a possibility that the sensitizing effect of adenosine on the C-fiber response to CO_2 involves releases of other mediators (e.g., histamine) from intermediate cells can not be ruled out in this study.

The mechanisms underlying the action of H^+ ions on the pulmonary C-fiber endings cannot be determined in this study, but several possible transduction mechanisms should be considered. The expression of acid-sensitive ion channels (ASICs) have been reported in rat dorsal root ganglion nociceptors (31), the counterpart of bronchopulmonary C-fiber afferents in other organ systems. The ASICs are H^+ -gated cation channels and members of the amiloride-sensitive Na^+ channel/degenerin family (31); the ASICs have an activation threshold of pH around or slightly below 7.0 and are rapidly inactivated following the activation by H^+ . A non-inactivating subunit of the ASICs expressed selectively in small-size sensory neurons was also cloned (30), but it is activated by a much lower pH. Whether these ASICs are present on pulmonary C-fiber endings is not yet known. A recent study using an isolated airway-nerve preparation demonstrated that an activation of transient receptor potential vanilloid type 1 (TRPV1) receptor is also involved in the stimulation of airway C-fiber afferents by sustained acidification of the airway tissue (19). Indeed, it is known that hydrogen ion can modulate the channel properties of the TRPV1 and enhance its sensitivity to capsaicin (1). Alternatively, it is also possible that certain chemical mediators (e.g., lipooxygenase metabolites, anandamide, etc.) are released from the surrounding tissue upon the action of H^+ , which in turn can either activate the TRPV1 receptor or lower its activation threshold.

The transient HPC, instead of the more conventional steady-state HPC, was chosen for testing the receptor sensitivity to CO_2 in this study because the considerably less amount of the CO_2 was inhaled during the brief duration of the HPC challenge. Therefore, the pulmonary C-fiber response to CO_2 is less likely to be influenced by the secondary effects of HPC such as changes in blood flow and pressure in pulmonary circulation and interstitium. Indeed, transient HPC induced a rapid, substantial, but readily reversible change in pulmonary venous blood pH (pH_a) without any sign of lingering systemic effects of HPC. We realize that the CO_2 concentration (30%) in the HPC gas mixture administered in this study was much higher than that encountered in normal physiological conditions. However, since only ~6 breaths of this gas mixture was administered, the highest alveolar CO_2 concentration during the HPC challenge only reached ~13%, accompanied by a decrease in pH in the arterial blood to ~7.1; the latter is certainly within the physiological range.

In conclusion, under normal conditions only a small percentage (< 10%) of the vagal bronchopulmonary C-fiber afferents are mildly activated by transient alveolar hypercapnia that lowered the arterial blood pH to ~7.1. However, both the percentage of C-fibers activated and

the sensitivity of the same afferents to CO₂ increased markedly when mucosal inflammation was induced in the airways. Under these conditions, the stimulatory effect of CO₂ is mediated through the action of H⁺ ions, probably by activating the H⁺-sensitive ion channels expressed on the terminal membrane of these sensory endings. In view of the high probability of simultaneous occurrence of inflammation and tissue acidosis in the airways, a potential involvement of this stimulatory effect on C-fiber afferents in the manifestation of various pulmonary pathophysiological responses should not be overlooked.

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References

1. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 1997;389:816–824. [PubMed: 9349813]
2. Coleridge JC, Coleridge HM. Afferent vagal C fibre innervation of the lungs and airways and its functional significance. *Rev Physiol Biochem Pharmacol* 1984;99:1–110. [PubMed: 6695127]
3. Coyle AJ, Ackerman SJ, Irvin CG. Cationic proteins induce airway hyperresponsiveness dependent on charge interaction. *Am Rev Respir Dis* 1993;147:896–900. [PubMed: 8385431]
4. Coyle AJ, Perretti F, Manzini S, Irvin CG. Cationic protein-induced sensory nerve activation: role of substance P in airway hyperresponsiveness and plasma protein extravasation. *J Clin Invest* 1994;94:2301–2306. [PubMed: 7527430]
5. Crone C and Levitt DG. Capillary permeability to small solutes. In: *Handbook of Physiology* Bethesda, MD: Am. Physiol. Soc, 1984, sect. 2, vol. IV, chap. 10, p. 411–466.
6. Delpierre S, Grimaud C, Jammes Y, Mei N. Changes in activity of vagal bronchopulmonary C fibres by chemical and physical stimuli in the cat. *J Physiol* 1981;316:61–74. [PubMed: 6798195]
7. Dickinson CJ and Paintal AS. Stimulation of type-J pulmonary receptors in the cat by carbon dioxide. *Clin Sci* 1970;38:33P.
8. Gordon T, Venugopalan CS, Amdur MO, Drazen JM. Ozone-induced airway hyperreactivity in the guinea pig. *J Appl Physiol* 1984;57:1034–1038. [PubMed: 6438025]
9. Gu Q, Kwong K, Lee LY. Ca²⁺ transient evoked by chemical stimulation is enhanced by PGE₂ in vagal sensory neurons: role of cAMP/PKA signaling pathway. *J Neurophysiol* 2003;89:1985–1993. [PubMed: 12612039]
10. Gu Q, Lee LY. Hypersensitivity of pulmonary C fibre afferents induced by cationic proteins in the rat. *J Physiol* 2001;537:887–897. [PubMed: 11744762]
11. Gu Q, Ruan T, Hong JL, Burki N, Lee LY. Hypersensitivity of pulmonary C fibers induced by adenosine in anesthetized rats. *J Appl Physiol* 2003;95:1315–1324. [PubMed: 12754169]
12. Ho CY, Gu Q, Hong JL, Lee LY. Prostaglandin E₂ enhances chemical and mechanical sensitivities of pulmonary C fibers. *Am J Respir Crit Care Med* 2000;162:528–533. [PubMed: 10934082]
13. 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]
14. Ho CY, Lee LY. Ozone enhances excitabilities of pulmonary C fibers to chemical and mechanical stimuli in anesthetized rats. *J Appl Physiol* 1998;85:1509–1515. [PubMed: 9760348]
15. Holtzman MJ, Cunningham JH, Sheller JR, Irsigler GB, Nadel JA, Boushey HA. Effect on ozone on bronchial reactivity in atopic and nonatopic subjects. *Am Rev Respir Dis* 1979;120:1059–1067. [PubMed: 507522]
16. Holtzman MJ, Fabbri LM, O'Byrne PM, Gold BD, Aizawa H, Walters EH, Alpert SE, Nadel JA. Importance of airway inflammation for hyperresponsiveness induced by ozone. *Am Rev Respir Dis* 1983;127:686–690. [PubMed: 6859651]
17. Holtzman MJ. Sources of inflammatory mediators in the lung: the role of epithelial and leukocyte pathways for arachidonic acid oxygenation. In: *Lung Biology in Health and Disease Series. Mediators*

of *Pulmonary Inflation*, edited by Bray MA and Anderson WH. New York: Dekker, 1991, vol. 54, chapt. 6, p. 279–325.

18. 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]
19. Kollarik M, Undem BJ. Mechanisms of acid-induced activation of airway afferent nerve fibres in guinea-pig. *J Physiol* 2002;543:591–600. [PubMed: 12205192]
20. Kwong K, Lee LY. PGE₂ sensitizes cultured pulmonary vagal sensory neurons to chemical and electrical stimuli. *J Appl Physiol* 2002;93:1419–1428. [PubMed: 12235043]
21. Kwong K, Lee LY. Prostaglandin E₂ potentiates a tetrodotoxin-resistant sodium current in capsaicin-sensitive pulmonary sensory neurons. *J Physiol* 2005;564:437–450. [PubMed: 15705651]
22. Lee LY, Gu Q, Gleich GJ. Effects of human eosinophil granule-derived cationic proteins on C-fiber afferents in the rat lung. *J Appl Physiol* 2001;91:1318–1326. [PubMed: 11509531]
23. Lee LY, Pisarri TE. Afferent properties and reflex functions of bronchopulmonary C-fibers. *Respir Physiol* 2001;125:47–65. [PubMed: 11240152]
24. **Lee LY and Undem BJ.** Bronchopulmonary vagal afferent nerves. In: *Advances in Vagal Afferent Neurobiology. Frontiers in Neuroscience Series*, edited by Undem BJ and Weinreich D. CRC Press: Taylor & Francis, 2005, chapt. 11, p. 279–313.
25. Nyce JW, Metzger WJ. DNA antisense therapy for asthma in an animal model. *Nature* 1997;385:721–725. [PubMed: 9034188]
26. Phillipson EA, Fishman NH, Hickey RF, Nadel JA. Effect of differential vagal blockade on ventilatory response to CO₂ in awake dogs. *J Appl Physiol* 1973;34:759–763. [PubMed: 4711584]
27. Polosa R, Rorke S, Holgate ST. Evolving concepts on the value of adenosine hyperresponsiveness in asthma and chronic obstructive pulmonary disease. *Thorax* 2002;57:649–654. [PubMed: 12096211]
28. Russell NJW, Raybould HE, Trenchard D. Role of vagal C-fiber afferents in respiratory response to hypercapnia. *J Appl Physiol* 1984;56:1550–1558.
29. Uchida DA, Ackerman SJ, Coyle AJ, Larsen GL, Weller PF, Freed J, Irvin CG. The effect of human eosinophil granule major basic protein on airway responsiveness in the rat in vivo. A comparison with polycations. *Am Rev Respir Dis* 1993;147:982–988. [PubMed: 8466137]
30. Waldmann R, Bassilana F, de Weille J, Champigny G, Heurteaux C, Lazdunski M. Molecular cloning of a non-inactivating proton-gated Na⁺ channel specific for sensory neurons. *J Biol Chem* 1997;272:20975–20978. [PubMed: 9261094]
31. Waldmann R, Champigny G, Bassilana F, Heurteaux C, Lazdunski M. A proton-gated cation channel involved in acid-sensing. *Nature* 1997;386:173–177. [PubMed: 9062189]
32. Wasserman K, Whipp BJ, Casaburi R, Beaver WL. Carbon dioxide flow and exercise hyperpnea. Cause and effect. *Am Rev Resp Dis* 1977;115:225–237. [PubMed: 326113]
33. Wu ZX, Morton RF, Lee LY. Role of tachykinins in ozone-induced airway hyperresponsiveness to cigarette smoke in guinea pigs. *J Appl Physiol* 1997;83:958–965. [PubMed: 9292485]

Fig. 1.

Change in arterial blood pH (pH_a) during transient alveolar hypercapnia (HPC). **A:** Experimental records illustrating a continuous change of pH in response to HPC in an anesthetized, open-chest, artificially ventilated rat (385 g). A CO_2 -enriched gas mixture (25% CO_2 , 21% O_2 , balance N_2) was administered via the respirator for 6 breaths (between the two arrows). P_t , tracheal pressure; CO_2 , CO_2 concentration measured in the tracheal cannula; because the display limit of the CO_2 monitor was only 13%, the inspired CO_2 concentration (25%) during the HPC challenge was not expressed to the full scale. A gray line was added to the CO_2 trace to depict the continuous change in end-tidal (alveolar) CO_2 concentration during the HPC challenge. pH_a , pH in the arterial blood ejected continuously from left ventricle. ABP, arterial blood pressure. Note that the spontaneous breathing, as indicated by the P_t signal, was elicited by the HPC challenge, presumably, as a result of the CO_2 -induced stimulation of central chemoreceptors. **B:** Group data showing the change in pH_a measured under the conditions described in A in 4 rats; BL, base-line; HPC, the lowest values of pH_a produced by the HPC challenge. **C:** Group data of pH_a measured in single arterial blood samples drawn during the base-line (BL) and at the end of the HPC challenge (HPC) in a different group of rats ($n = 7$). Data are means $\pm$ SE. *Significantly different from the base-line data.

Fig. 2.

Response of pulmonary C-fibers to transient alveolar hypercapnia (HPC). *A*: Experimental records illustrating the response to a right-atrial bolus injection of capsaicin (1 µg/kg) of a pulmonary C-fiber arising from the ending in the right middle lobe of an anesthetized, open-chest rat (412 g). *B*: response to lung inflation, which was produced by occluding the expiratory line of the respirator for 3 consecutive cycles and prolonged by turning off the respirator. *C*: response to HPC challenge, which was induced by administering a CO₂-enriched gas mixture (25% CO₂, 21% O₂, balance N₂) for 6 breaths (between the two arrows). Note the spontaneous ventilatory drive near the end of the HPC challenge. *D*: Group response indicated no statistically significant increase in the C-fiber activity (FA) generated by the HPC (25–30% CO₂) challenge. BL, base-line FA averaged over 20 s; HPC, the peak FA during the HPC challenge (2-s average). See legend of Fig. 1 for further explanation.

Fig. 3.

Experimental records illustrating the effect of intratracheal instillation of poly-L-lysine (PLL) on the response to HPC in a pulmonary C-fiber. *A*: response to an intravenous bolus injection of capsaicin (1 μ g/kg). *B*, *C* and *D*: response to a HPC challenge (between the two arrows) before, 20 and 100 min after the administration of PLL (0.25 mg/ml; 0.1 ml), respectively. Receptor location: right lower lobe. Rat weight: 430 g. See legends of Figs. 1 and 2 for further explanation.

Fig. 4.

Effect of airway inflammation on pulmonary C-fiber responses to HPC challenge. Effects of poly-L-lysine (PLL; 0.25 mg/ml, 0.1 ml; panel A), ozone exposure (O₃; 2.5 ppm for 30–45 min; panel B), intravenous infusion of adenosine (Ado; 40–120 µg/kg/min for 2 min; panel C) and prostaglandin E₂ (PGE₂; 1–2 µg/kg/min for 3 min; panel D) on pulmonary C-fiber responses to the HPC (25–30% CO₂, 21% O₂, balance N₂; 5–8 breaths) challenge. FA, fiber activity; BL, base-line FA averaged over 20 s; HPC, the peak FA during the HPC challenge (2-s average). Numbers of C-fibers studied were 8, 6, 15 and 12 for PLL, O₃, Ado and PGE₂, respectively. Recovery data were obtained 60–120, 20–30 and 20–30 min after the treatments of PLL, Ado and PGE₂, respectively; the recovery data were not obtained in the study of O₃. *Significantly different from the corresponding base-line. †Significantly different from the corresponding control response.

Fig. 5.

Experimental records illustrating the effect of intravenous infusion of adenosine (Ado) on a pulmonary C-fiber. *A*: response to an intravenous bolus injection of capsaicin (1 μ g/kg). *B*, *C* and *D*: response to a HPC (30% CO₂) challenge (between the two arrows) before, during and 20 min after the Ado infusion (120 μ g/kg/min; 2 min), respectively. A red line was added to the CO₂ trace in panels *B*, *C* and *D* to depict the continuous changes in end-tidal CO₂ concentration during the HPC challenge. Receptor location: right upper lobe. Rat weight: 385 g. See legend of Fig. 2 for further explanation.

Fig. 6.

Enhanced sensitivity of pulmonary C-fibers to HPC challenge generated by PLL and Ado was abolished by bicarbonate. **A:** Effect of intratracheal instillation of PLL (0.25 mg/ml; 0.1 ml) on pulmonary C-fiber responses to HPC challenge (30% CO₂, 21% O₂, balance N₂; 5–8 breaths) before and during infusion of NaHCO₃ (1.82 mmol/kg/min; 35 s). **B:** Effect of intravenous infusion of Ado (40–120 µg/kg/min; 2 min) on pulmonary C-fiber responses to HPC before and during infusion of NaHCO₃. FA, fiber activity; BL, base-line FA averaged over 20 s; HPC, the peak FA during the HPC challenge (2-s average). *Significantly different from the corresponding base-line. †Significantly different from the corresponding control

response. #Significantly different between the corresponding data of before and during infusion of NaHCO_3 . Data are means $\pm$ SE of 6 and 8 fibers in panels *A* and *B*, respectively.