Ozone enhances excitabilities of pulmonary C fibers to chemical and mechanical stimuli in anesthetized rats

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Ho, Ching-Yin, and Lu-Yuan Lee. Ozone enhances excitabilities of pulmonary C fibers to chemical and mechanical stimuli in anesthetized rats. J. Appl. Physiol. 85(4): 1509-1515, 1998.—Acute exposure to ozone (O3) enhances pulmonary chemoreflex response to capsaicin, and an increased sensitivity of bronchopulmonary C-fiber afferent endings may be involved. The present study was aimed at determining the effect of O3 on the responses of pulmonary C fibers to chemical and mechanical stimuli. A total of 31 C fibers were studied in anesthetized, open-chest, and vagotomized rats. During control, right atrial injection of a low dose of capsaicin abruptly evoked a short and mild burst of discharge (0.77 ± 0.28 impulses (imp)/s, 2-s average). After acute exposure to O3 (3 parts/million for 30 min), there was no significant change in arterial blood pressure, tracheal pressure, or baseline activity of C fibers. However, the stimulatory effect of the same dose of capsaicin on these fibers was markedly enhanced (6.05 ± 0.88 impulses/s; P < 0.01) and prolonged immediately after O3 exposure, and returned toward control in 54 ± 6 min. Similarly, the pulmonary C-fiber response to injection of a low dose of lactic acid was also elevated after O3 exposure. Furthermore, O3 exposure significantly potentiated the C-fiber response to constant-pressure (tracheal pressure = 30 cmH2O) lung inflation (control: 0.19 ± 0.07 imp/s; after O3: 1.12 ± 0.26 imp/s; P < 0.01). In summary, these results show that the excitabilities of pulmonary C-fiber afferents to lung inflation and injections of chemical stimulants are markedly potentiated after acute exposure to O3, suggesting a possible involvement of these afferents in the O3-induced changes in breathing pattern and chest discomfort in humans.

IT HAS BEEN DEMONSTRATED that acute exposure to ozone (O3), one of the major air pollutants in urban areas, induces transient bronchial hyperreactivity to a variety of bronchoactive substances (3, 7, 9, 11, 14, 16, 23). Reflex bronchoconstriction is probably involved because the increased bronchomotor responsiveness to histamine in human subjects after exposure to O3 was partially abolished by pretreatment with atropine (6, 11). Furthermore, the increased bronchomotor responses were also consistently associated with symptoms of bronchial irritation and cough on deep inspiration in these subjects (6, 9, 11). In conscious dogs, acute O3 exposure caused rapid, shallow breathing and increased tachypneic responses to inhaled histamine and prostaglandin F2α aerosols, and these augmented responses were almost completely eliminated when vagal conduction was blocked by cooling the exteriorized cervical vagus nerves to 0°C (17, 25), indicating a possible role of pulmonary afferents.

Morphological evidence has shown that >80% of afferent fibers in the vagus nerves are nonmyelinated C fibers (1, 4). It is known that these C-fiber affenti are involved in the entire respiratory tract and play an important role in regulating the airway functions in both physiological and pathophysiological conditions (4, 24). A recent study has shown that acute exposure to O3 potentiated the pulmonary chemoreflex responses to right atrial injections of specific stimulants of vagal pulmonary C-fiber afferents in anesthetized, spontaneously breathing rats (8). These enhanced reflex responses were completely abolished by perineural capsaicin treatment of both cervical vagus nerves to selectively block the C-fiber conduction, suggesting that an increase in the excitability of pulmonary C-fiber endings to these stimuli was probably involved. However, the definitive evidence obtained directly from electrophysiological recording of these afferents was still lacking. Hence the purpose of this study was to determine whether the pulmonary C-fiber afferent responses to injections of chemical stimulants and lung inflation are enhanced after acute O3 exposure in anesthetized rats.

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.

Male Sprague-Dawley rats (344–488 g) were anesthetized with intraperitoneal injection of a-chloralose (100 mg/kg; Sigma Chemical, St. Louis, MO) and urethan (500 mg/kg; Sigma Chemical) dissolved in a 2% borax (Sigma Chemical) solution; supplemental doses of the same anesthetics were given, 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 and for intravenous administration of pharmacological agents, respectively. The jugular venous catheter was advanced until its tip was slightly above the right atrium. Body temperature was maintained at ~36°C throughout the experiment with a servo-temperature controller (model K-20-F, K-Module) and a heating pad placed under the animal. The trachea was cannulated, and tracheal pressure (Ptr) was measured (Validyne MP-45–28) via a side port of the tracheal cannula. The rats were artificially ventilated with a respirator (UGO Basile 7025, Comerio-Varese, Italy); tidal volume (Vt) and respiratory frequency were set at 8–10 ml/kg and 44
breaths/min, respectively. After a midline thoracotomy was performed, the right vagus nerve was ligated just above the diaphragm to eliminate afferent signals arising from visceral organs below the diaphragm, and the left cervical vagus was sectioned. The expiratory outlet of the respirator was placed under 3 cmH₂O pressure to maintain a near-normal functional residual capacity.

The right cervical vagus nerve was separated from the carotid sheath and sectioned as rostrally as possible. The caudal end of the cut vagus nerve was placed on a small dissection platform and immersed in a trough of mineral oil held by tethered cervical skin; its nerve sheath was then removed. With the aid of a dissecting microscope and fine-tip forceps, a thin filament was teased away from the nerver trunk and placed on a platinum-iridium hook electrode. Action potentials were amplified (Grass P5–11K), 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.

Because vagal pulmonary C fibers usually have a sparse [-0.5 impulses (imp)/s] and irregular baseline discharge, hyperinflation of the lung (3–4 mL/Vt) was used as the first step in searching for these fibers. Once the afferent activity of a single unit was identified by hyperinflation, capsaicin (0.5–2 µg/kg) was injected via a jugular venous catheter into the right atrium. Only the fibers that responded to capsaicin within 1 s after the injection were studied. The signals of the afferent activities, Pter, and arterial blood pressure (Statham P23AA) were recorded on a Gould Thermal Writer (TW11) and on a videocassette recorder (Vetter 500H). Fiber activities (FAs) were analyzed later by a computer for each 0.5-s interval.

Each fiber was tested for its responses to right atrial injections of chemical stimulants and to lung inflation. Two chemical stimulants were chosen for this study: capsaicin and lactic acid. Capsaicin is known for its potency and selectivity in activating C-fiber endings (4). Lactic acid is a major product of anaerobic tissue metabolism and has recently been shown to cause a consistent and rapidly reversible stimulatory effect on pulmonary C fibers (13). Constant-pressure lung inflation was applied by inflating the lung with a constant airflow (14 mL/s) until Pter reached 30 cmH₂O and lung inflation was applied by inflating the lung with a synthetic airway. Two injections of chemical stimulants and to lung inflation. Two injections of chemical stimulants were made, and a pair of stimulating electrodes was then placed under the vagus nerve cranial to the exit of its pulmonary branches to deliver rectangular constant-current pulse (duration: 1 ms; intensity: 0.3–3.0 mA) generated by a pulse generator (A310 Accupuls, World Precision Instruments) and a stimulus-isolation unit (A360R-C, World Precision Instruments). Finally, the general locations of all fibers were identified by their responses to the gentle pressing of the lungs with a saline-wetted cotton Q-tip or a blunt-end glass rod. The exact distance between stimulating and recording electrodes for calculating the conduction velocity was measured postmortem. Animals were killed after the experiment by an intravenous injection of KCl.

A stock solution of capsaicin (200 µg/ml; Sigma Chemical) was prepared in a vehicle of 10% Tween 80, 10% ethanol, and 80% saline, and that of lactic acid (300 µg/ml; Sigma Chemical) was prepared by distilled water. A solution of the desired concentration was prepared daily with saline and distilled water, respectively, on the basis of the animal's body weight, and the volume of each bolus injection of these agents was kept at 0.2 ml.

We used a one-way analysis of variance for a mixed (random and fixed effects) model to analyze the data. The model is designed for analyzing matched or repeated-measures data obtained from a random sample of subjects (random effects) under a set of specific treatments (fixed effects); a level of P < 0.05 was considered significant. Data are expressed as means ± SE.

RESULTS

A total of 31 pulmonary C-fiber endings were studied in 27 anesthetized and open-chest rats; in 4 rats, 2 single pulmonary C fibers were recorded simultaneously from the same vagal filament. The conduction velocities were 0.83–1.85 m/s (1.11 ± 0.05 m/s; n = 23). The distribution of locations of these receptors was as follows: six in the upper lobe, six in the middle lobe, nine in the lower lobe, and one in the accessory lobe. The locations of the remaining nine fibers and the conduction velocities of eight of them were not determined because the recording of single-fiber signals could not be maintained at the end of the long experiments. Baseline activity was detectable in only 1 of the 31 C-fiber afferents studied (e.g., Fig. 1). The baseline activity averaged over a 10-s interval was 0.003 ± 0.003 imp/s (n = 31). After acute exposure to O₃ (3 ppm for 30 min), there was no significant change in the average baseline activity of these C fibers (0.02 ± 0.01 imp/s; P > 0.05), despite a slight increase in two receptors; there was no significant change in the baseline heart rate or the mean arterial blood pressure.

The response to capsaicin was always tested at an initial dose of 0.5 µg/kg. In 4 of the 31 C fibers, the dose was increased to 1.0 µg/kg when the initial dose failed to produce a clear stimulatory effect on these fibers. During control, injection of this low dose of capsaicin abruptly evoked a mild and short burst of discharge (e.g., Fig. 1); the difference between the peak activity
averaged over a 2-s duration after capsaicin and the baseline activity averaged over a 10-s duration (ΔFA) was 0.77 ± 0.28 imp/s (Figs. 2 and 3). However, the stimulatory effect of the same dose of capsaicin on these fibers was markedly enhanced (ΔFA: 6.05 ± 0.88 imp/s; n = 31; P < 0.01) within 5–20 min after the cessation of O₃ exposure (Figs. 1 and 2); the fiber responses increased in both the peak activity and the duration of firing (Fig. 2). The augmented response to lactic acid returned toward control levels (ΔFA: 0.85 ± 0.29 imp/s; Fig. 3) within 54 ± 6 min after the cessation of O₃ exposure in the 26 receptors tested. However, there was also a wide variation in this potentiating effect of O₃ among these receptors: a clear potentiating effect of O₃ on the response to lactic acid was found in 19 receptors, and the effect of O₃ was not detectable in the remaining 11 receptors.

Pulmonary C fibers were relatively insensitive to lung inflation; during control, only 13 of 29 pulmonary C fibers were activated by constant-pressure lung inflation at 30 cmH₂O. The ΔFA generated by lung inflation (the difference between the FA during lung inflation and the baseline activity, each averaged over a 10-s duration) was 0.19 ± 0.07 imp/s (e.g., Fig. 5). After O₃ exposure, the stimulatory effect of lung inflation was significantly increased (ΔFA: 1.12 ± 0.26 imp/s; n = 29; P < 0.01) (Figs. 3, 5, and 6) and returned toward control levels (ΔFA: 0.12 ± 0.08 imp/s; Fig. 3) within 30 ± 6 min after the cessation of exposure in the 10 fibers tested. However, there was also a wide variation in this potentiating effect of O₃ among these receptors: a clear potentiation after O₃ was found in 22 receptors, and no potentiating effect of O₃ was detected in the remaining 7 receptors.

RESULTS

Results obtained from this study show that responses of vagal pulmonary C-fiber afferents to chemical and
mechanical stimuli were markedly enhanced after acute exposure to O3. The augmented responses to both types of stimuli returned toward pre-O3 control levels within 1 h (range: 25–160 min) in most of the fibers studied, indicating the reversibility and the transient nature of the effect of O3 exposure.

These results indicate that the O3-induced increase in excitabilities of these C-fiber endings was independent of the types of stimuli applied. The observation that the excitability of these C-fiber endings to lung inflation was also markedly enhanced after O3 exposure is particularly interesting because these endings are, in general, relatively insensitive to changes in lung volume under normal conditions (4, 13). Furthermore, expansion of the lung is a natural stimulus and can occur in many physiological conditions (e.g., hyperventilation during heavy exercise, sighing, and so on). In fact, it has been previously reported by investigators from different laboratories that airway irritation and dyspnzic sensation were induced in healthy human subjects immediately after exposure to O3 (6, 9, 11); many of these subjects complained of substernal pain and coughed on taking a deep inspiration (9, 11), which resulted in a reduced inspiratory capacity (9). These findings clearly illustrated that airway irritation could be evoked in humans by an otherwise subthreshold stimulus after O3 exposure. The results obtained from the present study demonstrating an increased excitability of pulmonary C-fiber endings to lung inflation after O3 exposure have, therefore, provided direct electrophysiological evidence in support of these previous findings.

In a recent study, Hajj and Lee (8) reported that the pulmonary chemoreflex responses to capsaicin injection were potentiated after acute exposure to O3 (1.5 ppm, 1 h) in anesthetized, spontaneously breathing rats. These reflex responses, including the triad of apnea, bradycardia, and hypotension, are believed to be elicited from activation of pulmonary C-fiber afferents (4, 18). This notion is supported by the fact that these responses were completely abolished by perineural capsaicin treatment of both cervical vagi, which selectively blocked the conduction of C fibers (15, 18). A previous study by Lee et al. (17) has shown that the tachypneic response to inhalation challenge with histamine or prostaglandin F2 alpha aerosol was markedly potentiated after O3 exposure in conscious dogs. The results obtained in the present study clearly demonstrate the increased afferent responses of pulmonary C-fiber afferents to the same doses of the same chemical stimuli after O3 exposure and therefore are supportive of these earlier findings.

Fig. 3. Effects of O3 on pulmonary C-fiber responses to right atrial injections of capsaicin (0.5–1 µg/kg; A), lactic acid (0.1 mmol/kg; B), and lung inflation (Ptr = 30 cmH2O; C) in anesthetized, open-chest rats. ΔFA, difference between peak FA (averaged over 2-s intervals in responses to capsaicin and lactic acid injections and over 10-s interval in response to lung inflation) and baseline FA (averaged over 10-s intervals); open bars, control responses; solid bars, responses 5–40 min after cessation of O3 exposure (3 ppm, 30 min); shaded bars, responses during recovery. Values are means ± SE; n = 31 (A), 30 (B), and 29 (C) C fibers studied during control and after O3, and n = 26 (A), 8 (B), and 10 (C) C fibers studied during recovery. *P < 0.01.

Fig. 4. Effect of O3 on pulmonary C-fiber response to lactic acid injection in anesthetized, open-chest rats. FA was measured in 0.5-s intervals. Lactic acid (LA; 0.1 mmol/kg) was injected into right atrium at time 0 (arrow and vertical dashed line). ○, Control response; ●, response 15–30 min after cessation of O3 exposure (3 ppm, 30 min). Values are means ± SE of all 30 fibers from 26 rats.
Rapid, shallow breathing is a characteristic breathing pattern elicited by sustained stimulation of bronchopulmonary C-fiber afferents (4). Lee et al. (17) first reported that acute O₃ exposure induced rapid, shallow breathing and enhanced tachypneic responses to inhaled histamine and prostaglandin F₂α aerosols in conscious dogs; rapid, shallow breathing has also been reported in human subjects after exposure to O₃ (0.5 ppm, 2 h) (9). These responses were abolished when both exteriorized cervical vagus nerves were cooled to 0°C to completely block the conduction in the vagus nerves (17). Schelegle et al. (26) later demonstrated, by using a differential cooling technique, that the O₃-induced tachypnea persisted even after conduction in myelinated vagal axons was selectively blocked by cooling to 7°C in anesthetized dogs, suggesting an important role of bronchopulmonary C fibers. Indeed, in a companion study, Coleridge and co-workers (5) clearly demonstrated a mild stimulatory effect of O₃ (2–3 ppm) on bronchial C-fiber afferents. However, the focus and the design of the present study differed from those in their study in several ways. First, in this study, we were interested in how the sensitivities of pulmonary C-fiber afferents to other chemical stimuli were affected by acute exposure to O₃. In contrast, their experiment focused primarily on the stimulatory effect of O₃ alone during the exposure and did not study the change in sensitivity of these afferents after the O₃ exposure. Second, the effect of O₃ on the pulmonary C-fiber response to lung hyperinflation was not investigated in their study (5). Third, their results showed that O₃ stimulated primarily bronchial C fibers and exerted little or no stimulatory effect on pulmonary C fibers; the latter was confirmed in our experiment. Despite marked increases in the sensitivities of pulmonary C-fiber afferents to both chemical and mechanical stimuli, the baseline activities of these endings did not increase during or after the O₃ exposure (Figs. 1, 2, and 5). Consistent with this finding, exposure to O₃ at a total dose similar to that used in this study did not induce any significant change in the baseline breathing pattern in anesthetized rats, despite a markedly enhanced apneic response to capsaicin injection (8).

The possible mechanisms underlying the enhanced excitability of the pulmonary C-fiber endings after O₃ exposure were not determined in this study. It has been shown in many species that acute exposure to O₃ causes epithelial injury and mucosal inflammation in the airways (3, 12, 23). It is also known that certain inflammatory mediators, particularly the cyclooxygenase metabolites of arachidonic acid, can markedly enhance the sensitivity of cutaneous nociceptors, the counterpart of pulmonary C-fiber endings in the periph-

![Figure 5](image_url)

**Fig. 5.** Experimental records illustrating effect of O₃ on afferent response of a pulmonary C fiber arising from an ending in right lower lobe to constant-pressure lung inflation (Ptr = 30 cmH₂O) in an anesthetized, open-chest rat (420 g). A: control response. B: response 40 min after cessation of O₃ exposure (3 ppm, 30 min). C: response during recovery (70 min after cessation of exposure). Conduction velocity of fiber, 1.57 m/s. See legend of Fig. 1 for further explanation.

![Figure 6](image_url)

**Fig. 6.** Effect of O₃ on pulmonary C-fiber response to lung inflation in anesthetized, open-chest rats. FA was measured in 0.5-s intervals. Constant-pressure (Ptr = 30 cmH₂O) lung inflation was maintained for 10 s (between 2 arrows). ○, Control response; ●, response 15–40 min after cessation of O₃ exposure (3 ppm, 30 min). Values are means ± SE of all 29 fibers from 25 rats.
eral tissue, to various mechanical and chemical stimuli (19, 20, 22). In fact, the airway epithelium, which is the site of initial assault by O3, is also a primary cellular source of these mediators in the lung (10). In addition, elevated levels of several cyclooxygenase metabolites in the lung lavage fluid have already been documented after acute exposure to O3 in humans (27). Whether the endogenous release of cyclooxygenase metabolites was involved in the increased excitability of these afferent endings after O3 exposure remains to be determined.

Judging from the results obtained from this study, it appears that O3 exposure potentiates the C-fiber response to capsaicin to a greater degree than that to lactic acid (Figs. 2 and 4); the mechanism underlying this difference is not known. It is known, however, that the ion fluxes evoked by capsaicin and by protons can be differentially regulated in cultured rat dorsal root ganglion C neurons (2), suggesting that different transduction mechanisms are probably involved in the activation of C fibers by these two chemical substances. Indeed, the sensitivities to these two different chemical stimulants vary considerably among pulmonary C fibers in rats (unpublished observations). Another factor to be considered is that, although the sequence of testing these stimuli and lung inflation was randomized among fibers, the response to lactic acid was tested first after the O3 exposure in only 19% of the fibers studied. This difference in the sequence of challenges may partially contribute to the smaller potentiation of the responses to lactic acid, particularly because the potentiated responses of these C fibers gradually diminish over time after the O3 exposure.

In this study, the potentiated responses of most of the pulmonary C fibers to capsaicin and lactic acid recovered in less than an average of 60 min. In contrast, the potentiation of the apneic responses to the same stimuli, induced by the same total dose of O3 as that delivered in this study, was sustained for a substantially longer duration (>180 min) (8). Similar long-lasting effects of O3 exposure on the airways have also been reported by others (6, 16, 17, 23). The discrepancy between the results of this study and those of others may be related to the differences in O3 concentration and duration of exposure. Because of the difficulties involved in maintaining the recording of single-fiber signals for a sustained period of time, it was necessary to reduce the exposure duration and to raise the O3 concentration accordingly. Whether the same extent of mucosal injury is caused by the same total dose of O3 with different combinations of duration and O3 concentration is not known. On the other hand, we cannot rule out the possibility that other types of vagal afferents (e.g., bronchial C fibers) are also involved in potentiating the apneic responses after the acute O3 exposure.

In conclusion, these results show that the excitabilities of pulmonary C-fiber afferents to lung inflation and injections of chemical stimulants are markedly augmented after acute exposure to O3. This potentiating effect of O3 on pulmonary C-fiber sensitivity is reversible and is probably involved in the airway irritation shown immediately after the O3 exposure.

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