Stimulation of vagal pulmonary C fibers by inhaled wood smoke in rats

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Lai, C. J., and Y. R. Kou. Stimulation of vagal pulmonary C fibers by inhaled wood smoke in rats. J. Appl. Physiol. 84(1): 30–36, 1998.—This study investigated the stimulation of vagal pulmonary C fibers (PCs) by wood smoke. We recorded impulses from PCs in 58 anesthetized, open-chest, and artificially ventilated rats and delivered 6 ml of wood smoke into the lungs. Within 1 or 2 s after the smoke delivery, an intense and nonphasic burst of discharge ($\Delta = +7.4 \pm 0.7$ SE impulses/s, $n = 68$) was evoked in 60 of the 68 PCs studied and lasted for 4–8 s. This immediate stimulation was usually followed by a delayed and more sustained increase in C-fiber activity ($\Delta = +2.0 \pm 0.4$ impulses/s). The overall stimulation was not influenced by removal of smoke particulates ($n = 15$) or by pretreatment with vehicle ($n = 8$) for dimethylthiourea (DMTU; a hydroxyl radical scavenger) or indomethacin (Indo; a cyclooxygenase inhibitor). The immediate-phase stimulation was not affected by pretreatment with Indo ($n = 8$) but was largely attenuated by pretreatment with DMTU ($n = 12$) or by a combined treatment with DMTU and Indo (DMTU + Indo; $n = 8$). Conversely, the delayed-phase stimulation was partially suppressed either by DMTU or by Indo but was totally abolished by DMTU + Indo. These results suggest that 1) the stimulation of PCs is linked to the gas phase of wood smoke and 2) hydroxyl radical, but not cyclooxygenase products, is involved in the immediate-phase stimulation, whereas both metabolites are responsible for evoking the delayed-phase stimulation.

In addition to oxygen-reactive metabolites, inhalation of toxic smoke generated from combustion of materials is known to cause an increase in the release of other chemical mediators, including cyclooxygenase products (10, 13, 25). When the cyclooxygenase pathway is activated, arachidonic acid is metabolized to various types of prostaglandins and thromboxane (1). Cyclooxygenase products, either produced endogenously or administered exogenously, have been shown to stimulate lung vagal C fibers (5, 6, 11). However, whether these arachidonate metabolites are involved in the activation of lung vagal C fibers after smoke inhalation is not yet known.

In this study, we recorded afferent activity arising from vagal pulmonary C fibers in anesthetized rats to determine 1) whether these sensory nerve endings are stimulated by delivery of wood smoke into the lungs, 2) whether the gas phase of wood smoke is responsible for this afferent stimulation, and 3) whether $\cdot$OH and cyclooxygenase products are involved in this afferent stimulation.

METHODS

Sprague-Dawley rats (weight 334 ± 6 g) of either sex were anesthetized with intraperitoneal injection of chloralose (100 mg/kg) and urethane (500 mg/kg). A polyethylene catheter was inserted into the jugular vein and advanced until the tip was close to the right atrium for intravenous administration of pharmacological agents. The right femoral artery was cannulated for measuring arterial blood pressure. During the course of the experiments, supplemental doses of chloralose (20 mg·kg$^{-1}$·h$^{-1}$) and urethane (100 mg·kg$^{-1}$·h$^{-1}$) were administered to maintain abolition of pain reflexes induced by pinching the animal's tail. During the recording of vagal action potentials, the rats were paralyzed with pancuronium bromide (0.05 mg/kg iv; Orgon Teknika). Periodically, the effect of pancuronium was allowed to wear off so that the depth of anesthesia could be checked.

The rats were tethered in a supine position, and the trachea was cannulated below the larynx with a short tracheal tube via a tracheotomy. A midline thoracotomy was performed, and the edges of the rib cage were retracted. The lungs were ventilated by a rodent respirator (model 683, Harvard) at a constant volume of 2 ml. The frequency of the respirator was set at 65–75 breaths/min and was kept constant in each experiment. The expiratory outlet of the
respirator was placed under 3–4 cm of water to maintain a near-normal functional residual capacity. Tracheal pressure (Ptr; transpulmonary pressure in an open-chest preparation) was monitored by a pressure transducer (MP45–28, Validyne) via a side tap of the tracheal cannula. Body temperature was maintained at ~36°C throughout the experiment by means of a servo-controlled heating blanket.

Recording of afferent activity of vagal pulmonary C fibers. Afferent activity arising from pulmonary C fibers was recorded by using techniques described elsewhere (19). Briefly, a fine afferent filament was split from the desheathed nerve trunk of the right vagus and placed on a platinum-iridium recording electrode. Action potentials were amplified (P511K, Grass), monitored by an audio monitor (AM8, Grass), and displayed on an oscilloscope (model 420, Gould). The fine nerve filament was subdivided until activity from only one or two units was obtained. All physiological signals were recorded simultaneously by a thermal array recorder (TA11, Gould) and also recorded on tape (DR-890, Neurocorder) for later analysis.

Vagal pulmonary C fibers in rats usually have a sparse activity (<2 impulses/s) during eupneic artificial ventilation but can be activated by hyperinflation of the lungs (3, 20). Therefore, lung inflation was used as the first step to search for these pulmonary receptors; the lungs were hyperinflated in a stepwise manner to a maximum of four times the tidal volume (4 × VT; Fig. 1A) by occlusion of the expiratory line of the respirator. Once the presence of a suspected single unit was detected, capsaicin (1–2 µg/kg; Sigma Chemical), a potent chemical stimulant of C fibers, was injected as a bolus into the vein. Only units that were stimulated within 2 s after the injection were studied. Before the end of each experiment, the general locations of the C fibers studied were identified within the lung structure by gently probing the tissues with a polyethylene rod (diameter = 2 mm). Finally, the conduction velocity of the afferent fibers of 51 pulmonary C fibers was measured by a method described previously (3).

Generation and delivery of smoke. The electric furnace and the methods for generating wood smoke are described in detail in our previous study (14). In brief, 100 g of dry wood dust (lauan wood) were thermally decomposed by the furnace at a core temperature maintained at 500 ± 5°C for 5 min, and the effluent smoke was collected in a 25-liter plastic balloon attached to the furnace outlet. Gas-phase smoke was generated by passing the wood smoke through a standard glass fiber Cambridge filter, which removed ~99% of the smoke particulates (16). The smoke was sampled and analyzed for O2 (OM-11, Beckman), CO2 (LB-2, Beckman), CO (model 961, Neotronics), and particulate (P-5H2, Sibata) concentrations. Unfiltered smoke generated by this method contains ~1.5% O2–15% CO2–24% CO, and 25 µg/l particulates (14, 16). The gas-phase smoke contains similar concentrations of these gases but is free of particulates (16). Immediately after its generation, 6 ml of unfiltered smoke or gas-phase smoke at a temperature of ~25°C were delivered by the respirator in three ventilatory cycles by using a circuit similar to that described previously (19).

Pharmacological agents. Dimethylthiourea (DMTU, an -OH scavenger; Sigma Chemical) was dissolved in isotonic saline to a concentration of 500 mg/ml. Indomethacin (Indo; a cyclooxygenase inhibitor; Sigma Chemical) was first dissolved in polyethylene glycol and then diluted at 1:1 ratio in isotonic saline to a concentration of 5 mg/ml. Capsaicin solution (1–2 µg/ml) was made from a refrigerated stock solution (5 mg/ml), which was prepared by dissolving capsaicin in 10% ethanol, 10% polyoxyethylene sorbitan monoleate, and 80% saline.

Experimental procedures. A total of 58 rats was used in this investigation. Sixty-eight pulmonary C fibers were recorded and studied for their control responses to unfiltered wood smoke. In 15 pulmonary C fibers recorded from 15 rats, afferent responses to gas-phase smoke were compared with those to unfiltered smoke. In another 36 pulmonary C fibers recorded from 36 rats, challenges of unfiltered smoke were

![Fig. 1. Afferent responses of a vagal pulmonary C fiber to lung inflation (A), capsaicin (B), unfiltered wood smoke (C), and gas-phase smoke (D). A: lungs were hyperinflated to 4 times tidal volume. B: capsaicin (1 µg/kg) was injected into catheter (1st arrowhead) and flushed into vein (2nd arrowhead). C and D: 6 ml of unfiltered smoke or gas-phase smoke were delivered into lungs in 3 ventilatory cycles as indicated by horizontal bars. Twenty minutes elapsed between capsaicin injection and smoke delivery, and 30 min elapsed between 2 smoke deliveries. C fiber had a conduction velocity of 1.5 m/s. Ptr, tracheal pressure; AP, action potential; ABP, arterial blood pressure.](http://jap.physiology.org/)
Hyperinflated lungs up to 3 or 4 VT (Fig. 1A) and by bolus intravenous injection of capsaicin (1 or 2 mg/kg iv) before and after administration of a vehicle or these chemicals were also studied. Before each test of smoke delivery or capsaicin injection, the animal's lungs were hyperinflated (4 × Vr) to maintain a constant volume history. Challenges of unfiltered smoke and gas-phase smoke were alternated among the animals to achieve a balanced design. An interval of at least 30 min elapsed between the two deliveries of smoke to avoid tachyphylaxis; our preliminary study indicated that the C-fiber responses to smoke were reproducible when this period of recovery time was allowed. A 20-min period elapsed before the study was resumed after the administration of DMTU, Indo, or DMTU + Indo.

Data analysis and statistics. Neural activity of pulmonary C fibers, mean arterial blood pressure, and heart rate was measured at 1-s intervals. Ptr was measured on a breath-by-breath basis. Baseline data of these physiological parameters were calculated as the average values over the 10-s or 10-breathe period immediately preceding the smoke challenge. Pulmonary C fibers were judged to be stimulated by the smoke when the peak evoked discharge exceeded the baseline activity by at least 1 impulse/s. These physiological parameters were analyzed by using a computer equipped with an analog-to-digital converor (DASA 4600, Gould) and software (BioCybernatics 1.0). Results obtained from the computer analysis were routinely checked for accuracy with those calculated manually. Results were analyzed by a paired t-test or a two-way repeated-measures analysis of variance followed by Duncan’s test when appropriate. P < 0.05 was considered significant. All data are presented as means ± SE.

RESULTS

The baseline activity of the C fibers studied was irregular and sparse (0.5 ± 0.1 impulses/s, n = 68). The nerve endings were stimulated by hyperinflating the lungs up to 3 or 4 VT (Fig. 1A) and by bolus intravenous injection of capsaicin (Fig. 1B). The mean conduction velocity of the afferent fibers conducting impulses from 51 of the C fibers was 1.1 ± 0.1 m/s (range 0.5–2.1 m/s); the conduction velocity of the remaining 17 fibers was not measured. All C fibers studied were localized within the lung structure, and their physiological properties were consistent with those reported in rats (3, 20) and in other species (6).

Delivery of unfiltered wood smoke stimulated 60 of the 68 pulmonary C fibers studied. When the fibers were stimulated, an intense and nonphasic burst of discharge was evoked within 1 or 2 s after smoke delivery (Fig. 1C). This immediate increase in C-fiber activity quickly peaked in 1–2 s after smoke delivery and lasted for 4–8 s (Figs. 1C and 2A). After this immediate-phase stimulation, the evoked discharge of these pulmonary C fibers declined yet remained at a level higher than the baseline activity (Fig. 2A). This delayed and more sustained increase in C-fiber activity was not in phase with ventilatory cycles (Fig. 1C). The remaining eight pulmonary C fibers were not stimulated, either immediately or later, by delivery of unfiltered smoke. For the whole group of 68 pulmonary C fibers, the mean duration of the overall stimulation induced by unfiltered smoke was 25.5 ± 2.2 s (range: 0–75 s).

In 15 pulmonary C fibers that were stimulated by unfiltered wood smoke, afferent responses were compared with those evoked by the gas phase of wood smoke. Unfiltered and gas-phase smoke essentially produced a similar pattern of stimulation in the same 15 C fibers (Fig. 1). Statistical analysis revealed that mean responses of pulmonary C fibers to unfiltered wood smoke and to gas-phase smoke. Data are means ± SE of 15 C fibers studied. Mean fiber activity is plotted during control period (baseline) and in 10-s intervals after smoke challenge. No statistical significance (P > 0.05) was found in any paired comparison of values between 2 types of smoke within same time intervals.
significantly (Figs. 3 and 4). In the vehicle-treated group, a repeated smoke challenge evoked afferent responses of similar amplitude and time course in the same eight C fibers compared with their control responses. In contrast, in the DMTU-, Indo-, or DMTU + Indo-treated groups, a repeated smoke challenge evoked a milder afferent stimulation in each of the C fibers tested (Fig. 3). On average, the immediate responses of C fibers evoked during the first 10-s interval after smoke delivery were not altered by vehicle or Indo but were largely attenuated by DMTU or DMTU + Indo (Fig. 4). Furthermore, the delayed responses of C fibers evoked beyond that first 10-s interval after smoke delivery were not altered by vehicle, but were significantly diminished by DMTU or Indo, and were totally abolished by DMTU + Indo (Fig. 4). As a result, the average duration of the afferent stimulation produced by unfiltered smoke was not significantly affected by vehicle but was greatly shortened by DMTU, Indo, or DMTU + Indo (Fig. 5). At the end of the test period, all the pulmonary C fibers in the vehicle-, DMTU-, Indo-, and DMTU + Indo-treated
groups still responded to intravenous injection of capsaicin, and their average responses were not significantly altered by pretreatment with vehicle or these chemicals (Table 1).

Under controlled conditions, delivery of unfiltered wood smoke did not cause any detectable change in $P_{\text{tr}}$ (Fig. 3). The peak response of $P_{\text{tr}}$ after smoke delivery was $8.1 \pm 0.1 \text{ cmH}_2\text{O}$, which was not significantly different from its baseline ($8.0 \pm 0.1 \text{ cmH}_2\text{O}; P > 0.05; n = 58$). In contrast, mean arterial blood pressure initially increased from a baseline of $84.7 \pm 3.1 \text{ mmHg}$ to a peak of $89.4 \pm 3.5 \text{ mmHg} (P < 0.05; n = 58)$ at 5–9 s and subsequently decreased to its lowest level of $63.6 \pm 2.6 \text{ mmHg} (P < 0.01; n = 58)$ at 15–26 s after delivery of unfiltered smoke (Fig. 3). During the hypertensive and ensuing hypotensive period, mean heart rate decreased from a baseline of $338.1 \pm 8.8$ to $324.9 \pm 8.2$ and

![Graphs showing mean duration of stimulation](http://jap.physiology.org/)

**Fig. 5.** Effects of pretreatment with vehicle (A; $n = 8$), DMTU (B; $n = 12$), Indo (C; $n = 8$), or DMTU + Indo (D; $n = 8$) on mean duration of C-fiber stimulation induced by unfiltered wood smoke. Data are means ± SE. **Statistically different from values before pretreatment, $P < 0.05$.**

Table 1. Effects of pretreatment with vehicle, $\cdot OH$ scavenger, and cyclooxygenase inhibitor on peak responses of pulmonary C fibers to intravenous injection of capsaicin.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Vehicle</th>
<th>DMTU Pretreatment</th>
<th>Indo Pretreatment</th>
<th>DMTU + Indo Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>($n = 8$)</td>
<td></td>
<td>($n = 12$)</td>
<td>($n = 8$)</td>
<td>($n = 8$)</td>
</tr>
<tr>
<td>Impulses/s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before pretreatment</td>
<td>$10.9 \pm 2.3$</td>
<td>$9.2 \pm 1.7$</td>
<td>$17.4 \pm 2.9$</td>
<td>$16.0 \pm 3.7$</td>
</tr>
<tr>
<td>After pretreatment</td>
<td>$11.5 \pm 1.7$</td>
<td>$9.5 \pm 2.5$</td>
<td>$13.9 \pm 3.2$</td>
<td>$16.8 \pm 3.3$</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, no. of fibers or animals. DMTU, dimethylthiourea; Indo, indomethacin; DMTU + Indo, a combined treatment with DMTU and Indo. No statistical significance ($P > 0.05$) was found in any paired comparison of values before and after pretreatment.
fibers to capsaicin. The persistence of the C-fiber responses to capsaicin after pretreatment with these chemicals may suggest that the attenuation of the C-fiber responses to wood smoke observed after administration of these chemicals was not likely due to anesthetic or deleterious effects on these nerve endings.

A third possibility is that -OH and cyclooxygenase products may indirectly stimulate pulmonary C fibers through their bronchoconstrictive effects (1, 12). However, the smoke-evoked discharges of pulmonary C fibers that we observed were not modulated by ventilatory cycles. Furthermore, delivery of wood smoke did not cause any detectable change in Pfr in this study. Accordingly, this possibility is questionable.

The source of the origin of -OH and cyclooxygenase products cannot be determined in this study. Several investigators (22, 27, 30) have suggested that oxygen-reactive metabolites formed in lung tissues after inhalation of toxic smoke might originate from an exogenous source. The gas phase of wood smoke is known to contain high concentrations of free radicals and radical precursors (22), which may continuously generate -OH either in the smoke or when they reach lung tissues after smoke inhalation (22, 30). However, the particulate phase of wood smoke also contains free radicals (22) but did not appear to be a major contributor to the stimulation of pulmonary C fibers observed in this study. One plausible explanation for the difference in the contribution of gas and particulate phases of wood smoke is that these two smoke components might each have different accessibility to pulmonary C fibers, the nerve endings of which are believed to be located primarily in the alveolar region (6). Because of their gravitational settling and deposition in the central airways (9), smoke particulates presumably have less ability to reach the lung periphery compared with gas-phase smoke. The other plausible explanation is that the oxygen-reactive metabolites generated by these two smoke components may be different in their types, concentrations, and life spans. Although still not fully understood, the radical chemistry of the gas phase of wood smoke is known to be quite different from that of the particulate phase (22).

Both -OH and cyclooxygenase products may be formed and released endogenously in the lungs after delivery of wood smoke. Certain lung cells, such as polymorphonuclear leukocytes and alveolar macrophages, are primary oxygen radical releasers (8, 27) and were found to be activated after acute inhalation of toxic smoke (23, 27). Additionally, it is known that the lungs are a rich source of arachidonate products and the enzymes necessary for their metabolism (1). Previous studies indicated that significant amounts of oxygen-reactive metabolites are formed during the metabolism of arachidonic acid via cyclooxygenase (7) and that oxygen-reactive metabolites may increase the production of cyclooxygenase products (24). Therefore, these two metabolites may be interrelated in their biosynthesis. Because the involvement of cyclooxygenase products was observed only during the delayed-phase stimulation, it may suggest that a longer delay is required for the arachidonate metabolites to be produced and/or to act on C-fiber nerve endings after smoke delivery compared with -OH.

The time required to initiate the immediate-phase stimulation of pulmonary C fibers is very similar to that needed to evoke the slowing of respiration observed in our previous studies (14–16), thus confirming the importance of -OH in eliciting this respiratory reflex. In this study, pretreatment with DMTU did not totally abolish the immediate-phase stimulation, an observation that is in good agreement with the finding that a similar dose of DMTU could not completely prevent the C-fiber-mediated respiratory reflex evoked by wood smoke (15). It is speculated that oxygen-reactive metabolites other than -OH and/or smoke constituents that are not related to free radicals were also involved in this immediate-phase stimulation of C fibers. In our previous studies of the reflex responses (14–16), inhaled wood smoke generally evoked an immediate and prominent bradycardia and hypotension, coinciding with the slowing of respiration (occurred within 1 or 2 s). We have hypothesized that these immediate cardiovascular responses to wood smoke are also elicited by stimulation of lung vagal C fibers (14, 15). In this study, however, immediate cardiovascular responses to wood smoke were absent, probably because the right vagus nerve was sectioned. The mild hypertension and ensuing hypotension after smoke delivery observed in this study may be due to the activation of arterial chemoreceptors by the extremely low oxygen and high carbon dioxide concentrations in the smoke (14).

Inhalation of toxic smoke not only causes airspace irritations (2, 14, 17, 27) but also produces lung injury (4, 10, 13, 17, 25, 27, 30). Although the information regarding the inhalation injury of the lungs is relatively well established (27, 30), the mechanisms underlying the airway irritation induced by toxic smoke have been largely overlooked. It is generally believed that vagal pulmonary C fibers function as an important sensory system in detecting the onset of pathophysiological changes in the airways (6). Although oxygen-reactive metabolites and cyclooxygenase products have been strongly implicated in the pathogenesis of inhalation injury (4, 13, 25, 27, 30), their involvements in eliciting the sensory irritation of the airways after smoke inhalation are obscure. The observations made in this study provide the first electrophysiological evidence that vagal pulmonary C fibers play an important role in detecting the airway assault by wood smoke and that their functional significance is mediated through mechanisms involving -OH and cyclooxygenase products.

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REFERENCES


