Mechanisms of stimulation of vagal pulmonary C fibers by pulmonary air embolism in dogs

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Chen, H. F., B. P. Lee, and Y. R. Kou. Mechanisms of stimulation of vagal pulmonary C fibers by pulmonary air embolism in dogs. J. Appl. Physiol. 82(3): 765–771, 1997.—We investigated the involvement of the cyclooxygenase metabolites and hydroxyl radical (·OH) in the stimulation of vagal pulmonary C fibers (PCs) by pulmonary air embolism (PAE). Impulses were recorded from PCs in 51 anesthetized, open-chest, and artificially ventilated dogs. Fifty of 59 PCs were stimulated by infusion of air into the right atrium (0.2 ml·kg⁻¹·min⁻¹ for 10 min). As a group (n = 59), PC activity increased from a baseline of 0.4 ± 0.1 to a peak of 1.7 ± 0.2 impulses/s during the period from 1 min before to 2 min after the termination of PAE induction. In PCs initially stimulated by PAE induction, PAE was repeated after the intervening period of 1 min. The responses of PCs to PAE were not altered by saline vehicle but were abolished by ibuprofen and significantly attenuated by dimethylthiourea. Although hyperinflation of the lungs reversed the PAE-induced bronchomotor responses, it did not reverse the stimulation of PCs (n = 8). These results suggest that 1) cyclooxygenase products are necessary for the stimulation of PCs by PAE, whereas changes in lung mechanics are not, and 2) the functional importance of cyclooxygenase products may be mediated in part through the formation of ·OH.

PULMONARY MICROEMBOLISM is known to cause reflex tachypnea (1, 4, 12). Vagal lung C-fiber afferents are believed to play an important role in eliciting this respiratory response (1, 4, 12). Indeed, previous electrophysiological studies have demonstrated stimulation of vagal lung C-fiber nerve endings by emboli such as starch particles, plastic spheres, or glass beads (2, 3, 21). Although the physiological mechanisms of the stimulation remain unclear, several investigators have postulated that chemical mediators released locally in the lungs after microembolism may be responsible for the stimulation of vagal pulmonary C fibers (2, 7, 18).

Pulmonary microembolism has been found to cause an increase in the release of a variety of mediators, including cyclooxygenase products and reactive oxygen metabolites (5, 9, 10, 18, 29). When the cyclooxygenase system is activated, arachidonic acid is converted to prostaglandin H₂, which can be subsequently metabolized to various types of prostaglandins and thromboxane. Several cyclooxygenase products, when administered exogenously, have been shown to stimulate vagal lung C-fiber nerve endings (7, 13). On the other hand, superoxide anion, hydrogen peroxide, and hydroxyl radical (·OH) are the major reactive oxygen metabolites produced after pulmonary microembolization (18). Among them, ·OH, the reaction product of superoxide anion and hydrogen peroxide, is the most reactive free radical produced in the biological system (11). Recent studies have demonstrated that ·OH, generated by an ischemia-reperfusion process, can activate visceral C-fiber nerve endings in the gastrointestinal tract of cats (26) and in the heart of rats (28). Taken together, these findings suggest the possibility that the cyclooxygenase metabolites and ·OH may be involved in the stimulation of vagal lung C-fiber nerve endings induced by pulmonary microembolism. However, experimental evidence to support this possibility remains to be established.

The objective of the present study was to investigate the possible involvements of the cyclooxygenase system and ·OH in the stimulation of vagal pulmonary C fibers by pulmonary air embolism (PAE) in anesthetized dogs. PAE was chosen as the model of microembolism in this study because its effects are readily reversible within minutes after termination of induction (15, 23). To accomplish our objective, we compared the afferent responses of vagal pulmonary C fibers to PAE before and after systemic administration of a saline vehicle, a cyclooxygenase inhibitor [ibuprofen (Ibu)], or a ·OH scavenger [dimethylthiourea (DMTU)].

METHODS

Fifty-one dogs (8.5–20.0 kg) were anesthetized with an intravenous injection of thiotepal sodium (20 mg/kg; Abbott), followed by a combination of chloralose (50 mg/kg iv; Sigma Chemical) and urethan (500 mg/kg iv; Sigma Chemical). Supplemental doses of chloralose (15 mg·kg⁻¹·h⁻¹) and urethan (150 mg·kg⁻¹·h⁻¹) were administered to maintain abolition of the corneal and withdrawal reflexes during the course of the experiments. The femoral artery was cannulated for measurement of arterial blood pressure. Catheters (PE-240) were inserted into the right atrium and left ventricle via the femoral vein and carotid artery, respectively. During the recording of vagal action potentials, the dogs were paralyzed with pancuronium bromide (0.05 mg/kg iv; Organon Teknika). Periodically, the effect of pancuronium was allowed to wear off so that the depth of anesthesia could be checked.

After a midline incision was made in the neck, a short tracheal cannula was inserted just below the larynx, and a midline thoracotomy was performed. The lungs were ventilated (model 607, Harvard) with 65% O₂ at a frequency of 16–20 cycles/min and a tidal volume (V₁) of 12–15 ml/kg; both were kept constant during each experiment. CO₂ was mixed with the inspired gas when necessary to maintain end-tidal CO₂ concentration at ~5%. The expiratory outlet of the respirator was placed under 3–4 cmH₂O to maintain a near normal functional residual capacity. Respiratory flow was measured with a pneumotachograph (Fleisch no. 1) and a
flow transducer (model 17212, Gould). The flow signal was integrated to give Vt. Tracheal pressure (Ptr; i.e., transpulmonary pressure in open-chest preparation) and CO2 concentration were measured via side taps of the tracheal cannula by a pressure transducer (MP45-28, Validyne) and a capnograph (model 9000, Biochek), respectively. Total lung resistance (RL) and dynamic lung compliance (Cdyn) were determined by using the subtraction method (20). All physiological signals were recorded by a thermal array recorder (model TA11, Gould) and also recorded on tape (model DR-890, Recorder) for later analysis.

Recording of afferent activity of vagal pulmonary C fibers. Afferent activity arising from pulmonary C fibers was recorded by using techniques previously described (14, 16). 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 (model P511K, Grass), monitored by an audio amplifier (model AM8, Grass), and displayed on an oscilloscope (model 1425, Gould). The fine nerve filament was subdivided until the activity from a single unit was obtained. We used lung inflations (4 × Vt) as the first step to search for lung C fibers. Pulmonary C fibers were identified by their immediate response (within 1–3 s) to right atrial injection of capsaicin (5 µg/kg; Sigma Chemical) and their lack of response to left ventricular injection (6, 14, 16). Before the end of each experiment, the receptor’s location was identified within the lung structure by gentle hand palpation. Finally, the conduction velocity of afferent fibers of 30 pulmonary C fibers was measured by a method described previously (6).

Induction of PAE. PAE was induced by a constant infusion of air (0.2 ml·kg⁻¹·min⁻¹) into the right atrial catheter by an infusion pump (model 101, NanJou) for a 10-min period. The infusion rate thus ranged from 1.7 to 4 ml/min, depending on the body weight of each individual animal. Each study of PAE challenge consisted of a 5-min baseline period, a 10-min period during PAE induction, followed by a 5-min recovery period after the end of air infusion.

Experimental procedures. A total of 64 pulmonary C fibers were recorded from 51 dogs. In five pulmonary C fibers, impulses were continuously recorded over two consecutive 20-min periods to serve as the control. The remaining 59 pulmonary C fibers were first studied for their control responses to PAE. Subsequently, the challenge of PAE was repeated in 9, 11, and 12 pulmonary C fibers at 10 min after pretreatment with saline vehicle, Ibu (20 mg/kg), and DMTU (50 mg/kg), respectively, to investigate the involvements of the cyclooxygenase system and ·OH. In another eight pulmonary C fibers, PAE challenge was repeated and a brief hyperinflation of the lungs (4 × Vt) was performed at the termination of the second PAE induction to examine whether the increased activity of pulmonary C fibers was associated with the changes in RL and Cdyn after embolization. Ibuprofen (Sigma Chemical) and DMTU (Aldrich), dissolved in isotonic saline, were slowly injected into the right atrium over a 2-min period. These doses of Ibuprofen and DMTU have been previously shown to abolish the pulmonary responses to embolization (5) and the cardiac vagal C-fiber responses to reactive oxygen metabolites (27), respectively. Before each 20-min period of continuous recording or each challenge of PAE, the animal’s lungs were hyperinflated (4 × Vt) to establish a constant volume history. To confirm that pulmonary C fibers remained active, right atrial injection of capsaicin (5 µg/kg) was performed at 10 min after the end of PAE induction. Because air emboli last for ~20 min (23), at least 20 min were allowed to elapse between two challenges of PAE. Animals received one to three challenges of PAE to obtain control responses, depending on the number of pulmonary C fibers studied in each individual animal, but received only one challenge of PAE after pretreatment with saline, Ibuprofen, or DMTU. Results were discarded for those pulmonary C fibers that became inactive during the test and/or were unresponsive to capsaicin at the end of the test period.

Data analysis and statistics. Neural activity of pulmonary C fibers and mean arterial blood pressure were measured at 1-s intervals. RL and Cdyn were measured on a breath-by-breath basis. Baseline data of these physiological parameters were calculated as the average values over the 30-s period immediately preceding the PAE induction. Peak responses were measured as the peak values averaged over a 30-s period after the PAE induction. Pulmonary C fibers were judged to be activated by PAE when the peak response exceeded its baseline activity by at least 0.5 impulses/s. These physiological parameters were analyzed by using a computer equipped with an analog-to-digital convertor (model DASA 4600, Gould) and software (version 1.0, BioCybernatics). Results obtained from the computer analysis were routinely checked with those obtained by manual calculation for accuracy. Results were analyzed by a paired t-test and one-way or two-way repeated-measures analysis of variance followed by Tukey’s test when appropriate. P < 0.05 was considered significant. All data are presented as means ± SE.

RESULTS

The baseline activity of the 64 vagal pulmonary C fibers studied (0.4 ± 0.1 impulses/s, n = 64) was irregular and sparse. Pulmonary C fibers were stimulated by hyperinflating the lungs up to 3 or 4 Vt; they were also stimulated within 1–3 s by right atrial injections of capsaicin (Fig. 1A) but not by left ventricular injections (Fig. 1B). The locations of these pulmonary C fibers within the lung structure were identified by direct palpation. The conduction velocity of the afferent fibers conducting impulses from 30 of these pulmonary C fibers was 1.2 ± 0.1 m/s (range 0.6–2.0 m/s). The physiological properties of these pulmonary C fibers are consistent with those previously reported in dogs (6, 7, 14, 16) and in other species (7).

To study the changes in activity of pulmonary C fibers that occurred spontaneously under our experimental conditions, impulses of five pulmonary C fibers were recorded over two consecutive 20-min periods. Neural activity of these pulmonary C fibers did not change significantly during the two periods of continuous recording (Fig. 2A).

Induction of PAE stimulated 50 of the 59 pulmonary C fibers studied. The stimulation started 1.9 ± 0.1 min (0.9–2.7 min) after the onset of air infusion (Fig. 2B). When stimulated, these pulmonary C fibers fired irregularly, and the evoked discharge was not in phase with the ventilatory cycle (Fig. 1, C and D). On average, the evoked discharge of these pulmonary C fibers progressively increased from a baseline of 0.4 ± 0.1 impulses/s to a peak of 1.7 ± 0.2 impulses/s (n = 59) during the period from 1 min before to 2 min after the termination of PAE induction (Fig. 2B). They then gradually declined to their baseline activity within 5–10 min after the termination of PAE induction.

Of the 50 pulmonary C fibers stimulated by the first induction of PAE, 9, 11, and 12 pulmonary C fibers received a second challenge of PAE after the animals had pretreated with saline vehicle, Ibuprofen, and DMTU.
respectively. Ten minutes after pretreatment with saline or these chemicals, the baseline activity of pulmonary C fibers did not change significantly (Figs. 2B, 3, and 4). In the vehicle-treated group, a repeated challenge of PAE induced afferent responses of very similar amplitude and time course in the same pulmonary C fibers compared with their control responses (Fig. 2B). In contrast, a repeated challenge of PAE was unable to stimulate any of the pulmonary C fibers tested in the Ibu-treated group, and the overall stimulation of pulmonary C fibers induced by PAE was totally inhibited by the pretreatment (Fig. 3A). In the DMTU-treated group, a repeated challenge of PAE induced a milder stimulation in each of the pulmonary C fibers tested, and the overall stimulation of pulmonary C fibers was markedly suppressed by the pretreatment (Fig. 3B). Because the time at which peak activity occurred varied among pulmonary C fibers, the peak response was measured in each pulmonary C fiber, and the average data are shown in Fig. 4. Statistical analysis revealed that the average peak responses of pulmonary C fibers to PAE were not altered by pretreatment with saline (Fig. 4A) but were abolished by pretreatment with Ibu (Fig. 4B) and attenuated by pretreatment with DMTU (Fig. 4C). At the end of the test period, all the pulmonary C fibers in saline-, Ibu-, and DMTU-treated groups could still respond to the right atrial injection of capsaicin (Table 1). In 10 other pulmonary C fibers that were stimulated by the first induction of PAE and received pretreatment with saline, Ibu, or DMTU, they became inactive during the second challenge of PAE and were unresponsive to capsaicin at the end of the test period. Presumably, these 10 pulmonary C fibers had lost their viability.

Induction of PAE did not significantly affect the mean arterial blood pressure (before vs. 10 min after the
onset of PAE induction, 122.8 ± 2.9 vs. 118.1 ± 3.6 mmHg; n = 59). However, induction of PAE consistently caused an increase in RL and a decrease in Cdyn (Figs. 5 and 6). At 1.5–2.5 min after the onset of embolization, RL began to increase, which reached its peak 5–9 min after the onset of embolization and remained elevated for the remaining test period. In a similar time course, Cdyn was decreased by the induction of PAE (Fig. 5). The PAE-induced increase in RL was not significantly altered by pretreatment with saline vehicle or DMTU (Fig. 5, A and C) but was attenuated by pretreatment with Ibu (Fig. 5B). In the remaining eight pulmonary C fibers that were stimulated by the first induction of PAE, a brief hyperinflation of the lungs (4 × VT) was performed at the termination of the second PAE induction. Hyperinflation of the lungs largely restored RL and Cdyn to their baseline values, whereas it did not reverse the PAE-induced stimulation (Fig. 6).

DISCUSSION

Results of the present study demonstrated that a majority of vagal pulmonary C fibers studied (85%) were stimulated by PAE and that the stimulation was not due to the progressive changes in fiber activity that occurred spontaneously under our experimental conditions because not any detectable response was found in the five pulmonary C fibers tested as the time control (Fig. 2A). This is the first experimental evidence that air emboli lodged in the pulmonary vessels can stimulate pulmonary C fibers. These results are in general agreement with those reported by other investigators using emboli such as starch particles, plastic spheres, or glass beads (2, 3, 21). In addition, we have demonstrated that when two challenges of PAE separated by at least 20 min were induced, afferent responses similar in both intensity and time course were produced in the same pulmonary C fibers. The reproducibility of the afferent responses to PAE, therefore, allowed us to investigate the effects of Ibu or DMTU on the PAE-induced stimulation of pulmonary C fibers.

We found that pretreatment with Ibu totally abolished the afferent responses of pulmonary C fibers to PAE and that DMTU attenuated the responses of pulmonary C fibers by 62.2%. These results suggest that both the cyclooxygenase system and OH may play important roles in the stimulation of pulmonary C fibers by PAE. In fact, these two factors have also been
shown to participate in the process of the activation of afferent C-fiber nerve endings in other organs. In cats, the afferent responses of sympathetic visceral C-fiber nerve endings to abdominal ischemia are attenuated by either cyclooxygenase blockade (17) or inhibition of \( \cdot \)OH production (26). In rats, cardiac vagal C-fiber nerve endings are stimulated by an ischemia and reperfusion of the heart (28). The activation of these cardiac vagal afferents at the onset of ischemia is totally prevented by cyclooxygenase blockade, whereas the activation at reperfusion is completely abolished by inhibition of \( \cdot \)OH production (28). Hence, it appears that these two factors are important activators for a variety of afferent C-fiber nerve endings. The exact sources of these two factors are not well understood. However, it is known that the lungs are a rich source of arachidonate products and the enzymes necessary for their metabolism (5, 18). Furthermore, circulating leukocytes and possibly lung cells have been suggested to be possible sources for the production of oxygen radicals after embolization (9, 18, 29). Indeed, both in vivo and in vitro studies have demonstrated activation of the cyclooxygenase pathway and leukocytes after PAE by measurement of concentrations of 6-ketoprostaglandin \( F_1\alpha \) and thromboxane \( B_2 \) and by leukocyte counts in the plasma (10, 18, 29).

Our results suggest that there is an overlap of functional contributions of the cyclooxygenase system and \( \cdot \)OH to the afferent responses of pulmonary C fibers to PAE. Similar findings have also been reported in the study of other physiological responses. Longhurst and co-workers (17) and Stahl and co-workers (26) reported that more than half of the visceral C-fiber responses to abdominal ischemia are reduced by either cyclooxygenase blockade or inhibition of \( \cdot \)OH production. In studies of tissue injury in the lungs (19), heart (24), and brain (30), the responses produced under various pathological conditions could be prevented by either cyclooxygenase inhibitors or by oxygen radical scavengers. One plausible explanation for the overlap of functional contributions is that the cyclooxygenase system and \( \cdot \)OH are interrelated in their biochemical pathways. For example, it has been shown that significant amounts of oxygen radicals are formed during the metabolism of arachidonic acid via cyclooxygenase (8, 24, 30). Furthermore, some cyclooxygenase products have been demonstrated to induce adherence and aggregation of leukocytes (25), which are known to be the prime factor for the increased release of oxygen radicals after PAE (18, 29). Thus cyclooxygenase blockade by Ibu could possibly inhibit not only the production of prostaglandins and thromboxanes but also the generation of \( \cdot \)OH associated with the cyclooxygenase pathway. However, scavenging \( \cdot \)OH by DMTU presumably affects only the latter. Another plausible explanation is that cyclooxygenase products and \( \cdot \)OH are interrelated in their physiological effects. For example, some investigators (18, 26) have postulated that one mediator must act synergistically with the other to manifest either's functional contribution to the responses. Therefore, eliminating either of these two participants would prevent or attenuate the overall response. Collectively, it would be assumed that the cyclooxygenase system is essential for the activation of pulmonary C fibers by PAE and that its functional importance is mediated in part through the involvement of \( \cdot \)OH in our experimental model.

Several mechanisms have been postulated to explain how pulmonary microembolism stimulates pulmonary C fibers, including 1) a direct stimulation by the released mediators (2, 7, 18), 2) a mechanical stimulation by an increase in interstitial fluid volume (18, 22), and 3) a mechanical stimulation by airway constriction (2). Therefore, the involvement of cyclooxygenase prod-

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**Table 1. Average peak responses of vagal pulmonary C fibers to right atrial injection of capsaicin**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Responses 10 min Before First PAE Induction, impulses/s</th>
<th>Responses 10 min After End of Second PAE Induction, impulses/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline treated</td>
<td>9</td>
<td>18.1 ± 3.3</td>
<td>16.2 ± 2.8</td>
</tr>
<tr>
<td>Ibu treated</td>
<td>11</td>
<td>22.7 ± 3.4</td>
<td>16.8 ± 3.0</td>
</tr>
<tr>
<td>DMTU treated</td>
<td>12</td>
<td>19.4 ± 3.0</td>
<td>15.4 ± 2.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of fibers. PAE, pulmonary air embolism; Ibu, ibuprofen; DMTU, dimethylthiourea. *Significantly different from responses before first PAE induction, \( P < 0.05 \).
ucts and ·OH may possibly arise from their direct actions on pulmonary C fibers or their ability to induce further releases of other chemical mediators to stimulate pulmonary C fibers. It is also plausible that neither cyclooxygenase products nor ·OH directly mediates the PAE-induced stimulation of pulmonary C fibers. However, baseline levels of cyclooxygenase products and ·OH (27) might nonspecifically raise the sensitivity of C-fiber nerve endings. Consequently, administration of Ibu and DMTU might lower the baseline levels of these metabolites and thereby make pulmonary C fibers less responsive to PAE-related stimulus. The possibility of an increase in interstitial fluid volume should also be considered because both cyclooxygenase products and ·OH after embolization can increase vascular permeability (18). In the present study, PAE elicited an increase in RL and a decrease in Cdyn, a finding consistent with that reported in our previous study (15). However, pretreatment with Ibu totally abolished the afferent responses of pulmonary C fibers, whereas it only partially attenuated the bronchomotor responses to PAE. Furthermore, pretreatment with DMTU attenuated the afferent responses of pulmonary C fibers while yielding no effect on the bronchomotor responses to PAE. Moreover, hyperinflation of the lungs restored the increase in RL and the decrease in Cdyn to their baseline values, but it did not reverse the PAE-induced stimulation of pulmonary C fibers. These observations suggest that the bronchomotor responses to PAE may be due to constriction and/or closure of small airways and regional atelectasis in the lungs (20) and that the cyclooxygenase system, but not ·OH, is involved in these responses. In addition, the dissociation of the

![Fig. 5. Bronchomotor responses to PAE before and after pretreatment with saline vehicle (A), Ibu (B), and DMTU (C). Data represent means ± SE averaged over 30 s; n, no. of dogs. RL, total lung resistance; Cdyn, dynamic lung compliance. Dashed lines, termination of PAE induction. *Significantly different from responses before pretreatment.](http://jap.physiology.org/)

![Fig. 6. Effects of hyperinflation of lungs on C fiber (A) and bronchomotor responses (B) to PAE. Baseline values were measured as data averaged over 30s immediately preceding PAE induction. Hyperinflation (HI; 4 x tidal volume) was performed at termination of PAE induction. Responses before and after HI were measured as values averaged over 30 s immediately before and after HI. Values are means ± SE averaged from 8 C fibers from 8 dogs. NA, neural activity. *Significantly different from baseline values, P < 0.05.](http://jap.physiology.org/)
relationship between the afferent and bronchomotor responses to PAE may imply that the involvements of cyclooxygenase products and -OH in the stimulation of pulmonary C fibers are not likely mediated through bronchoconstriction.

Armstrong and Miller (3) initially attempted to study the mechanisms underlying the stimulation of pulmonary C fibers by pulmonary microembolism. They demonstrated that platelet depletion prevented the increased activity of pulmonary C fibers after glass-bead microembolization in rabbits (3), suggesting a central role for platelet aggregation. Although not specifically identified as the mediator(s) involved in this electrophysiological study (3), many mediators such as cyclooxygenase metabolites, oxygen radicals, histamine, and serotonin could be released as a consequence of platelet aggregation (18). In their studies, Armstrong and co-workers (1, 4) showed that the reflex tachypneic response to glass-bead microembolization in rabbits was totally prevented by platelet depletion and partially attenuated by pretreatment with a serotonin-receptor antagonist. The tachypneic response to microembolization in rabbits has been shown to be a reflex elicited by the stimulation of pulmonary C fibers (12). Therefore, their findings may indirectly suggest that the C-fiber stimulation after embolization is mediated in part by the effects of serotonin associated with platelet aggregation. In this study, we made no attempt to investigate the involvement of serotonin because pulmonary C fibers are relatively insensitive to serotonin in dogs (7). However, these findings (1, 4) that indomethacin or aspirin (two other cyclooxygenase inhibitors) completely abolished the reflex tachypneic response to pulmonary C fibers by pulmonary microembolism. They demonstrated that platelet depletion prevented the increased activity of pulmonary C fibers by a single breath of cigarette smoke in dogs. Therefore, their findings may indirectly suggest that the C-fiber innervation of the lungs and airways and its functional significance in eliciting this respiratory reflex in their embolic model.

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REFERENCES


