Vagal and mediator mechanisms underlying the tachypnea caused by pulmonary air embolism in dogs

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Chen, H. F., and Y. R. Kou. Vagal and mediator mechanisms underlying the tachypnea caused by pulmonary air embolism in dogs. J Appl Physiol 88: 1247–1253, 2000.—We investigated the vagal and mediator mechanisms underlying the tachypnea caused by pulmonary air embolism (PAE) in anesthetized and spontaneously breathing dogs. PAE was induced by infusion of air into the right atrium (0.2 ml·kg−1·min−1 for 10 min). The first PAE induction caused an increase in respiratory frequency accompanied by a decrease in tidal volume in each of the 30 animals studied. Subsequently, animals were evenly divided into five groups, and a second PAE induction was repeated after various experimental interventions. The tachypneic response to PAE was not significantly altered by pretreatment with a saline vehicle but was largely attenuated by either perivagal capsaicin treatment (a technique that selectively blocks the conduction of unmyelinated C fibers), pretreatment with ibuprofen (a cyclooxygenase inhibitor), or pretreatment with dimethylthiourea (a hydroxyl radical scavenger). Ultimately, the tachypneic response was nearly abolished by a bilateral cervical vagotomy. These results suggest that 1) lung vagal unmyelinated C-fiber afferents play a predominant role in evoking the reflex tachypneic response to PAE and 2) both cyclooxygenase products and hydroxyl radical are important in eliciting this vagally mediated response.

PULMONARY AIR EMBOLISM (PAE) occurs in a number of clinical situations and is known to cause tachypnea (17, 24). The physiological mechanisms underlying the PAE-induced tachypnea are not fully understood. A previous investigation in dogs (20) demonstrates that the PAE-induced tachypnea is totally abolished by a bilateral cervical vagotomy, suggesting that this response is a reflex mediated by lung vagal afferents. Two recent electrophysiological studies in dogs reveal that lung vagal C-fiber nerve endings (7) and pulmonary rapidly adapting receptors (8) are stimulated by PAE. Additionally, both cyclooxygenase products and hydroxyl radical participate in the activation of lung vagal C-fiber nerve endings by PAE (7), whereas the former mediators, but not the latter, contribute to the stimulation of pulmonary rapidly adapting receptors (8). C-fiber nerve endings and rapidly adapting receptors are supplied by lung vagal unmyelinated and myelinated afferents, respectively, and are believed to play an important role in eliciting respiratory reflexes under various pathophysiological conditions (9, 11, 30). Cyclooxygenase products and hydroxyl radical are chemical mediators, the release of which in the lungs has been shown to be increased by PAE (13, 22, 28). Thus questions still remain as to whether one or both types of lung vagal afferents and chemical mediators are involved in eliciting the tachypneic response to PAE.

To differentiate the role of lung vagal unmyelinated C-fiber afferents and myelinated afferents in evoking respiratory reflexes, previous investigators (15, 18, 21) have employed perivagal capsaicin treatment to produce a differential vagal block. This technique is based on the fact that application of capsaicin, a chemical extracted from hot peppers, directly to the peripheral nerves blocks the conduction of unmyelinated C fibers but does not affect the conduction of myelinated fibers (5, 25). Therefore, when capsaicin is applied perineurally to both cervical vagi, it selectively blocks the respiratory reflexes resulting from stimulation of C-fiber nerve endings (15, 18, 21).

The objectives of this study were twofold: 1) to determine the relative contribution of lung vagal unmyelinated C-fiber afferents and myelinated afferents to, and 2) to assess the role of cyclooxygenase products and hydroxyl radical in, the PAE-induced tachypnea in anesthetized dogs. To accomplish our objectives, we compared the ventilatory responses to PAE before and after perivagal capsaicin treatment or bilateral cervical vagotomy and also before and after systemic administration of a saline vehicle, a cyclooxygenase inhibitor (ibuprofen), or a hydroxyl radical scavenger (dimethylthiourea).

METHODS

Dogs (9.2–15.4 kg) of both sexes were anesthetized with an intravenous injection of thiopental 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). During the experiment, the depth of anesthesia was constantly checked; supplemental doses of chloralose (15 mg·kg−1·h−1) and urethan (150 mg·kg−1·h−1) were administered intravenously to maintain abolition of the corneal and withdrawal reflexes. Body temperature was maintained at...
The femoral artery was cannulated for measuring arterial blood pressure. A catheter (PE-240) was inserted into the right atrium via the right jugular vein for measuring arterial blood pressure. A catheter (PE-240) was inserted into the right atrium via the right jugular vein for administration of pharmacological agents and for induction of PAE. A midline incision was made in the neck, and a segment (~3 cm) of each vagus nerve was carefully isolated from the common carotid artery for later use. A short tracheal cannula was inserted just below the larynx via a tracheostomy, through which animals breathed spontaneously in a supine position. Respiratory flow ($V\dot{}$) was measured with a pneumotachograph (Fleisch no. 1) coupled with a differential pressure transducer (model MP45–12, Validyne). The flow signal was integrated to give tidal volume ($V_t$). Tracheal pressure ($P_t$) was monitored by another differential pressure transducer (model MP45–28, Validyne) via a side tap of the tracheal cannula. All physiological signals were recorded on a chart recorder (model RS3200 or TA240, Gould) and a tape recorder (model DR-890, Neurorecorder) for later analysis.

Perivagal capsaicin treatment. The technique of perivagal capsaicin treatment was modified from that employed by previous investigators (15, 16, 18). In brief, a segment (~4 mm) of each cervical vagus nerve was wrapped in a cotton strip that was presoaked in capsaicin solution (6 mg/ml). After 20 min, when the reflex apneic response to the right atrial injection of capsaicin (5 µg/kg) had been abolished, the cotton strips were removed; this dose of capsaicin injection is known to stimulate pulmonary vagal C-fiber nerve endings and to evoke resultant pulmonary reflexes in dogs (9–11). Solutions of capsaicin were made daily from a refrigerated stock solution (6 mg/ml; Sigma Chemical) that was prepared by dissolving capsaicin into a solvent containing 10% ethanol, 10% Tween 80, and 80% saline. To determine whether this treatment affected conduction in myelinated fibers, the reflex apneic response induced by inflating the lungs to a value of $P_t$ (20 cmH$_2$O) was also compared before and 20 min after perivagal capsaicin treatment.

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, Nan J ou) for a 10-min period. The infusion rate thus ranged from 1.8 to 3.1 ml/min, depending on the body weight of individual animal. Each study of PAE challenge consisted of a 5-min baseline period, a 10-min period during PAE induction, and then a 15-min recovery period after the end of air infusion.

Experimental procedures. Thirty dogs were first studied for their control responses to PAE. Subsequently, these animals were randomly and evenly divided into five groups, and the challenge of PAE was repeated after the following experimental interventions: group 1, pretreatment with saline vehicle; group 2, perivagal capsaicin treatment; group 3, pretreatment with ibuprofen (20 mg/kg; Sigma Chemical); group 4, pretreatment with dimethylthiourea (50 mg/kg; Sigma Chemical); group 5, bilateral cervical vagotomy. Ibuprofen and dimethylthiourea, dissolved in isotonic saline, were slowly injected into the right atrium over a 2-min period. Perivagal capsaicin treatment, vagotomy, and pretreatment with a saline vehicle or drugs were made 30 min before the onset of the second PAE induction. In groups 1–4, the reflex apneic responses induced by right atrial injection of capsaicin (5 µg/kg) and by hyperinflation the lungs ($P_t$ = 20 cmH$_2$O) were studied 10 min before the first PAE induction and 15 min after the end of the second PAE induction. Six minutes before each PAE challenge, the animal’s lungs were hyperinflated (4 × $V_t$) to establish a constant volume history. Because air emboli last for ~5 min (26), 35 min were allowed to elapse between two challenges of PAE.

Data analysis and statistics. Inspiratory duration, expiratory duration, respiratory frequency ($f$), $V_t$, and $V_t$ were all analyzed on a breath-by-breath basis and were measured in 30-s intervals. Mean arterial blood pressure was measured in 1-s intervals. Baseline data of these physiological parameters were calculated as the average values over 10 consecutive 30-s periods immediately preceding the PAE induction. Peak responses in $f$ or $V_t$ were measured as the maximal or minimal values averaged over six consecutive 30-s periods after the PAE induction and expressed as percentage of baseline values. 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, BioCybernetics). 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. $P < 0.05$ was considered significant. All data are presented as means ± SE.

RESULTS

During control, induction of PAE caused a tachypneic response (Fig. 1, A and B), which started within 2.1 ± 0.1 min ($n = 30$) after the onset of air infusion. On average ($n = 30$), the $f$ progressively and significantly increased from a baseline of 21.4 ± 0.8 breaths/min to a peak of 52.8 ± 2.9 breaths/min during the period from 1 min before to 3 min after the termination of PAE induction (Figs. 1D, 2, and 3). The $f$ then gradually declined to its baseline value within 8–14 min after the termination of PAE induction (Fig. 1C). In a similar time course, the $V_t$ significantly decreased from a baseline of 140.8 ± 5.4 ml to a maximal reduction of 117.7 ± 7.0 ml before it gradually returned to its baseline value (Figs. 1E, 2, and 3).

Twenty-five minutes after perivagal capsaicin treatment or intravenous injection of saline vehicle, ibuprofen, or dimethylthiourea, the baseline $f$ and $V_t$ did not significantly ($P > 0.05$) change in these groups of animals (Figs. 1, 2, and 3A). However, 25 min after bilateral cervical vagotomy, animals displayed a slow and deep respiration so that the baseline $f$ decreased and the baseline $V_t$ increased (Fig. 3B). In animals pretreated with saline vehicle, a repeated challenge of PAE induced ventilatory responses of a very similar magnitude and time course compared with their control responses (Fig. 1, D and E). In contrast, the ventilatory responses to the second PAE induction were markedly suppressed in animals pretreated with perivagal capsaicin (Fig. 2A), ibuprofen (Fig. 2B), or dimethylthiourea (Fig. 3A). Additionally, the tachypneic response to the second PAE induction was prevented in vagotomized animals (Fig. 3B). Instead, these animals responded to the PAE induction with an increase in $V_t$ (Fig. 3B).

Because the time at which peak responses occurred varied among the animals, the peak increase or de-
Fig. 1. Ventilatory responses to pulmonary air embolism (PAE). A: baseline recorded 1 min before PAE induction. B: responses recorded 10 min after onset of PAE induction. C: recovery of responses recorded 15 min after termination of PAE induction. PAE was induced by infusion of air (0.2 ml·kg\(^{-1}\)·min\(^{-1}\) for 10 min) into right atrium. \(V_\text{\的速度}\), respiratory flow; \(V_T\), tidal volume; ABP, arterial blood pressure. D and E are mean ventilatory responses to 2 consecutive inductions of PAE separated by 35 min in 1 group of animals pretreated with saline vehicle before second PAE induction. Period of PAE induction is indicated between 2 dashed lines. Values are means ± SE of 6 dogs.

Fig. 2. Mean ventilatory responses to 2 consecutive inductions of PAE separated by 35 min in 2 groups of dogs. Animals received perivagal capsaicin treatment (A) or pretreatment with ibuprofen (B) before second PAE induction. Period of PAE induction is indicated between 2 dashed lines. Values in each group are means ± SE of 6 dogs.
crease in f or Vt was measured in each animal, and the average data are shown in Fig. 4. Statistical analysis revealed that the peak increase in f produced by PAE was not significantly affected by pretreatment with saline vehicle, but was significantly attenuated by pretreatment with perivagal capsaicin, ibuprofen, or dimethylthiourea, and was nearly abolished by vagotomy (Fig. 4). Furthermore, the maximal reduction in

![Graph showing ventilatory responses to PAE inductions](image)

**Fig. 3.** Mean ventilatory responses to 2 consecutive inductions of PAE separated by 35 min in 2 groups of dogs. Animals received pretreatment with dimethylthiourea (A) or bilateral cervical vagotomy (B) before second PAE induction. Period of PAE induction is indicated between 2 dashed lines. Values in each group are means ± SE of 6 dogs.

![Graph showing averaged peak increase in respiratory frequency and maximal decrease in tidal volume](image)

**Fig. 4.** Averaged peak increase in respiratory frequency and maximal decrease in tidal volume produced by 2 consecutive inductions of PAE in 5 groups of dogs: A: saline-treated group. B: perivagal capsaicin-treated group. C: ibuprofen-treated group. D: dimethylthiourea-treated group. E: vagotomy group. Values in each group are means ± SE of 6 dogs. ∆, change. Dashed lines were added to indicate the 100% level. *Significantly different from responses produced by first PAE induction, P < 0.05.
In this study, the PAE-induced tachypnea was prevented by bilateral cervical vagotomy (Fig. 2A), confirming that this response was mediated through lung vagal afferents as suggested by other investigators (20). Additionally, we showed that perivagal capsaicin treatment largely reduced the tachypneic response to PAE (Fig. 2B). It has been shown that this capsaicin treatment blocks the conduction of unmyelinated C fibers but does not affect the conduction of myelinated fibers (5, 25). Indeed, the capsaicin treatment selectively abolished the reflex apnea resulting from stimulation of lung vagal C-fiber afferents by capsaicin injection but did not affect the reflex apnea originating from activation of lung vagal myelinated afferents (pulmonary stretch receptors) by hyperinflation (Table 1). These results suggest that lung vagal C-fiber afferents play a primary role in eliciting the PAE-induced tachypnea. This notion is supported by our recent observation (7) that these lung unmyelinated sensory nerve endings are activated by a same method of PAE induction. Our results are relevant to the findings reported by Hartridge et al. (15), who demonstrated that, in unanesthetized spontaneously breathing decerebrate cats, perivagal capsaicin treatment eliminated the tachypnea caused by an increase in pulmonary vascular pressure subsequent to pulmonary vascular congestion, a situation that may also be produced by pulmonary embolism (22). Two previous studies investigated the C-fiber mechanism in the tachypnea induced by other emboli. Whitteridge (29) reported that the tachypneic response to starch emboli persisted when vagal myelinated fibers were differentially blocked by low temperature, suggesting the involvement of vagal C-fiber afferents. Guz and Trenchard (14) showed that the tachypneic response to microsphere emboli in rabbits was unaffected by anodal polarization block of vagal myelinated fibers, indicating the exclusive role of vagal C-fiber afferents. Whether the difference in the C-fiber contribution between our study and that of Guz and Trenchard was due to the dissimilarity in the embolic or animal model is not known. However, it is clear that lung vagal C-fiber afferents are important in eliciting tachypnea during various types of pulmonary microembolism. On the other hand, the small and residual PAE-induced tachypneic response after perivagal capsaicin treatment (Fig. 2A) presumably originated from the participation of lung vagal myelinated afferents. In fact, PAE has been shown to stimulate pulmonary rapidly adapting receptors (8) and inhibit pulmonary stretch receptors (19), both of which have been postulated to contribute to the elicitation of tachypnea during various types of pulmonary microembolism (2, 19, 23).

We further demonstrated that pretreatment with either ibuprofen or dimethylthiourea markedly suppressed the tachypneic response to PAE (Fig. 3), suggesting that both cyclooxygenase products and hydroxyl radical may play important roles in eliciting this reflex response. The exact sources of these two chemical mediators are not well understood. However, it is known that the lungs are a rich source of arachidonate products and the enzymes necessary for their metabo-

Table 1. Average apneic responses to right atrial injection of capsaicin and to lung hyperinflation before and after inductions of pulmonary air embolism in animals receiving various experimental interventions

<table>
<thead>
<tr>
<th>Group</th>
<th>Capsaicin Injection (Apneic Duration/Baseline TE)</th>
<th>Lung Hyperinflation (Apneic Duration/Baseline TE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before first PAE After second PAE</td>
<td>Before first PAE After second PAE</td>
</tr>
<tr>
<td>Saline treated</td>
<td>6.7 ± 1.1  7.6 ± 1.9</td>
<td>74.3 ± 16.5  82.4 ± 9.9</td>
</tr>
<tr>
<td>Capsaicin treated</td>
<td>10.3 ± 3.0  1.2 ± 0.1*</td>
<td>86.5 ± 12.2  72.5 ± 7.5</td>
</tr>
<tr>
<td>Ibuprofen treated</td>
<td>7.7 ± 1.9  8.2 ± 1.9</td>
<td>92.3 ± 18.2  84.0 ± 10.5</td>
</tr>
<tr>
<td>Dimethylthiourea treated</td>
<td>6.7 ± 2.1  4.3 ± 1.3</td>
<td>79.6 ± 11.2  70.2 ± 8.5</td>
</tr>
</tbody>
</table>

Values are means ± SE for six animals. Dose of capsaicin injected was 5 µg/kg. Lung hyperinflation was performed to increase tracheal pressure to 20 cmH₂O. Responses were measured 10 min before the first pulmonary air embolism (PAE) induction and 15 min after the end of the second PAE induction. *Significantly different from corresponding responses before first PAE induction, P < 0.05.

DISCUSSION

Results of this study demonstrated that PAE caused an increase in f accompanied by a decrease in Vt in anesthetized dogs. These results are in general agreement with those reported by previous investigators using air emboli (20) or other emboli such as starch particles, plastic spheres, or glass beads (1, 3, 14, 22, 29). In addition, we demonstrated that when two challenges of PAE separated by 35 min were induced, ventilatory responses similar in both magnitude and time course were produced in the same animals (Fig. 1, D and E). The reproducibility of the ventilatory responses to PAE, therefore, allowed us to investigate the effects of vagotomy, perivagal capsaicin treatment, ibuprofen, or dimethylthiourea on the PAE-induced tachypnea.

Vt produced by PAE was not significantly affected by pretreatment with saline vehicle, perivagal capsaicin, or dimethylthiourea, but was prevented by pretreatment with ibuprofen, and was converted into a significant increase by vagotomy (Fig. 4).

In the perivagal capsaicin-treated group, the reflex apneic response induced by right atrial injection of capsaicin, which had been totally abolished 20 min after the treatment, remained absent at the end of the observation period for the second PAE challenge (Table 1). Conversely, this reflex apneic response was not significantly affected by pretreatment with saline vehicle, ibuprofen, or dimethylthiourea (Table 1). Furthermore, the reflex apneic response induced by hyperinflation of the lungs was not significantly changed by any of these four experimental interventions (Table 1).

Induction of PAE did not significantly alter the mean arterial blood pressure (before vs. 10 min after the onset of PAE induction, 110.9 ± 2.6 vs. 114.5 ± 2.7 mmHg; n = 30; P > 0.05).
sitive to serotonin in dogs (11). However, these findings indicating the PAE-induced tachypnea, are relatively insensitive to serotonin because lung vagal C-fiber afferents, the major type of pulmonary receptors mediating the reflex tachypnea in their embolic model. It is conceivable that cyclooxygenase inhibitors would be of clinical importance if similar mechanisms also operate in patients displaying PAE-induced tachypnea. When their tachypneic response was prevented, vagotomized animals conversely responded to PAE with a large increase in VT (Fig. 3B), a result similar to the finding reported by Armstrong and Kay (1), who used glass beads as the emboli. The mechanism responsible for this nonvagal response is not known at present. Armstrong and Kay postulated that arterial chemoreceptors might be a possible origin. It is well known that hypoxia is a major consequence of PAE and that it may contribute to excitatory effects on breathing, as manifested by the excitatory response after bilateral vagotomy. It is interesting to note that this large increase in VT was not seen in intact animals in this study, despite the fact that most of them had a suppressed tachypneic response to PAE after experimental interventions. It appears that there are different controls for VT during PAE and lung vagal afferents might provide a dominant and inhibitory influence on VT during PAE in intact animals. Thus this nonvagal response of VT to PAE might be revealed after removing the inhibitory influence by vagotomy. If this were the case, it is unlikely that the lung vagal C-fiber afferents would be the candidate to exert the inhibitory influence because perivagal capsaicin-treated animals did not display such an increase in VT after PAE (Fig. 2A).

In summary, lung vagal unmyelinated C-fiber afferents play a predominant role in evoking the reflex tachypneic response to PAE. Additionally, both cyclooxygenase products and hydroxyl radical are important in eliciting the reflex tachypneic response to PAE. It is speculated that lung myelinated afferents may also be involved because perivagal capsaicin treatment did not totally prevent this vagally mediated response.

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