Different Roles of Two Subgroups of Lung Vagal C-Fiber Afferents in the Tachypneic Response to Pulmonary Air Embolism in Dogs

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Abstract

It is known that lung vagal C-fiber afferents play an important role in eliciting the tachypneic response to pulmonary air embolism (PAE), and can be subgrouped as those with low resistance (LRC) and those with high resistance (HRC) to perivagal capsaicin. In this study, we investigated the relative contributions of vagal LRC and HRC C-fiber afferents to the PAE-induced tachypneic response. Phrenic activity was recorded from 10 anesthetized, paralyzed, and artificially ventilated dogs. PAE was induced by infusion of air into the vein (2 ml/min, 1 ml/kg). During control conditions, induction of PAE produced a shortening in expiratory duration with no significant change in inspiratory duration, resulting in tachypnea. The PAE-induced tachypneic response was totally abolished by perivagal capsaicin treatment with a method (capsaicin concentration, 6 mg/ml; treatment duration, 25-30 min) that blocks the conduction of LRC C-fiber afferents, but not that of HRC C-fiber afferents. This tachypneic response was not affected by cooling of both vagi to a temperature (4.5 °C) that blocks the conduction of HRC C-fiber afferents, but not that of LRC C-fiber afferents. A bilateral cervical vagotomy virtually eliminated this tachypneic response. These results suggest that LRC C-fiber afferents are responsible for eliciting the reflex tachypneic response to PAE, whereas HRC C-fiber afferents play no vital role.

Key Words: tachypnea, pulmonary microembolism, perivagal capsaicin, vagal cooling, dogs

Introduction

Pulmonary air embolism (PAE) occurs in a number of clinical situations (13, 21) and is known to cause tachypnea (6,15,16). The physiological mechanisms underlying PAE-induced tachypnea are not fully understood. Previous investigations in dogs demonstrated that PAE-induced tachypnea is totally abolished by a bilateral cervical vagotomy (6, 15, 16) or is largely reduced by perivagal capsaicin treatment (6). The latter technique is known to preferentially block impulse propagation in unmyelinated C fibers, but does not interfere with neural conduction of myelinated fibers (3, 22, 24). These observations suggest the important role of lung vagal C-fiber afferents in eliciting reflex tachypnea following PAE. Indeed, am electrophysiological study in dogs revealed that lung vagal C-fiber sensory nerve endings are stimulated by PAE (4).

Lung vagal C-fiber afferents play an important role in eliciting respiratory reflexes under various pulmonary pathophysiological conditions (8). We recently reported (5) that lung vagal C-fibers afferents can be further categorized as those with low resistance (LRC) and those with high resistance (HRC) to perivagal capsaicin. The former group can be blocked

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by perivagal capsaicin treatment with a concentration of less than 6 mg/ml, whereas the latter group can only be blocked by capsaicin at a concentration > 10 mg/ml (5). Coincidentally, the former group can be blocked by cooling of both vagi to a temperature range of -2 to 2 °C, whereas the latter group can be blocked by a temperature range of 2 to 6 °C (5). These two ranges of temperature can also block the conduction of myelinated fibers because the temperature requires to completely block the conduction of myelinated fibers is even higher (7-10 °C) (9,12). Thus, LRC C-fiber afferents differ from HRC C-fiber afferents due to their afferent activity being blocked at relatively low concentrations of perivagal capsaicin and at relatively low temperatures of vagal cooling. Although the physiological properties of these two subgroups of lung vagal C-fiber afferents are clearly distinct, whether they have different roles in eliciting the PAE-induced reflex tachypnea is not known.

The present study was undertaken to investigate the relative contributions of LRC and HRC lung vagal C-fibers afferents to PAE-induced tachypneic response in anesthetized dogs.

Materials and Methods

Animal Preparation

Adult dogs (10-16 kg) were anesthetized with an intravenous (i.v.) injection of thiopental sodium (20 mg/kg; Abbott), followed by a combination of chloralose (50 mg/kg, i.v.; Sigma) and urethane (500 mg/kg, i.v.; Sigma). Supplemental doses of chloralose (15 mg/kg per hr) and urethane (150 mg/kg per hr) were administered to maintain abolition of the corneal and withdrawal reflexes during the course of the experiments. The femoral artery was calculated to measure arterial blood pressure. A catheter (PE-240) was inserted into the right jugular vein with its tip close to the right atrium for intravenous injection of pharmacological agents. During the experiment, the dogs were paralyzed with pancuronium bromide (0.05 mg/kg, i.v.; Organon Teknica). Periodically, the effect of pancuronium was allowed to wear off so that the depth of anesthesia could be checked. Throughout the experiment, body temperature was maintained at ~ 36 °C by means of a servo heating blanket.

After a midline incision was made in the neck, a short tracheal cannula was inserted just below the larynx. The lungs were ventilated (Harvard 607) with 65% O_2 at a frequency of 16-20 cycles/min and a tidal volume (V_T) of 12-15 ml/kg; both were kept constant during each experiment. CO_2 was mixed with the inspired gas when it was necessary to maintain an end-tidal CO_2 concentration at about 5%. Tracheal pressure (P_{tr}) and CO_2 concentration were measured via side taps of the tracheal cannula by a pressure transducer (Validyne MP45-28) and a capnograph (Biochem 9000), respectively. All physiological signals were recorded by a thermal array recorder (Gould TA11) and also recorded on tape (Neurocorder DR-890) for later analysis.

Recording of Phrenic Nerve Activity

The method for recording phrenic nerve activity has been described in detail previously (16). In brief, a rootlet of the phrenic nerve (C5) was exposed in the neck by a ventral approach. The nerve was cut, and the sheath of its proximal end was retracted. It was immersed in a pool of warm mineral oil, then placed on a pair of silver electrodes. The nerve signals were amplified (Grass P511K), monitored by an audio amplifier (GMSS AM8), displayed on an oscilloscope (Gould 1425), and integrated.

Perivagal Capsaicin Treatment and Vagal Cooling

The procedures for perivagal capsaicin treatment and for vagal cooling have been described in detail elsewhere (6, 11,14,17-19). In brief, with the aid of a microscope, both cervical vagus nerves were desheathed over a 5-7 cm long segment. Strips of cotton soaked in capsaicin solution (6 mg/ml) were wrapped around the desheathed portion of the nerve for 25-30 min and then removed. Capsaicin (Sigma) was dissolved in 2.5% ethanol, 10% Tween 80, and 87.5% isotonic saline, as described previously (7). This dose of capsaicin and this treatment time are known to block the conduction of LRC, but not HRC, C-fiber afferents (5). For vagal cooling, both vagus nerves were placed in a groove (1 cm long) of a copper tube and covered with agar (4% in saline). Care was taken to avoid producing any tension in the nerve. A thermocouple was glued to the copper tube to measure the temperature. The coolant (ethylene glycol) was pumped through a copper tube, kept at a constant preset temperature. The temperature was progressively lowered to 4.5 °C and was continuously recorded. At this cooling temperature, the conduction of LRC C-fiber afferents is known to be preserved, whereas the conduction of HRC C-fiber afferents is preferentially blocked (5). To ensure the effectiveness of these two interventions, the reflex apneic responses induced by intravenous injection of a small dose of capsaicin (5 μg/kg) and by hyperinflation of the lungs (3 x tidal volume) were also studied before and after perivagal capsaicin treatment, as well as before and during vagal cooling. Capsaicin- and hyperinflation-induced apneic responses are thought to originate

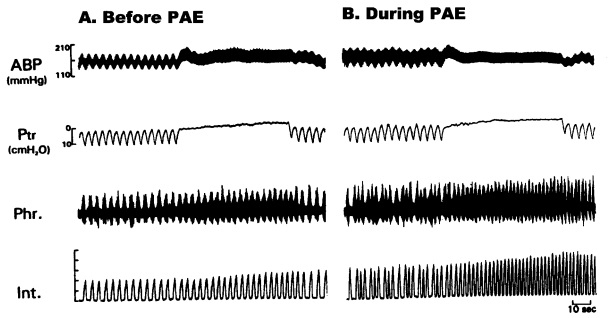

Fig. 1. Response of phrenic nerve to pulmonary air embolism (PAE) in an anesthetized dog. A: baseline recorded 3 min before PAE induction; B: responses recorded 5 min after onset of PAE induction. PAE was induced by infusion of air (2 ml/min for 7 min) into vein. ABP, arterial blood pressure; P_{tr}, tracheal pressure; Phr., raw phernic activity; Int., integrated phrenic activity. Note that the tachypneic response to PAE was obvious, with or without mechanical ventilation.

from stimulation of lung vagal unmyelinated and myelinated afferents, respectively (8).

Induction of PAE

PAE was induced by infusion of air through the catheter in the right jugular vein at a rate of 2 ml/min with a Harvard infusion pump (model 901). The infusion was stopped when the cumulative infusion volume reached 1 ml/kg.

Experimental Protocol

A total of 10 dogs were used in this study. Phrenic responses to PAE challenges were studied during control conditions, after perivagal capsaicin treatment, during vagal cooling, and after bilateral vagotomy. Each study of PAE challenge consisted of a 5-min baseline period followed by a PAE induction period of 5-8 min, depending on the body weight of the animals. Since air emboli last for < 5 min (23), at least 40 min were allowed to elapse between two challenges of PAE. The sequences of the studies of perivagal capsaicin treatment and vagal cooling were alternated among the animals in order to balance the design. After the end of the observation period, both vagi were washed several times with warm saline (~36 °C) to reverse the blocking effects of perivagal capsaicin treatment or vagal cooling. The reversibility was confirmed by the recovery of the reflex apneic responses induced by intravenous capsaicin injection (5 μg/kg) and by lung inflation (3 x tidal volume).

Data Analysis and Statistics

The duration of the phrenic burst (T_I), the period between bursts (T_E), and the respiratory frequency were measured on a breath-by-breath basis. These physiological parameters were analyzed using a computer equipped with an analog/digital convertor (Gould DASA 4600) and software (BioCybernatics, 1.0). Results obtained from the computer analysis were routinely checked with those obtained by manual calculation for accuracy. Results were analyzed by a repeated measures two-factor analysis of variance (ANOVA) followed by Fisher's least significant difference procedure when appropriate. One factor was the effects of PAE, while the other factor was the effects of various vagal interventions. $P < 0.05$ was considered significant. All data are presented as mean ± SE.

Results

During control conditions, induction of PAE produced an increase in respiratory frequency in all animals studied. When responding, the phrenic nerve activity began to increase within 1-3 min, reached its peak within 4-7 min, and returned to the baseline within 20 min after the air infusion. Typical recordings of the tachypneic response to PAE are shown in Fig. 1. As shown, by using cessation of the mechanical ventilator to remove the involvement of myelinated afferents, it was demonstrated that PAE-induced tachypnea may be mediated by vagal nonmyelinated C-fiber afferents. Further analysis revealed that the PAE-induced increase in respiratory frequency was mainly due to a shortening in T_E with no significant change in T_I (Fig. 2). Overall, the characteristics of the tachypneic response to PAE were similar to those described previously (6, 15, 16).

Before perivagal capsaicin treatment, intravenous injection of a small dose of capsaicin elicited an apneic response (10.7 ± 0.8 s; n = 10) (Fig. 3A). After the neural conduction of the vagal LRC C-fiber afferents was selectively blocked by perivagal capsaicin treatment, either the apneic response to intravenous capsaicin or the tachypneic response to PAE was totally abolished (Fig. 3). On the other hand, before vagal cooling, lung hyperinflation elicited an apneic response (19.7 ± 1.2 s; n = 10) (Fig. 4A). When the temperature of the nerves was lowered to 7 °C to differentially block the neural conduction of the vagal myelinated afferents, the apneic response to lung hyperinflation was completely prevented (Fig. 4B). When the temperature of the nerves was further lowered to 4.5 °C to produce an additional blockade of the neural conduction of the HRC C-fiber afferents, the tachypneic response to PAE still persisted (Fig. 4D). Statistical analysis revealed that induction of PAE caused no significant change in T_I, T_E, and respiratory frequency after perivagal capsaicin

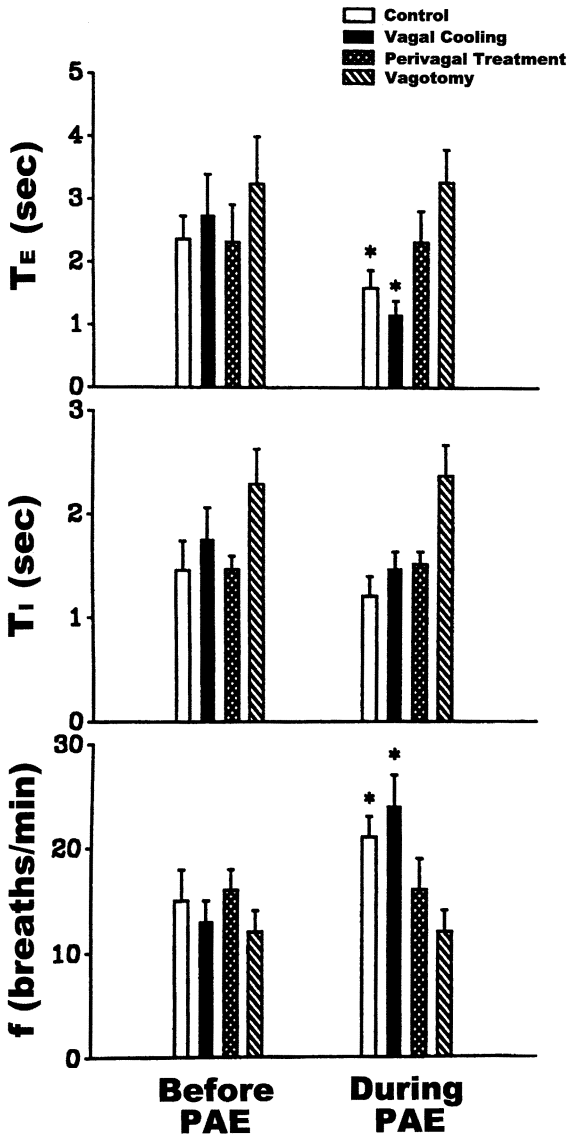

Fig. 2. Comparisons of the peak phrenic nerve activity in response to pulmonary air embolism (PAE) under various experimental conditions. Open columns: control, n = 10; solid columns: during vagal cooling to 4.5 °C, n = 10; crosshatched columns: after perivagal capsaicin treatment, n = 10; hatched columns: after vagotomy, n = 7. T_E, period between bursts; T_I, duration of phrenuc burst; f, respiratory frequency. *, $p < 0.05$ compared with values measured before PAE.

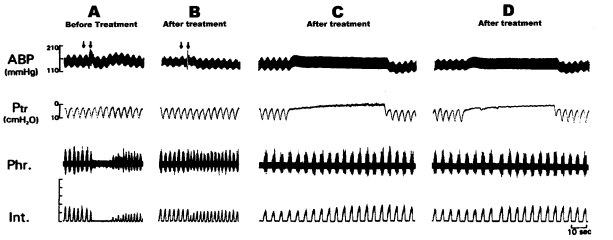

Fig. 3. Effects of perivagal capsaicin treatment on apneic response to intravenous capsaicin injection (A and B) and on tachypneic response to pulmonary air embolism (PAE) (C and D) in an anesthetized dog. Perivagal capsaicin treatment consisted of a capsaicin concentration of 6 mg/ml and a treatment duration of 30 min. In panels A (before treatment) and B (after treatment), capsaicin was injected into catheter at first arrow and flushed at second. Panels C and D were recorded after perivagal treatment, and were recorded 3 min before and 5 min after PAE induction, respectively. Note that both apneic response to intravenous capsaicin injection and tachypneic response to PAE were abolished by perivagal treatment. See legend of Fig. 1 for further explanations.

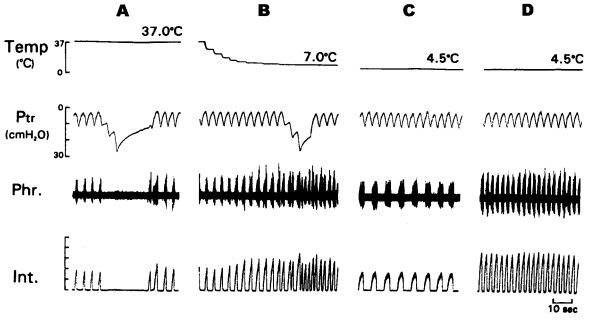

Fig. 4. Effects of vagal cooling on apneic response to lung hyperinflation (A and B) and on tachypneic response to pulmonary air embolism (PAE) (C and D) in an anesthetized dog. Coolant temperature was lowered from 37 to 7 °C and to 4.5 °C. In panels A (before cooling) and B (during cooling to 7 °C), lungs were inflated to 3 x tidal volume. Panels C and D were recorded during cooling (4.5 °C) and recorded 3 min before and 5 min after PAE induction, respectively. Note that, during vagal cooling, apneic response to lung hyperinflation was abolished, yet tachypneic response to PAE persisted. Temp, cooling temperature. See legend of Fig. 1 for further explanations.

treatment, whereas it still significantly decreased T_E and increased respiratory frequency during cooling of both vagi to 4.5 °C (Fig. 2). Finally, in vagotomized animals, induction of PAE virtually had no effect on T_I, T_E, and respiratory frequency (Fig. 2), and the PAE-induced tachypneic response was totally eliminated.

Discussion

Results of this study demonstrate that PAE causes a shortening of T_E with no significant change in T_I, resulting in a tachypneic response. These results are in general agreement with those reported by previous investigators using air emboli (6, 15, 16) or other emboli such as starch particles, plastic spheres, or glass beads (1, 2, 10, 20, 25). In addition, we demonstrated that this tachypneic response can be prevented by a perivagal capsaicin treatment that is sufficient to block the conduction of LRC, but not HRC, C-fiber afferents. Conversely, this tachypneic response is preserved by cooling of both vagi to a temperature that is sufficient to block the conduction of HRC, but not LRC, C-fiber afferents. These results

suggest that LRC C-fiber afferents are responsible for eliciting the tachypneic response to PAE, whereas HRC C-fiber afferents play no vital role.

Two previous studies investigated the C-fiber mechanism in tachypnea induced by other emboli. Whitteridge (25) 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 (10) showed that the tachypneic response to microsphere emboli in rabbits was unaffected by anodal polarization blockage of vagal myelinated fibers, indicating the exclusive role of vagal C-fiber afferents. It appears that this type of pulmonary receptors is important in eliciting a tachypneic response to various forms of pulmonary embolism. We previously reported (5) that lung vagal C-fiber afferents can be categorized into LRC and HRC C-fiber afferents, both of which are stimulated by intravenous capsaicin injection and have conduction velocities within the range of unmyelinated fibers. These two criteria are regarded as a conventional method for the identification of lung vagal C-fiber afferents (8). Therefore, whether one or both subgroups of C-fiber afferents are involved in eliciting the C fiber-mediated respiratory reflexes would then become a subject of interest. Additionally, in anesthetized and spontaneously breathing dogs, we recently (6) showed that the PAE-induced tachypneic response is largely reduced by perivagal capsaicin treatment at a capsaicin dose (6 mg/ml) that can block the conduction of LRC, but not HRC, C-fiber afferents. It is thus very plausible that the small residual response evoked by PAE after perivagal capsaicin treatment in that study (6) was due to the contribution of HRC C-fiber afferents. However, this possibility can be ruled out by the findings of the present study in which the cooling of both vagi to 4.5 °C did not affect the PAE-induced tachypneic response. Whether the difference in the C-fiber contribution between this and our previous study (6) is due to the difference in the animal model is not known. However, it is clear that LRC C-fiber afferents are important in eliciting tachypnea during PAE in both animal models.

The lack of functional significance of HRC C-fiber afferents is not unique to the tachypneic response to PAE. In this study, the apneic response to intravenous injection of capsaicin was completely abolished by perivagal capsaicin treatment at a capsaicin dose of 6 mg/ml, suggesting that HRC C-fiber afferents also play no role in eliciting this response. In our previous study, we found that 82% of the lung vagal C-fiber afferents studied are LRC, whereas the remaining are HRC (5). Therefore, the lack of functional significance of HRC C-fiber afferents could be due to the fact that the total number of these afferents is relatively small as compared to the total number of LRC C-fiber afferents. Alternatively, HRC C-fiber afferents may have physiological functions that are distinct from those of LRC C-fiber afferents. Further investigations will be required to explore the latter possibility.

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