Stimulation of breathing by activation of pulmonary peripheral afferents in rabbits

J. YU, J. F. ZHANG, AND E. C. FLETCHER
Pulmonary Division, Department of Medicine, University of Louisville, Louisville, Kentucky 40292

Yu, J., J. F. Zhang, and E. C. Fletcher. Stimulation of breathing by activation of pulmonary peripheral afferents in rabbits. J. Appl. Physiol. 85(4): 1485–1492, 1998.—Respiratory response to selective activation of vagal afferents in the peripheral airways was investigated in anesthetized, open-chest, and artificially ventilated rabbits. Phrenic activity was used as an index of central respiratory drive before and after injection of hypertonic saline (8.1%, 0.1 ml) into the periphery of the lung to stimulate the afferents. The amplitude of “integrated” phrenic activity and phrenic burst rate increased by 19 ± 3.4 and 53.7 ± 12.7% (n = 23; P < 0.001), respectively. The response peaked at 5.5 ± 1.6 s and returned to the baseline at 7 min (median) after the injection. The magnitude of the response was positively related to the concentration of injected NaCl. The response could not be elicited by injection of normal saline and was abolished by vagotomy. Because artificial ventilation caused phrenic activity to be entrained with the ventilator, respiratory drive was further assessed after the ventilator was stopped. Again, neural hyperpnea and tachypnea were observed. Because activation of a small fraction of the pulmonary peripheral afferents resulted in vigorous stimulation of respiratory drive, we speculate that initiation of this reflex may contribute to hyperpnea and tachypnea under both physiological and pathophysiological conditions. Vagal reflex; peripheral lung receptors; C fibers; rapidly adapting receptors

HYPERPNEA AND TACHYPNEA are a common finding in patients with cardiopulmonary diseases involving the lung parenchyma. There have been many attempts to identify the responsible mechanism. Hypoxia, hypercapnia, and activation of pulmonary C fibers (J receptors) are suggested as contributing factors. However, hyperpnea and tachypnea still exist after arterial Po2 is corrected. Hyperpnea is often accompanied by hypocapnia instead of hypercapnia. Activation of C fibers produces rapid shallow breathing (4), which cannot explain hyperpnea. In an animal model of lung disease (2, 9), vagal afferents are found to mediate an alteration of breathing pattern (also see reviews in Refs. 3 and 18). Despite vigorous efforts, the mechanisms that produce hyperpnea have not been completely identified. We hypothesize that there are vagal afferents in the lung periphery that evoke hyperpnea and tachypnea. In the present study, we measured phrenic nerve discharge as an index of respiratory drive and examined the effects of selectively activating vagal afferents located in peripheral airways. We found that activating the afferents can cause neural hyperpnea and tachypnea.

METHODS

General. Experiments were conducted on male New Zealand White rabbits (body weight, 2.0–2.6 kg). The rabbits were initially anesthetized with ketamine (37.5 mg/kg) and xylazine (5 mg/kg) intramuscularly, and surgical anesthesia was maintained by additional doses (10 mg) of pentobarbital sodium intravenously. After completion of the surgery, anesthesia was maintained with α-chloralose (1%) and urethane (10%) by intravenous infusion at 1.4–2.0 ml/h. Measurements were made at least 90 min after the last dose of pentobarbital was given.

The trachea was cannulated low in the neck, and the lungs were ventilated by a small-animal ventilator (model 683, Harvard) in which the expiratory outlet was connected to 3–4 cmH2O of positive end-expiratory pressure (PEEP). Airway pressure was monitored by a pressure transducer attached to a sidearm of the tracheal tube. Tidal volume was set at 10 ml/kg body wt. Airway gas was monitored periodically by an infrared analyzer (model LB-2, SensorMedics). Ventilatory frequency was adjusted to maintain peak airway Pco2 constant at ~40 Torr; this may have been an underestimation of alveolar Pco2. The chest was opened by a midline incision. The opening was covered with saline-soaked gauze to prevent drying of the tissue. The femoral artery was cannulated for blood pressure monitoring and for arterial blood samples to monitor Pao2, pH, and Pco2. Blood pressure, airway pressure, and phrenic activity and its time-averaged signals were recorded by a thermorecorder (Astro-Med Dash IV).

Stimulation of peripheral pulmonary receptors. To determine whether activation of pulmonary receptors in peripheral airways can reflexly stimulate breathing, we injected hypertonic saline directly into the lung, monitoring phrenic activity as an index of respiratory drive. We injected 0.1 ml (0.2 ml in a few cases) of 8.1% NaCl (osmolality = 2,770 mosM) into the lung parenchyma (5–7 mm under the surface) through a 30-gauge needle.

Phrenic nerve recordings. The phrenic nerve from C8 (right or left) was separated from the surrounding tissue and transected. The nerve sheath was removed, and the central end of the nerve was placed on a bipolar silver electrode. The electrode was connected to a high-impedance probe (model HIP 511) connected to a Grass (model P 511) amplifier. Nerve activity was amplified and displayed on an oscilloscope, as well as monitored by a loudspeaker. The raw nerve signal and its “integrated signal,” i.e., moving time-averaged signal (model 7P3D integrator, Grass; time constant, 0.2 s) were recorded to assess respiratory activity. The amplitude and rate of phrenic burst were examined. The steepest slope of the integrated neurogram was also measured as an index of respiratory drive. The best time constant for time-averaged integration is 0.1 s (7). Shorter or longer time constants would produce too much noise or dampen the signals, respectively. Because the Grass (model 7P3D) integrator does not provide 0.1 s, we chose 0.2 s. Therefore, an underestimation of the values of amplitude and slope could be expected because of the longer time constant to dampen the signals. In addition, the long time constant may have the effect of making amplitude dependent on inspiratory time and expiratory time. However, this should not alter our data substantially. The respiratory response to local injection of hypertonic saline was examined before and after vagotomy. The response to 0.9% NaCl (0.1 ml) was also examined as the vehicle control.

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Data are presented as means ± SE. A Student's paired t-test was used to compare two groups of data from the same animals. The paired Wilcoxon test was used to compare the percent change of a variable from its control value. A P value < 0.05 was considered as statistically significant.

RESULTS

In 17 open-chest and artificially ventilated rabbits, we examined phrenic activity in response to hypertonic saline injection into the lung periphery. We tested both lungs in 6 of the 17 rabbits and, therefore, obtained 23 data points. During control, the phrenic burst rate was 16.3 ± 0.5 bursts/min (n = 23). The phrenic burst was usually linked to the ventilator cycle in a 1:1 ratio, occurring during the deflation phase of the ventilator. After injection, breathing frequency (bursts/min) increased in 10 of the 17 rabbits. On average, it increased

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Fig. 1. Increased phrenic activity by injection of 8.1% NaCl (0.1 ml) into periphery of left lung in an anesthetized, open-chest, and artificially ventilated rabbit. Shown are electroneurogram of left phrenic nerve (ENG), "integrated" (time-averaged) electroneurogram [ENG (integ)], airway pressure (Paw), and blood pressure (BP). Two event marks (Mark) at top denote start and end of injection. Note that phrenic activity increased immediately (first breath). Breathing rate was still linked to ventilator cycle. Phrenic nerve gave 2 bursts, although fused, per ventilator cycle in some breaths. Also note that there was no significant change in blood pressure after injection of hypertonic saline.

Fig. 2. Injection of 0.9% NaCl (0.1 ml) into periphery of left lung did not change breathing pattern (A); injection of 8.1% (0.1 ml) NaCl increased phrenic activity (B); and increased activity was blocked after vagotomy (C). Three event marks at top denote insertion of needle into lung, and start and end of injection.
by 53.7 ± 12.7% (n = 23; P < 0.01). In most cases, the phrenic bursts remained in phase with the ventilator cycles. The amplitude of each burst (measured from the time-averaged neurogram) increased by 19 ± 3.4% (P < 0.001). The slope of the phrenic neurogram also increased by 20.8 ± 5.1% (P < 0.05). The excitatory effects often began within the first breath, peaked at 5.5 ± 1.6 s after injection, and returned to the baseline at 7 min (medium). While phrenic activity was stimulated, there was no significant change in blood pressure (Fig. 1), which averaged 69.7 ± 1.4 and 66.9 ± 1.9 mmHg during control and during the peak of the respiratory response, respectively. In 11 rabbits we also examined phrenic activity before and after injecting normal saline. We did not observe any changes in breathing pattern after injection of normal saline. In 12 rabbits (4 of the 12 rabbits had both lungs tested), we examined phrenic responses to hypertonic saline injection before and after cervical vagotomy. The excitatory response to hypertonic injection was abolished by bilateral vagotomy (Figs. 2 and 3).

In a separate group of rabbits, the dose-response relationship between the concentration of NaCl and phrenic activity was examined. The concentrations of NaCl injected were 0.9, 4.05, 8.1, and 16.2%. There was a positive relationship between NaCl concentration and the phrenic burst rate. There was no effect at 0.9%; the rate doubled in two of five rabbits at 4.05 and 8.1%, respectively; and it doubled in four of five rabbits at 16.2%. The amplitude of phrenic activity in response to different concentrations of NaCl is illustrated in Fig. 4.

Because vagotomy altered the breathing pattern, it can be argued that the abolishment of the respiratory response to hypertonic saline injection after vagotomy is due to the alteration of the breathing pattern. To further confirm the role of the vagus nerves in the excitatory lung reflex, we examined the phrenic response to hypertonic saline (8.1%, 0.1 ml) injected into both lungs, one of which was denervated by vagotomy. The response to the injection was compared between the two lungs. We observed the excitatory lung reflex to hypertonic saline injection in the vagus-intact lung but not in the vagotomized lung (Fig. 5). Thus a vagally mediated reflex is firmly established by our results in a unilaterally vagotomized preparation.

Because phrenic activity was entrained with the ventilator during mechanical ventilation, phrenic activity was further assessed in eight rabbits under free-running conditions by stopping the ventilator for ~60 s. The phrenic activity in response to the injection of hypertonic saline when the ventilator was stopped was compared with that without injection under the same conditions. After the ventilator was stopped, phrenic burst occurred spontaneously. During control free-running conditions, phrenic amplitude gradually increased without significant changes in burst rate (Fig. 6A). During the same free-running conditions, injection...
of hypertonic saline increased phrenic burst rate, amplitude, and slope (Fig. 6B). The increased phrenic activity caused by hypertonic saline during free-running conditions was abolished by bilateral vagotomy (Fig. 6D). The increases in phrenic amplitude, slope, and burst rate during the peak response (averaged over 3 breaths indicated by the second group of three arrows in Fig. 6B) from the control (averaged over 3 breaths indicated by first group of 3 arrows in Fig. 6B) were compared with the increases at corresponding time points during control free running conditions (indicated by the 2 groups of 3 arrows in Fig. 6A). The same comparison was made after bilateral vagotomy (Fig. 6, C and D). After vagotomy the corresponding time points were chosen according to the time points when the vagus nerves were intact, i.e., those time points in

Fig. 6B. The group data for the phrenic responses are summarized in Fig. 7.

DISCUSSION

Excitatory effects on breathing by activation of lung receptors have been observed during injection of stimulants into the circulation (intravenous or right atrial) or by direct airway application (aerosol inhalation or instillation) (8, 12, 14, 16, 17, 21). Intravenous injection of stimulants will activate all the receptors (including those in large airways) accessible to the circulation (17), although it preferentially stimulates receptors located in the periphery of the lung. On the other hand, aerosol inhalation will preferentially stimulate receptors in the central airways. In the present study we
used a new method to selectively stimulate pulmonary receptors in the lung periphery by direct injection of hypertonic saline into the lung of the rabbit. We found that activating receptors located in the peripheral airways initiated a reflex that induced neural hyperpnea and tachypnea. These excitatory effects are dose related; that is, the higher the injected concentration of NaCl, the greater the effect. The afferent arm of the reflex appears to be in the vagus nerves. This "excitatory lung reflex" is different from other reflexes described in the literature.

Activation of receptors located in the peripheral airway induced neural hyperpnea and tachypnea, exhibited by increases in the amplitude and slope of the phrenic neurogram and in the phrenic burst rate in artificially ventilated rabbits. Because, under artificial ventilation, phrenic activity was entrained with the ventilator, it could be argued that the neural hyperpnea (increases in phrenic amplitude and slope) is due to an artifact. That is, when the linkage between phrenic bursts and ventilator cycles is present, an increase in the frequency of phrenic bursts could be forced to manifest itself as an increase in amplitude. However, the amplitude and slope still increased after local injection of hypertonic saline while respiratory rhythm was free running. This response firmly establishes that neural hyperpnea is a feature of activation of pulmonary afferents.

The excitatory lung reflex can be evoked by stimulation of a small fraction of the pulmonary afferents. As a rough estimate, the volume of the rabbit lungs is ~40 ml after the lungs have been excised and passively deflated. The amount of hypertonic saline injected was 0.1 ml, which is only 0.25% of the total lung volume. However, the area of the lung affected by the injection may have been substantially larger. The volume of fluid is far larger than an acinus; thus liquid is likely to be forced up the airway. On the basis of our data, it must be assumed that the stimulation may have involved several airway generations. At this point, we do not know the extent that hypertonic saline was distributed. At any rate, only a small fraction of the receptors should have been activated in our study. Full activation of all the receptors would likely exert a much more intense effect on breathing. Thus understanding this reflex may elucidate an important mechanism for many physiological and pathological processes. The following is our analysis in identifying the responsible afferents.

Sympathetic afferents supply the lungs (13) and have limited influence on breathing (3, 19). Stimulation of these afferents does not affect diaphragm activity (5) or even may inhibit it (13). Furthermore, the excitatory lung reflex was abolished by vagotomy in our study. Therefore, the excitatory lung reflex is not sympathetically mediated but is vagally mediated. This vagally mediated reflex is further confirmed by our results in unilaterally vagotomized preparation.

Airways and lungs are richly innervated by vagal nerves (3, 14, 18, 25). There are at least four different types of vagal receptors in the airways and the lungs, pulmonary slowly adapting stretch receptors (PSRs), rapidly adapting receptors (RARs), bronchial C fibers, and pulmonary C fibers. All of the pulmonary vagal afferents identified so far are believed to modify breathing.

Activation of PSRs inhibits breathing (3, 18), which is opposite of what we observed. However, the stimulation of breathing can be explained by a decrease in PSR activity. It is possible that the injected hypertonic...
Reflexes during sleep and waking may contribute to hyperpnea and tachypnea generated in the lungs. Although we have not identified vagally mediated and different from any known reflex and reflexes initiated by pulmonary afferents. This used to further explore the mechanism for activation of ways. The response was neural hyperpnea and tachy- selectively stimulate afferents in the peripheral air- types of pulmonary afferent are responsible for the reflex. Although there is no direct evidence to prove this, our present data are not inconsistent with this notion. Therefore, the role of RARs in this reflex needs to be further explored.

Stimulation of C fibers can accelerate the breathing rate (4, 10, 20). In addition, pulmonary C fibers are found in the periphery of the lung and are numerous. Thus it is possible that C fibers are responsible for the excitatory lung reflex. However, the reflex responses to instillation of hypertonic saline into the airway (16), intravenous injection of hypertonic saline (15), or activation by injection of extraneous substance (1) include apnea, which usually is followed by rapid shallow breathing in addition to the decreased heart rate and blood pressure. It has been reported that activation of C fibers by right atrial injection of stimulants in rabbits can induce rapid shallow breathing (22). Clearly, the response is different from what we observed. However, the possibility exists that C fibers in the peripheral lung have different effects on breathing from the C fibers located in a central airway.

Another explanation of the observation is that we had activated some undifferentiated vagal afferents in the lungs that do not fall into any category known to the investigators. Indeed, existence of such vagal afferents has been described (3). In addition, we could not exclude the possibility that more than one type of pulmonary afferent is responsible for the reflex.

In short, we used a new method (local injection) to selectively stimulate afferents in the peripheral airways. The response was neural hyperpnea and tachy- pnea, similar to the response in many disease states, such as pulmonary edema. Perhaps, this method can be used to further explore the mechanism for activation of and reflexes initiated by pulmonary afferents. This excitatory lung reflex observed in the present study is vagally mediated and different from any known reflex generated in the lungs. Although we have not identified the responsible afferents, it is possible that initiation of this reflex may contribute to hyperpnea and tachy- pnea both during some physiologic conditions and in pulmo- nary disease.

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