Excitatory lung reflex may stress inspiratory muscle by suppressing expiratory muscle activity

J. Yu, Y. Wang, G. Soukhova, L. C. Collins, and J. C. Falcone

Excitatory lung reflex may stress inspiratory muscle by suppressing expiratory muscle activity. J Appl Physiol 90: 857–864, 2001.—Recently, a vagally mediated excitatory lung reflex (ELR) causing neural hyperpnea and tachypnea was identified. Because ventilation is regulated through both inspiratory and expiratory processes, we investigated the effects of the ELR on these two processes simultaneously. In anesthetized, open-chest, and artificially ventilated rabbits, we recorded phrenic nerve activity and abdominal muscle activity to assess the breathing pattern when the ELR was evoked by directly injecting hypertonic saline (8.1%, 0.1 ml) into lung parenchyma. Activation of the ELR stimulated inspiratory activity, which was exhibited by increasing amplitude, burst rate, and duty cycle of the phrenic activity (by 22 ± 4, 33 ± 9, and 57 ± 11%, respectively; n = 13; P < 0.001), but suppressed expiratory muscle activity. The expiratory muscle became silent in most cases. On average, the amplitude of expiratory muscle activity decreased by 88 ± 6% (median). Injection of H2O2 into the lung parenchyma produced similar responses. By suppressing expiration, the ELR produces a shift in the workload from expiratory muscle to inspiratory muscle. Therefore, we conclude that the ELR may contribute to inspiratory muscle fatigue, not only by directly increasing the inspiratory activity but also by suppressing expiratory activity.

Ventilatory failure; pulmonary reflex; lung receptors; vagal afferents

Respiratory centers effectively control minute ventilation through feedback via input from many sources. Vagal afferents play an important role in this regard (7, 8, 13, 17, 21, 26, 30). Neural control of ventilation is regulated through two closely coordinated processes, inspiration and expiration. A balance between these two processes gives harmony, whereas an imbalance produces discord. During quiet breathing, inspiration is an active process, whereas expiration is passive (6). Expirations become active when respiratory activity increases (6), either during physiological conditions such as exercise (1) or as a consequence of pathological conditions such as chronic obstructive pulmonary disease (COPD) (15). In addition, expiratory (abdominal) muscles participate in defense reflexes, such as coughing and sneezing (5, 14).

Recently, our laboratory identified an excitatory lung reflex (ELR), which is vagally mediated (30). Activation of this reflex causes neural hyperpnea and tachypnea, manifested by increases in amplitude and burst rate of phrenic output. Activation of this reflex also increases the duty cycle of phrenic activity when the lungs are motionless in open-chest, anesthetized, and paralyzed rabbits (28). Therefore, activation of this reflex could promote inspiratory muscle fatigue, which is observed more frequently in severe pulmonary diseases. In addition, this reflex can be activated by common mediators released during many cardiopulmonary diseases, such as H2O2 (22). It is possible, therefore, that activation of this reflex is one of the factors contributing to inspiratory muscle fatigue and ventilatory failure in pulmonary patients.

Ventilatory stimuli activate inspiratory and expiratory muscles differently. For example, exercise stimulates both inspiration and expiration (1), as does increased Pco2, decreased Po2, and activation of muscle afferents (11, 12, 29). Activation of baroreceptors inhibits both inspiratory and expiratory muscle activity, whereas withdrawal of baroreceptor gives the opposite effect (4). On the other hand, activation of the Hering-Breuer reflex by inflation of the lung stimulates expiration while suppressing inspiration, whereas deflation of the lung produces the reverse effects (3, 20). Therefore, it is important to know how these two processes, inspiration and expiration, are affected by a reflex to fully assess the role of the reflex in ventilatory control. The present study examines the effects of the ELR on inspiratory and expiratory activities simultaneously. Our results show that activation of the ELR stimulated inspiration but suppressed expiration. Thus activation of the ELR may contribute to inspiratory muscle fatigue by inhibiting expiratory muscle activity in addition to stimulating inspiratory muscle activity.
METHODS

Experiments were conducted on 18 male, New Zealand White rabbits (body wt, 2.0–2.4 kg). The rabbits were surgically anesthetized with pentobarbital sodium intravenously (30 mg/kg). After completion of the surgery, anesthesia was maintained with a mixture of α-chloralose (1%) and urethane (10%) by intravenous infusion (~1.6 ml/h). Measurements were made at least 90 min after the final dose of pentobarbital.

The trachea was cannulated low in the neck, and the lungs were ventilated by a ventilator (model 683, Harvard Apparatus) in which the expiratory outlet was connected to a positive end-expiratory pressure of 3 cmH₂O. Airway pressure was monitored by a pressure transducer attached to a side arm of the tracheal tube. Tidal volume was set at 10 ml/kg body wt. The chest was then opened by a midline incision. The opening was covered with saline-soaked gauze to prevent dehydration of the underlying tissue. The femoral artery was cannulated to obtain arterial blood samples. Airway pressure and phrenic nerve and abdominal muscle activities were recorded by a thermorecorder (Astro-Med, Dash IV). Because the expiratory muscles are often quiet during rest, ventilation frequency was adjusted to produce an active abdominal muscle activity, which resulted in a slight hypoventilation. On the average, ventilator frequency was at 13.9 ± 0.4 cycles/min, ranging from 11 to 17 cycles/min; arterial blood gases had an arterial Po₂ of 88.2 ± 11.2 Torr, arterial PCO₂ of 46.2 ± 2.3 Torr, and pH of 7.34 ± 0.03.

The electromyogram of the phrenic nerve was recorded. The right or left phrenic nerve was separated from the surrounding tissue and transected. The central end of the nerve was desheathed and placed on a bipolar silver electrode connected to a high-impedance probe (model HIF 511) and then to a Grass (P 511) amplifier. Nerve activity was monitored by a loudspeaker, and its “integrated signals,” i.e., moving-time-averaged signals (7P3D integrator, Grass; time constant, 50 ms), were recorded (9). The electromyogram (EMG) of the external oblique abdominal muscle was recorded from bipolar needle electrodes inserted into the muscle. The EMG signals were preamplified and band-pass filtered between 30 and 3,000 Hz. The processed signals were then passed through a moving-time average integration (7P3D integrator, Grass; time constant, 50 or 200 ms) and recorded by the thermorecorder (Astro-Med, Dash IV). Amplitudes of electromyogram and EMG activity were taken as the peak height above the baseline (electrical zero) of the moving-time-averaged signal. The ELR was evoked by directly injecting stimulants, such as hypertonic saline (8.1% NaCl, 0.1 ml) or H₂O₂ (in 0.1 ml of 0.9% NaCl) into the lung parenchyma. The method has been described in previous reports (22, 30). The phrenic nerve and abdominal muscle activities were examined in response to local injection of the stimulants.

To eliminate the possibility of the reflex response being evoked via input of proprioceptors located in the inspiratory muscles, we compared the ELR before and after blocking the proprioceptive input by bilateral section of phrenic nerves and local anesthetics to block intercostal nerves. Lidocaine (2%) was injected bilaterally along the ribs (at 2 to 8 near spinal cord) where intercostal nerves were running. In addition, we examined the changes in breathing pattern in response to electrical stimulation of the sciatic afferent nerve, which is known to stimulate inspiratory muscle activity (29).

The method of nerve stimulation is the same as reported previously (29). In brief, the left sciatic nerve was separated from its surrounding tissues and sectioned. The central end was placed on a stimulating electrode, which is connected to a stimulating isolation unit. The stimulating current used was 130 times the twitch threshold.

Data are presented as means ± SE. Each data point for respiratory variables was the average of three ventilatory cycles. A Student’s paired t-test was used to compare two sets of data from the same animal. 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 statistically significant.

RESULTS

During resting control, inspiratory and expiratory activities were entrained to the ventilator cycles (Figs. 1–4). The phrenic burst (inspiratory activity) was in a relation of 1:1 (Figs. 1, 3, and 4) or 2:1 (Fig. 2) to the ventilator cycle, as was the expiratory (abdominal muscle) activity. In general, phrenic bursts occurred out of phase with the increase in airway pressure, whereas the expiratory muscle activity occurred almost in phase with increased airway pressure (Figs. 1–4). Expiratory muscle activity was verified by its responses to inflate the lung at a constant pressure of 15 to 20 cmH₂O and then to deflate the lungs to the atmospheric pressure in each rabbit. Hyperinflation of the lung caused inspiratory activity to cease while stimulating the expiratory activity. Conversely, deflation of the lung stimulated inspiratory activity while suppressing expiratory activity (Fig. 1).

Injection of hypertonic saline into the lung parenchyma (n = 13) stimulated phrenic nerve activity immediately by increasing both its amplitude and burst rate (Figs. 2 and 3) (30). Blood pressure increased slightly from 79 to 84 mmHg (P < 0.05). In addition, hypertonic saline suppressed expiratory muscle activity almost completely (Figs. 2, 3, and 5). Sometimes hypertonic saline injection produced one or two coughs, as evidenced by vigorous contractions of the abdominal muscle, which were followed by suppression of expiratory muscle activity (Fig. 3). The time courses for activation of inspiratory muscle and suppression of expiratory muscle were very similar. The responses started immediately with peak effects at 4.7 ± 0.7 s for stimulation of phrenic activity and at 6.9 ± 1 s for suppression of expiratory muscle. These effects lasted 114 and 200 s (medians), respectively. The percent changes in amplitude of phrenic nerve and abdominal muscle activities and their burst rates, as well as inspiratory duty cycle (assessed from the phrenic nerve activity), are illustrated in Fig. 5. In most cases, expiratory muscle activity disappeared after bilateral vagotony. Therefore, the inhibitory effects of the ELR on expiratory muscle activity cannot be examined after vagotony. No changes in inspiratory and expiratory activities were observed after local injection of normal saline (0.9% NaCl) (Fig. 4).

In 12 rabbits, H₂O₂ (in 0.1 ml of 0.9% NaCl: 10 μmol in 8 rabbits and 100 μmol in 4 rabbits) was injected into the lung parenchyma. The responses to the two different doses of H₂O₂ were the same, although the response tended to be greater at the higher dose. The
data are pooled for analysis. H$_2$O$_2$ produced similar reflex effects as those evoked by hypertonic saline. It immediately stimulated inspiratory activity and suppressed expiratory activity (Fig. 3). Blood pressure increased in five rabbits and did not change in seven rabbits. On average, it increased from 76 to 80 mmHg. In general, the responses evoked by H$_2$O$_2$ were weaker than those evoked by hypertonic saline injection. That is, the increases in amplitude and burst rate of phrenic and abdominal muscle activities were less (Fig. 5), the latency-to-peak response was longer, and the periods of stimulated inspiratory activity and suppressed expiratory activity were shorter. The time courses of inspiratory and expiratory responses to H$_2$O$_2$ were similar, with peaks at 10.5 ± 1.0 and 15.4 ± 1.2 s for stimulation of phrenic nerve activity and for suppression of expiratory muscle activity, respectively. These effects lasted 55 and 92.5 s (medians).

In an additional five rabbits, we examined the reflex before and after bilateral section of phrenic nerves and lidocaine blockade of intercostal nerves. No significant differences in the reflex effects were observed. After sectioning the phrenic nerve and blocking the intercostal nerves, activation of pulmonary vagal afferents by hypertonic saline still evoked ELR. Inspiratory (phrenic nerve) activity increased, and expiratory (abdominal muscle) activity decreased (Fig. 6). In four rabbits, we also examined inspiratory and expiratory activities in response to electrical stimulation of the central end of the sciatic nerve. In all experiments, afferent input
DISCUSSION

The present studies confirm our previous reports that the ELR increases phrenic amplitude, burst rate, and inspiratory duty cycle (28, 30). The results advance our understanding and extend the previous reports to demonstrate that the ELR can be elicited under hypoventilated conditions with slight hypoxemia and hypocapnia, which is a common clinical presentation in patients with COPD. The ELR can be repeatedly evoked by repeated injections (Refs. 22, 30, and present results). More importantly, our present work further demonstrates that activation of the ELR, either by hypertonic saline or by H$_2$O$_2$, suppressed expiratory activity as well as stimulated inspiratory activity. The resultant effect is to shift the respiratory workload from expiratory muscles to the inspiratory muscles. Therefore, it is possible that the ELR, under certain conditions (such as lung diseases), may contribute to inspiratory muscle fatigue by suppressing expiratory muscle activity.

Our animal preparation preserves proprioceptive input that occurs as the inspiratory muscles contract. Because the chest of the rabbit was open, during contraction the tension generated in inspiratory muscles (diaphragmatic and intercostal muscles) would be less and the shortening of the muscles would be greater. Thus the proprioceptive inputs during muscle contraction are different from those in the closed-chest preparation. It can be argued that the changes in respiratory pattern, such as suppression of expiratory muscle activity, in response to local injections of stimulants (hypertonic saline or H$_2$O$_2$) may result from alterations in the inputs from proprioceptors in inspiratory mus-
Fig. 4. Effects on breathing pattern of injecting normal saline (0.9% NaCl, 0.1 ml) into the lung parenchyma. There were no stimulatory effects on phrenic activity and no inhibitory effects on abdominal muscle activity. Solid squares are as defined in Fig. 2.

Fig. 5. Percent changes in respiratory variables from control in response to injections of hypertonic saline (8.1% NaCl, 0.1 ml; A) or \( \text{H}_2\text{O}_2 \) (10 or 100 μmol in 0.1 ml of normal saline; B) into the lung parenchyma. Values are means ± SE. The control values are calculated as 100% and are marked as 0. Ea, amplitude of myogram of the expiratory muscle (abdominal muscle); Ef, burst rate of abdominal myogram; Ia, amplitude of neurogram of inspiratory nerve (phrenic nerve); If, burst rate of phrenic neurogram; DC, inspiratory duty cycle (assessed from phrenic neurogram).

Fig. 6. Inspiratory and expiratory responses to hypertonic saline injection (8.1%) before (A) and after (B) blockage of proprioceptive input from the inspiratory muscles. Note that the responses are the same. Phrenic nerve activity (amplitude) increased, while abdominal muscle activity (amplitude) decreased in both cases. Values are means ± SE. *Significant difference \( P < 0.05; n = 5 \) compared with control (baseline).
cles during contraction. To eliminate this possibility, we examined the reflex before and after blocking the proprioceptive inputs by bilateral section of the phrenic nerves and blocking intercostal nerves with local anesthetics. After blocking the proprioceptive inputs, we still observed the reflex stimulation of phrenic activity and suppression of abdominal muscle activity. There was no significant difference in the magnitude of the responses before and after blocking of the inputs (Fig. 6). Furthermore, in the same preparation, electrical stimulation of the sciatic nerve, which also stimulates inspiratory muscle activity, did not suppress but stimulated expiratory muscle activity (Fig. 7). These responses were consistent with those in closed-chest dogs as reported in a previous study (29). Because the ELR is initiated from activation of vagal afferents in the lung, any influence from the proprioceptive inputs in the inspiratory muscles would be secondary. Therefore, it is unlikely that the suppression of expiratory muscle activity after injection of stimulants into the lung is due to the alteration of proprioceptor input from the inspiratory muscles. The influence from the proprioceptors, if any, may not be primary but modulatory. Thus we believe that suppression of expiratory muscle activity is a part of the ELR.

Our results show that expiratory muscle activity in the rabbit is similar to that observed in other species, such as the dog (12), the cat (4), and the piglet (25). The expiratory muscles are stimulated by mechanosensitive input from vagal afferents and are suppressed by bilateral vagotomy (Refs. 3, 20, 27, and present results). The inputs from pulmonary stretch receptors (PSRs) may be responsible for the excitatory effect on the expiratory muscles (20). Hyperinflation of the lung, which suppressed phrenic activity, activated expiratory muscle (Fig. 1). It is possible that the observed suppression of expiratory muscle activity during activation of the ELR could result from a decrease in PSR activity downstream from the injection point, because of blockage of the airways by the injectate. However, as analyzed and addressed in a previous report (30), the injected volume is very small (0.1 ml), and removal of background activity from a small fraction of pulmonary afferents, such as PSRs, cannot produce such an enormous reflex effect. In addition, injected normal saline (0.9% NaCl) at the same volume (0.1 ml), which would produce the same blockade and the same reduction of PSR activity downstream from the injection point, does not evoke the reflex (Fig. 4). Therefore, it is likely that activation of vagal afferents, rather than withdrawal of input from PSRs, is responsible for initiating the ELR that suppresses expiratory muscle activity.

Time is required to empty the lung. The greater the inspired volume, the longer the time required for passive expiration. As the breathing rate increases, expiratory time shortens, resulting in less time available for expiration. Eventually, there would be a limiting point at which further shortening of the expiratory time produces hyperinflation of the lung (dynamic hyperinflation or intrinsic positive end-expiratory pressure), because the lung does not have enough time to return to functional residual capacity. The resulting hyperinflation would stress inspiratory muscles (see below for possible mechanisms). In patients with pulmonary diseases, such as COPD, as lung mechanics deteriorate (decreased lung compliance and increased airway resistance) (18), more energy is required to achieve a given level of ventilation. These conditions may significantly stress inspiratory muscles. Many of these patients demonstrate an active expiration (15), which can be a compensatory mechanism. The forces generated by expiratory muscles can help reduce end-expiratory volume. The smaller the end-expiratory volume (the closer the lung volume moves toward the residual volume at the end of expiration), the greater the passive outward recoil force of the chest wall, and
the lesser the energy requirement during initial inspiration for a given tidal volume. Expiratory muscle contraction can accelerate expiratory airflow and thus help empty the lungs and unload inspiratory muscle. The expiratory muscles serve as a reserve for ventilation. As ventilatory demand increases, the expiratory muscles are recruited to participate in breathing to meet the increased ventilatory needs.

Our present and previous studies (28, 30) show that activation of the ELR produces neural hyperpnea and increased inspiratory duty cycle. Together, these two factors will increase the inspiratory effort quotient, promoting inspiratory muscle fatigue (2, 28). Activation of the ELR can also produce tachypnea (Ref. 30 and present results), which adds more stress on inspiratory muscle by increasing dead space ventilation. Furthermore, activation of the ELR, as demonstrated in the present study, may contribute to inspiratory muscle fatigue by another mechanism, the suppression of the expiratory muscle activity. As stated above, reduction of expiratory muscle activity during active expiration can stress the inspiratory muscles. This can be achieved by 1) shifting the workload from the expiratory muscles to inspiratory muscles and 2) producing dynamic hyperinflation of the lungs. Hyperinflation not only makes inspiratory muscle operate at a higher volume position at which the muscle fiber deviates from the optimal length-tension relationship, but also puts the respiratory system on the flat part of the compliance curve, which requires more energy to do the same amount of work (16). In addition, at high lung volume, the pressure output generated by inspiratory muscle under a given inspiratory neural output is reduced (10); therefore, dynamic lung inflation may actually compromise the ventilatory function of inspiratory muscles. Clearly, all of these ventilatory effects induced by the ELR could work together to stress the inspiratory muscles and promote inspiratory muscle fatigue.

Reactive oxygen species are believed to be responsible for many diseases involving different organ systems (31). For example, H2O2 and hydroxyl free radicals are associated with certain pulmonary diseases (see review in Ref. 19), especially those that involve the lung parenchyma. Directly applying H2O2 to the surface of the heart initiates a cardiac reflex (24). H2O2 is also known to stimulate afferents in the gastrointesti nal tract to cause reflex effects (23). Employing local injection techniques, we were able to directly deliver H2O2 to the vicinity of pulmonary receptors, and we have shown that H2O2 can evoke the ELR as hypertonic saline does (Ref. 22 and present results). Because H2O2 (a product of neutrophil respiratory burst) is a key mediator in pulmonary diseases, results from the present study lend support to the claim that the ELR may play an important role in pulmonary pathological processes.

In summary, the present study demonstrates that initiation of the ELR by local injection of hypertonic saline or H2O2 (a common mediator released during many cardiopulmonary diseases) suppresses expiratory activity as well as increases inspiratory activity (neural hyperpnea and tachypnea) and duty cycle. All of these reflex effects may promote and exacerbate inspiratory muscle fatigue. Therefore, activation of the ELR may be one of the factors that significantly contributes to causing ventilatory failure in many cardiopulmonary diseases.

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REFERENCES