Phasic pulmonary stretch receptor feedback modulates both eupnea and gasping in an in situ rat preparation

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Harris, Michael B., and Walter M. St.-John. Phasic pulmonary stretch receptor feedback modulates both eupnea and gasping in an in situ rat preparation. Am J Physiol Regul Integr Comp Physiol 289: R450–R455, 2005. First published April 14, 2005; doi:10.1152/ajpregu.00750.2004.—The perfused in situ juvenile rat preparation produces patterns of phrenic discharge comparable to eupnea and gasping in vivo. These ventilatory patterns differ in multiple aspects, including most prominently the rate of rise of inspiratory activity. Although we have recently demonstrated that both eupnea and gasping are similarly modulated by a Hering-Breuer expiratory-promoting reflex to tonic pulmonary stretch, it has generally been assumed that gasping was unresponsive to afferent stimuli from pulmonary stretch receptors. In the present study, we recorded eupneic and gasp-like efferent activity of the phrenic nerve in the in situ juvenile rat perfused brain stem preparation, with and without phrenic-triggered phasic pulmonary inflation. We tested the hypothesis that phasic pulmonary inflation produces reflex responses in situ akin to those in vivo and that both eupnea and gasping are similarly modulated by phasic pulmonary stretch. In eupnea, we found that phasic pulmonary inflation decreases inspiratory burst duration and the period of expiration, thus increasing burst frequency of the phrenic neurogram. Phasic pulmonary inflation also decreases the duration of expiration and increases the burst frequency during gasping. Bilateral vagotomy eliminated these changes. We conclude that the neural substrate mediating the Hering-Breuer reflex is retained in the in situ preparation and that the brain stem circuitry generating the respiratory patterns respond to phasic activation of pulmonary stretch receptors in both eupnea and gasping. These findings support the homology of eupneic discharge patterns in the reduced in situ preparation and eupnea in vivo and disprove the common supposition that gasping in an in situ rat preparation produces patterns of phrenic discharge comparable to eupnea in vivo (35–39). Although the supposition that motor patterns expressed by any model system can be identical to eupnea or gasping in vivo must be made with caution, a strong body of evidence supports the homology between respiratory patterns in vivo and in situ (25–27, 32, 38, 44). We found changes in phrenic discharge in response to tonic lung inflation in the in situ preparation and, moreover, found that these responses were similar in eupnea and gasping. These findings were in direct contrast to the commonly stated conclusion that gasping is insensitive to vagal afferent feedback from pulmonary stretch receptor mechanisms.

Hering-Breuer; slowly adapting pulmonary stretch receptors; vagus; gasping

EUPNEA AND GASPING ARE GENERATED within the brain stem by intrinsic mechanisms. Eupnea is the motor pattern that results in stable and sustained ventilation and long-term gas exchange. Gasping consists of brief maximal inspiratory efforts that, although not an appropriate mechanism for long-term ventilation, can promote gas exchange over a short term to provide “autoresuscitation” (35, 38).

Afferent fibers from slowly adapting pulmonary stretch receptors project to the brain stem through the vagus nerve. Pulmonary afferent information is integrated within the brain stem and modulates the activity of brain stem respiratory neurons (2, 8, 9, 13, 17, 22, 23, 43, 45). The most commonly recognized influences of such pulmonary stretch receptor feedbacks are the Hering-Breuer reflexes. In many species, stimuli that mimic tonic activation of pulmonary stretch receptors alter the duration of expiration with little or no influence on either the inspiratory duration or tidal volume (5, 15, 22–24, 28, 42). The characteristics of changes in ventilatory activity by stimuli that mimic phasic activation of slowly adapting pulmonary stretch receptors depend on the timing of stimulation. In general, phasic activation during the first portion of inspiration causes no change in inspiratory activity, whereas this input arriving during the last phase of inspiration is inhibitory, resulting in a shortening of the duration of the inspiratory phase. This phasic pulmonary inflation in inspiration also decreases the duration of expiration and increases the frequency of breathing (8, 22, 23, 30, 45).

We have recently evaluated whether the neural substrate mediating Hering-Breuer-type modulation by tonic pulmonary inflation is retained in the in situ preparation of the juvenile rat. This in situ preparation exhibits an incrementing pattern of phrenic discharge similar to eupnea in vivo (25–27, 32, 38, 44). With severe hypoxia or ischemia, the eupnea-like discharge pattern is replaced by a decrementing pattern similar to gasping in vivo (35–39). Although the supposition that motor patterns expressed by any model system can be identical to eupnea or gasping in vivo must be made with caution, a strong body of evidence supports the homology between respiratory patterns in vivo and in situ (25–27, 32–39, 44). We found changes in phrenic discharge in response to tonic lung inflation in the in situ preparation and, moreover, found that these responses were similar in eupnea and gasping. These findings were in direct contrast to the commonly stated conclusion that gasping is insensitive to pulmonary stretch receptor feedback (e.g., Refs. 1, 17–20, 32). Indeed, as discussed in our previous report, sensitivity to vagal feedback has been used as a criterion for distinguishing eupnea from gasping, although our results suggest that this is not an accurate supposition (12).

In the present investigation, we sought to assess further the homology of the phrenic neurogram in the reduced in situ preparation and breathing in vivo by reproducing reflexive changes in burst activity in response to phasic pulmonary stretch synchronized with inspiration. Given that tonic pulmonary inflation produces Hering-Breuer-type modulation of gasping in the in situ preparation, the present investigation was also designed to determine whether gasping is altered by phasic pulmonary inflation.

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METHODS

General. Eleven juvenile male Sprague-Dawley rats (80–120 g) were used. Only minor changes have been made from procedures described previously (12, 36–39, 44). All experimental protocols were approved by the Institutional Animal Care and Use Committee of Dartmouth College. Briefly, rats were anesthetized with halothane (5% in O₂), bisected below the diaphragm, immersed in ice-cold artificial cerebrospinal fluid, and decerebrated at a precollicular level. All brain areas rostral to the colliculi were removed by aspiration. The right phrenic nerve was sectioned at the level of the diaphragm and dissected rostrally. The descending aorta was freed from other tissue, and a catheter was inserted into the aorta (double lumen, size 4.0 French), advanced rostrally, and tied in place. The aortic catheter was connected to a peristaltic pump delivering a perfusate (see below), which was equilibrated with 95% O₂-5% CO₂. Perfusion was connected to a peristaltic pump delivering a perfusate (see below), and was equilibrated with 95% O₂-5% CO₂. Perfusion pressure was maintained at ~70 mmHg by manually adjusting the rate of the perfusion pump. With this preparation, oxygenation and removal of carbon dioxide are dependent on the constituents of the perfusate and are independent of pulmonary ventilation.

Phrenic activity was monitored by a bipolar glass suction electrode, amplified, filtered (3 Hz to 3 kHz), integrated (50-ms time constant), and recorded to a computer data-acquisition system. Gallamine triethiodide was added to the perfusate in increments until spontaneous respiratory movements ceased; 60–80 mg/l were usually required. The temperature of the brain stem was monitored and maintained at 29.5–30.5°C by adjusting the temperature of the perfusate. Once burst activity was observed, preparations were left for 45 min before experimental protocols were begun.

Perfusate. The perfusate contained the following in distilled water (in mM): 1.25 MgSO₄, 1.25 KH₂PO₄, 5.0 KCl, 25 NaHCO₃, 125 NaCl, 2.5 CaCl₂, 10 dextrose, and 0.1785 Ficoll 70. Ficoll was included as an osmotic agent. Although not measured at the time of experiment, system calibration showed that the perfusate equilibrated with 95% O₂-5% CO₂ entered the aorta at 31°C and had a PO₂, PCO₂, pH, and HCO₃ concentration of ~600 Torr, 33 Torr, 7.4, and 23 mM, respectively.

Phasic pulmonary stretch receptor activation. The trachea was cannulated and attached to a flow of air via a servo-controlled respirator. The respirator used a butterfly valve to alter the resistance of the system to inflate the lungs with an incremental pressure transducer that tracked tracheal pressure during the phrenic cycle. The output of the pressure transducer was regulated by the servo-respirator to limit maximum tracheal pressure to ~12 cmH₂O. Maximum tracheal pressure was also limited by a bypass tube between the tracheal cannula and the bottom of a graduated cylinder. Approximately 10 cm of water in the cylinder acted as a pressure release “valve” to prevent overinflation of the lungs.

A side arm of the tracheal cannula was attached to a differential pressure transducer on the pressure source (7). The beginning and end of each burst were identified from the integrated phrenic neurogram. In cases where phrenic activity was below the command threshold, the valve was open and tracheal pressure approximated atmospheric pressure. The pressure closed in proportion to the phrenic command; as it closed, tracheal pressure was elevated toward (but never reached) a source pressure of ~20 cmH₂O above atmospheric pressure.

Analysis. The beginning and end of each burst were identified from the raw and moving averages of phrenic nerve recordings with the use of computer-aided threshold detection (DATAPAC, Run Technologies). Mean durations of phrenic bursts (neural inspiration; Tᵢ), the period between bursts (neural expiration; Tₑ), and the total “ventilatory” cycle (the period from burst onset to the next consecutive burst onset; Tᵣᵣ) were calculated for each burst. The averages of 30 consecutive phrenic phasic bursts and 3–5 consecutive gasps provided values for subsequent analyses. Variables associated with patterns of phrenic burst activity indicative of eupnea and gasping were compared as independent phenomena between conditions of pulmonary inflation by a series of one-way repeated-measures ANOVAs to determine an influence of pulmonary inflation. Data are presented as means ± SE of all preparations during a given condition. Significant differences were attributed to a P value of <0.05.
RESULTS

Eupnea. When perfused with artificial cerebrospinal fluid equilibrated with 95% O₂-5% CO₂, the eupneic pattern of the phrenic burst discharge was characterized by an incrementing onset and rapid offset (Fig. 1A). The shape of phrenic bursts was consistent between preparations and within a given preparation over time. Randomization of the order of inflation treatment controlled for time-dependent variations. Compared with cycles with lung inflation, the average TI and TE and the TTOT were all significantly increased when lung inflation was withheld (Figs. 2, A and B, and 3). Hence, the burst frequency was significantly lower when the phrenic-triggered ventilator was bypassed and tracheal pressure was approximately equal to atmospheric pressure. The burst amplitude was not significantly different for cycles with or without lung inflation.

Gasping. Perfusion with the hypoxic solution transformed the phrenic burst pattern in a manner characteristic of the conversion from eupnea to gasping. Gasplike bursts differed from eupneic bursts by having a shorter duration and greater peak height and, most notably, by having a rapid onset and offset (Fig. 1B).
or gasping. The frequencies of gasps and eupneic bursts were the same. As in our previous study (12), reperfusion with a hyperoxic solution was commenced after a series of three to five gasps to facilitate recovery of eupneic burst patterns.

During hypoxic gasping, periodic lung inflations during Ti caused significant declines in Ti and Ttot compared with cycles without lung inflation (Figs. 2, C and D, and 3). Hence, the frequency of phrenic bursts was significantly greater during cycles with lung inflation. Neither the duration of Ti nor the burst amplitude was altered by lung inflation in gasping (Fig. 3).

Vagotomized preparations. Variables of the burst pattern were not assessed in the same preparations before and after vagotomy. Thus the influence of vagotomy per se is not addressed in these studies. In vagotomized preparations, there were no alterations in phrenic burst pattern between the presence and absence of phasic pulmonary inflation, during eupnea or gasping.

DISCUSSION

Phrenic-triggered pulmonary inflation in the in situ preparation altered the phrenic burst pattern in a manner consistent with the Hering-Breuer inspiratory-termination reflex during eupnea in vivo. We presume that pulmonary inflation altered the phrenic pattern through phasic activation of slowly adapting pulmonary stretch receptors, via a Hering-Breuer inspiratory-termination reflex. Because this reflex is mediated by afferents in the vagus nerve, our presumption is supported by the absence of an influence of phrenic-triggered ventilation on phrenic discharge following vagotomy (3, 4, 12, 16, 30). Confirming what has been shown many times in a variety of experimental preparations, lung inflation or the absence thereof caused no change in any variable of integrated phrenic activity in vagotomized preparations.

Our data also illustrate that phasic pulmonary inflation results in an increase in respiratory frequency in this preparation due to a shortening of both inspiratory and expiratory durations. Assuming that pulmonary volumes do not fall below functional residual capacity during expiration, the shortening of expiratory duration reflects the well-described linkage of inspiratory and expiratory durations in eupnea (8). Similarly, decreases in breathing frequency produced by expiratory lengthening that follows vagotomy are commonly reported and attributed to the removal of vagal facilitatory mechanisms (10, 11). Thus, beyond an influence on individual burst pattern, pulmonary inflation has an influence on the overall expression of inspiratory and expiratory durations in the in situ preparation in a manner similar to breathing in vivo.

Exposing the in situ preparation to hypoxic conditions that produce gasping in vivo induced a gasplike phrenic burst pattern. In all cases during the present investigation, hypoxia produced a burst pattern characterized by rapid onsets and decrementing profiles, with peak activity occurring within the first 40% of Ti. In contrast, eupneic bursts had incremental onsets, with peak amplitudes occurring in the last half of Ti. These observations confirm previous studies and support the conclusion that the gasplike patterns of phrenic discharge present in the perfused rat preparation are homologous to gasping in vivo (12, 36–41). However, it is to be noted that, in contrast to lower frequencies of gasping, compared with eupnea in vivo, frequencies were the same during eupnea and gasping in situ. In a previous detailed examination (33, 34), our group found that this difference between in vivo and in situ results is due to the hypothermia at which in situ preparations are studied. With elevations of the temperature of the in situ preparation above 34°C, the frequency of gasping was less than that of eupnea, as is found with in vivo preparations (33).

The gasping pattern differed between conditions where phrenic-triggered pulmonary inflation was present or absent. This difference was not present after vagotomy. These data indicate that the gasplike phrenic patterns in this preparation are modulated by phasic pulmonary inflation in a manner similar to that occurring during the eupnea-like pattern. Specifically, mechanisms that cause increases in respiratory frequency with phasic pulmonary inflations during periods of eupnea-like burst discharges are preserved during the generation of gasplike patterns. Yet, the linkage of durations of Ti and Tτ, described above for eupnea, cannot directly account for the change in frequency of gasping when lung inflation was withheld. As noted in RESULTS, the durations of Ti were not significantly different for cycles with or without lung inflations in gasping. Two factors might account for the change in duration of expiration in gasping. First, inspiration duration of gasping did decline in some preparations and/or during some gasping cycles with lung inflation, resulting in a nonsignificant trend toward reductions in Ti and peak amplitude with lung inflation (Fig. 2). Thus, with more trials, a statistically significant difference in Ti during inflation and no inflation cycles might emerge. Second, many studies document that events in Ti can result in changes of variables during Tτ that appear independent of any changes in the durations of either Ti or Tτ (e.g., Refs. 8, 14). This detail concerning changes, or the lack thereof, in inspiratory duration should not obscure the most important finding of the present study, namely that both eupnea and gasping are modified by the phasic activation of pulmonary stretch receptors. As noted in the Introduction, gasping in vivo is frequently stated to be insensitive to and not influenced by vagal feedback (1, 17–20, 32). Yet, as we have reviewed in our earlier paper (12), the origin of this conclusion is unclear. Indeed, results from in vivo studies, although limited, demonstrate that vagal feedback mechanisms alter phrenic discharge in gasping. In fact, in the earliest study of gasping, Lumsden (16) found that both vagal blockade and vagal stimulation altered the frequency of gasps (12). In a more contemporary study with decerebrated, paralyzed, and ventilated cats, our group (41) reported that withholding lung inflation during gasping altered peak phrenic activity and the period between phrenic bursts. However, because arterial blood pressure was altered concomitantly when lung inflation was withheld, we considered that these findings were inconclusive concerning the role of the Hering-Breuer reflex in modulating gasping.

One study reported a seeming lack of influence of pulmonary inflations on gasping (29). However, in this study, evaluations were performed during the recovery from prolonged
cerebral ischemia and apnea. During this recovery, ventilatory activity commenced as gasps that were ultimately succeeded by eupnea. Not only were gasps insensitive to manipulations of lung volume but an inhibition of eupneic phrenic discharge with lung inflation was also absent for several minutes after eupnea returned. Hence, this study would imply that the prolonged cerebral ischemia had suppressed synaptic mechanisms responsible for brain stem processing of afferent stimuli from pulmonary stretch receptors. These synaptic mechanisms only recovered with time, and, because gasping returned before eupnea, any differential recovery of the Hering-Breuer reflex reflects time rather than the respiratory pattern.

Since first introduced, a number of studies have been devoted to comparing the patterns of the in situ perfused preparations of the neonatal and juvenile rat with eupnea and gasping in vivo. On the basis of numerous criteria, these in vivo and in situ patterns have been found to be identical, albeit when corrected for the hypothermia in which in situ preparations have been maintained. Included in these criteria have been the patterns of cranial and spinal neural activities in Ti and Tr, high-frequency oscillations in these neural activities, changes in activities following brain stem transections, and responses to chemo- and mechanoreceptor stimuli (12, 36–40, 44). Hence, evidence is compelling that eupnea and gasping in the in situ preparations accurately reflect these same patterns in vivo. Results of the present study also confirm the limited in vivo observations that, like a number of other sensory afferents, feedback from pulmonary stretch receptors modulates the respiratory cycle in gasping (12, 16, 21, 46).

The present results have significant implications for interpretation of findings from en bloc in vitro preparations of the neonatal rat. The relationship between the rhythmic discharges of this preparation and eupnea or gasping in vivo or in situ is controversial (see Refs. 1, 12, 17–20, 31–38 for discussion). Recently, phasic lung inflation in a lung-attached in vitro neonate rat preparation was demonstrated to shorten the respiratory period (18–20). Using a presumption that gasping is not modulated by pulmonary stretch receptor feedback, the authors present these observations as validation that this in vitro preparation exhibits eupnea and not gasping. On the basis of our present and previous results in situ and previous results in vivo, this presumption concerning the in vitro preparation cannot be supported. Thus modulation of an in vivo fictive respiratory pattern by tonic or phasic lung inflation cannot exclusively link such a pattern to eupnea or exclude it as gasping.

In conclusion, phrenic-triggered phasic pulmonary inflation in the in situ preparation altered the eupneic pattern of phrenic bursts in a manner consistent with the Hering-Breuer inspiratory-termination reflex during eupnea in vivo. In addition, pulmonary inflation altered the durations of expiration and total cycle duration and frequency of phrenic bursts during both eupnea and gasping. Our results indicate that the neural elements underlying the modulation of breathing by phasic pulmonary stretch are preserved in the in situ preparation and support a conclusion that both eupnea and gasping are similarly modulated by pulmonary stretch receptor feedback. These observations support the homology of burst patterns generated by the in situ perfused preparation and breathing in vivo and contradict the generally held supposition that gasping is insensitive to such feedback.

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