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

Michael B. Harris and Walter M. St.-John

Department of Physiology, Dartmouth Medical School, Dartmouth Hitchcock Medical Center, Borwell Building, Lebanon, New Hampshire 03756

Submitted 4 March 2003; accepted in final form 5 March 2003

Harris, Michael B. and Walter M. St.-John. Tonic pulmonary stretch receptor feedback modulates both eupnea and gasping in an in situ rat preparation. Am J Physiol Regul Integr Comp Physiol 285: R215–R221, 2003. First published March 6, 2003; 10.1152/ajpregu.00112.2003.—The perfused in situ juvenile rat brain stem preparation produces phrenic discharge patterns comparable to eupnea and gasping in vivo. These ventilatory patterns of eupnea and gasping differ in multiple aspects, including most prominently the rate of rise of inspiratory activity. Because gasping, but not eupnea, appeared similar after vagotomy in spontaneous breathing preparations, it has been assumed that gasping was unresponsive to afferent stimuli from pulmonary stretch receptors. In the present study, efferent activity of the phrenic nerve was recorded during eupnea and gasping in the in situ juvenile rat preparation. Gasping was induced in hypoxic-hypercapnia or ischemia. An increase in the pressure of tonic pulmonary inflation from 1 to 10 cm H2O caused a prolongation of the duration between phrenic bursts in both eupnea or 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 responds to tonic activation of pulmonary stretch receptors in a similar manner in eupnea and gasping. These findings support the homology of eupnea-like phrenic discharge patterns in the reduced in situ preparation and eupnea in vivo and disprove the common supposition that gasping is insensitive to vagal afferent feedback from pulmonary stretch receptor mechanisms.

Hering-Breuer; slowly adapting pulmonary stretch receptors; vagus; eupnea; gasp

The perfused in situ juvenile rat brain stem preparation exhibits an incrementing phrenic discharge pattern similar to eupnea in vivo (15–17, 28). With severe hypoxia or ischemia, the eupnea-like discharge pattern is replaced by a decrementing pattern similar to gasping in vivo (28, 29, 31).

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, 6, 7, 9, 35). The most commonly recognized influences of pulmonary stretch receptor feedback on euepneic breathing are the Hering-Breuer reflexes. Phasic pulmonary inflation and vagal feedback act primarily to regulate the resting breathing pattern by modulating the duration and volume of inspiration (3, 4; see Refs. 5 and 13 for review). Tonic pulmonary inflation and vagal feedback primarily regulate the duration of expiration and have little or no influence on either the inspiratory duration or tidal volume (5, 8, 13, 19, 33). Ventilatory responses to stimuli that mimic tonic slowly adapting pulmonary stretch receptor activation are described as a Hering-Breuer expiratory-promoting reflex. The duration of expiration generally increases and the breathing frequency declines with treatments such as tonic pulmonary inflation, airway occlusion at end inspiration, or tonic vagal stimulation in rats and most other mammals (3, 4, 6–8, 13, 39). In the present investigation, we sought to further assess the homology of phrenic neurogram in the reduced in situ preparation and breathing in vivo by reproducing reflexive changes in burst activity in response to tonic pulmonary inflation. The respiratory pattern of eupnea is markedly different from that of gasping (22, 24). One generally held distinction between eupnea and gasping has been that the phrenic neurogram during gasping is unresponsive to afferent stimuli that markedly alter the euepneic respiratory pattern (1, 22). The generality of this supposition has been questioned (11, 32, 37), yet unresponsiveness to afferent stimuli, in particular vagal pulmonary stretch receptor feedback, has been considered a prime characteristic of gasping (1, 22). The origin of this generally held belief is curious as responsiveness of gasping to vagal feedback was described during early studies of gasping (9).

In the present investigation, we used the in situ perfused brain stem preparation of the juvenile rat to assess the validity of the supposition that pulmonary receptor feedback does not modulate gasping. We test the null hypothesis that eupnea, but not gasping, is altered by changes in tonic pulmonary inflation that presumably influence pulmonary stretch receptors. In addition, we determine the influence of vagotomy on the response of both eupnea and gasping to pulmonary inflation.

Address for reprint requests and other correspondence: M. B. Harris, Dept. of Physiology, Dartmouth College, Borwell Bldg., Dartmouth Hitchcock Medical Center, One Medical Center Dr., Lebanon, NH 03756 (E-mail: michael.b.harris@dartmouth.edu).

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GASPING IS MODULATED BY TONIC PULMONARY STRETCH RECEPTOR FEEDBACK

METHODS

General. Nineteen juvenile male Sprague-Dawley rats (80–180 g) were used. These experiments conform with the “Guiding Principles for Research Involving Animals and Human Beings” of the American Physiological Society. Only minor changes have been made from procedures described previously (15–18, 28). Briefly, rats were anesthetized with halothane (5% in O2, bisected below the diaphragm, immersed in ice-cold artificial cerebral spinal fluid (aCSF; described below), and decerebrated at a precollicular level. All of the brain rostral to the colliculi was 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 Fr), advanced rostrally, and tied in place. The aortic catheter was connected to a peristaltic pump delivering aCSF, which was equilibrated with 95% O2-5% CO2. Perfusion was commenced and modulated until phrenic activity occurred with a eunвечник rampike pattern. Phrenic activity was monitored by a bipolar glass capillary suction electrode, amplified (1st stage by WPI DAM 50 differential amplifier, 2nd stage by Gould Universal AC amplifier), filtered (3 Hz–3 kHz, at 2nd stage), integrated (Charles Ward moving averager; 50-ms time constant), and recorded to a computer data-acquisition system. Gallamine triethiodide (20 mg/ml) was added to the perfusate in increments of 1 ml until spontaneous respiratory movements cease; 60–80 mg/l was usually required. The temperature of the brain stem was monitored and maintained at 29.5–30.5°С by adjusting the temperature of the perfusate. For simplicity, eunвечник-like and gasplike patterns of phrenic discharge in the reduced preparation are hereafter referred to as eunвечник and gasping.

Perfusate. The aCSF 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. When equilibrated with 95% O2-5% CO2, the eunвечник pattern of the phrenic burst discharge was characterized by an incrementing onset and rapid offset (Fig. 1 A). The shape of phrenic bursts appeared to be consistent between preparations and within a given preparation over time.

In the present investigation, reperfusion was commenced after a series of three to five gasps.

Analysis. Patterns of phrenic burst activity were recorded during eupnea (n = 11) and hypoxic (n = 11) or ischemic (n = 9 of the 11) gasping, while the lungs were inflated by tonic tracheal pressures of either 1 or 10 cmH2O. Treatments were presented in random order. Phrenic burst activity was recorded in eight additional preparations under the same conditions, after bilateral vagotomy.

The beginning and end of each burst were identified as the point where the integrated record of burst activity first rose from the neutral baseline and the point where it returned, respectively. Mean durations of phrenic bursts (neural inspiration; Ti), the period between bursts (neural expiration; Te), and the total ventilatory cycle (Ttot; the period from burst onset to the next consecutive burst onset), as well as the peak height of the burst (burst amplitude), were defined from the integrated recordings. Ten consecutive eunвечник phrenic bursts or three to five consecutive gasps were analyzed. Patterns of phrenic burst activity indicative of eunвечник, and hypoxic or ischemic gasping were compared between conditions of pulmonary inflation by one-way repeated-measures ANOVA and Student-Newman-Keuls pairwise multiple comparison procedures.

RESULTS

Eupnea. When perfused with aCSF equilibrated with 95% O2-5% CO2, the eunвечник pattern of the phrenic burst discharge was characterized by an incrementing onset and rapid offset (Fig. 1A). The shape of phrenic bursts appeared to be consistent between preparations and within a given preparation over time.

![Fig. 1](http://ajpregu.physiology.org/) Typical recording of integrated phrenic nerve discharge from the in situ juvenile rat preparation during perfusion with a solution equilibrated with either 95% O2-5% CO2 (A) or 8% O2-7% CO2 (B), or during ischemia (C). Note the incrementing “eupneic-like” activity presented in A and the “gasplike” decrementing burst profile associated with either hypoxic-hypercapnia or ischemia (B and C). Scale bar, 0.5 s.
During eupnea, the average \( T_I, T_E, \) and \( T_{TOT} \) were 700 ± 58, 1,560 ± 226, and 2,261 ± 199 ms, respectively, when the preparation was exposed to a tonic tracheal pressure of 1\( \text{cmH}_2\text{O} \). Both \( T_E \) and \( T_{TOT} \) were greater during pulmonary inflation to a tonic tracheal pressure of 10\( \text{cmH}_2\text{O} \) (Figs. 2 and 3; \( P < 0.016 \)). \( T_I \) was unchanged (Fig. 3).

**Gasping.** Either ischemia or perfusion with the hypoxic solution transformed phrenic burst pattern in a manner characteristic of the conversion from eupnea to gasping. Gasplike bursts generally differed from eupneic bursts by having a shorter duration and greater peak height and, most notably, by having a rapid onset and decrementing profile (Fig. 1, A and C).

During hypoxic gasping, the average durations of \( T_I, T_E, \) and \( T_{TOT} \) were 542 ± 42, 3,062 ± 246, and 3,605 ± 276 ms, respectively, when tonic tracheal pressure was set at 1\( \text{cmH}_2\text{O} \) (Figs. 2 and 3). Both \( T_E \) and \( T_{TOT} \) were greater during tonic pulmonary inflation with 10\( \text{cmH}_2\text{O} \) tracheal pressure (Figs. 2 and 3) (\( P = 0.040 \) and \( P = 0.038 \), respectively); \( T_I \) was unchanged.

During ischemic gasping, the average durations of \( T_I, T_E, \) and \( T_{TOT} \) were 561 ± 45, 2,537 ± 181, and 3,102 ± 172 ms, respectively, when lungs were exposed to a tonic inflation pressure of 1\( \text{cmH}_2\text{O} \). Both \( T_E \) and \( T_{TOT} \) were again increased when tonic tracheal pressure was 10\( \text{cmH}_2\text{O} \) (Fig. 3) (\( P < 0.001 \) and \( P < 0.001 \), respectively); \( T_I \) was unchanged.

**Vagotomized preparations.** During eupnea, after vagotomy, the average durations of \( T_I, T_E, \) and \( T_{TOT} \) were 781 ± 60, 2,166 ± 1,656, and 2,941 ± 626 ms, respectively, when tonic tracheal pressure was 1\( \text{cmH}_2\text{O} \). In the present investigation, variables of the burst pattern were not assessed in the same preparations before and after vagotomy, although this has been investigated in a prior study (21). After vagotomy, the only change in phrenic burst pattern accompanying elevation of tonic tracheal pressure, under any condition, was a 14\% decrease in \( T_E \) and an 11\% decrease in \( T_{TOT} \) (\( P = 0.011 \) and \( P = 0.009 \), respectively) observed during hypoxic gasping (Fig. 4)

**DISCUSSION**

Changing tracheal pressure between 1 and 10\( \text{cmH}_2\text{O} \) in the in situ preparation altered the phrenic pattern in a manner consistent with the Hering-Breuer expiratory-promoting reflex observed during eupnea in vivo in rats and other mammals (5, 7, 8, 13, 19, 20, 33, 34). Thus we presume that increasing tracheal pressure altered the phrenic pattern through tonic activation of slowly adapting pulmonary stretch receptors via the Hering-Breuer reflex mechanism. As this reflex is mediated by the vagus nerve, our presumption is supported by the absence of an influence on phrenic discharge of a change in tracheal pressure after vagotomy.

Our results suggest that the neural substrate mediating the Hering-Breuer reflex in vivo is retained in the in situ preparation. Thus these observations lend further support to the conclusion that the eupnea-like pattern of phrenic discharge present in the perfused preparation is homologous to eupnea in vivo.

Exposing the in situ preparation to either hypoxia or ischemia, conditions that produce gasping in vivo, induced a gasplike phrenic burst pattern. In all cases during the present investigation, hypoxia or ischemia produced a burst pattern characterized by rapid onsets and decrementing profiles, with peak activity occurring within the first 40\% of \( T_I \); eupneic bursts had incrementing onsets with peak amplitudes occurring in the last half of \( T_I \). 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 (reviewed in 26, 28, 29, 31).

During either hypoxia or ischemia, the gasping pattern was different between conditions where tracheal pressure was set at 1 and 10\( \text{cmH}_2\text{O} \). This difference was absent after vagotomy. These data indicate that the gasplike phrenic patterns are modulated by tonic pulmonary inflation in a manner similar to that occurring during the eupnea-like pattern. The most important finding of the present study is that both eupnea
and gasping are modified by the activation of pulmonary stretch receptors. This finding is important as the gasping respiratory pattern is generally considered to be insensitive to modulatory influences and specifically regarded as uninfluenced by vagal feedback (1, 10, 11, 20, 22). Our results demonstrate that the presumption concerning gasping is not accurate.

The presumption that gasping is insensitive to vagal feedback and pulmonary stretch is often stated, but its origin is not clear. In a detailed characterization of gasping resulting from brain stem transection, Lumsden (9) concluded that the only nerve having any specific action on gasping was the vagus. Lumsden reports observing an instance during which “while pure gasping was occurring, the vagi were frozen...[and] gasping continued at a markedly increased rate (16 vs. 10 gasps/min). Similarly, Lumsden noted that “stimulation of the vagi very definitely inhibited the gasping...[while] subsequent section of the vagi at once stopped this inhibition and gasping commenced.” He surmised that “vagal impulses when acting directly on the gasping center tend to inhibit gasps, while vagotomy releases them” and concluded the specific effect of vagal impulses on the gasping center is inhibitory. Moreover, in a study using decerebrate, paralyzed, and ventilated cats, withholding lung inflation during gasping altered peak phrenic activity and the period between phrenic bursts (32). However, as arterial blood pressure was altered concomitantly when lung inflation was withheld, these findings were considered as inconclusive concerning the role of the Hering-Breuer reflex in modulating gasping.

**Critique of methods.** Tracheal pressures used to inflate the lungs were within the physiological range (12). Increasing tracheal pressure from 1 to 10 cmH2O caused a change in inflation of the lungs. Although not measured, pulmonary inflation was slight and appeared to be nonuniform and well below vital capacity. The absolute magnitude of the expiratory facilitation observed in the present investigation was less pronounced than that noted in other studies where rats have been exposed to vagal stimulation, tracheal occlusion, or lung inflation (7, 34). Larger inflation pressures or pulmonary inflation by fluid injection may
have resulted in larger and more uniform pulmonary inflation and, potentially, could produce greater changes in the pattern of phrenic bursts. Regardless, the mild degree of inflation employed in the present study was sufficient to alter eupneic phrenic burst pattern in a consistent manner. Thus the relatively mild stimulus intensity chosen was appropriate to initiate a response and to demonstrate the presence of a Hering-Breuer expiratory-promoting reflex in the in situ preparation. Using these methods, our results indicate that stimulus intensities sufficient to modulate eupneic phrenic discharge pattern were also sufficient to alter phrenic discharge during gasping.

It could be argued that, although the gasplike burst pattern observed in this in situ perfused preparation is altered by pulmonary inflation, such patterns are not homologous to gasping in vivo. However, this argument would ignore a now extensive body of findings as to the similarity of fictive gasping in situ and gasping in vivo. Included in this evidence are the following changes, compared with eupnea, on exposure to severe hypoxia or ischemia in vivo and in situ: 1) similar alterations to a decrementing discharge pattern of the phrenic nerves, 2) a similar reduction or elimination of neural activities during neural expiration, 3) a similar shift to the high-frequency oscillations in inspiratory neural activities, and 4) similar patterns after transections of the brain stem at the pontomedullary junction (27–31). Thus we submit that the in situ preparation does exhibit distinctly different patterns of ventilatory activity comparable to eupnea and gasping in vivo.

Perspectives
A switching model that describes the genesis of gasping predicts that hypoxia or ischemia alters the breathing pattern from eupnea to gasping by the oxygen-sensitive suppression of postsynaptic inhibition. More specifically, inhibition impinging on neurons of the gasping center-pre-Bötzinger complex is reduced, allowing the release of pacemaker mechanisms for gasping from the pontomedullary neuronal circuit for eupnea (23, 26, 31). In a similar context, we have recently reported that, as opposed to eupnea, the neurogenesis of gasping is not dependent on inhibitory synaptic transmission (29, 31).
Hering-Breuer reflexes require integration of vagal afferent information by neurons in discrete regions, including the nucleus tractus solitarius, and involve both excitatory and inhibitory synaptic transmission (2, 6, 7, 14, 34, 35). Furthermore, it is apparent that Hering-Breuer-type expiratory promotion persists during gasping. This may reflect the influence of excitatory synaptic transmissions that persist despite oxygen-sensitive suppression of postsynaptic inhibition. If such modulation is specifically dependent on inhibitory mechanisms, however, such mechanisms must persist to some degree during gasping. We believe that either of two interrelated factors might account for these seemingly conflicting observations. First, a complete removal of all inhibition may not be necessary to release gasping and, while depressed, a sufficient stimulus may transiently reactivate specific neuronal pathways that include inhibitory synaptic transmission. Evidence for the former is the observation that stimulation of the superior laryngeal nerve causes a diminution in frequency during hypoxia-induced gasping (11). In addition, tissue conditions that restrict postsynaptic inhibition may not be uniformly present during the relatively brief periods of hypoxia or ischemia. Tissue PO2 and pH in this preparation are normally relatively homogeneous (36). Conceivably, tissue conditions could become heterogeneous during hypoxia or ischemia, conserving critical inhibitory processes in neuron populations involved in the integration of pulmonary afferent information, while restricting inhibitory processes in other regions. Measurements of such heterogeneity, however, have not been made.

The present results have significant implications for interpretation of findings from en bloc in vitro preparations of the neonatal rat. Since first introduced, the relationship between the rhythmic discharges of this preparation and eupnea or gasping in vivo or in situ has been controversial (see Refs. 1, 21, 22, and 25 for discussion). Recently, lung inflation or electrical stimulation of vagal afferents in a lung-attached in vitro neonate rat preparation was demonstrated to prolonged expiration (1, 10). Given the presumption that gasping is not modulated by pulmonary stretch receptor feedback, the authors interpret this finding as strengthening the link between fictive respiration in the in vitro preparation and eupnea in vivo. Our results demonstrate that lung inflation prolongs expiration during both eupnea and gasping. Thus modulation of an in vitro fictive respiratory pattern by lung inflation cannot exclusively link such a pattern to eupnea or exclude it as gasping.

Our results indicate that the neural elements underlying the modulation of breathing by pulmonary stretch are preserved in this preparation and support a conclusion that both eupnea and gasping are similarly modulated by pulmonary stretch receptor feedback. These observations contradict the generally held supposition that gasping is insensitive to such feedback.

We are grateful for the assistance of J. F. R. Paton in the formulation of this study.

This research was supported by National Heart, Lung, and Blood Institute Grant HL-26091.

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