Phrenic motoneuron discharge during sustained inspiratory resistive loading

STEVE ISCOE
Department of Physiology, Queen's University, Kingston, Ontario K7L 3N6, Canada

Iscoe, Steve. Phrenic motoneuron discharge during sustained inspiratory resistive loading. J. Appl. Physiol. 81(5): 2260–2266, 1996.—I determined whether prolonged inspiratory resistive loading (IRL) affects phrenic motoneuron discharge, independent of changes in chemical drive. In seven decerebrate spontaneously breathing cats, the discharge patterns of eight phrenic motoneurons from filaments of one phrenic nerve were monitored, along with the global activity of the contralateral phrenic nerve, transdiaphragmatic pressure, and fractional end-tidal CO₂ levels. Discharge patterns during hyperoxic CO₂ rebreathing and breathing against an IRL (2,500–4,000 cmH₂O·l⁻¹·s) were compared. During IRL, transdiaphragmatic pressure increased and then either plateaued or decreased. At the highest fractional end-tidal CO₂ common to both runs, instantaneous discharge frequencies in six motor axons were greater during sustained IRL than during rebreathing, when compared at the same time after the onset of inspiration. These increased discharge frequencies suggest the presence of a load-induced nonchemical drive to phrenic motoneurons from unidentified source(s).

CONTRADICTION RESULTS EXIST concerning the effects of diaphragmatic fatigue on the electromyographic (EMG) activity of spontaneously contracting ischemic canine diaphragm; it may either decrease (29) or increase (30). In the former study, section of the phrenic nerve innervating the isometrically contracting strip of diaphragm increased the activities of the contralateral diaphragm and the ipsilateral efferent phrenic nerve; this suggests that phrenic afferents activated by phrenic receptors inhibit phrenic motoneurons innervating both hemidiaphragms. On the other hand, diaphragmatic activity of the isotonically contracting diaphragm in the latter study increased. The divergent results between the two studies may reflect not just differences in mode of contraction but also the moderate to severe respiratory acidosis [pH 7.22 and 7.05 at arterial PₐCO₂ (PₐCO₂) of 55 and 76 Torr, respectively] in the former study; in the latter, arterial pH was low (−7.29) and PₐCO₂ was normal (−40 Torr). Both groups, however, attributed their results to activation of afferents in the phrenic nerves.

The canine diaphragm is supplied by the internal mammary, costophrenic, and phrenic arteries (4). Perfusion increases when the diaphragm is subjected to external loading (20) and during fatiguing contractions (28). Thus, under conditions in which the diaphragm is loaded, as commonly occurs, diaphragmatic perfusion is probably adequate; hence, loading, not ischemia, may represent a more appropriate stimulus to diaphragmatic receptors.

The objective of the present study was to determine whether sustained inspiratory resistive loading (IRL) elicits changes in phrenic motoneuron activity independent of changes in chemical drive. Decerebrate cats were used to avoid anesthesia-induced depression of spinal or supraspinal reflex control of phrenic motoneurons. Discharge patterns were monitored during both IRL and CO₂ rebreathing, the latter being used to permit differentiation of the effects of the load from those secondary to CO₂ accumulation caused by IRL-induced hypoventilation. The discharge patterns were compared at similar fractional end-tidal CO₂ levels (FETCO₂). Both IRL and CO₂ rebreathing were conducted under hyperoxic conditions, which reduce or silence carotid chemoreceptor discharge (18).

METHODS

Experiments were conducted on 10 cats of either sex weighing 3.4–4.0 kg. After induction of anesthesia with an intravenous injection of a mixture of alfaxalone and alfadalone acetate (9 and 3 mg/kg, respectively; Saffan, Pitman-Moore), a tracheal cannula was inserted just below the larynx; anesthesia was maintained with halothane in oxygen. A femoral artery was cannulated to monitor blood pressure. After the external carotid arteries were ligated, the cat was placed in a stereotaxic frame and decerebrated at the midcerebellar level; halothane was then discontinued, and the cat breathed room air.

The cat was then rotated to the supine position. Two catheters with attached latex balloons were placed in the abdomen and middle third of the esophagus to measure abdominal (Pab) and esophageal (Pes) pressures, respectively. The pressure difference, Pab – Pes, was taken as transdiaphragmatic pressure (Pdi).

Pools, filled with warmed mineral oil, were made for the phrenic nerves from the surrounding skin flaps. Both C₅ branches of the phrenic nerves were isolated and desheathed but left intact. The left phrenic nerve was placed over bipolar silver electrodes and its activity was amplified, band-pass filtered (0.03–10 kHz), and integrated (Paynter filter, time constant 100 ms); both raw and integrated signals were displayed on an oscilloscope and chart recorder. Phrenic activity served as an index of inspiratory drive.

A filament from the right phrenic nerve was dissected and placed across bipolar electrodes to test for activity. Unitary recordings allowed analysis of discharge frequencies (mean, peak, and instantaneous) of individual motor axons and determination of whether recruitment of previously inactive motor axons occurred, neither of which is possible when the phrenic neurogram or diaphragmatic EMG activity is recorded. When easily discriminated unit activity was present, the distal end of the filament was cut and wrapped around the proximal electrode; the distal electrode was then grounded to obtain a monopolar recording. To preserve input from diaphragmatic receptors, no more than approximately one-third of the right phrenic nerve was dissected and tested for filaments containing unitary activity.

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Once a satisfactory signal from an individual axon was obtained during “resting” ventilation, hyperoxic CO2 rebreathing was started. (Previous experience indicated that motion of the cat, and therefore loss of the filament, was less likely during CO2 rebreathing than during breathing against the IRL.) To reduce the rate of increase of CO2 and to permit averaging of discharge frequency data from several breaths at a given FETCO2, the volume of the rebreathing bag (initial concentrations 5% CO2-95% O2) was ≈2 liters. CO2 rebreathing continued until a FETCO2 of 0.10-0.12 was attained. Rebreathing was then discontinued, and the cat was allowed to breathe room air for 10–15 min. After the inspirate was changed to 50% O2-50% N2 and it was verified that this did not affect phrenic discharge (2–3 min), an IRL of 2,500–4,000 cmH2O·l−1·s−1 was added. The magnitude of IRL was chosen to elicit a substantial increase in Pdi but minimize motion of the cat; these values differed among cats. The maximum FETCO2 attained during IRL before loss of the filament determined the value of FETCO2 at which discharge patterns on IRL and CO2 rebreathing were compared.

All signals (Pdi, FETCO2, integrated phrenic activity, and activity of the phrenic motor neuron) were recorded on paper (model TA2000, Gould) and stored on tape (model DR886, NeuroCorder). Unitary discharges were monitored continuously on the chart recorder to verify that the recording originated from the same axon despite changes in spike amplitude (Fig. 1) associated with motion of the filament on the electrode. Mean discharge frequency was determined as the number of spikes less one per burst, divided by the duration of the burst. Peak frequency was computed as the reciprocal of the shortest interspike interval during a breath.

Values are expressed as means ± SD, and comparisons were made by using the appropriate tests (as described in RESULTS) for parametric or nonparametric data; a P value < 0.05 was considered significant.

RESULTS

All decerebrate cats, while breathing against the IRL and during CO2 rebreathing, had intermittent locomotor-type movements of the forelimbs. In 10 cats, recordings were obtained from 27 filaments but retained in only eight filaments in seven cats. Of the eight filaments, seven contained a spontaneously active unit; in the remaining filament, the unit was recruited soon after the onset of CO2 rebreathing and IRL. These eight filaments provided sufficient data to allow comparison of axonal discharge frequencies during both IRL and CO2 rebreathing. In these filaments, one additional unit was recruited during IRL. In the other 19 filaments, 5 instances of unambiguous recruitment occurred.

Breathing against the IRL continued until motion of the cat caused loss of the filament, thus terminating the run. The durations of breathing against the IRL varied greatly among cats: 5, 6, 7, 7, 18, 22, 22, and 133 min, corresponding to FETCO2 of 0.077, 0.104, 0.085, 0.078, 0.100, 0.081, 0.112, and 0.138. The durations of CO2 rebreathing were (same order as for IRL) 18, 10, 7, 13, 25, 10, 12, and 23 min. Application of IRL resulted in a rapid increase in Pdi to values averaging 53 ± 26 cmH2O; Pdi was either sustained (n = 3) or decreased (n = 4) during IRL. Measurements of Pdi were unsuccessful in one cat.

Control values of inspiratory, expiratory, and total breath duration (Ti, Te, and Tr, respectively) and respiratory frequency were 0.73 ± 0.16 s, 1.18 ± 0.43 s, 1.91 ± 0.55 s, and 33.7 ± 9.5 breaths/min, respectively. At the time of measurement of instantaneous discharge frequencies of phrenic motoneuronal discharge (see below), Ti decreased during rebreathing (0.57 ± 0.17 s) and increased on IRL (1.00 ± 0.19 s) [both P < 0.05; 1-way analysis of variance (ANOVA)]; Te did not change significantly during rebreathing (1.00 ± 0.68 s) but increased during IRL (1.59 ± 0.24 s; P < 0.05, Kruskal-Wallis 1-way ANOVA). Respiratory frequency did not change during rebreathing (45.9 ± 19.7 breaths/min) but fell during IRL (25.2 ± 5.0 breaths/min; P < 0.05, Kruskal-Wallis 1-way ANOVA).

The discharge patterns of one phrenic motor axon during control breathing (FETCO2 = 0.03), CO2 rebreathing (FETCO2 = 0.11), and breathing against the IRL (FETCO2 = 0.11) are shown in Fig. 1. During both rebreathing and breathing against the IRL, all aspects of respiratory output (unitary activity, whole phrenic nerve activity, and Pdi) increased. Mean and peak discharge frequencies of the motor axon depicted in Fig. 1 are plotted vs. FETCO2 in Fig. 2. At any given FETCO2, the mean and peak discharge frequencies were greater during breathing against the IRL. During resting ventilation, mean and peak discharge frequencies averaged 10.7 ± 4.0 and 14.2 ± 3.7 spikes/s (n = 7). During CO2 rebreathing, these values increased to 18.7 ± 2.7 and 29.5 ± 8.7 spikes/s; during IRL, they increased to 26.0 ± 4.2 and 57.2 ± 30.5 spikes/s (n = 8; 1 unit was recruited by CO2 rebreathing.
and IRL). These increases during IRL compared with those during CO₂ rebreathing, although significant (\(P < 0.05\), paired \(t\)-test) were due in part to the prolongation of Ti associated with breathing against an IRL (Fig. 1).

To permit comparisons of discharge frequencies during CO₂ rebreathing and breathing against IRL despite differences in Ti, I plotted instantaneous discharge frequencies vs. time during inspiration, at identical FETCO₂ values. Four examples are depicted in Fig. 3. Instantaneous discharge frequencies were greater during IRL than during CO₂ rebreathing in two motoneurons (Fig. 3, A and D). In the last half of inspiration during IRL, instantaneous frequency was irregular, with high frequencies evident (Fig. 3, A, B, and D; and one other unit).

This impression of higher discharge frequencies at iso-CO₂ levels was confirmed by comparing instantaneous discharge frequencies at end inspiration of six breaths during CO₂ rebreathing with those of six breaths at the same FETCO₂ during IRL. Frequencies during IRL were measured at the same time after the onset of inspiration as during CO₂ rebreathing. Of the eight motoneurons, six increased their discharge frequencies significantly (\(P < 0.01\), paired \(t\)-test or Mann-Whitney). In the other two, the discharge frequencies did not differ (\(P > 0.05\), paired \(t\)-test). For all eight motoneurons, the average instantaneous discharge frequency at iso-CO₂ and isotime increased significantly on IRL, from 23.4 ± 8.4 to 31.8 ± 8.3 spikes/s (\(P = 0.002\), paired \(t\)-test).

The higher frequencies during IRL compared with those during CO₂ rebreathing were not a function of time breathing against the IRL or rebreathing CO₂. In Fig. 3A, FETCO₂ was 0.11; the cat had rebreathed CO₂ for 23 min and breathed against the IRL for 47 min. In Fig. 3D, FETCO₂ was 0.07; the cat had rebreathed CO₂ for 76 s and breathed against the IRL for 93 s. The insets in Fig. 3, A–D, show six superimposed traces, three during CO₂ rebreathing and three during IRL, of integrated phrenic activity during the same breaths from which were measured the instantaneous discharge frequencies depicted. During IRL, inspiration was prolonged and peak integrated phrenic activity

![Fig. 2](https://example.com/fig2.png)

**Fig. 2.** Mean (A) and peak (B) discharge frequency of unit depicted in Fig. 1 as function of fractional end-tidal CO₂ concentration during CO₂ rebreathing (●) and IRL (○). Bars are 1 SD.

![Fig. 3](https://example.com/fig3.png)

**Fig. 3.** Instantaneous discharge frequencies of 4 phrenic motor axons from 4 different cats during 3 consecutive breaths during CO₂ rebreathing (●) and 3 consecutive breaths during IRL (○) at iso-CO₂ (values of fractional end-tidal CO₂ (FETCO₂) are indicated). Insets, traces of integrated phrenic activity for 6 breaths analyzed.
reached larger values. However, this difference cannot be attributed just to the prolongation of inspiration because the discrepancy between integrated phrenic activity was present even before the end of inspiration during CO₂ rebreathing. Integrated phrenic activity, measured at end inspiration during CO₂ rebreathing and at the same time during IRL doubled, on average, in six of the eight runs; in the other two, the shape of the integrated phrenic activity changed from a ramp to one convex to the time axis.

Changes in Pdi as a function of time for the same four cats depicted in Fig. 3 are shown in Fig. 4; the panels in Figs. 3 and 4 correspond to the same cats. Asterisks in Fig. 4 for both the CO₂ rebreathing and IRL responses indicate the times at which measurements of instantaneous discharge frequency at iso-CO₂ levels presented in Fig. 3 were made.

**DISCUSSION**

During prolonged breathing against severe IRL, six of eight phrenic motoneurons increased their instantaneous discharge frequencies compared with those during CO₂ rebreathing at iso-CO₂ levels. These results are similar to those of Osborne and Road (22), who observed that diaphragmatic EMG activity of anesthetized rabbits on IRL, with supplemental oxygen, is maintained despite a drop in inspiratory pressure. They are also reminiscent of those of Teitelbaum et al. (30), who observed a bilateral increase in diaphragmatic EMG during ischemia of the left hemidiaphragm in spontaneously breathing anesthetized dogs. They attributed the results to activation of phrenic afferents acting on supraspinal structures because both respiratory timing and alae nasi activity were altered.

Although there are many studies of the respiratory responses to IRL (see Ref. 32 for review), few provide data in which the effects of IRL are compared with CO₂ “controls.” Lopata et al. (19) concluded that reductions in inspiratory flow (and prolonged Ti) and abdominal muscle contraction in human subjects breathing against IRL improved diaphragmatic contractility (due to changes in the force-velocity and force-length relationships, respectively). In that study, the emphasis was on the role of respiratory mechanics in load compensation and not on the control of inspiratory motoneuron discharge. Although the decerebrate cats of my study also had prolonged Ti with reduced inspiratory flows, they exhibited an increased rate of rise of integrated phrenic activity (or instantaneous discharge frequency of individual motor axons) (Fig. 3), unlike the human subjects of Lopata et al. who had reduced rates of rise of integrated diaphragmatic activity while breathing against an IRL.

The cats of this study generated Pdi of ~50 cmH₂O, comparable to those generated by rabbits breathing against IRL (1, 22) but less than the maximum observed in human subjects (9). Despite the increase in phrenic activity, evident in both individual phrenic motoneurons and whole phrenic activity, Pdi declined in four of the seven cats in which it was measured. The reasons for this decline have been discussed by Osborne and Road (22); a depression of diaphragmatic contractility by CO₂ is unlikely because a decline in Pdi was not always apparent even at high FETCO₂ (e.g., at FETCO₂ = 0.11 in Fig. 4A). The decline in some cats may be related to a shift to other inspiratory muscles or an increase in end-expiratory lung volume because of failure of the abdominal muscles (21).

Source of increased phrenic motoneuron activity. Compared with instantaneous discharge frequencies during CO₂ rebreathing, IRL either increased (n = 6) or did not affect (n = 2) instantaneous discharge frequencies of phrenic motoneurons at a given FETCO₂; these results are reflected in the discharge of the whole phrenic.
nerve. This result does not support Younes’ conclusion (32) that “the increase in intensity of respiratory muscle activation (i.e., rate of rise) in conscious preparations [in response to loads] is almost entirely due either to chemical feedback or to cortical influences.” The data from the present study, in which cortical influences were removed by decerebration and comparisons of discharge patterns made at iso-CO₂ levels, indicate that an additional factor(s) contributes to increased phrenic motoneuron discharge. The source(s) of this increased drive is unclear but could include pulmonary, diaphragmatic, and intercostal and abdominal muscle (spindles and tendon organs) receptors.

In cats lightly anesthetized with pentobarbitone, increases in pulmonary slowly adapting receptor (SAR) feedback cause progressively more phrenic motoneuron discharge during inspiratory cycles with, compared with those without, lung inflation (7). In my cats, the increase in instantaneous phrenic motoneuron discharge during IRL was similar to the effects of SAR feedback because the disparity between phrenic discharge increased during inspiration (Fig. 3). However, for SARs to increase phrenic motoneuronal discharge during IRL requires that, at a given CO₂ level and inspired volume, they fire more during IRL than during CO₂ rebreathing. Although there are no data to support or refute this hypothesis, the smaller inspired volumes, lower flows, and reduced end-expiratory lung volumes during IRL (19) should all cause less SAR feedback. However, the increased transpulmonary pressures during IRL and distortion of the rib cage associated with breathing against loads are associated with activation of SAR with atypical discharge patterns (15), the effects of which on phrenic motoneuron discharge are unknown.

Pulmonary receptors, rather than diaphragmatic spindles, also appear to be responsible for the first-breath increase in phrenic or diaphragmatic motor activity during augmented inspiratory efforts. Shannon and Zechman (26) observed a first-breath increase in the mean discharge frequency of diaphragmatic motor units of anesthetized cats to added inspiratory flow resistances, but this increase was abolished by bilateral vagotomy. Inspiratory resistances also fail to elicit changes in diaphragmatic motor unit activity in vagotomized anesthetized rabbits (24).

The role of diaphragmatic receptors during sustained IRL has yet to be investigated. Typically, their discharge patterns have been studied during the first or second loaded breaths, before respiratory drive could change, and have been compared with those of un-loaded breaths. There are few diaphragmatic spindles, and, hence, their effects have generally been discounted. Moreover, they stop firing when unloaded by diaphragmatic contraction (2). However, they could exert an effect during IRL-induced decreases in end-expiratory lung volume, when the diaphragm is stretched.

Golgi tendon organs constitute a large fraction of the mechanoreceptors in the feline diaphragm (2) but are unlikely to explain the increase in phrenic motoneuron discharge observed in the present study. During inspiratory efforts against an occluded airway, which activates these receptors, the peak discharge frequencies of diaphragmatic motor units of unanesthetized rabbits do not change even though inspiratory duration more than doubles; when the rabbits were anesthetized, discharge frequencies increase by 56%, although inspiratory duration increases by 200% (24). After vagotomy, occlusion either does not affect or, at increased levels of respiratory drive, inhibits diaphragmatic motor unit discharge (24). These results are similar to those of Cuénod (6) and Corda et al. (5) in cats.

A more likely source of increased respiratory drive is from diaphragmatic receptors with small myelinated (group III) afferents; these constitute about one-third of all myelinated afferents in the feline phrenic nerve (8). Many respond weakly to contraction of the diaphragm, are located in the muscular rather than tendinous portion of the diaphragm, and also respond to injection of lactic acid and bradykinin (2). They excite inspiratory neurons of the dorsal and ventral respiratory groups (27) and, hence, would also increase phrenic activity. Although diaphragmatic contraction elicits only small increases in their discharge (2), their responses could be potentiated by even modest increases in concentrations of catabolites during loading; they thus could exert a disproportionate effect on phrenic motoneurons during loaded breathing, even if catabolite concentrations increase very little or not at all.

The diaphragm contains receptors that respond to metabolic conditions (2, 14, 17). But did IRL cause metabolic conditions that would have activated these afferents? Although I have no data to answer this question directly, three factors, combined with the hyperoxia used in my experiments, make it unlikely that IRL caused diaphragmatic ischemia. First, contraction was submaximal. Second, it was intermittent; perfusion would have occurred during expiration. Third, IRL (20) and prolonged fatiguing contractions (28) increase diaphragmatic blood flow substantially. Additional indirect evidence suggests that diaphragmatic ischemia, and fatigue, did not occur during IRL. When diaphragmatic fatigue is produced in cats by direct electrical stimulation of the muscle, the duration of the respiratory cycle (Tᵣ) increases along with the advent of discharge of small myelinated and unmyelinated afferents (16). In my cats, however, an increase in Tᵣ was observed in only one cat just before motion terminated the run. Thus IRL in my experiments was unlikely to have caused local metabolic conditions sufficient to have activated phrenic thin myelinated and unmyelinated afferents.

Activation of caudal intercostal afferents (23) causes a short-latency increase in phrenic activity (for review, see Ref. 25). If these receptors were repeatedly activated while breathing against an IRL occurred, they could contribute to increased phrenic motoneuron activity. However, activation of intercostal (and possibly abdominal) tendon organ afferents decreases activity of medullary inspiratory neurons and phrenic motoneurons, but selective activation of spindle afferents has no effect (3). Moreover, vagotomy abolishes the immediate
increase in diaphragmatic activity in response to inspiratory resistances, despite the presence of intact thoracic roots (26). Thus thoracic mechanoreceptors appear unlikely, on the basis of these early responses, to be responsible for the increase in phrenic motoneuron discharge observed in the present experiments. However, the role of mechanoreceptors of the chest wall remains unexamined during prolonged loading.

Comparison to limb motoneurons. My results differ from those from motoneurons or motor units of limb muscles that decrease their discharge frequencies during maximal (e.g., Ref. 31) or submaximal (12) sustained voluntary contractions, which cause ischemia. Receptor endings responsive to metabolic conditions in the muscle (13) inhibit the motoneurons (11, 31), reducing their discharge frequencies. This has been elegantly demonstrated by Gandevia et al. (10); during maximal contraction of the adductor pollicis in human subjects, the fall in discharge frequency of the motor units is prevented by local anesthetic blockade of small myelinated and unmyelinated afferents. Even though the diaphragm also contains receptors responding to metabolic conditions (14, 17), comparisons of phrenic with limb motoneurons may be inappropriate because, as indicated above, diaphragmatic contractions are intermittent, not sustained, and loading increases, not decreases, diaphragmatic perfusion (20, 28). A reflex inhibition of phrenic motoneurons by phrenic small myelinated and unmyelinated afferents, if the appropriate spinal circuitry exists, may only occur when perfusion is compromised (e.g., cardiac failure).

In conclusion, hyperoxic IR in decerebrate spontaneously breathing cats increases the discharge of phrenic motoneurons; this increase cannot be explained by the load-induced hypercapnia. The sources of this excitatory drive to the phrenic during IR are unknown.

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Address for reprint requests: S. Iscoe, Dept. of Physiology, Queen’s University, Kingston, Ontario Canada K7L 3N6.

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