Respiratory muscle reserve in rats during heavy exercise

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THE RESPIRATORY SYSTEM must generate adequate ventilation to maintain normal arterial blood-gas and pH homeostasis. A robust system has evolved that involves complex neural integration of respiratory muscle output to achieve effective chest wall displacement and subsequent pulmonary ventilation and gas exchange. Although the diaphragm is considered the primary inspiratory muscle of ventilation during quiet breathing, other respiratory muscles are also recruited (4). The relative contribution of these inspiratory muscles varies depending on posture, states of consciousness, and metabolic demand (10). The integrated responses by the respiratory muscles not only maintain arterial blood-gas and pH status but also minimize the work (or oxygen cost) of breathing (3).

During heavy exercise, minute ventilation increases substantially in response to increased metabolic demand. This exercise hyperpnea is associated with an increased work of breathing, as evidenced by a significant increase in respiratory muscle oxygen consumption (1), increased blood flow to the diaphragm (13), increased oxygen extraction by the diaphragm (16), and increased diaphragm muscle glycogen utilization (11).

The increased work of breathing during exercise is reflected in the significant increases in rat diaphragm muscle oxidative enzyme capacity as a consequence of whole body endurance training (7). This adaptation enhances aerobic production of ATP and delays the onset of skeletal muscle fatigue (5). However, despite these significant alterations in indexes of respiratory muscle work and positive adaptations associated with endurance exercise, it is unclear whether the respiratory pump muscles actually impose a limitation on peak whole body oxygen consumption (V\textsubscript{O\textsuperscript{2}peak}).

Therefore, the purpose of this study was to determine whether decreasing the amount of available respiratory muscle mass by either unilateral or bilateral diaphragm paralysis decreases V\textsubscript{O\textsuperscript{2}peak} in healthy untrained rats. We hypothesized that if the respiratory muscles as a whole reached their maximum capacity during heavy exercise, loss of one-half or all of the diaphragm, in healthy rats would have a deleterious impact on V\textsubscript{O\textsuperscript{2}peak}.

METHODS

Animals. Adult male Sprague-Dawley rats (wt 280–380 g) were randomly assigned to either a Sham (n = 6), unilateral (n = 7), or bilateral (n = 7) denervation (Dnv) group. The studies were approved by the University at Buffalo Institutional Animal Care and Use Committee. Rats were maintained on an alternating 12:12-h light-dark cycle and provided with water and commercial rat chow ad libitum.

Measurement of V\textsubscript{O\textsuperscript{2}peak}. All rats, drug free, were habituated to treadmill walking over a 1-wk period before measurement of V\textsubscript{O\textsuperscript{2}peak}. Measurement of V\textsubscript{O\textsuperscript{2}peak} was carried out in all animals before and 2 wk after surgery. Each rat ran on a modular treadmill (Columbus Instruments) at an initial speed of 10 m/min, 20% grade, followed by an increase in speed of 6 m/min every 3 min to the point of exhaustion (i.e., rats could no longer keep up with the belt speed). Airflow into the treadmill chamber was regulated at 5 l/min by a needle valve flowmeter. Gas sampling from the treadmill chamber was continuous at a rate of 400 ml/min and analyzed by a CO\textsubscript{2} (model CD-3A, Ametek) and O\textsubscript{2} (model S-3A, Ametek) analyzer. The analyzers were calibrated before each test with room air and a calibrated reference gas (17.6% O\textsubscript{2}-2.11% CO\textsubscript{2}-balance N\textsubscript{2}). Oxygen consumption was calculated as described by Brooks and White (2) and converted to standard temperature and pressure, dry (STPD).

Surgical procedures. After anesthesia with a mixture of xylazine (10 mg/kg) and ketamine (60 mg/kg) given intraperitoneally (0.1 ml/100 g), the phrenic nerves were isolated ventrally in the lower neck without compromise to the
overlying sternomastoid muscles. In the Sham group, the phrenic nerves were left intact; whereas in the unilateral Dnv group, a 2- to 3-mm segment of the right phrenic nerve was cut. The wound was closed with sutures and treated with a topical antibiotic cream (0.2% nitrofurazone), and the animals were allowed to recover. To ensure that the rats survived bilateral Dnv while under anesthesia, a thin strand of silk thread was looped under the intact left phrenic nerve. After the right phrenic nerve was cut, the incision was carefully closed while the ends of the thin silk strand looped around the left phrenic nerve were exteriorized. The two ends of the thread were pulled after the rats recovered partially from anesthesia, thereby achieving bilateral hemidiaphragm paralysis. All rats were injected once subcutaneously with 0.25 cc of antibiotic (Crystiben).

Verification of Dnv. After measurement of postsurgical $V_{\text{O}_2}\text{peak}$ 2 wk after surgery, each rat was reanesthetized as described above. Both halves of the diaphragm were widely exposed through an abdominal incision. Fine-wire electrodes were implanted in the costal region of the left and right hemidiaphragm. The electromyographic (EMG) signals were amplified, band-pass filtered between 100 Hz and 3 kHz, and then recorded on a chart recorder (model 5000, AstroMed). To verify Dnv under conditions of increased respiratory drive, diaphragm EMG was recorded while the animal made spontaneous efforts against an occluded airway for a period of 25–30 s.

Statistical analysis. A one-way analysis of variance with repeated measures was used to compare differences in $V_{\text{O}_2}\text{peak}$ among the Sham and the unilateral and bilateral Dnv groups before and after surgery. $P < 0.05$ was considered statistically significant.

RESULTS

Mean body weights of the Sham and Dnv groups are listed in Table 1. All rats gained weight over the 2-wk postsurgical period, ranging from 8.5% in the bilateral Dnv group to 11.4% in the unilateral Dnv group.

Presence or absence of Dnv was verified in all rats. In the Sham animals during spontaneous inspiration, visual signs of shortening of both sides of the diaphragm were associated with EMG activity (Fig. 1A). In the unilateral Dnv group (Fig. 1B), EMG activity in the right hemidiaphragm was absent and corresponded with visual signs of muscle paralysis (lengthening of the Dnv right hemidiaphragm during contraction of the contralateral side). In the bilateral Dnv group, EMG activity was absent in both hemidiaphragms but was present during inspiration in the parasternal intercostal (Fig. 2).

Pre- and postsurgical running time to exhaustion (Table 2) and maximal running speed did not differ in either the Sham or unilateral Dnv group. Additionally, compared with presurgery, postsurgical $V_{\text{O}_2}\text{peak}$ did not change in either the Sham or unilateral Dnv group (Table 2). The presurgery $V_{\text{O}_2}\text{peak}$ in the bilateral Dnv group averaged 92 ml·kg$^{-1}$·min$^{-1}$ and did not differ from that in either the Sham or unilateral Dnv groups. However, unlike in the latter two groups, $V_{\text{O}_2}\text{peak}$ decreased by $\sim$19% ($P < 0.05$) after bilateral Dnv. The decrease in $V_{\text{O}_2}\text{peak}$ was associated with a reduction in the running time to exhaustion (Table 2) and a similar percent decrease in maximum running speed. $V_{\text{O}_2}\text{peak}$ values in each animal are shown in Fig. 3.

Figure 1. Electromyographic (EMG) tracing of left and right hemidiaphragm from anesthetized Sham-operated (A) and unilaterally denervated (Dnv; B) rats.

Figure 2. EMG tracing of parasternal intercostal (A), right (B), and left hemidiaphragm (C) from an anesthetized, bilaterally Dnv rat.

<p>| Table 1. Body weight before and 2 wk after Sham, unilateral, and bilateral Dnv surgery |
|-----------------------------------|--------|--------------|------------------|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Presurgery</th>
<th>Postsurgery</th>
<th>%Change from presurgery</th>
</tr>
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<tbody>
<tr>
<td>Sham</td>
<td>6</td>
<td>321 ± 14</td>
<td>354 ± 11</td>
<td>10.3</td>
</tr>
<tr>
<td>Unilateral Dnv</td>
<td>7</td>
<td>317 ± 12</td>
<td>353 ± 8</td>
<td>11.4</td>
</tr>
<tr>
<td>Bilateral Dnv</td>
<td>7</td>
<td>331 ± 3</td>
<td>359 ± 6</td>
<td>8.5</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE; n, no. of rats. Sham, phrenic nerves intact; Dnv, hemidiaphragm denervation.
We hypothesized that the loss of one hemidiaphragm would significantly decrease exercise V˙O2peak as a result of decreased respiratory muscle reserve. However, despite unilateral hemidiaphragm paralysis, the rats achieved a V˙O2peak similar to that obtained before surgery. With complete diaphragm paralysis, V˙O2peak was decreased by ~19%. Therefore, the results of this study strongly suggest that the respiratory musculature has an adequate reserve to account for the loss of one-half of the diaphragm during maximal exercise.

Table 2. V˙O2peak and running time to exhaustion before and after Sham, unilateral, and bilateral Dnv surgery.

<table>
<thead>
<tr>
<th>Group</th>
<th>V˙O2peak (ml/kg/min)</th>
<th>Time to exhaustion (min)</th>
</tr>
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<tbody>
<tr>
<td>Sham</td>
<td>86.0 ± 13.4</td>
<td>142 ± 1.8</td>
</tr>
<tr>
<td>Unilateral Dnv</td>
<td>85.0 ± 5.2</td>
<td>142 ± 0.46</td>
</tr>
<tr>
<td>Bilateral Dnv</td>
<td>91.8 ± 5.6</td>
<td>124 ± 0.53</td>
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Values are means ± SE; n = 6 Sham and 7 unilateral and bilateral Dnv animals. Significant difference from control, P < 0.05.

DISCUSSION

Previous studies have examined the effect of diaphragm paralysis on resting arterial blood gases and respiratory rate in rats. In unanesthetized rats with bilateral hemidiaphragm paralysis, respiratory rate increased significantly shortly after bilateral hemidiaphragm paralysis was achieved by spinal cord section. In the intact rat, the respiratory muscles maintain adequate ventilation in response to altered breathing patterns. This alteration in recruitment strategy may also serve as a mechanism to increase breathing rate and recruit other respiratory muscles to maintain adequate ventilation under conditions where ventilation is inadequate. We hypothesized that the increase in ventilation to match increased metabolic demand in the bilaterally paralyzed rat would be less than that in the Sham animals.

For the rat to achieve a similar V˙O2peak with unilateral and bilateral Dnv, the animal must alter its breathing strategy and recruit other respiratory muscles to maintain adequate ventilation under conditions where ventilation is inadequate. We hypothesized that the increase in ventilation to match increased metabolic demand in the bilaterally paralyzed rat would be less than that in the Sham animals.

However, it is possible that the increase in ventilation to match increased metabolic demand in the bilaterally paralyzed rat may be less than that in the Sham animals.

As breath-by-breath ventilation in the Sham animals is higher than in the paralyzed rats, it is possible that the increase in ventilation to match increased metabolic demand in the bilaterally paralyzed rat may be less than that in the Sham animals.

To compensate for the loss of the diaphragm, the rat relies on other respiratory muscles. The diaphragm is primarily responsible for the respiratory muscles, and the other respiratory muscles have a lower reserve. Therefore, the loss of the diaphragm results in a decrease in respiratory muscle reserve. However, the other respiratory muscles have a lower reserve, and the increase in ventilation to match increased metabolic demand in the bilaterally paralyzed rat may be less than that in the Sham animals.

As breath-by-breath ventilation in the Sham animals is higher than in the paralyzed rats, it is possible that the increase in ventilation to match increased metabolic demand in the bilaterally paralyzed rat may be less than that in the Sham animals.
The recruitment strategy the rats in the present study adopt to compensate for the loss of one or both hemidiaphragms during exercise is unknown. However, on the basis of data obtained from resting rats, we can safely hypothesize that recruitment of both inspiratory and expiratory accessory respiratory muscles is greater. Further studies are necessary to determine the precise recruitment pattern employed by rats to achieve the necessary exercise hyperpnea.

In addition to alterations in recruitment strategy during breathing, other adaptations may occur to optimize ventilation. The effectiveness of the diaphragm depends, in part, on the extent of fiber shortening, which results in flattening of the diaphragm and subsequent lung expansion. Loss of an active hemidiaphragm will result in lengthening of the paralyzed muscle, thereby altering the extent of diaphragm flattening. In a separate group of rats, the concentration of collagen in the Dnv hemidiaphragm was increased by 40% 2 wk after Dnv (L. Gosselin, unpublished observation). Such an increase presumably results in increased stiffness of the Dnv hemidiaphragm and may assist diaphragm flattening during contraction of the intact contralateral side. However, further studies are required to support this idea.

The nature of the reduction in VO\textsubscript{2peak} is not entirely clear. Assuming all other factors remain constant, alveolar minute ventilation would have to decrease ∼35–45% to cause a 20% reduction in VO\textsubscript{2peak}. There was no difference in the slope of the oxygen cost of running before or after bilateral phrenic nerve transection. Thus the reduction in VO\textsubscript{2peak} in the bilaterally Dnv rats appears to be due primarily to a decrease in maximal running speed and time to exhaustion. Because arterial blood gases were not measured in this study, it is unknown whether these rats were hypoxic during treadmill running. Another possibility for the reduction in VO\textsubscript{2peak} is that efficiency of ventilation is markedly decreased without the diaphragm, and therefore the accessory chest wall muscles have a higher metabolic demand that “steals” blood flow from the locomotor skeletal muscles. We think this is unlikely because additional blood generally reserved for the diaphragm should be available for any increased work by the accessory respiratory muscles. This point is highly speculative, and future studies are required before any conclusive statement can be made.

The effectiveness of the diaphragm depends, in part, on its ability to generate force. A modest reduction (15–25%) in maximal specific force of the diaphragm muscle has been observed in several experimental models, including chronic obstructive pulmonary disease (14), hypothyroidism (9), aging (8, 20), and malnutrition (15). It has been speculated that such a reduction may significantly impair pulmonary gas exchange, especially during periods when the ventilatory demand is high. However, the results of the present study suggest that despite a 50% reduction in the available mass of the diaphragm, an adequate reserve exists in the remaining respiratory musculature to compensate for this loss. Moreover, even with complete diaphragm paralysis, rats are still able to achieve 80% of their presurgical VO\textsubscript{2peak} (with the diaphragm intact). These findings highlight the remarkable reserve of the respiratory muscle system to alter its recruitment strategy to compensate for the loss of part or all of the diaphragm.

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