Respiratory muscle reserve in rats during heavy exercise

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Gosselin, L. E., D. Megirian, J. Rodman, D. Mueller, and G. Farkas. Respiratory muscle reserve in rats during heavy exercise. J. Appl. Physiol. 83(4): 1405–1409, 1997.—The extent to which the respiratory pump muscles limit maximal aerobic capacity in quadrupeds is not entirely clear. To examine the effect of reduced respiratory muscle reserve on aerobic capacity, whole body peak oxygen consumption (V˙O₂peak) was measured in healthy Sprague-Dawley rats before and after Sham, unilateral, or bilateral hemidiaphragm denervation (Dnv) surgery. V˙O₂peak was determined by using a graded treadmill running test. Hemidiaphragm paralysis was verified after testing by recording the absence of electromyographic activity during inspiration. Before surgery, V˙O₂peak averaged 86, 87, and 92 ml·kg⁻¹·min⁻¹ for the Sham, unilateral, and bilateral Dnv groups, respectively. Two weeks after surgery, there was no significant change in V˙O₂peak for either the Sham or unilateral Dnv group. However, V˙O₂peak decreased ~19% in the bilateral Dnv group 2 wk after surgery. These findings strongly suggest that the pulmonary system in rats is designed such that during heavy exercise, the remaining respiratory pump muscles are able to compensate for the loss of one hemidiaphragm, but not of both.

hemiparalysis; diaphragm; denervation; peak oxygen consumption

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˙O₂peak).

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˙O₂peak 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˙O₂peak.

METHODS

Animals. Adult male Sprague-Dawley rats (wt 280–380 g) were randomly assigned to either a Sham (n = 7), 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˙O₂peak. All rats, drug free, were habituated to treadmill walking over a 1-wk period before measurement of V˙O₂peak. Measurement of V˙O₂peak 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 CO₂ (model CD-3A, Ametek) and O₂ (model S-3A, Ametek) analyzer. The analyzers were calibrated before each test with room air and a calibrated reference gas (17.6% O₂-2.11% CO₂-balance N₂). 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 VO$_{2\text{peak}}$ 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 VO$_{2\text{peak}}$ 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 VO$_{2\text{peak}}$ did not change in either the Sham or unilateral Dnv group (Table 2). The presurgery VO$_{2\text{peak}}$ 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, VO$_{2\text{peak}}$ decreased by ~19% ($P < 0.05$) after bilateral Dnv. The decrease in VO$_{2\text{peak}}$ was associated with a reduction in the running time to exhaustion (Table 2) and a similar percent decrease in maximum running speed. VO$_{2\text{peak}}$ values in each animal are shown in Fig. 3.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Presurgery</th>
<th>Postsurgery</th>
<th>%Change from presurgery</th>
</tr>
</thead>
<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 ± SE; n, no. of rats. Sham, phrenic nerves intact; Dnv, hemidiaphragm denervation.

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

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

We hypothesized that the loss of just one hemidiaphragm would significantly decrease exercise \( \text{V} \dot{\text{O}} \text{2peak} \) as a result of decreased respiratory muscle reserve. However, despite unilateral hemidiaphragm paralysis, the rats achieved a \( \text{V} \dot{\text{O}} \text{2peak} \) similar to that obtained before surgery. With complete diaphragm paralysis, \( \text{V} \dot{\text{O}} \text{2peak} \) was decreased by \( \sim 19\% \). Therefore, the results of this study strongly suggest that the respiratory musculature in rats has an adequate reserve to account for the loss of one-half of the diaphragm during maximal exercise, but not for both halves.

Previous studies have examined the effect of diaphragm paralysis on resting arterial blood gases and ventilatory response in rats. In unanesthetized rats with hemidiaphragm paralysis achieved by spinal cord hemisection, respiratory rate increased significantly from 78 breaths/min before surgery to 104 breaths/min 24 h after surgery (6). Pressures of arterial oxygen \( (\text{PaO}_2) \) and carbon dioxide \( (\text{PaCO}_2) \) were essentially unaffected (6). However, in unanesthetized rats with bilateral hemidiaphragm paralysis, \( \text{PaO}_2 \) significantly decreased by \( \sim 11 \) Torr, whereas \( \text{PaCO}_2 \) increased \( \sim 3 \) Torr (17). These changes in arterial blood gases were attributed to alterations in breathing pattern. At rest, bilaterally phrenicotomized rats responded by increasing breathing frequency at the expense of a lower tidal volume, thus increasing dead space ventilation (18).

For the rat to achieve a similar \( \text{V} \dot{\text{O}} \text{2peak} \) with unilateral Dnv, or to achieve even the 80% of \( \text{V} \dot{\text{O}} \text{2peak} \) observed after bilateral Dnv, the animal must increase ventilation to match increased metabolic demand. We were unable to measure minute ventilation in our system and therefore do not know to what extent peak exercise minute ventilation is affected as a consequence of unilateral Dnv. The results of this study suggest peak exercise minute ventilation in unilaterally Dnv rats is affected minimally, at worst. Maskrey et al. (18) reported that in awake, bilaterally Dnv rats exposed to hypoxia, minute ventilation increased to \( \sim 87\% \) of that observed in Sham animals. The increase in ventilation, however, appeared largely to be because of an increase in frequency rather than in tidal volume \( (\text{VT}) \) because the maximal \( \text{VT} \) in the bilaterally Dnv rats was only \( \sim 73\% \) of that in Sham animals.

To compensate for the loss of the diaphragm, the rat alters its breathing strategy and recruits other respiratory muscles to maintain adequate ventilation. At rest, bilaterally Dnv rats increase activity of the internal and external intercostal muscles during inspiration (19). In addition, activity of the external and internal oblique muscles also increases during expiration, presumably to cause caudal displacement of the lower rib cage and thus assist lung deflation. This alteration in recruitment pattern may also serve as a mechanism to increase breathing frequency. Changes in recruitment strategy during breathing at rest are also noted in the canine Dnv model. Although paralysis of a hemidiaphragm does not appear to alter the resting length of the intact hemidiaphragm, EMG activity of the intact hemidiaphragm increases significantly during inspiration and is associated with greater shortening (12). With bilateral paralysis, costal diaphragm end-expiratory length increases slightly from control, and costal shortening is significantly less compared with control and unilateral paralysis. Other respiratory muscles are also recruited to compensate for the loss of the diaphragm (12). For example, shortening of the transverse abdominis is significantly higher than control by 22.7 and 42.5% after unilateral and bilateral hemidiaphragm paralysis, respectively. Compared with control, shortening of the parasternal intercostal muscles also is significantly higher after both unilateral and bilateral hemidiaphragm paralysis. Because of the altered recruitment strategy, dogs at rest are able to maintain normal resting end-tidal CO2 levels after both unilateral and bilateral hemidiaphragm paralysis.

Table 2. \( \text{V} \dot{\text{O}} \text{2peak} \) and running time to exhaustion before and 2 wk after Sham, unilateral, and bilateral Dnv surgery.

<table>
<thead>
<tr>
<th>Group</th>
<th>Presurgery</th>
<th>Postsurgery</th>
<th>%Change From Presurgery</th>
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<tr>
<td></td>
<td>( \text{V} \dot{\text{O}} \text{2peak}, \text{ml·kg}^{-1}·\text{min}^{-1} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>86.2 ± 1.3</td>
<td>89.2 ± 5.2</td>
<td>+3.5</td>
</tr>
<tr>
<td>Unilateral Dnv</td>
<td>87.5 ± 3.2</td>
<td>88.4 ± 5.6</td>
<td>+1.0</td>
</tr>
<tr>
<td>Bilateral Dnv</td>
<td>91.8 ± 4.3</td>
<td>74.5 ± 3.5*</td>
<td>−18.8</td>
</tr>
<tr>
<td></td>
<td>Time to exhaustion, min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>14.2 ± 0.48</td>
<td>14.2 ± 0.48</td>
<td>0</td>
</tr>
<tr>
<td>Unilateral Dnv</td>
<td>14.0 ± 0.22</td>
<td>14.0 ± 0.53</td>
<td>0</td>
</tr>
<tr>
<td>Bilateral Dnv</td>
<td>13.4 ± 0.53</td>
<td>12.0 ± 0.82*</td>
<td>−10.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 6 \) Sham and 7 unilateral and bilateral Dnv rats, respectively. \( \text{V} \dot{\text{O}} \text{2peak} \), peak \( \text{O}2 \) consumption. *Significantly different from control, \( P < 0.05 \).
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 VO2peak 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 VO2peak. There was no difference in the slope of the oxygen cost of running before or after bilateral phrenic nerve transection. Thus the reduction in VO2peak 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 VO2peak 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 accessory respiratory muscles is 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 VO2peak (with the diaphragm intact). These findings highlight the remarkable reserve of the respiratory musculature, as well as the ability of the pulmonary system to alter its recruitment strategy to compensate for the loss of part or all of the diaphragm.

The authors are grateful to Dr. Frank Cerny for his input on this project.

This work was supported in part by grants from the American Federation for Aging Research and National Institutes of Health Grants HD-07423 and HL-43865.

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Received 10 April 1997; accepted in final form 23 July 1997.

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