Cardiovascular effects of the respiratory muscle metaboreflexes in dogs: rest and exercise

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Rodman, Joshua R., Kathleen S. Henderson, Curtis A. Smith, and Jerome A. Dempsey. Cardiovascular effects of the respiratory muscle metaboreflexes in dogs: rest and exercise. J Appl Physiol 95: 1159–1169, 2003. First published May 16, 2003; 10.1152/japplphysiol.00258.2003.—In awake dogs, lactic acid was injected into the phrenic and deep circumflex iliac arteries to elicit the diaphragm and abdominal muscle metaboreflexes, respectively. At rest, injections into the phrenic or deep circumflex iliac arteries significantly increased mean arterial blood pressure 21 ± 7% and reduced cardiac output 6 ± 2% and blood flow to the hindlimbs 20 ± 9%. Simultaneously, total systemic, hindlimb, and abdominal expiratory muscle vascular conductances were reduced. These cardiovascular responses were not accompanied by significant changes in the amplitude or timing of the diaphragm electromyogram. During treadmill exercise that increased cardiac output, hindlimb blood flow, and vascular conductance 159 ± 106, 276 ± 309, and 299 ± 90% above resting values, lactic acid injected into the phrenic or deep circumflex iliac arteries also elicited pressor responses and reduced hindlimb blood flow and vascular conductance. Adrenergic receptor blockade at rest eliminated the cardiovascular effects of the respiratory muscle metaboreflex. We conclude that the cardiovascular effects of respiratory muscle metaboreflex activation are similar to those previously reported for limb muscles. When activated via metabolite production, the respiratory muscle metaboreflex may contribute to the increased sympathetic tone and redistribution of blood flow during exercise.

diaphragm; lactic acid; afferent

FREE NERVE ENDINGS IN LIMB MUSCLE with centrally projecting axons depolarize when exposed to lactic acid, arachidonic acid, capsaicin, bradykinin, and potassium (22, 23, 24, 37, 41). Sufficient activation of limb muscle metaboreceptors in anesthetized animals reflexively increases arterial pressure, stroke volume, heart rate (HR), and renal vascular resistance (6, 17, 38). Type IV afferents have also been identified in the diaphragm (19, 48). Furthermore, phrenic type IV afferent activity was shown to increase at the onset of electrically induced diaphragm fatigue in the anesthetized rat (16, 20).

The aforementioned findings in anesthetized animals point to a potent role for respiratory muscle metaboreflexes, similar to those for limb muscle, in determining the distribution of blood flow when the diaphragm or accessory muscles of respiration undergo fatiguing contractions as occurs during heavy exercise (4, 21). However, it is not known whether there are cardiovascular effects of specific metaboreflex activation from the respiratory muscles in awake animals. This information is crucial to our understanding of the physiological role played by respiratory muscle metaboreflex feedback because anesthesia is known to have a significant inhibitory effect on respiratory and cardiovascular reflexes elicited by small-fiber afferents (19, 48). Furthermore, we wished to determine the effectiveness of the respiratory muscle metaboreflex on vascular conductance in exercising muscle.

Accordingly, the present study sought to determine whether diaphragm or abdominal expiratory muscle metaboreflex activation via lactic acid injection would increase blood pressure and reduce cardiac output (CO) or specific tissue blood flow in an awake animal at rest or during exercise. We also determined whether the cardiovascular effects of specific respiratory muscle metaboreflex activation were mediated by the sympathetic nervous system.

METHODS

Chronic Instrumentation

All surgical and experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin-Madison and conducted in accordance with the American Physiological Society’s “Guiding Principles in the Care and Use of Animals.” Ten female mongrel dogs weighing between 20 and 25 kg were trained to lie quietly on a bed and run on a motorized treadmill. After training, two surgical procedures separated by at least 2 wk were required to instrument the dogs for study. General anesthesia and strict sterile techniques were used; appropriate antibiotic and analgesics were used postoperatively. Ul-
transonic flow probes were installed around the ascending aorta, the deep circumflex iliac (DCI) artery, and terminal aorta (types 20A, 1.5RB, and 10A, respectively; Transonic Systems, Ithaca, NY). Catheters were placed in 1) the abdominal branch of the left phrenicobdominal artery such that the tip of the catheter was lateral to the junction with the phrenic artery and communication with the aorta was maintained; 2) the right DCI artery with the ligature between the abdominal aorta and catheter insertion site; and 3) the abdominal aorta via the inferior mesenteric artery; this catheter was used for control injections and systemic blood pressure measurement. Two pairs of bipolar electrodes were sewn into the crural portion of the right hemidiaphragm for measurement of muscle electrical activity. All cables, catheters, and electrode wires were exteriorized dorsally a few centimeters lateral to the lower thoracic spine.

All signals were digitized at 64 Hz and stored on the hard drive of a personal computer for subsequent analysis and on a polygraph (AstroMed K2G, West Warwick, RI). All ventilatory, blood flow, and blood pressure data were analyzed on a breath-by-breath or beat-by-beat basis by use of custom analysis software developed in our laboratory.

**Protocols**

The first experimental session was performed 72 h after the second surgery. Experimental testing sessions were separated by 3–4 days with no more than seven trials performed per session. Each animal performed the protocols described below over the course of 2–3 wk.

*Respiratory muscle metaboreflex at rest and exercise.* Boluses of dilute lactic acid solution were injected into the phrenic and DCI arteries separately to activate the diaphragm and abdominal expiratory muscle metaboreflexes, respectively. Lactic acid was chosen as the stimulus because it is normally produced by muscle during exercise in an intensity-dependent manner and also increases the firing of diaphragm and limb muscle type IV afferents (10, 37). The stock solution of lactic acid [30% (L(+)-lactic acid, Sigma Chemical, St. Louis, MO] was diluted with saline, sterile filtered, and then injected into the phrenic or DCI artery followed by a 5-ml flux of sterile heparinized saline. The initial dose used was 0.5 ml of 0.001 N lactic acid; if this dose did not produce a pressor response, then the volume of acid was doubled. If this dose had no effect, the concentration was increased to 0.01 N and the volume was reduced to 0.5 ml. Lactic acid injections were separated by ~10 min. If a dog showed any sign of perceiving the lactic acid injection such as movement of the head, trunk, limbs, or eyes, the data were discarded. A similar injection protocol was used during 2–3 min of steady-state treadmill exercise (3.5–5.5 km/h, 5% grade).

It is unlikely that our lactic acid injections (mean pH = 2.3) precisely mimicked the changes in acidity experienced at the site of the respiratory muscle metaboreceptors during heavy-intensity exercise (also see Discussion). On the basis of measurements of limb muscle interstitial pH during similar lactic acid injections into isolated hindlimb muscle (37), it is likely that diaphragm and abdominal muscle pH values were only reduced a few tenths of one unit below baseline.

**Role of the sympathetic nervous system in the respiratory muscle metaboreflex.** To determine whether the cardiovascular effects of respiratory muscle metaboreflex activation were mediated by the sympathetic nervous system, phrenic and DCI lactic acid injections were performed at rest before and after pharmacological blockade of the sympathetic nervous system in five dogs. Pharmacological blockade of the sympathetic nervous system was established by using phenolamine and propranolol (2 mg/kg iv each; Sigma Chemical). One control lactic acid injection was performed to confirm the pressor response, after which the blockers were administered. Thirty minutes after phenolamine and propranolol administration, lactic acid injections at the same dose as the preblock control tests were administered. In three of the dogs, we also confirmed the adequacy of sympathetic blockade by a lack of a pressure response to an intravenous bolus injection of 75 μg of phenylephrine, a dose that clearly elicited increases in MAP (23 ± 15 mmHg) before blockade.

**Control injections.** Three types of control injections were performed to determine whether the cardiovascular changes observed after lactic acid injection were in fact due to metaboreflex effects originating from the respiratory muscles: 1) lactic acid injections identical in volume and concentration to those eliciting transient pressor responses when made into the phrenic or DCI arteries were given intravenously via a forelimb vein to test for possible contributions from the heart, lungs, or carotid bodies, i.e., effects that might be expected if significant amounts of lactic acid recirculated after injection into the phrenic or DCI artery. 2) Saline injections equal in volume to the effective dose of lactic acid were made into the phrenic and DCI arteries to test for nonspecific effects arising from volume or pressure changes resulting from the injections. 3) Lactic acid injections equal in volume and concentration to the effective dose found for the diaphragm were made into the abdominal aortic catheter whose tip was just caudal to the origin of the phrenicobdominal artery. This addressed whether cardiovascular effects occurred because of a portion of the phrenic catheter lactic acid injection spilling over into the systemic arterial circulation via the thoracic aorta.

**Microsphere distributions.** As a terminal study, the animals were anesthetized, and 2.5 million 15-μm microspheres (2 ml) were injected into the phrenic or DCI arteries in a manner identical to the lactic acid injections. A few minutes after microsphere injection, the animals were euthanized and ~1-cm² tissue samples were obtained bilaterally in tissues throughout the body including the skin, mesentery, intercostals, gastrocnemius, gracilis, sartorius, vastus medialis, semimembranosus, quadratus lumborum, psoas major, rectus abdominus, internal and external oblique, transversus abdominus, kidneys, and costal and crural diaphragm. A blank tissue sample was obtained before microsphere injection. The tissue samples were prepared according to BioPAL protocol (BioPAL, Worcester, MA; also see Ref. 34). Briefly, samples were freed of connective tissue, rinsed with sansSaline solution to remove surface contaminants, minced, and placed into preweighed plastic vials, reweighed for sample wet weight, and then dried overnight. The dried samples were then sent to BioPAL for subsequent neutron activation and analysis via standard gamma-counting methods.

**Data Analyses**

The transient pressor responses elicited by the lactic acid injections were analyzed with custom-made computer software on a beat-by-beat basis for CO, HR, MAP, specific tissue blood flows, and vascular conductances. Crural diaphragm electrical activity (EMGdi) was amplified, band-pass filtered, rectified, moving time averaged (100-ms time constant, models BMA-931 and MA-821RSP, CWE, Ardmore, PA), and subsequently analyzed on a breath-by-breath basis for integrated activity, peak activity, and rate of rise.

For each individual variable, the mean values obtained during the preinjection period and at two time points during...
the postinjection period were compared by using a one-way ANOVA with repeated measures. Tukey’s post hoc tests with \( P < 0.05 \) were considered significant. The preinjection period values for each variable consisted of average values over the 30 s before injection. The postinjection metaboreflex period values for each cardiovascular variable were the average of five heartbeats, specifically the beat with the highest mean blood pressure value plus the two beats before and two beats after it. The heartbeats chosen were constrained to be within 20 s of completion of the acid injection. This time period was chosen to minimize secondary baroreflex effects caused by the lactic acid-induced pressor response. Diaphragm EMG variables were the mean of two consecutive breaths, both early in the pressor response (initial increase in BP) and at the peak of the pressor response.

The cardiovascular data for the exercising lactic acid injection trials were analyzed similarly to those at rest, except that, owing to the increased HR during exercise, the postinjection metaboreflex period values for each variable were the average of the heartbeat with the peak mean blood pressure and the three beats before and three beats after it.

The data confirming sympathetic blockade were analyzed during the 2 min immediately preceding and the 20–50 s period immediately following intravenous injection of phenylephrine. The 20- to 50-s period postinjection was chosen because this was the time period on nonblocked days in which the peak cardiovascular effects were observed.

RESULTS

Distribution of Microsphere Injections

Microspheres injected into the phrenic artery catheter (\( n = 3 \)) were only detected in the diaphragm. When expressed in counts per gram of tissue dry weight, a much greater percentage of the total counts (i.e., number of microspheres) was found in the ipsilateral crural region (53 ± 12%) compared with the ipsilateral costal (23 ± 7%) or contralateral crural (21 ± 6%) and costal regions (3 ± 4%). Microspheres injected into the DCI catheter (\( n = 3 \)) were found bilaterally and confined to the three most internal abdominal expiratory muscles with 43 ± 14% in the ipsilateral transverse abdominis, 14 ± 4% in the contralateral transverse abdominis, 25 ± 8% in the ipsilateral internal oblique, 5 ± 3% in the contralateral internal oblique, 10 ± 5% in the ipsilateral external oblique, and 3 ± 4% in the contralateral external oblique. Microspheres injected into the phrenic or DCI arteries were not found in any nonrespiratory muscles or other tissues. In addition, microspheres injected into the DCI were not detected in the diaphragm, and microspheres injected into the phrenic catheter were not detected in the abdominal expiratory muscles. In summary, these microsphere data support the contention that lactic acid injections into the phrenic or DCI arteries targeted primarily the diaphragm or abdominal expiratory muscles, respectively.

Diaphragm Metaboreflex at Rest

The typical response to injection of lactic acid into the diaphragm via the phrenic artery in an awake, resting dog is shown in Fig. 1. The bolus of lactic acid caused a rapid increase in MAP that was concomitant with, or slightly preceded by, decreases in specific tissue blood flows (hindlimbs and abdominal expiratory...
muscles) and CO. Arterial pressure and blood flow changes began 6 ± 4 s after the start of lactic acid injection. The peak changes in arterial pressure and specific tissue blood flows were reached 8 ± 3 s after they began to change and returned to baseline 9 ± 3 s after the peak changes for an average response duration of 17 ± 4 s.

**MAP.** The values obtained for resting MAP in the present study (legend of Fig. 2) are similar to values previously reported in awake, resting dogs (25, 31). Injection of lactic acid into the phrenic artery catheter at rest significantly increased MAP (+21 ± 7%; \( P = 0.002 \)) (Fig. 2).

**CO, HR, and stroke volume.** Resting CO, HR, and stroke volume in the present study (legend of Fig. 2) are similar to values previously reported in awake, resting dogs (25, 31). Coincident with the 21% increase in MAP elicited by lactic acid injection into the phrenic catheter, CO decreased significantly (−6 ± 2%; \( P = 0.001 \)) (Fig. 2). There was no significant effect on HR (−3 ± 5%; \( P = 0.234 \)) or stroke volume (−2 ± 5%; \( P = 0.614 \)) during the same time period (Fig. 2).

**Specific tissue blood flows and vascular conductances.** Resting terminal aorta blood flow, as well as total systemic and terminal aorta vascular conductance reported in the present study (legend of Fig. 2), are similar to values previously reported in awake, resting dogs (25, 31). Resting hindlimb and abdominal expiratory muscle blood flows averaged 20 and 1% of CO, respectively. Diaphragm metaboreflex activation significantly reduced hindlimb blood flow (−20 ± 9%; \( P = 0.007 \)), whereas abdominal expiratory muscle blood flow remained unchanged (−7 ± 8%; \( P = 0.103 \)) (Fig. 2). Total systemic (−22 ± 6%; \( P < 0.001 \)), hindlimb (−31 ± 9%; \( P < 0.001 \)), and abdominal expiratory muscle (−23 ± 10%; \( P = 0.011 \)) vascular conductances all decreased significantly (Fig. 2).

**Abdominal Expiratory Muscle Metaboreflex at Rest**

The typical response to injection of lactic acid into the abdominal expiratory muscles via the DCI artery in an awake, resting dog is shown in Fig. 3. Boluses of lactic acid injected into the DCI artery elicited similar responses to that seen after injection into the phrenic artery, including a rapid increase in MAP that was concomitant with, or slightly preceded by, decreases in specific tissue blood flows (hindlimbs and abdominal expiratory muscles) and CO. These changes began 6 ± 6 s after the start of lactic acid injection. The peak changes in MAP and specific tissue blood flows were reached 5 ± 1 s after they began to change and returned back to baseline 10 ± 2 s after the peak changes for a total duration of 15 ± 3 s.

**MAP.** Injection of lactic acid into the right DCI artery in resting dogs significantly increased MAP (+19 ± 5%; \( P = 0.011 \)) (Fig. 4).

**CO, HR, and stroke volume.** Coincident with the 19% increase in MAP after lactic acid injection into the DCI artery in resting dogs, CO was significantly decreased (−7 ± 2%; \( P = 0.001 \)) (Fig. 4). In contrast, there were no significant effects on HR (−3 ± 6%; \( P = 0.520 \)) or stroke volume (−4 ± 8%; \( P = 0.366 \)) during the same time period (Fig. 4).

**Specific tissue blood flows and vascular conductances.** Abdominal expiratory muscle metaboreflex activation significantly decreased hindlimb blood flow (−18 ± 13%; \( P = 0.024 \)), hindlimb vascular conductance (−33 ± 13%; \( P = 0.010 \)), and total systemic conductance (−22 ± 4%; \( P = 0.008 \)) (Fig. 4). Although there was a trend toward decreases in abdominal expiratory muscle blood flow in the contralateral DCI (−9 ± 11%; \( P = 0.264 \); 5 of 7 dogs showed decreases) and conductance (−25 ± 11%; \( P = 0.121 \); 7 of 7 showed decreases), neither achieved statistical significance (Fig. 4).

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**Fig. 2.** Diaphragm metaboreflex data at rest in 8 dogs. Each symbol represents the average value obtained in an individual animal with the group mean value indicated by the heavy horizontal line. *Significant change from preinjection value, \( P < 0.05 \). HR, heart rate; SV, stroke volume. Preinjection group mean ± SD values were as follows: MAP = 93 ± 13 mmHg; HR = 101 ± 22 beats/min; SV = 26 ± 9 ml; CO = 2,550 ± 500 ml/min. Blood flows: terminal aorta (HL) = 500 ± 100 ml/min and deep circumflex iliac (Ab) = 24.0 ± 15.4 ml/min. Conductances: total systemic (Sys) = 28.9 ± 7.2 ml-min⁻¹-mmHg⁻¹, HL = 5.5 ± 1.0 ml-min⁻¹-mmHg⁻¹, and Ab = 0.265 ± 0.164 ml-min⁻¹-mmHg⁻¹.
Control Testing

None of the control injections caused an increase in blood pressure or reduction in specific tissue blood flows. Four of ten dogs showed consistent increases in terminal aorta blood flow and concomitant systemic hypotension with the systemic arterial or intravenous lactic acid injections, likely a direct vasodilatory effect of the acid on hindlimb vascular smooth muscle.

Effects of Sympathetic Blockade

In resting, recumbent dogs, intravenous phentolamine plus propranolol significantly reduced MAP (−10 ± 4%; \( P = 0.03 \)) and hindlimb vascular conductance (−19 ± 6%; \( P = 0.05 \)). The mean responses to phenylephrine injection in the three dogs tested before blockade was a 23 ± 15% increase in MAP, a 19 ± 9% decrease in HR, a 4 ± 2% decrease in CO, and a 39 ± 7% decrease in hindlimb blood flow. An identical injection of phenylephrine performed on a different day after phentolamine propranolol administration had minimal and inconsistent effects on the aforementioned four variables: 5 ± 5% increase in MAP; 1 ± 4% increase in HR; 1 ± 5% increase in CO; 8 ± 19% increase in hindlimb blood flow.

The cardiovascular responses to diaphragmatic metaboreflex activation via lactic acid injection into the phrenic artery before and after sympathetic blockade are shown for one trial in an awake, resting dog in Fig. 5, A and B, respectively, with group mean values presented in Table 1. These data show that adrenergic receptor blockade completely eliminated the cardiovascular responses to phrenic artery lactic acid injection.

Effects of Exercise

The values for CO, stroke volume, hindlimb blood flow, and hindlimb vascular conductance obtained in the present study (legend of Fig. 6) are similar to values previously reported in dogs exercising at similar HRs (25, 28, 31). On average, CO, stroke volume, and HR increased 159, 69, and 49%, respectively, above

![Fig. 3. Polygraph tracing of 1 abdominal expiratory muscle metaboreflex trial (lactic acid injection into the deep circumflex iliac artery) in an awake, resting dog. Note the decreases in specific tissue blood flows despite the increased MAP. Arrow indicates beginning of lactic acid injection.](image)

![Fig. 4. Abdominal expiratory muscle metaboreflex data at rest in 4 dogs. Each symbol represents the average value obtained in individual animals with the group mean value indicated by the heavy horizontal line. *Significant change from preinjection value, \( P < 0.05 \). Preinjection group mean ± SD values were as follows: MAP = 87 ± 11 mmHg; HR = 116 ± 27 beats/min; SV = 25 ± 1 ml; CO = 2,680 ± 500 ml/min. Blood flows: HL = 600 ± 210 ml/min and Ab = 27.1 ± 16 ml/min. Conductances: Sys = 30.8 ± 3.5 ml·min⁻¹·mmHg⁻¹, HL = 6.8 ± 1.8 ml·min⁻¹·mmHg⁻¹, and Ab = 0.319 ± 0.0018 ml·min⁻¹·mmHg⁻¹.](image)
resting levels during steady-state treadmill exercise. From rest to exercise, hindlimb and abdominal muscle blood flow increased 276 and 7%, respectively, and hindlimb and abdominal muscle vascular conductances increased 299 and 10%.

**Respiratory Muscle Metaboreflex During Exercise**

The typical response to injection of lactic acid into the diaphragm via the phrenic artery in an exercising dog is shown in Fig. 6 and into the abdominal expiratory muscles via the DCI artery in Fig. 7. Boluses of lactic acid injected into the phrenic or DCI artery during exercise caused a rapid increase in MAP that was concomitant with or slightly preceded by decreases in specific tissue blood flows (terminal aorta and DCI artery) and CO. The rise in arterial pressure and reductions in muscle blood flows with lactic acid injection into the respiratory muscles during exercise began 5 ± 7 s after the start of injection. The peak changes in arterial pressure and specific tissue blood flows were reached 9 ± 3 s after they began to change and returned back to baseline 12 ± 4 s after the peak changes for an average duration of 22 ± 6 s.

Given the small number of observations of lactic acid-induced metaboreflex trials obtained during exercise and the fact that the effects of diaphragm and abdominal expiratory muscle metaboreflex activation were qualitatively similar at rest, metaboreflex responses obtained from both sets of muscles during exercise were combined for statistical purposes.

**MAP.** Injection of lactic acid into the diaphragm or abdominal expiratory muscles in dogs exercising on a treadmill significantly increased MAP (+17 ± 10%; *P* = 0.036) (Fig. 8).

**CO, HR, and stroke volume.** Coincident with the 17% increase in MAP after respiratory muscle metaboreflex activation with lactic acid injection during exercise, CO fell significantly (−9 ± 1%; *P* = 0.027) (Fig. 8). There were no significant effects on HR (−2 ± 8%; *P* = 0.723) or stroke volume (−3 ± 7%; *P* = 0.573) during the same time period (Fig. 8).

**Specific tissue blood flows and vascular conductances.** Injections of lactic acid into the diaphragm or abdominal muscles during exercise reduced hindlimb (−13 ± 9%; *P* = 0.042) and abdominal expiratory muscle (−9 ± 9%; *P* = 0.120) blood flows, but only the reduction in hindlimb flow reached statistical significance (Fig. 8). Hindlimb and abdominal muscle blood flows decreased in four of five dogs. Lactic acid injections during exercise significantly decreased total systemic (−18 ± 4%; *P* = 0.014), hindlimb (−23 ± 10%; *P* = 0.002), and abdominal expiratory muscle (−23 ± 8%; *P* = 0.029) conductances (Fig. 8).
Diaphragm Metaboreflex Effects on Breath Timing and EMGdi

The respiratory effects of diaphragm metaboreflex activation at rest are summarized in Table 2 with a typical response illustrated in Fig. 1. There were no significant effects of the diaphragm metaboreflex on breath timing. At the beginning of the pressor response, there was a trend toward a reduction in respiratory motor output (EMGdi) in four of five dogs, but these reductions were mostly <10% of baseline and statistically insignificant. At the peak of the pressor response, four of five dogs showed an increase in EMGdi, but again most of these increases were in the 5–10% range and the mean change was insignificant. There were no trends or significant changes in breath timing throughout the time course of the pressor response.

DISCUSSION

In awake dogs, lactic acid injected into the phrenic or DCI artery significantly increased MAP, while concomitantly reducing CO and hindlimb blood flow and vascular conductance both at rest and during mild- to moderate-intensity treadmill exercise. At rest, adrenergic receptor blockade eliminated the cardiovascular effects of lactic acid injection. Accordingly, we attribute the effects of injecting lactic acid into the phrenic or DCI arteries on blood pressure, CO, specific tissue blood flows, and vascular conductances to metaboreflexes originating in the diaphragm and abdominal expiratory muscles that increase sympathetic vasoconstrictor outflow. Significant reductions also occurred in hindlimb blood flow and vascular conductance when the respiratory muscle metaboreflexes were activated during exercise, thereby demonstrating that the strength of the reflex vasoconstriction was sufficient to overcome the abundance of metabolic vasodilators being produced in contracting limb muscles.

Evidence for a Respiratory Muscle Metaboreflex

There are several lines of evidence in anesthetized animals suggesting that the observed cardiovascular...
responses to injection of lactic acid into the diaphragm and expiratory muscles were probably mediated by unmyelinated phrenic afferents (10, 16, 20; see Introduction). Hussain et al. (17) investigated the systemic effects of phrenic artery capsaicin injections in anesthetized dogs. Like the present study, they reported a pressor response and decreased vascular conductance in renal and mesentery vascular beds, but unlike our findings they showed no effect on hindlimb blood flow or vascular conductance. It is unlikely that differences in the level of stimulation accounted for the discrepant results regarding the effects on limb vasculature because the magnitude of the increases in blood pressure via diaphragm metaboreflex activation was similar between the two studies. Perhaps lactic acid and capsaicin do not stimulate the same populations or types of muscle afferents.

Table 2. Effects of the diaphragm metaboreflex on breath timing and EMGdi at rest

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Post-Lactic Acid Injection (early)</th>
<th>Post-Lactic Acid Injection (peak)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1, s</td>
<td>1.58 ± 0.48</td>
<td>1.58 ± 0.29</td>
<td>1.67 ± 0.43</td>
</tr>
<tr>
<td>T2, s</td>
<td>1.68 ± 0.77</td>
<td>1.89 ± 1.19</td>
<td>1.83 ± 1.28</td>
</tr>
<tr>
<td>fb, breaths/min</td>
<td>20.6 ± 7.7</td>
<td>19.5 ± 6.0</td>
<td>19.7 ± 6.6</td>
</tr>
<tr>
<td>Int. EMGdi, au</td>
<td>0.68 ± 0.15</td>
<td>0.62 ± 0.26</td>
<td>0.81 ± 0.30</td>
</tr>
<tr>
<td>Rate of rise of EMGdi, au/s</td>
<td>1.17 ± 0.64</td>
<td>1.05 ± 0.61</td>
<td>1.24 ± 0.74</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>90 ± 8</td>
<td>96 ± 10</td>
<td>105 ± 12*</td>
</tr>
</tbody>
</table>

Values are group means ± SD (n = 5). Control, 30 s period before lactic acid injection; postinjection (early) values, 2-breath average during the initial rise in arterial pressure (duration 3.8 ± 0.8 s); postinjection (peak) values, the 2-breath average during the peak in arterial pressure (duration 3.6 ± 1.0 s). T1, inspiratory time; T2, expiratory time; fb, breathing frequency; Int. EMGdi, integrated diaphragm electrical activity; rate of rise of EMGdi, within-breath rate of rise of diaphragm EMG (peak/T1); au, arbitrary units. *Significantly different from preinjection.

Limbo vs. Respiratory Muscle Metaboreflex

As predicted, eliciting the respiratory muscle metaboreflex produced effects that were quite similar to the limb muscle metaboreflex in terms of the increased MAP and vasoconstriction evident in several systemic vascular beds (6, 28, 29). Similarly in humans, time-dependent increases in muscle sympathetic nerve activity and reductions in leg vascular conductance occurred in response to rhythmic handgrip (27, 42, 46) or diaphragmatic contractions (40, 43).

In contrast to a previously reported increase in CO on activation of the limb muscle metaboreflex via terminal aorta occlusion in exercising dogs (2), we found a small but consistent reduction in CO during diaphragm or abdominal expiratory muscle metaboreflex activation. We attribute our observed reduction in CO to the accompanying increase in systemic vascular resistance, with relatively small sympathetically mediated inotropic or chronotropic effects on the heart. Despite this difference between findings in limb compared with respiratory muscle metaboreflexes, we do not think they are due to fundamental differences between the reflexes from the two different sets of muscles. Specifically, careful inspection of the CO response to hindlimb occlusion in the exercising dog (2) reveals an early reduction as MAP began to rise; this occurred over the same time period we observed CO to fall after phrenic artery lactic acid injection. It was only over time as MAP rose further that CO increased during limb vascular occlusion. Accordingly, if our diaphragm metaboreflex had been sustained for a longer time period (as with the limb vascular occlusion), we might have also observed an eventual increase in CO.

Respiratory Muscle Metaboreflex Effects on Contracting Limb Muscle

Whether increased sympathetic activity results in vasoconstriction to contracting muscle depends on the
relative balance struck between adrenergic vasoconstrictor activity and local vasodilator in relative balance struck between adrenergic vasoconstriction and arterial pressure on activation of the metaboreflex.

In the present study, exercise was of mild to moderate intensity (1.6-fold increase in CO above rest) but clearly caused accumulation of local muscle metabolites as indicated by the threefold increase in terminal aorta vascular conductance (legend of Fig. 8). Activation of the respiratory muscle metaboreflex via lactic acid injection into the diaphragm or abdominal expiratory muscles was sufficient to consistently reduce exercising hindlimb vascular conductance. Furthermore, blood flow was also reduced to the exercising hindlimb despite the concomitant increase in MAP. These findings demonstrate that contracting locomotor muscles in the exercising dog are not immune to sympathetically mediated vasoconstriction, at least that induced by respiratory muscle metaboreflex activation. It is likely that the vasoconstriction caused by respiratory muscle metaboreflex activation in contracting limb muscle vasculature would have been less effective at higher exercise intensities.

Potential Role of Respiratory Muscle Metaboreflex and Sympathetic Activation on Blood Flow Distribution During Whole Body Exercise

Under what conditions might the respiratory muscle metaboreflex be activated during physiological exercise, and what influence would it have on the distribution of CO?

Two types of evidence suggest that the diaphragm or expiratory muscles must perform fatiguing levels of work before activation of the respiratory muscle metaboreflex and increased sympathetic vasoconstrictor outflow. First, Hill (16) and Jamieson and Balzamo (20) used phrenic nerve or diaphragm electrical stimulation to show that phrenic type IV afferents began to increase their activity only with the onset of diaphragmatic task failure. Second, in humans breathing against a resistive load, muscle sympathetic nerve activity did not increase nor did resting limb vascular conductance decrease until the diaphragm fatigued or expiratory muscles reached task failure (8, 40, 43). The diaphragm has been shown to fatigue after whole body exercise but only when the exercise intensity exceeded 80% of maximal oxygen uptake and was sustained until exhaustion (4, 21). Whether the expiratory muscles fatigue during heavy exercise is not known. Thus it seems likely that respiratory muscle metaboreflexes will only be activated in healthy individuals under normoxic conditions during high-intensity, sustained, whole body exercise. Respiratory muscle fatigue, and thus respiratory muscle metaboreflex activation, may occur at lower exercise intensities during exercise in hypoxic environments (3) or in patients with congestive heart failure (30) or chronic obstructive pulmonary disease (15) who expend high levels of energy to breathe, often combined with a compromised CO.

Once the diaphragm or expiratory muscle metaboreceptors are activated during heavy exercise, will they cause a redistribution of blood flow from the locomotor muscles to the respiratory muscles? Both the respiratory and locomotor muscles undergo substantial elevations in blood flow and vascular conductance during heavy and maximum exercise compared with rest (26). Significantly unloading the work of breathing in humans during maximal exercise by using a mechanical ventilator increased blood flow and vascular conductance to the working limbs (12), despite a concomitant reduction in CO (13). Furthermore, increasing the work of breathing during maximal exercise via resistive loading reduced limb blood flow and vascular conductance (12).

These data demonstrate that changes in the work of breathing can influence blood flow to the exercising limb, but two key questions remain unresolved. First, are the vasoconstriction and dilatation effects seen with changing respiratory muscle work mediated specifically by respiratory muscle metaboreflex activation and deactivation? Certainly this seems feasible given the present findings, although it is possible that changes in respiratory muscle work exert their systemic effects via baroreceptor resetting, an effect known to occur during limb muscle metaboreflex activation (32). Second, we do not know whether the contracting respiratory muscles would undergo less vasoconstriction than contracting limb locomotor muscles once sympathetic vasoconstrictor outflow increases during heavy exercise. There is clear evidence in anesthetized animals that both diaphragm and resting limb muscle vascular beds are responsive to modulations in sympathetic tone (7, 18), although recent evidence shows that isolated diaphragm vessels undergo less vasoconstriction in response to norepinephrine than those from limb muscles (1). However, these data do not speak to the relative sensitivities of respiratory vs. locomotor muscle vascular beds during in vivo whole body exercise.

Dissociation Between the Ventilatory and Cardiovascular Responses During Respiratory Muscle Metaboreflex Activation

In contrast to the significant cardiovascular effects of diaphragm metaboreflex activation observed in our awake dogs, lactic acid injected into the phrenic artery had no consistent effects on breath timing or electrical activity of the contralateral crural diaphragm. The lack of diaphragm metaboreflex-induced respiratory effects despite significant blood pressure elevation is in contrast to previous studies using anesthetized dogs or cats, with most of the studies reporting pressor responses but also significant increases in respiratory muscle electrical activity and breathing frequency. These previous reports also reveal that the direction of the respiratory motor output response to activation of type III and IV phrenic afferents depends on the type of
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stimulus used. Electrical stimulation (36), pharmacological stimulation with capsaicin (18, 35) or bradykinin (47), and diaphragm ischemia (44) all increased amplitude and frequency of phrenic nerve or diaphragm EMG activity. Motor activity increased to varying degrees in most inspiratory muscles including the diaphragm, parasternals, and abductor muscles of the upper airways (18, 47). On the other hand, Jammes and Balzammo (20) showed that when type IV phrenic afferents were stimulated in anesthetized cats via lactic acid injections, phrenic motor activity was either inhibited or showed no change. These findings are similar to what we report in the present study measuring EMGdi in the awake dog. Jammes and Balzammo also reported that diaphragm fatigue produced via repeated, direct electrical stimulation of the diaphragm increased phrenic afferent firing but reduced phrenic motor nerve activity and caused bradypnea.

Our findings of a dissociation between the cardiovascular and ventilatory response to phrenic afferent stimulation are in agreement with some recent reports on the effects of limb muscle mechano- and metaboreceptor stimulation in anesthetized, decerebrate cats. Whereas substantial increases in MAP and HR were obtained consistently in response to limb metaboreflex (11) or mechanoreflex activation (14), significant increases in ventilation were inconsistent. Taken together, these findings of qualitative disparities in ventilatory vs. cardiovascular reflex effects imply quite different sensitivities of the two control systems in response to the same sensory inputs.

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DISCLOSURES

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