Skeletal muscle chemoreflex and pH in exercise ventilatory control

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Oelberg, David A., Allison B. Evans, Mirko I. Hrovat, Paul P. Pappagianopoulos, Samuel Patz, and David M. Systrom. Skeletal muscle chemoreflex and pH in exercise ventilatory control. J. Appl. Physiol. 84(2): 676–682, 1998.—To determine whether skeletal muscle hydrogen ion mediates ventilatory drive in humans during exercise, 12 healthy subjects performed three bouts of isotonic submaximal quadriceps exercise on each of 2 days in a 1.5-T magnet for 31P-magnetic resonance spectroscopy (31P-MRS). Bilateral lower extremity positive pressure cuffs were inflated to 45 Torr during exercise (BLPPex) or recovery (BLPPrec) in a randomized order to accentuate a muscle chemoreflex. Simultaneous measurements were made of breath-by-breath expired gases and minute ventilation, arterialized venous blood, and by 31P-MRS of the vastus medialis, acquired from the average of 32 radio-frequency pulses at a repetition rate of 2.5 s. With BLPPex, end-exercise minute ventilation was higher (53.3 ± 3.8 vs. 37.3 ± 2.2 l/min; \( P < 0.0001 \)), arterialized PCO₂ lower (33 ± 1 vs. 36 ± 1 Torr; \( P = 0.0009 \)), and quadriceps intracellular pH (pHi) more acid (6.44 ± 0.07 vs. 6.62 ± 0.07; \( P = 0.004 \)), compared with BLPPrec. Minute ventilation decreased with BLPPex but did not change arterialized pH. For each subject, pHi was linearly related to minute ventilation during exercise but not to arterIALIZED pH. These data suggest that skeletal muscle hydrogen ion contributes to the exercise ventilatory response.

METHODS

Subjects. Twelve healthy adult subjects (7 men and 5 women), ages 24–51 yr [mean 33 ± 3 (SE) yr], were recruited from a hospital advertisement and gave consent to participate in this prospective, randomized, controlled investigation. All subjects were lifetime nonsmokers, with no known medical illness, and were taking no medication. Pulmonary function tests (spirometry, plethysmography, and single-breath diffusing capacity for CO) and a resting 12-lead electrocardiogram were performed within 1 wk of the study and were normal for all subjects. All participating subjects were asked to abstain from alcohol and caffeinated beverages for at least 12 h and strenuous physical activity for at least 24 h before the study and to have a light meal at least 2 h before testing. The study was approved by the Massachusetts General Hospital Subcommittee on Human Studies.

Despite much work in the area, the mechanisms whereby minute ventilation (Ve) is tightly linked to rapidly changing metabolic demands of exercising muscle remain uncertain (15). One explanation offered for intense exercise is lactic acid stimulation of the carotid bodies (51), but, under certain conditions, changes in arterial blood lactate and Ve can be uncoupled (6, 23, 26). This has led to a renewed interest in alternative pathways for ventilatory control during exercise.

One such pathway is the muscle chemoreflex or metaboreflex where by-products of exercising muscle metabolism are believed to stimulate group IV unmyelinated nerves communicating with regions of the central nervous system important in cardiorespiratory regulation. Evidence for such a reflex is strongest for cardiovascular function (1, 14), but a growing body of literature suggests it may be important for control of ventilation as well (5, 12, 16, 29, 32, 35, 42, 46, 52, 53). The presence of neural afferents that stimulate respiration has been established from several animal studies (5, 29, 32, 35, 42, 46, 52). The most compelling evidence comes from experiments involving crosscirculation of a neurally intact isolated hindlimb (29, 32, 52).

In an animal model, several metabolites produced by exercising skeletal muscle have been shown to stimulate ventilation via group IV afferents, including potassium (35, 52) and the cyclooxygenase products of arachidonic acid (41). Lactic acid seems to be the most potent metaboreflex stimulus, however (41, 42). In exercising humans, inhibition of limb venous return by positive pressure is associated with increased ventilation (12, 13, 16, 40, 45, 53), but the nature of the stimulus is unknown.

Until recently, measurement of skeletal muscle metabolite concentrations relevant to the chemoreflex required invasive and potentially destructive biopsy and electrode techniques, which are not particularly amenable to human study. 31P-magnetic resonance spectroscopy (31P-MRS), on the other hand, allows safe, continuous, noninvasive measurement of exercising skeletal muscle intracellular pH (pHi) in humans (2, 11, 49, 50). Most of the phosphorus in muscle is intracellular, and a 31P-MRS spectrum shows peaks for ATP, phosphocreatine (PCr), and Pi, the areas of which are proportional to their concentrations. As a function of decreasing pHi, protonated P becomes diprotonated and the net Pi peak resonates closer to that of PCr in a graded fashion (37). Pi measured by 31P-MRS is nearly identical to that measured by microelectrodes from a single cell (3, 22). We have recently adapted a metabolic cart for use in the fringe field of a 1.5-T magnet, allowing simultaneous measurement of large metabolic cart for use in the fringe field of a 1.5-T magnet, allowing simultaneous measurement of large muscle pH and breath-by-breath ventilation (19). We use these techniques in the present study to determine whether skeletal muscle pHi mediates the ventilatory chemoreflex in humans.
Exercise test protocol. Each subject completed two exercise protocols, separated by at least 5 days, by using an experimental setup that has been previously described (19). On each day, three bouts of constant-load exercise were performed, with 30 min of recovery time between bouts. Each bout consisted of 3 min of isotonic bilateral leg extension against a constant load equal to 40% of a previously determined maximal voluntary contraction. Leg extension was at 1 Hz and a duty cycle of 0.5, with the aid of auditory feedback.

On one day, bilateral lower extremity positive pressure (BLPP) was applied throughout each exercise bout and the first minute of recovery by using proximal thigh cuffs inflated to 45 Torr (BLPPex). On the other day, BLPP was applied only after the first minute of recovery for each exercise bout (BLPPrec). The order of BLPPex and BLPPrec was randomly assigned.

Physiological measurements. Expired gases and V̇e were measured by breath by using a commercially available metabolic cart (SensorMedics 2900c, Yorba Linda, CA) throughout 2 min of rest, exercise, and recovery.

Arterialized venous blood (21) samples were obtained from a superficial dorsal hand vein 20-gauge Teflon catheter. Heated water bottles were placed over the dorsum and volar aspects of the hand to maintain surface temperature between 41 and 44 °C. After the catheter dead space was cleared, 1-ml samples were collected over 30 s at 1-min intervals for 2 min of rest and 3 min of exercise. One-milliliter samples were collected at 30-s intervals for the first 3 min of recovery and then each minute for the remainder of the 10-min recovery period. Blood was analyzed for arterialized pH (pHa) and arterialized Pco2 (PaCO2) by using a pH/blood-gas analyzer (model 178, Ciba Corning Diagnostics, Norwood, MA) within 30 min of collection (34). Blood lactate concentration was measured by an enzymatic technique (Analox Instruments, Lunenburg, MA).

31P-MRS spectra were acquired from the vastus medialis by using an 8-cm-diameter radio-frequency surface coil fastened 7–8 cm proximal to the superior aspect of the patella overlying the anteromedial right thigh. The radio-frequency pulse length was adjusted to provide maximum signal at a repetition time (TR) of 2.5 s. By using a TR of 15 s, spectra were acquired over a 6-min baseline period before the test protocol. During the protocol, 31P-MRS spectra were typically collected from the average of four free induction decays, i.e., at 10-s intervals. One thousand twenty-four sample points were collected with a sweep width of 2.5 kHz.

A Borg category scale (8) was used to quantify the sensation of leg discomfort during and after exercise. Data analysis. Gas-exchange data were averaged over contiguous 30-s intervals. O2 uptake (V̇O2) and CO2 output (V̇CO2) were derived from standard formulas.

Processing of 31P-MRS data consisted of applying a 12-Hz Gaussian filter and zero filling before Fourier transformation. The spectra were uniformly phased as a group. Spectra were then averaged over contiguous 30-s intervals during rest, exercise, and the first 2 min of recovery; thereafter, they were averaged over contiguous 60-s intervals. δH was determined by measuring the chemical shift of the Pi peak relative to PCr according to the following equation, which was determined in our laboratory: δH = 6.85 + log [δ(3.56 – δH)/(δH – 5.64)], where δH is the chemical shift between the median area of PCr and Pi peaks in parts per million. Partial saturation of metabolite concentrations during the test protocol (TR = 2.5 s) was corrected by using peak areas obtained from the average of the 15-s TR spectra. Corrected PCr and Pi peaks were integrated, and the ratio of PCr area to that of Pi (PCr/Pi) was used as an estimate of phosphorylation potential of skeletal muscle mitochondria.

Unless otherwise stated, data are expressed as means ± SE. Comparisons among resting, exercise, and recovery data were made by using the Student’s two-tailed t-test. Correlations between continuous variables were determined by simple linear regression. Statistics were performed by using Statview software (Abacus Concepts, Berkeley, CA). P < 0.05 was considered significant.

RESULTS

At end exercise, V̇O2 was not affected by BLPPex (806 ± 30 vs. 762 ± 26 ml/min, BLPPex and BLPPrec, respectively; P > 0.05). PCr/Pi, however, was slightly reduced at end exercise with BLPPex (0.6 ± 0.1 vs. 0.8 ± 0.1; P < 0.05) but was not different before each subsequent bout of exercise or during recovery. V̇E was 43% higher at end exercise (53.3 ± 3.8 vs. 37.3 ± 2.2 l/min; P < 0.0001) during BLPPex compared with BLPPrec (Fig. 1). At end exercise, V̇E/V̇CO2 was higher (48 ± 2 vs. 41 ± 1; P < 0.0001) and PaCO2 (Fig. 1) lower (33 ± 1 vs. 36 ± 1 Torr; P = 0.0009) with BLPPex. The increase in V̇E at end exercise was mediated by changes in both respiratory rate (40 ± 2 vs. 34 ± 2 breaths/min; P = 0.002) and tidal volume (1.43 ± 0.10 vs. 1.13 ± 0.06 liters; P = 0.0007). The relative hyperventilation with BLPPex persisted for the first minute of recovery.

Although blood lactate concentration was slightly higher at end exercise with BLPPex, pHa was not different (Fig. 2). Blood lactate continued to rise on both days during the early recovery period at a time when V̇E was rapidly returning toward the resting level.

Skeletal muscle pHi became more acidic at end exercise with BLPPex (6.44 ± 0.07 vs. 6.62 ± 0.07; P = 0.004), and the difference persisted into early recovery (Fig. 3). The pattern of pHi fall and recovery mirrored in a reciprocal fashion the ventilatory response. V̇E was linearly related to pHi, but not to pHa, during exercise for individual subjects. Slopes and intercepts of the relationships are shown in Table 1 and are illustrated for two subjects in Fig. 4.

The sensation of leg discomfort was slightly higher with BLPPex at end exercise (Fig. 3) but was not different at any point during recovery. The difference in leg discomfort between BLPPex and BLPPrec at end exercise did not correlate with the difference in V̇E.

When data for BLPPex and BLPPprec were pooled, mean resting blood lactate concentrations for the second and third exercise bouts were elevated vs. the first bout (2.3 ± 0.2 vs. 1.2 ± 0.1 mM; P = 0.0003). Ve, pHi, and pHa, however, had returned to their respective baseline values. At end exercise, blood lactate for second and third bouts was higher compared with the first bout (3.9 ± 0.2 vs. 3.1 ± 0.2 mM; P < 0.05), but no difference was identified for V̇E, pHi, or pHa.

DISCUSSION

The control of ventilation during heavy exercise cannot be fully explained by lactic acid stimulation of arterial chemoreceptors (15). The present investigation was designed to evaluate an alternative neural pathway for exercise respiratory control, the ventilatory...
chemoreflex. It was postulated that, if skeletal muscle hydrogen ion is an important stimulus to the afferent limb of such a reflex, it should be so under conditions where pH\textsubscript{a} is not. The major finding of the investigation was a hyperventilatory response to BLPP during exercise and early recovery, which was not due to changes in pH\textsubscript{a}, pain, or central motor command induced by BLPP, but which was related to acid changes within the exercising muscle itself.

The application of positive pressure to the exercising limb has been used previously to investigate ventilatory control during exercise (4, 12, 13, 16, 40, 45, 48, 53). Hyperventilation has been found if the exercise is sufficiently intense to produce lactic acidemia (16, 45, 53). An older study that utilized limb positive pressure during mild exercise, but did not measure blood lactate, showed slight decreases in ventilation (4). This might suggest that muscle acidosis is necessary for the ventilatory metaboreflex.

During recovery from exercise, BLPP has been associated with an increase (40) or decrease (27, 43) in ventilation. Piepoli et al. (40) studied upper extremity exercise, whereas other investigators (27, 43) used leg exercise. Greater muscle acidosis is elicited by arm exercise at the same or lower workloads, compared with leg exercise (7, 39), and may explain these discrepant ventilatory results. Prior work therefore suggests, but does not prove, that muscle acidosis is an important stimulus to the ventilatory chemoreflex during exercise. To our knowledge, there is as yet no direct evidence to support this hypothesis in humans.

The mechanism by which BLPP decreases exercising skeletal muscle pH\textsubscript{i} was not specifically addressed by...
this study. Increased intracellular hydrogen ion results, during exercise, from narrowing of the strong ion difference by lactate anion accumulation and potassium efflux, increased concentration of weak acids, and elevated PCO₂ (33). The 45 Torr of positive pressure used in this study has been associated with a 12–15% reduction in muscle blood flow (44), and consequent reduction in O₂ may have increased lactate production and decreased in situ lactate metabolism (25). The depressed skeletal muscle PCR/Pi ratio, an index of a relatively stressed bioenergetic state (31) during BLPPex would support this mechanism. It is also tempting to speculate that transient slowing of venous return from exercising limb to the central circulation increased extracellular fluid lactate and PCO₂, which, in turn, blunted their respective active transport (28) and diffusion across the sarcolemma.

If skeletal muscle pH is an independent ventilatory stimulus during heavy exercise, it must be capable of influencing ventilation when other potential pathways such as lactic acidosis, central motor command, and noxiation cannot be implicated. In the present study, BLPP during exercise led to slightly higher concentrations of arterialized blood lactate, a finding noted by others (16, 53). The modest elevation in blood lactate at end exercise is an unlikely explanation for the observed hyperventilatory response, however. Casaburi et al. (10) have shown that, in normal humans, a 0.5 mM difference in arterial lactate concentration (which occurred between BLPPex and BLPPrec in the present study) would be expected to increase VE by only 3–4 l/min at most, whereas the observed difference was 16 l/min. In addition, during recovery, arterialized blood lactate continued to rise while VE was falling precipitously. Dissociation between blood lactate and VE was also identified when the three bouts of exercise were evaluated separately: blood lactate failed to return to baseline before repeat exercise bouts, whereas VE and pH, did. Similarly, end-exercise blood lactate was higher with repeat exercise bouts, but VE and pH, were not. In addition, relatively greater exercise hypocapnia with BLPP should have markedly blunted carotid chemoreceptor activity (47). Finally, and perhaps most importantly, if relative hyperventilation with BLPP were due to lactic acidosis, it should have been mediated by acidemia, but BLPP had no effect on pH during exercise. We conclude, as have others (6, 23, 24, 26, 38), that ventilatory drive during intense exercise is not solely mediated by lactic acid stimulation of arterial chemoreceptors. In fact, Pan et al. (38) showed that heavy exercise hyperventilation is accentuated, rather than attenuated, by carotid body denervation.

Could relative hyperventilation have resulted from the painful influence of limb positive pressure, as suggested by Comroe and Schmidt (13)? The chemoreflex in cat and noxiation are thought to be mediated by the same or closely related group IV unmyelinated afferents (30, 36). In the present study, leg discomfort was in fact slightly more pronounced at end exercise with BLPP but was not different during recovery when relative hyperventilation persisted. Therefore, leg dis-

**Table 1. Individual VE-pHi relationship during exercise**

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age, yr</th>
<th>Gender</th>
<th>Slope, l·min⁻¹·pH unit⁻¹</th>
<th>Intercept, l/min</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31</td>
<td>M</td>
<td>-19</td>
<td>153</td>
<td>0.59</td>
<td>0.0002</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
<td>F</td>
<td>-32</td>
<td>325</td>
<td>0.55</td>
<td>0.0006</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>M</td>
<td>-23</td>
<td>200</td>
<td>0.65</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>M</td>
<td>-23</td>
<td>423</td>
<td>0.36</td>
<td>0.03</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>F</td>
<td>-12</td>
<td>107</td>
<td>0.79</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6</td>
<td>51</td>
<td>M</td>
<td>-21</td>
<td>186</td>
<td>0.45</td>
<td>0.0061</td>
</tr>
<tr>
<td>7</td>
<td>34</td>
<td>M</td>
<td>-26</td>
<td>203</td>
<td>0.65</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>F</td>
<td>-38</td>
<td>292</td>
<td>0.66</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>9</td>
<td>31</td>
<td>M</td>
<td>-26</td>
<td>206</td>
<td>0.77</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>10</td>
<td>39</td>
<td>F</td>
<td>-17</td>
<td>139</td>
<td>0.60</td>
<td>0.0002</td>
</tr>
<tr>
<td>11</td>
<td>23</td>
<td>M</td>
<td>-57</td>
<td>462</td>
<td>0.67</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>12</td>
<td>23</td>
<td>F</td>
<td>-19</td>
<td>154</td>
<td>0.74</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

VE, minute ventilation; pHᵢ, intracellular pH; M, male; F, female.
comfort alone probably cannot explain the hyperventilatory response associated with BLPP. It is also conceivable that the hyperventilatory response to BLPP ex in the present study was mediated by receptors sensitive to changes in either pressure or volume within the limb capacitance vessels rather than by acid changes in the muscle. If this were the case, BLPP at 45 Torr applied during recovery should have led to relative hyperventilation. No difference was found for either $\dot{V}E$ or $P_{ACO2}$ during BLPP rec, however. Additionally, Piepoli et al. (40) have shown that limb circulatory occlusion at 200 Torr at rest is not associated with an increase in ventilation.

If perception of muscular effort with BLPP were greater, it might have been associated with increased muscle activation and concurrent stimulation of ventilation, a mechanism known as central motor command (17). This is a plausible mechanism, which may have contributed to the hyperventilatory response during exercise with BLPP because the sense of leg discomfort was higher and $PCr/Pi$, lower in this setting. Additionally, the persistent BLPP-associated hyperventilation in recovery when motor activation has ceased may have resulted from afterdischarge mediated by central motor command (18). In the present study, the difference in leg discomfort at end exercise was small compared with the difference in $\dot{V}E$, and the two were not correlated. Therefore, central motor command was probably not the predominant mechanism mediating the hyperventilatory response induced by BLPP.

A reflex evoked by a decrease in skeletal muscle pH i probably best explains hyperventilation during and after intense exercise when venous return to the central circulation is partially occluded. In this study, skeletal muscle pH i was more acid and ventilation greater during BLPP, and the two variables correlated well for individual subjects. Rotto et al. (42) have demonstrated that lactic acid injected into the femoral artery of anesthetized cats can increase activity of group IV neural afferents and augment ventilation, in keeping with the hypothesis that muscle pH mediates the ventilatory chemoreflex. The present investigation was designed to measure muscle pH i; however, the postulated chemoreflex is probably mediated more directly by muscle extracellular pH where the neural afferents are situated. Use of pH as a surrogate marker for extracellular pH is supported by a recent study by Evans et al. (20), who demonstrated a relationship between muscle pH and extracellular pH in a rat model of exercise. On the other hand, other investigators have demonstrated that other metabolites can stimulate this reflex, such as potassium (35, 52) and the cyclooxygenase products of arachidonic acid (41), and the present investigation cannot rule out a role for these variables.

The skeletal muscle chemoreflex is probably not the only mechanism capable of modulating the exercise ventilatory response. Perhaps the best evidence for the existence of redundant pathways derives from experiments involving paraplegics, in whom a near-normal ventilatory response to electrically stimulated exercise has been observed (9). Our data suggest, however, that exercise hyperventilation is induced by a chemoreflex stimulated by muscle acidosis.

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![Fig. 4. Plot of $\dot{V}E$ vs. quadriceps pH i during exercise in 2 representative individuals. Data for all 3 bouts of exercise performed with BLPP ex (□) and with BLPP rec (○) are included for each. Data for a 23-yr-old man are shown in top panel ($y = 462 - 57x; r = 0.67, P < 0.0001$), and data for a 50-yr-old woman are shown in bottom panel ($y = 107 - 12x; r = 0.79, P < 0.0001$).](http://jap.physiology.org/)


