Muscle metaboreflex improves O$_2$ delivery to ischemic active skeletal muscle

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O’Leary, Donal S., Robert A. Augustyniak, Eric J. Ansorge, and Heidi L. Collins. Muscle metaboreflex improves O$_2$ delivery to ischemic active skeletal muscle. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1399-H1403, 1999.—Ischemia of active skeletal muscle elicits a powerful pressor response, termed the muscle metaboreflex. We recently reported that the muscle metaboreflex pressor response acts to partially restore blood flow to the ischemic active skeletal muscle. However, because this reflex is activated by reductions in O$_2$ delivery rather than blood flow per se, gain of the muscle metaboreflex as analyzed on the basis of blood flow alone may underestimate its true strength if this reflex also acts to increase arterial O$_2$ content. In conscious dogs chronically instrumented to measure systemic arterial pressure, cardiac output, and hindlimb blood flow, we activated the muscle metaboreflex via graded, partial reductions in hindlimb blood flow during mild (3.2 km/h) and moderate (6.4 km/h, 10% grade) workloads. At rest, during free-flow exercise, and with metaboreflex activation, we analyzed arterial blood samples for Hb concentration and O$_2$ content and compared muscle metaboreflex gain calculations based on the ability to partially restore flow with those based on the ability to partially restore O$_2$ delivery (blood flow $\times$ arterial O$_2$ content). During both mild and moderate exercise, metaboreflex activation caused significant increases in arterial Hb concentration and O$_2$ content. Metaboreflex gain quantified on the ability to partially restore O$_2$ delivery was significantly greater than that based on restoration of blood flow during both mild and moderate workloads ($0.52 \pm 0.10$ vs. $0.39 \pm 0.08$, $P < 0.05$, and $0.61 \pm 0.05$ vs. $0.46 \pm 0.04$, $P < 0.05$, respectively). We conclude that the muscle metaboreflex acts to increase both arterial O$_2$ content and blood flow to ischemic muscle such that when combined, O$_2$ delivery is substantially increased and metaboreflex gain is greater when analyzed with a more integrative approach.

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mild exercise hindlimb perfusion must be reduced below a clear threshold before metaboreflex responses occur, during moderate exercise no apparent metaboreflex threshold exists, e.g., any reduction in hindlimb blood flow elicits reflex responses. Thus, at heavier workloads, the muscle metaboreflex may be tonically active and may contribute to the increase in sympathetic activity.

Whether the muscle metaboreflex acts to increase blood Hb levels has not been investigated. It is assumed that the metaboreflex pressor response is linked to O₂ delivery rather than blood flow, if even moderate increases in Hb concentration occur with metaboreflex activation, then the strength of control of the muscle metaboreflex may be underestimated via analysis of control of blood flow alone; that is, because O₂ delivery is a multiplicative function of arterial O₂ content and blood flow, even small increases in arterial O₂ content coupled with moderate increases in blood flow can elicit significant increases in O₂ delivery. In the present study, we measured arterial Hb levels and O₂ content during activation of the muscle metaboreflex and compared the metaboreflex closed-loop gains in terms of the ability to partially restore blood flow versus O₂ delivery. We found that increases in arterial Hb levels do occur with metaboreflex activation and that they act to increase significantly the functional gain of the metaboreflex in the ability to partially restore the deficit in O₂ delivery.

METHODS

Experiments were performed on six mongrel dogs (3 males, 3 females; 22–27 kg) selected for their willingness to run on a motor-driven treadmill. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee and conform to National Institutes of Health guidelines.

Surgical preparation. The animals were prepared in a series of surgical sessions with at least 1 wk allowed between surgeries and between the last surgery and the first experiment, as described in detail previously (10, 12, 17). Briefly, the animals were prepared with blood flow transducers (Transonic Systems) on the ascending aorta and the terminal aorta to monitor cardiac output (CO) and terminal aortic blood flow (TAQ), respectively. Distal to the TAQ transducer, a vascular occluder was implanted to mechanically restrict blood flow to the hindlimbs. Catheters were implanted in the aorta proximal to the flow probe, in the right jugular vein (advanced to the atrial-caval junction), and in side branches of the femoral artery and vein to measure systemic arterial pressure (SAP), central venous pressure (CVP), and femoral arterial pressure (FAP) and to infuse drugs unrelated to the present study, respectively. For studies unrelated to the present investigation, in five animals a blood flow transducer was also placed on the left renal artery, and in all animals aortic pacing electrodes were sutured to the apex of the left ventricle. All catheters and wires were tunneled subcutaneously and exteriorized between the scapulae. After each surgical procedure was completed, buprenorphine (0.015 mg/kg iv) and acepromazine (0.1 mg/kg im) were administered for analgesia and sedation, respectively. The animals were treated with cephalixin (500 mg iv) immediately pre- and postoperatively and with cephalotaxin (30 mg/kg po, bid) postoperatively to avoid infection.

Experimental procedures. All experiments were performed after the animals had fully recovered from surgery and were active, afebrile, and of good appetite. The animal was brought to the laboratory and allowed to roam freely for ~20 min. The animal was then directed to the treadmill, the blood flow transducers were connected to a flowmeter (Transonic Systems), and the catheters were connected to pressure transducers (Spectromed 10 E.Z. or Transpac IV, Abbott Laboratories). Heart rate was measured from the CO signal. All data were sampled with a laboratory computer at 1,000 Hz, and beat-by-beat mean values were saved to a hard disk for subsequent analysis.

The animals exercised at mild (3.2 km/h, 0% grade) or moderate (6.4 km/h, 10% grade) workloads. After all variables reached steady state, the terminal aortic vascular occluder was partially inflated to increase terminal aortic vascular resistance. After all variables had again reached steady state (3–6 min), another increase in vascular resistance was induced. At rest, during steady-state free flow exercise, and at steady-state with each level of partial vascular occlusion, arterial blood samples were drawn and blood gases, Hb concentration, and O₂ content were measured using a Radiometer ABL 500 blood gas analyzer interfaced to a Radiometer OSM3 hemoximeter.

Analysis of restoration of blood flow and O₂ delivery by the muscle metaboreflex. The objectives of this experiment were to determine 1) whether the muscle metaboreflex elicits increases in arterial Hb concentration and O₂ content and 2) whether the gain of the muscle metaboreflex in terms of the ability to partially restore O₂ delivery to the ischemic skeletal muscle exceeds that based on restoration of blood flow alone. Metaboreflex gain based on the ability to partially restore blood flow was calculated as described by O’Leary and Sheriff (12). Briefly, with each level of partial vascular occlusion, total hindlimb vascular resistance (R total) is increased. R total is calculated as (SAP – CVP)/TAQ. This includes both the mechanical (e.g., vascular occluder) and vascular components of the hindlimb vascular resistance. With each level of partial vascular occlusion, the predicted level of TAQ (TAQp) was calculated as TAQp = SAP/Ri, where SAP is the initial level of SAP before each level of occlusion. Thus the closed-loop gain of the muscle metaboreflex in the ability to partially restore blood flow to the ischemic active skeletal muscle (Gflow) can be calculated on the basis of the observed TAQ (TAQo) and TAQp, as Gflow = ([TAQo – TAQp]/[TAQo – TAQi]), where TAQ is the initial level of TAQ prior to each step increase in Ri. For example, if the reflex pressor response increased TAQo such that only one-half of the predicted increase in TAQ occurred, then Gflow = 0.5.

An analogous equation was used to calculate the closed-loop gain of the muscle metaboreflex based on the ability to restore O₂ delivery (G O₂). O₂ delivery to the hindlimbs was calculated as arterial O₂ content × TAQ. Thus the predicted and observed levels of O₂ delivery were calculated on the basis of the analogous values of TAQ and the observed levels of arterial O₂ content. The predicted level of O₂ delivery was calculated as the predicted level of TAQ times the O₂ content observed during free-flow exercise (e.g., no occlusion). Thus, if no changes in O₂ content occurred with metaboreflex activation, then G O₂ would equal Gflow. However, if the muscle metaboreflex acts to increase arterial O₂ content (e.g., via increases in blood Hb concentration or P O₂), then G O₂ would exceed Gflow.

Statistical analysis. The steady-state levels of all hemodynamic variables were averaged over 1 min. Immediately after this 1-min interval, the blood samples were drawn. At each workload, the predicted versus observed levels of TAQ and O₂
Table 1. Average values at rest and during free-flow exercise and levels observed during maximal
level of metaboreflex activation during mild and moderate exercise

<table>
<thead>
<tr>
<th></th>
<th>3.2 km/h (0% Grade)</th>
<th>6.4 km/h (10% Grade)</th>
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<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
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<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
</tr>
<tr>
<td>SAP, mmHg</td>
<td>93.0 ± 2.3</td>
<td>92.1 ± 2.4</td>
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<tr>
<td>HR, beats/min</td>
<td>90.2 ± 7.3</td>
<td>120.5 ± 7.5*</td>
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<tr>
<td>CO, l/min</td>
<td>3.71 ± 0.37</td>
<td>5.41 ± 0.55*</td>
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<tr>
<td>TAQ, l/min</td>
<td>0.69 ± 0.06</td>
<td>1.34 ± 0.14*</td>
</tr>
<tr>
<td>Arterial Po2, mmHg</td>
<td>99.3 ± 2.9</td>
<td>103.2 ± 1.6</td>
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<tr>
<td>[Hb], gm%</td>
<td>12.3 ± 0.4</td>
<td>13.1 ± 0.4</td>
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<tr>
<td>Arterial O2 content, vol%</td>
<td>17.0 ± 0.6</td>
<td>17.8 ± 0.5</td>
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<td>95.7 ± 3.6</td>
<td>105.9 ± 2.2*</td>
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<td></td>
<td>90.5 ± 7.5</td>
<td>181.2 ± 6.3*</td>
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<td>3.83 ± 0.29</td>
<td>9.33 ± 0.30*</td>
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<td>0.72 ± 0.06</td>
<td>3.13 ± 0.07*</td>
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<td></td>
<td>101.4 ± 1.6</td>
<td>103.2 ± 1.5</td>
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<tr>
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<td>12.6 ± 0.1</td>
<td>14.0 ± 0.2*</td>
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<td>17.0 ± 0.1</td>
<td>19.0 ± 0.2*</td>
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</table>

Values are means ± SE. During maximum level of metaboreflex activation (MR Max), levels were observed during mild (3.2 km/h, 0% grade) and moderate (6.4 km/h, 10% grade) exercise. SAP, systemic arterial pressure; HR, heart rate; CO, cardiac output; TAQ, terminal aortic blood flow; [Hb], Hb concentration. *P < 0.05, rest vs. exercise; †P < 0.05, exercise vs. MR Max.

delivery were compared during metaboreflex activation via Student’s t-tests. The effects of rest, exercise, and metaboreflex activation on arterial Po2, Hb concentration, and O2 content were compared by analysis of variance for repeated measures, and individual means were compared by the test for simple effects. The mean levels of Go2 and G flow were compared using Student’s t-tests. Five experiments were performed on four animals during moderate exercise during both mild and moderate workloads. No significant changes in arterial Po2 occurred with either the transition from rest to exercise or with metaboreflex activation during either mild or moderate workloads.

Figure 1 shows the average values of metaboreflex gain in terms of the ability to partially restore blood flow (Gflow) and O2 delivery (Go2) to the ischemic muscle during mild and moderate exercise. During both mild and moderate exercise, Go2 was significantly greater than Gflow. There were no significant differences between workloads on the levels of either Gflow or Go2.

DISCUSSION

The major new finding of this study is that in conscious dogs during dynamic exercise metaboreflex activation causes significant increases in arterial Hb concentration and O2 content such that the gain of the muscle metaboreflex quantified on the basis of the ability to partially restore O2 delivery is greater than that based on the restoration of blood flow.

The muscle metaboreflex is elicited by the activation of metaboreceptors within the skeletal muscle (14, 15). A number of putative substances have been implicated in initiating this reflex, including H+, lactate, and diprotonated phosphate (4, 5, 13, 20, 21). Using the same animal model as in the present study, Sheriff et al. (19) investigated whether the reflex response to skeletal muscle ischemia was due to insufficient washout of substances that activate metaboreceptors versus insufficient delivery of O2. They disassociated blood flow from O2 delivery via decreasing arterial O2 content with carbon monoxide and found that, based on blood flow, the relationship between the reflex response (e.g., increases in SAP and blood flow to the ischemic muscle was shifted toward higher flows, whereas the relationship between SAP and O2 delivery was not affected. They concluded that the muscle metaboreflex is elicited when O2 delivery falls below a threshold level, thereby causing the accumulation of metabolites due to the lack of sufficient O2.

Recently, O’Leary and Sheriff (12) quantified the extent to which the muscle metaboreflex can partially restore blood flow to the ischemic muscle as a method of assessing the closed-loop gain of the reflex. They con-
cluded that the muscle metaboreflex acts with a closed-loop gain of ~0.4–0.5, meaning that ~40–50% of the blood flow deficit is corrected by the reflex. That study, like the present study, relied on TAQ as an index of blood flow to skeletal muscle. In dogs, ~85% of iliac blood flow is directed to skeletal muscle at rest (2), and with the large increases in TAQ with even mild dynamic exercise this fraction must increase considerably. Thus the partial restoration of TAQ observed previously (12) is likely directed to the active skeletal muscle. However, Sheriff et al. (19) previously demonstrated that the reflex is activated by reductions in O2 delivery rather than blood flow per se. In the present study we found that with metaboreflex activation significant increases in arterial Hb concentration and O2 content occurred such that, when combined with the partial restoration of blood flow, metaboreflex gain calculated in the basis of the ability to restore O2 delivery was on average one-third greater than that based only on flow during both mild and moderate exercise. It should be noted that G02 may be underestimated in this experiment due to possible movement of fluid from the interstitium into the capillaries within the ischemic hindlimb because of the fall in hindlimb arterial pressure that occurs with partial vascular occlusion (8, 18, 24). This fluid movement would cause a decrease in Hb concentration rather than the observed increase. In settings in which the metaboreflex may be tonically active without partial vascular occlusion (e.g., severe exercise), G02 may be even greater.

Our data indicate that the mechanism mediating the rise in arterial O2 content with metaboreflex activation was the increase in Hb concentration because no change in arterial Po2 occurred. A likely mechanism for this increase in Hb concentration is metaboreflex-induced constriction of the spleen. In dogs, the spleen acts as a reservoir of red blood cells, and marked increases in hematocrit can occur with splenic constriction. Vatner et al. (22) observed that during severe exercise in dogs hematocrit increased from 40 to 49% and that this increase was abolished by splenectomy. Thus it is likely that with metaboreflex activation sympathetic activity to the spleen is increased. A previous study from our laboratory (17) showed that with metaboreflex activation right atrial pressure is maintained or increased despite the rise in cardiac output that would, by itself, decrease filling pressure, thereby indicating that this reflex increased central blood volume mobilization. Vasoconstriction of the compliant splanchnic circulation (which includes the spleen) could cause both increased blood volume mobilization and increased blood Hb concentration. Vatner et al. (22) also observed that during severe exercise after splenectomy much greater vasoconstriction in the mesenteric and renal vascular beds occurred. Inasmuch as the increase in arterial pressure with the exercise was identical before and after splenectomy, this greater sympathoexcitation after splenectomy is likely not a consequence of the arterial baroreflex; rather, it is possible that the delivery of O2 to the active skeletal muscle was decreased after splenectomy because no increase in hematocrit occurred with exercise. Reduced O2 delivery to the active skeletal muscle could elicit an increased sympathetic activity via the muscle metaboreflex.

In summary, muscle metaboreflex activation during dynamic exercise elicits a pressor response that acts to partially restore blood flow to the ischemic active skeletal muscle. In addition, this reflex also acts to increase blood Hb concentration and arterial O2 content. The combination of the increased flow coupled with the increased arterial O2 content results in a greater metaboreflex gain when analyzed on the basis of the ability to partially restore O2 delivery than that calculated only on the basis of blood flow. We believe that this integrative approach to analysis of the muscle metaboreflex better reflects the true strength of the reflex in the intact animal.

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