Muscle metaboreflex activation during dynamic exercise vasoconstricts ischemic active skeletal muscle


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Submitted 26 August 2015; accepted in final form 12 October 2015

Kaur J, Machado TM, Alvarez A, Krishnan AC, Hanna HW, Altamimi YH, Senador D, Spranger MD, O’Leary DS. Muscle metaboreflex activation during dynamic exercise vasoconstricts ischemic active skeletal muscle. Am J Physiol Heart Circ Physiol 309: H2145–H2151, 2015. First published October 16, 2015; doi:10.1152/ajpheart.00679.2015.—Metabolite accumulation due to ischemia of active skeletal muscle stimulates group III/IV chemosensitive afferents eliciting reflex increases in arterial blood pressure and sympathetic activity, termed the muscle metaboreflex. We and others have previously demonstrated sympathetically mediated vasoconstriction of coronary, renal, and forelimb vasculatures with muscle metaboreflex activation (MMA). Whether MMA elicits vasoconstriction of the ischemic muscle from which it originates is unknown. We hypothesized that the vasodilation in active skeletal muscle with imposed ischemia becomes progressively restrained by the increasing sympathetically vasoconstricted muscle during MMA. We activated the metaboreflex during mild dynamic exercise in chronically instrumented canines via graded reductions in hindlimb blood flow (HLBF) before and after α1-, adrenergic blockade [propranolol (50 μg/kg)], β-adrenergic blockade [propranolol (2 mg/kg)], and α1 + β-blockade. Hindlimb resistance was calculated as femoral arterial pressure/HLBF. During mild exercise, HLBF must be reduced below a threshold level before the reflex is activated. With initial reductions in HLBF, vasodilation occurred with the imposed ischemia. Once the muscle metaboreflex was elicited, hindlimb resistance increased. This increase in hindlimb resistance was abolished by α1-adrenergic blockade and exacerbated after β-adrenergic blockade. We conclude that metaboreflex activation during submaximal dynamic exercise causes sympathetically mediated α-adrenergic vasoconstriction in ischemic skeletal muscle. This limits the ability of the reflex to improve blood flow to the muscle.

exercise pressor reflex; ischemic skeletal muscle; muscle blood flow; metabolic vasodilation; sympathetically mediated vasoconstriction; β2-mediated vasodilation; α1- and β-blockade

NEW & NOTEWORTHY

We found that the muscle metaboreflex elicits vasoconstriction within the same ischemic active skeletal muscle from which the reflex originates, thereby creating a limiting positive feedback, which essentially amplifies the reflex responses. This vasoconstriction could limit the ability of the reflex to restore flow to ischemic muscle.

WHEN O2 DELIVERY TO THE active muscle is insufficient to meet O2 demands, metabolites (e.g., H+, adenosine, lactic acid, etc.) accumulate and activate chemosensitive group III and IV afferents (1, 6, 18, 21, 31, 37–39). Activation of these sensory nerves elicits reflex increases in sympathetic outflow, heart rate (HR), cardiac output (CO), ventricular contractility, and arterial blood pressure, termed the muscle metaboreflex (10, 16, 33, 38, 40, 42, 44).

When the muscle metaboreflex is engaged in normal subjects during submaximal dynamic exercise, the reflex raises arterial blood pressure primarily by increasing CO (10, 16, 34, 36, 40). This increase in CO raises the total blood flow available for tissue perfusion and thereby improves blood flow to ischemic muscle (12, 27, 29, 32). O’Leary and Sheriff (29) concluded that metaboreflex-mediated increases in CO and arterial pressure restore ~50% of the blood flow deficit induced by imposed partial reductions in muscle blood flow during dynamic exercise in canines. Similarly, Rowell et al. (32) concluded that metaboreflex activation during dynamic leg exercise in humans partially restores blood flow to ischemic active muscle. Sheriff et al. (35) concluded that the substances responsible for initiating the reflex accumulate due to reduced O2 delivery rather than a failure of adequate washout. As blood flow, and thereby the O2 delivery, to active muscle becomes insufficient, muscle afferent activity rises, and the metaboreflex elicits increases in CO, which partially restores the muscle blood flow. Therefore, the muscle metaboreflex is often regarded as a flow-sensitive, flow-raising reflex: engaged by suboptimal muscle blood flow and acting to increase total body blood flow (i.e., CO), which partially restores blood flow to underperfused active skeletal muscle (12, 27, 29, 32). Recently, our laboratory (19) concluded that muscle metaboreflex activation induces epinephrine release, causing β2-mediated vasodilation within skeletal muscle. This may be another potential mechanism that improves blood flow to ischemic muscle during exercise.

We and others have shown that metaboreflex activation elicits vasoconstriction of coronary, renal, and forelimb vasculatures (3, 4, 8, 22, 23). Furthermore, Joyner (17) suggested that the muscle metaboreflex may vasoconstrict ischemic active muscle as venous O2 saturation significantly declined with metaboreflex activation during rhythmic forearm exercise in humans. Whether the muscle metaboreflex vasoconstricts the ischemic active muscle from which it originates is debatable (26). The reflex increases in CO and mean arterial pressure (MAP) partially restore blood flow and O2 delivery to ischemic muscle (12, 27, 29, 32). However, this restoration could be attenuated by vasoconstriction within ischemic muscle. To address this controversy, we investigated the changes in vascular tone within ischemic muscle during metaboreflex activation. We hypothesized that with imposed reductions in skeletal muscle blood flow during exercise, metabolic vasodilation in ischemic skeletal muscle would be progressively restrained by...
metaboreflex-mediated increase in sympathetic activity. This vasoconstriction would be attenuated after α-blockade and potentiated after β-blockade.

METHODS

Experimental subjects. Six adult mongrel canines (~19–24 kg) of either sex were selected for the study. All animals were acclimatized to the laboratory surroundings and willing to run on a motor-driven treadmill. All methods and procedures used in the study were approved by the Institutional Animal Care and Use Committee of Wayne State University and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All animals exercised voluntarily during experimentation; no negative reinforcement techniques were used.

Surgical procedures. For each of the three surgical procedures, animals were sedated with acepromazine (0.4–0.5 mg/kg im) and received preoperative analgesics [carprofen (2.0 mg/kg iv), buprenorphine (0.01 mg/kg im), and fentanyl (75–125 µg/h, 72 h, transdermal delivery)]. Anesthesia was induced with ketamine (5.0 mg/kg iv) and diazepam (0.22 mg/kg iv) and maintained with isoflurane gas (1–3%). For postoperative care, animals were closely monitored and given acepromazine (0.5 mg/kg iv) and buprenorphine (0.05 mg/kg iv) as needed. To avoid acute postoperative infections, cefazolin (antibiotic, 30 mg/kg po bid) was administered prophylactically for the entire term of the experimental protocol. Animals recovered for 2 wk after each surgery.

In the first surgical procedure, the thoracic cavity was opened via a left thoracotomy (third/fourth intercostal space) approach, and the pericardium was cut to expose the heart. A perivascular flow probe (20PAU, Transonic Systems) was placed around the ascending aorta to measure CO. Due to the anatomic limitation for the aortic flow probe placement, coronary circulation was not included in the CO measurements. In dogs at rest, myocardial blood flow is ~6% of the total CO, and this fraction remains relatively unchanged across workloads (13, 24). A telemetry blood pressure transmitter (TA11 PA-D70, Data Sciences) was tethered subcutaneously at the height of the left ventricular apex and two intercostal spaces caudal to the thoracotomy incision. The tip of the pressure transducer catheter was inserted and secured inside the left ventricle to measure left ventricular pressure. Three pacing electrodes were secured to the right ventricular free wall for experiments unrelated to the present investigation. All wires were tunneled subcutaneously and exteriorized between the scapulae. The pericardium was reaproximated, and the chest was closed in layers.

In the second surgical procedure, an incision was made on the left flank cranial to the iliac crest to expose the abdominal aorta and left renal artery. Perivascular flow probes (Transonic Systems) were positioned around the terminal aorta (10PAU) and left renal artery (4PSB) to measure hindlimb blood flow (HLBF) and renal blood flow, respectively. All side branches of the terminal aorta between the iliac arteries and aortic flow probe were ligated and severed. Two hydraulic occluders (8–10 mm, DocXS Biomedical Products) were placed around the terminal aorta just distal to the flow probe. A 19-gauge polyvinyl catheter (Tygon, S54-HL, Norton) was inserted into a side branch of the aorta cranial to the flow probe to measure systemic arterial pressure. A second 19-gauge polyvinyl catheter was inserted into a side branch of the aorta caudal to the occluders to measure arterial pressure below the occluders. When the catheterization of a caudal aortic branch was not possible in this surgery, a catheter was placed into a side branch of the femoral artery in a third procedure. All instrumentation was tunneled subcutaneously and exteriorized between the scapulae, and the abdomen was closed in layers. For experiments unrelated to this study, a catheter was inserted into the right jugular vein (n = 2) and a hydraulic occluder was placed around each common carotid artery in a separate procedure.

Data acquisition. Each animal was brought into the laboratory and allowed to roam freely and acclimate for ~10–20 min after which it was directed onto the treadmill. The flow probe cables were connected to flowmeters (TS420, Transonic Systems). The two arterial catheters were aspirated, flushed, and connected to pressure transducers (Transpac IV, ICU Medical). All hemodynamic variables were monitored as real-time waveforms by a data-acquisition system (LabScribe2, iWorx) and recorded for subsequent offline analysis.

Experimental procedures. All experiments were performed after animals had fully recovered from surgery (i.e., were active and had a good appetite). Each experiment began with the animal standing still on the treadmill until all resting hemodynamic data were stable (typically 5–10 min). The treadmill was turned on, and the speed was gradually increased to 3.2 km/h at 0% grade. To activate the muscle metaboreflex, HLBF was reduced to ~40% of free-flow exercise levels via graded reductions (by partial inflation of terminal aortic occluders). Free-flow exercise and each level of vascular occlusion were maintained until all hemodynamic parameters reached steady state (typically 3–5 min). Control experiments were repeated in the same animals after α1-adrenergic blockade [prazosin (50 µg/kg intra-arterial)], β1-adrenergic blockade [propranolol (2 mg/kg intra-arterial)], and α1 + β1-blockade on separate days. Drugs were administered 20–30 min before the experiment, and subsequent experiments were not performed for at least 48 h. The large vasoconstrictor effect of phenylephrine (4 µg/kg) was completely abolished with a 50 µg/kg dose of prazosin. Preliminary studies showed that a 2 mg/kg dose of propranolol completely abolished the vasodilatory response caused by isoprotenerol (0.5 µg/kg). Animals performed the same exercise workload after each blockade as during control experiments.

Data analysis. MAP, femoral arterial pressure, HR, CO, and HLBF were continuously recorded during each experimental procedure. Hindlimb vascular resistance was calculated as femoral arterial pressure/HLBF. Vascular conductance to all vascular beds with the exception of the hindlimb [termed nonischemic vascular conductance (NIVC)] was calculated as follows: NIVC = (CO – HLBF)/MAP. One-minute averages of all variables were taken during steady state at rest, free-flow exercise, and each graded reduction in HLBF. Data were analyzed as initially described by Wyss et al. (44). Previous studies have shown that during mild exercise in canines, HLBF must be reduced below a clear threshold before the muscle metaboreflex is activated (4, 35, 44). Initial reductions in HLBF did not evoke metaboreflex responses, whereas reductions in HLBF below the threshold resulted in a significant pressor response. Therefore, the data were approximated to two linear regression lines: 1) an initial slope line that includes free-flow exercise and each reduction in blood flow that did not elicit reflex response and 2) a pressor slope line that includes reductions in HLBF that resulted in large pressor response (Fig. 2). The threshold for metaboreflex activation was approximated as the intersection of the initial and pressor slope lines. Mean values were averaged across all animals to obtain the sample mean of the study. Each animal served as its own control.

Statistical analysis. All hemodynamic data are reported as means ± SE. An α-level of P < 0.05 was used to determine statistical significance. Averaged responses for each animal were analyzed with Systat software (Systat 11.0). Two-way ANOVA with repeated measures was used to compare hemodynamic data for time and/or condition effects. In the event of a significant time-condition interaction, individual means were compared using the test for simple effects.

RESULTS

Figure 1 shows 5-s averages of MAP, CO, HLBF, and hindlimb resistance in a control experiment during rest, mild (3.2 km/h) exercise, and graded reductions in HLBF. From rest to exercise, hindlimb resistance decreased and HLBF increased along with the rise in CO. Initial graded reductions in HLBF did not evoke changes in MAP or CO but caused further...
describes decreases in hindlimb resistance. When reductions in HLBF elicited the metaboreflex, marked increases in MAP and CO occurred, and, despite the continued progressive hindlimb ischemia, hindlimb vascular resistance rose, indicating that vasoconstriction occurred.

Figure 2 shows the relationships between MAP versus HLBF (top) and hindlimb resistance versus HLBF (bottom) from a control experiment. Figure 3 shows average hindlimb resistance responses as a function of HLBF in control and after \( \alpha_1 \)-blockade, \( \beta \)-blockade, and \( \alpha_1 + \beta \)-blockade.

**Control.** Initial reductions in HLBF caused a significant decrease in hindlimb vascular resistance. Once HLBF was reduced below threshold, there was a significant increase in hindlimb resistance.

\( \alpha_1 \)-Blockade. Initial HLBF reductions above the threshold caused a significant decrease in hindlimb resistance as in control. However, when HLBF was reduced below threshold, the increase in resistance observed in control was abolished, revealing a continued decrease in resistance. The magnitude of hindlimb resistance during maximal metaboreflex activation was significantly lower than control.

\( \beta \)-Blockade. During free-flow exercise, hindlimb resistance was significantly higher than control. With initial reductions in HLBF, hindlimb resistance significantly decreased to a level not different from control. After metaboreflex activation, there was a significant increase in hindlimb resistance. The magnitude of resistance at maximal metaboreflex activation was significantly larger than in control.

\( \alpha_1 + \beta \)-Blockade. With initial reductions in HLBF, hindlimb resistance decreased to a level not different from control. After metaboreflex activation, there was a small increase in hindlimb resistance, which was not different from control.

Figure 4 shows average slopes of the relationship between hindlimb vascular resistance versus hindlimb blood flow during the initial and pressor responses in control and after \( \alpha_1 \)-blockade, \( \beta \)-blockade, and \( \alpha_1 + \beta \)-blockade. In control, the initial slope was significantly different from the pressor slope. After \( \alpha_1 \)-adrenergic blockade, the initial and pressor slopes were not significantly different from each other. The initial slope after \( \alpha_1 \)-blockade was similar to that in control, whereas the pressor slope was reversed and significantly different from control. After \( \beta \)-blockade, the initial and pressor slopes were significantly different from each other. The pressor slope was markedly larger (more negative) than in control. After \( \alpha_1 + \beta \)-blockade, the two slopes were significantly different from each other but not from control.

Table 1 shows mean hemodynamic data during rest, mild exercise, and muscle metaboreflex activation in control experiments and after \( \alpha_1 \)-blockade, \( \beta \)-blockade, and \( \alpha_1 + \beta \)-blockade. From rest to exercise in control, there were significant increases in MAP, CO, HR, NIVC, and HLBF. With muscle metaboreflex activation, there were further significant increases in MAP, CO, HR, and NIVC. After \( \alpha_1 \)-adrenergic blockade, resting MAP was significantly lower, whereas HR and NIVC were significantly higher than in control. With mild exercise, there was a significant increase in all variables, and...
MAP, HR, and NIVC were significantly higher than control exercise levels. Muscle metaboreflex activation after \( \alpha_1 \)-blockade resulted in substantial increases in MAP, CO, HR, and NIVC with a significantly attenuated pressor response compared with control. After \( \beta \)-adrenergic blockade, all parameters increased with exercise, but the levels of CO, HR, NIVC, and HLBF were significantly lower than those observed during control experiments. Muscle metaboreflex activation significantly increased MAP, CO, and HR, whereas NIVC was unchanged, and all parameters were significantly attenuated compared with metaboreflex responses in control. After \( \alpha_1 + \beta \)-blockade, resting MAP and NIVC were lower than in control. From rest to exercise, CO, HR, NIVC, and HLBF significantly increased, whereas MAP remained unchanged and was significantly lower than in control. Muscle metaboreflex activation led to an increase in MAP, CO, and HR, but all responses were significantly lower than in control.

DISCUSSION

The major new finding of the present study is that muscle metaboreflex-induced increases in sympathetic activity cause vasoconstriction of the ischemic active skeletal muscle from which the metaboreflex originates. Inasmuch as metaboreflex-induced increases in CO and arterial blood pressure partially restore blood flow to ischemic muscle, sympathetically mediated vasoconstriction would limit the ability of the metaboreflex to improve blood flow to ischemic muscle.

The muscle metaboreflex is activated when the \( O_2 \) supply to the muscle is unable to meet the metabolic demands of active muscle (35, 44). During mild exercise in canines, initial reductions in HLBF did not cause any marked increases in MAP, CO, or HR, indicating an adequate \( O_2 \) supply to working muscle. With further reductions in HLBF, the \( O_2 \) supply becomes insufficient to meet the \( O_2 \) demand of active muscle, leading to an accumulation of metabolites (\( H^+ \), lactic acid, diprotonated phosphate, etc.) (6, 31, 37–39). As a result, the muscle metaboreflex is activated, and marked elevations in MAP, CO, HR, and ventricular contractility are observed (8–10, 33, 40, 44). Thus, during mild dynamic exercise in canines, there is a clear threshold before the muscle metaboreflex is activated (Fig. 2).

In control, initial reductions in HLBF resulted in metabolic vasodilation, as indicated by a decrease in hindlimb vascular resistance (Fig. 3). When reductions in HLBF below the threshold elicited the metaboreflex, metabolic vasodilation was opposed by vasoconstriction, as indicated by an increase in vascular resistance within ischemic muscle. After \( \alpha_1 \)-blockade, the vasoconstriction observed during metaboreflex activation in control was abolished, revealing a continued vasodilation in the ischemic vasculature with further reductions in HLBF. These findings indicate that muscle metaboreflex activation induces \( \alpha_1 \)-mediated sympathetic vasoconstriction within ischemic skeletal muscle. After \( \beta \)-blockade, metaboreflex activation caused a significantly larger vasoconstriction in the hindlimb vasculature than in control. These findings are in agreement with our previous work demonstrating metaboreflex-induced \( \beta_2 \)-mediated vasodilation in ischemic skeletal muscle (19). After \( \alpha_1 + \beta \)-blockade, metaboreflex activation resulted in a vasoconstriction not significantly different from control. This could be due to the contribution of other hemodynamic factors, such as vasopressin, endothelin, neuropeptide Y, etc., and/or a potential \( \alpha_2 \)-mediated vasoconstriction (5, 7, 11, 15, 28). Therefore, the vasomotor tone of ischemic muscle with muscle metaboreflex activation during submaximal exercise is a combined result of a complex interplay between metabolic vasodilation and neurogenic and circulating vaso-
constrictor factors as well as possible local factors released from the endothelium. In control experiments, the prevailing neurogenic vasoconstriction results in frank vasoconstriction within the ischemic vasculature.

We and others have shown that metaboreflex activation elicits vasoconstriction of coronary, renal, and forelimb vasculatures (3, 4, 8, 22, 23). Whether the muscle metaboreflex vasoconstricts ischemic muscle itself has been controversial (17, 26, 29). Muscle metaboreflex activation during dynamic exercise in canines markedly increases MAP and CO, which restores about half of the blood flow deficit to ischemic skeletal muscle (29). In humans, Rowell et al. (32) showed that metaboreflex activation induces about half of the blood flow deficit to ischemic skeletal muscle. If no sympathetic vasoconstriction occurred with metaboreflex activation, the amount of blood flow restoration to the ischemic vasculature would have been larger than 50%. Therefore, muscle metaboreflex-induced neurogenic vasoconstriction limits the ability of the reflex to restore blood flow to muscle.

Vasoconstriction within ischemic muscle would result in a decrease in muscle blood flow, which would lead to exaggerated metaboreflex activation. Therefore, this becomes a positive feedback loop wherein vasoconstriction decreases the blood flow to the already ischemic muscle, further activating muscle afferents and causing a larger vasoconstriction. However, the positive feedback could either become run-away positive feedback, where each cycle would lead to exaggerated metaboreflex activation and a larger vasoconstriction, or be-

![Fig. 4. Initial and pressor slope responses of hindlimb vascular resistance during muscle metaboreflex activation (n = 6) in control (open bars), after α1-blockade (hatched bars), β-blockade (cross-hatched bars), and α1 + β-blockade (solid bars). *P < 0.05 vs. the initial slope; †P < 0.05 vs. the control setting.]

**Table 1. Mean hemodynamic values during rest, free-flow exercise, and MMA during control experiments and after α1-blockade, β-blockade, and α1 + β-blockade**

<table>
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<tr>
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<th>Control</th>
<th>α1-Blockade</th>
<th>β-Blockade</th>
<th>α1 + β-Blockade</th>
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<tr>
<td>Mean arterial pressure, mmHg</td>
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<td></td>
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<td></td>
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<tr>
<td>Rest</td>
<td>88.4 ± 1.9</td>
<td>81.4 ± 1.4†</td>
<td>84.4 ± 2.2</td>
<td>76.9 ± 2.0†</td>
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<tr>
<td>Exercise</td>
<td>92.1 ± 1.2∗</td>
<td>83.5 ± 1.6†</td>
<td>90.3 ± 3.5*</td>
<td>81.5 ± 1.9†</td>
</tr>
<tr>
<td>MMA</td>
<td>143.6 ± 1.6∗</td>
<td>119.5 ± 3.4†</td>
<td>114.4 ± 5.4†</td>
<td>113.7 ± 4.8†</td>
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<td>Cardiac output, l/min</td>
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<td></td>
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<td>Rest</td>
<td>2.75 ± 0.15</td>
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<td>3.71 ± 0.25†</td>
<td>4.03 ± 0.24*</td>
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<tr>
<td>MMA</td>
<td>6.50 ± 0.27∗</td>
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<tr>
<td>Rest</td>
<td>69.9 ± 4.2</td>
<td>83.2 ± 5.5†</td>
<td>70.4 ± 3.1</td>
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<td>112.9 ± 4.8†</td>
<td>95.3 ± 1.0†</td>
<td>106.6 ± 4.7*</td>
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<tr>
<td>MMA</td>
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<td>159.4 ± 8.6†</td>
<td>104.3 ± 4.3†</td>
<td>119.6 ± 6.7†</td>
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<td>Nonischemic vascular conductance, ml-min⁻¹-mmHg⁻¹</td>
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<td></td>
<td></td>
<td></td>
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<td>Rest</td>
<td>24.3 ± 1.3</td>
<td>28.1 ± 2.2†</td>
<td>24.6 ± 1.2</td>
<td>27.9 ± 1.8†</td>
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<tr>
<td>Exercise</td>
<td>34.2 ± 1.7∗</td>
<td>39.6 ± 2.6†</td>
<td>29.7 ± 1.3†</td>
<td>36.3 ± 1.7*</td>
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<tr>
<td>MMA</td>
<td>41.4 ± 1.7∗</td>
<td>50.8 ± 1.7†</td>
<td>31.5 ± 1.3†</td>
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<td>Hindlimb blood flow, l/min</td>
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<td></td>
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<tr>
<td>Rest</td>
<td>0.60 ± 0.07</td>
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<td>Exercise</td>
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<td>1.02 ± 0.12†</td>
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Values are means ± SE; n = 6 animals. MMA, maximal metaboreflex activation. ∗P < 0.05 vs. previous setting in same experiment; †P < 0.05 vs. control settings.
come limiting positive feedback, where the stimulus and response progressively become smaller and reach a plateau. Whether this positive feedback loop becomes a run-away cycle of continuing amplified responses or serves as an amplifier of the initial responses that reaches a plateau depends on the magnitude of the change in sympathetic activity engendered by the reflex and the efficacy of this change in sympathetic activity on blood flow to ischemic muscle. That is, if the rise in sympathetic activity elicited by a 1-unit decrease in muscle blood flow evokes vasoconstriction in muscle that causes a <1 unit fall in flow, then the positive feedback will cycle with ever-decreasing amplitude and eventually plateau at a heighten- ened response. If, however, the fall in blood flow caused by the rise in sympathetic activity is greater than the change in flow that caused the rise in sympathetic activity itself, then this becomes a run-away positive feedback with ever-increasing responses eventually leading to complete vasoconstriction. Using the average results in our study, the pressor slope for the relationship between the imposed decrease in HLBf and the observed increase in hindlimb vascular resistance was ~36 units, which indicates that for every liter per minute decrease in flow, there would be a 36 mmHg·l⁻¹·min⁻¹ increase in resistance. This 36 mmHg·l⁻¹·min⁻¹ increase in resistance caused by metaboreflex activation would itself cause a decrease in flow. At the threshold level of HLBf, an increase in hindlimb resistance by 36 mmHg·l⁻¹·min⁻¹ would cause a 0.54 l/min further decrease in flow if pressure remained constant. This decrease in flow would additionally increase resistance by 19.7 mmHg·l⁻¹·min⁻¹, which, in turn, would decrease flow by 0.15 l/min and so forth. With each cycle, the increase in the stimulus and the resultant reflex response become progressively smaller, and the positive feedback will eventually reach a steady state. Whereas a variety of factors can affect the hindlimb resistance response, our results indicate that this is a limiting positive feedback that is a stable system, resulting in amplified gain rather than run-away instability.

In canines, nearly half of the CO at rest perfuses skeletal muscle (14), and, during exercise, all of the rise in CO is directed to active skeletal muscle (24). When further increases in CO become limited (e.g., during severe exercise, heart failure, after β-blockade, etc.), peripheral vasoconstriction is the only mechanism available to increase arterial pressure. Since active skeletal muscle constitutes a large proportion of total vascular conductance during severe exercise, ischemic active muscle is the likely target for progressively larger vasoconstriction. Several studies have indicated that metabolic byproducts in skeletal muscle reduce neurogenic vasoconstriction, termed functional sympatholysis (30, 41). However, to some extent, the conclusions may also be dependent on methods of data analysis (25). In pathophysiological states, functional sympatholysis may be reduced (41, 43), which may thereby heighten the vasoconstrictor responses in ischemic muscle in these settings. This would further limit O₂ delivery to ischemic muscle, leading to an exaggerated activation of the muscle metaboreflex, stimulating a dangerous positive feedback loop. To what extent this metaboreflex positive feedback approaches run-away levels in pathophysiological states is unknown. Fundamentally, this feedback could impair the ability to exercise and contribute to exercise intolerance.

**Limitations.** Arterial baroreflex buffers about one-half of the muscle metaboreflex-induced pressor response, and this occurs primarily by inhibition of peripheral vasoconstriction (20). In our study, MAP during muscle metaboreflex activation after α₁- and β-blockade was significantly smaller than in control. This lower arterial pressure could potentially result in a reduced buffering by the baroreflex and cause larger metaboreflex-induced peripheral vasoconstriction. A larger vasoconstrictor drive could limit the hindlimb vasodilation we observed with metaboreflex activation after α₁-blockade and could exaggerate the vasoconstriction we observed after β-blockade. Our observation of continued hindlimb vasodilation after α₁-blockade despite possible larger sympathetic activation would argue in favor of our conclusion that sympathetic vasoconstriction occurs in the ischemic hindlimb during metaboreflex activation. The larger vasoconstriction seen after β-blockade may have been exaggerated due to the lower arterial pressure and less baroreflex buffering of metaboreflex responses.

**ACKNOWLEDGMENTS**

The authors thank Jody Helme-Day and Audrey Nelson for expert technical assistance and animal care.

**GRANTS**

This work was supported by National Heart, Lung, and Blood Institute Grant HL-55473.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


