Muscle metaboreflex activation during dynamic exercise evokes epinephrine release resulting in β2-mediated vasodilation

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Kaur J, Spranger MD, Hammond RL, Krishnan AC, Alvarez A, Augustyniak RA, O’Leary DS. Muscle metaboreflex activation during dynamic exercise evokes epinephrine release resulting in β2-mediated vasodilation. Am J Physiol Heart Circ Physiol 308: H524–H529, 2015. First published December 24, 2014; doi:10.1152/ajpheart.00648.2014.—Muscle metaboreflex-induced increases in mean arterial pressure (MAP) during submaximal dynamic exercise are mediated principally by increases in cardiac output. To what extent, if any, the peripheral vasculature contributes to this rise in MAP is debatable. In several studies, we observed that in response to muscle metaboreflex activation (MMA; induced by partial hindlimb ischemia) a small but significant increase in vascular conductance occurred within the nonischemic areas (calculated as cardiac output minus hindlimb blood flow and termed nonischemic vascular conductance; NIVC). We hypothesized that these increases in NIVC may stem from a metaboreflex-induced release of epinephrine, resulting in β2-mediated dilation. We measured NIVC and arterial plasma epinephrine levels in chronically instrumented dogs during rest, mild exercise (3.2 km/h), and MMA before and after β-blockade (propranolol; 2 mg/kg), α1-blockade (prazosin; 50 μg/kg), and α1 + β-blockade. Both epinephrine and NIVC increased significantly from exercise to MMA: 81.9 ± 18.6 to 141.3 ± 22.8 pg/ml and 33.8 ± 1.5 to 37.6 ± 1.6 ml·min⁻¹·mmHg⁻¹, respectively. These metaboreflex-induced increases in NIVC were abolished after β-blockade (27.6 ± 1.8 to 27.5 ± 1.7 ml·min⁻¹·mmHg⁻¹) and potentiated after α1-blockade (36.6 ± 2.0 to 49.7 ± 2.9 ml·min⁻¹·mmHg⁻¹), while α1 + β-blockade also abolished any vasodilation (33.7 ± 2.9 to 30.4 ± 1.9 ml·min⁻¹·mmHg⁻¹). We conclude that MMA during mild dynamic exercise induces epinephrine release causing β2-mediated vasodilation.

Several studies have suggested that the muscle metaboreflex improves blood flow to the ischemic working muscle (9, 30, 32, 35, 47). However, very few studies have investigated the effect of muscle metaboreflex activation on blood flow to nonischemic active muscle and the results are equivocal. Using the model originally developed by Wyss et al. (47) to investigate the metaboreflex (graded reductions in hindlimb blood flow), Mittelstadt et al. (28) showed that metaboreflex activation during moderate exercise in canines causes forelimb (a nonischemic active tissue) vasoconstriction; however, forelimb blood flow still increased due to the large pressor response (the rise in arterial pressure was greater than the fall in forelimb vascular conductance, and therefore forelimb blood flow increased). In contrast, Augustyniak et al. (3) did not find a significant decrease in forelimb conductance with metaboreflex activation during moderate exercise.

In previous studies in normal animals, we often observed a small but statistically significant metaboreflex-mediated increase in the total nonischemic systemic vascular conductance (NIVC) (conductance to all vascular beds excluding the hindlimbs) during submaximal dynamic exercise (4, 21, 36). In contrast, after induction of heart failure this small vasodilation is reversed to a substantial metaboreflex-mediated peripheral vasoconstriction due to much greater reflex increases in sympathetic activity (16). Therefore there appears to be both vasodilator as well as vasoconstrictor processes elicited by metaboreflex activation. Whereas many studies have shown that activation of the muscle metaboreflex can elicit increases in sympathetic activity and peripheral vasoconstriction (4, 16, 46), the mechanisms mediating this vasodilation are unknown. Increases in sympathetic activity to the adrenal gland increase epinephrine release, which can elicit substantial vasodilation, especially so in the skeletal muscle (5, 22, 25, 26). However, whether epinephrine release contributes to muscle metaboreflex-induced peripheral vasodilation is unknown. In the present study we tested the hypothesis that during metaboreflex activation epinephrine is released and causes β2-mediated peripheral vasodilation.

METHODS

Experimental subjects. Eleven adult mongrel canines (~20–25 kg) of either sex were selected for the study. All animals were acclimated to the laboratory surroundings and willing to run on a motor-driven treadmill. All the methods and procedures employed in the study were approved by the Institutional Animal Care and Use Committee (IACUC) of Wayne State University and complied with the National Institutes of Health Guide to the Care and Use of Laboratory Animals. All animals exercised voluntarily during experimentation; no negative reinforcement techniques were utilized.

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Surgical procedures. For each of the two surgical procedures, the 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 [100–175 μg/h (72 h) transdermal delivery]. Anaesthesia 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 buprenorphine and acepromazine (0.05 and 0.5 mg/kg, respectively) as needed. To avoid acute postoperative infections, cefazolin (antibiotic, 30 mg/kg iv) was administered pre- and postoperatively. Cephalexin (antibiotic, 30 mg/kg po bid) was administered prophylactically for the entire term of the experimental protocol.

In the first surgical procedure, the thoracic cavity was opened via a left thoracotomy (3rd/4th intercostal space) approach, and the pericardium was cut to expose the heart. A perivascular flow probe (10PAU, Transonic Systems) was positioned around the ascending aorta to measure CO. For studies unrelated to the present investigation, in some animals (n = 6) a telemetry blood pressure transmitter (TA11 PA-D70, DSI) was placed in the left ventricle, a flow probe was placed on the left circumflex artery (n = 5), and pacing wires were secured to the free wall of the right ventricle (n = 6) as described previously (16). The pericardium was reapproximated, and the wires were tunneled subcutaneously and exteriorized between the scapulae. 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. A perivascular flow probe (10PAU, Transonic Systems) was positioned around the terminal aorta to measure hindlimb flow (HLBF). For studies unrelated to the present investigation, a blood flow transducer was also placed on the left renal artery. All side branches of the terminal aorta between the iliac arteries and the 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 flow probe and, for unrelated studies, a hydraulic occluder was also placed on the left renal artery. A 19-gauge polyvinyl catheter (Tygon, SS4-HL, Norton) was inserted into a side branch of the aorta cranial to the flow probe to measure systemic arterial pressure. All instrumentation was tunneled subcutaneously and exteriorized between the scapulae, and the abdomen was closed in layers. Last, in some animals (n = 6), a midline neck incision was made to expose and catheterize the right jugular vein for unrelated studies.

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 flow meters (TS420, Transonic Systems). The arterial catheter was aspirated, flushed, and connected to a pressure transducer (Transpac IV, ICU Medical). All hemodynamic variables were monitored as real-time waveforms by a data-acquisition system (LabScribe, iWorx) and recorded for subsequent off-line analysis.

Experimental procedures. All experiments were performed after the animals had fully recovered from surgery (i.e., were active, afebrile, and of 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. The muscle metaboreflex was then engaged via graded reductions in exercise and each level of vascular occlusion were maintained until all parameters reached steady state (typically 3–5 min). In a subset of experiments in some animals (n = 5), arterial blood samples were drawn for analysis of arterial epinephrine levels at rest, during free-flow exercise, and at peak metaboreflex activation. Epinephrine levels were measured by high-performance liquid chromatography with electrochemical detection (Waters, Milford, MA). Control experiments (n = 11) were repeated in same animals after β-adrenergic blockade [2 mg/kg intra-arterial (ia) propranolol; n = 7], α1-adrenergic blockade (50 μg/kg ia prazosin; n = 6), and α1 + β blockade (n = 6) on separate days. The drugs were administered 30 min before each experiment, and subsequent experiments were not performed for at least 48 h.

Data analysis. Each animal served as its own control. CO, HLBF, heart rate (HR), and mean arterial pressure (MAP) were continuously recorded during each experimental procedure. Other hemodynamic parameters were calculated during off-line data analysis [e.g., total vascular conductance (TVC) and NIVC]. TVC and NIVC were calculated as CO/MAP and (CO – HLBF)/MAP, respectively. One-minute averages of all variables were taken during steady state at rest, during free-flow exercise, and after metaboreflex activation. Mean values were averaged across all animals to obtain the sample mean of the study.

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). A two-way ANOVA with repeated measures was used to compare hemodynamic data for time and/or conditional effects. In the event of a significant time-condition interaction, individual means were compared using the test for simple effects.

RESULTS

Figure 1 shows 2 min of steady-state levels of MAP, CO, NIVC, and HLBF during rest, mild (3.2 km/h) exercise, and muscle metaboreflex activation in a control experiment. From rest to exercise, there is negligible change in MAP but a moderate increase in CO, NIVC, and HLBF. Muscle metaboreflex activation during exercise resulted in large increases in MAP and CO with a small increase in NIVC.

Figure 2 shows the average arterial epinephrine levels during rest, mild exercise, and metaboreflex activation in control experiments. There was no change in epinephrine levels from rest to exercise; however, with metaboreflex activation, arterial epinephrine levels increased by ~75%.

Figure 3 shows mean values of MAP, CO, HR, and NIVC during rest, mild exercise, and metaboreflex activation in control and after β-blockade, α1-blockade, and α1 + β-blockade.

Control (n = 11). MAP, CO, HR, and NIVC significantly increased from rest to exercise and furthermore from exercise to muscle metaboreflex activation.

β-Adrenergic blockade (n = 7). CO, HR, and NIVC significantly increased from rest to exercise; however, the magnitudes of these responses were significantly lower compared with control exercise levels. There was no change in MAP from rest to exercise. Muscle metaboreflex activation significantly increased MAP, CO, and HR while NIVC was unaffected. The magnitudes of responses during muscle metaboreflex activation for all parameters were significantly attenuated compared with control responses during metaboreflex activation.

α1-Adrenergic blockade (n = 6). Resting MAP was significantly lower while HR was significantly higher compared with control. CO, HR, and NIVC significantly increased from rest to exercise, while there was no change in MAP. Muscle metaboreflex activation caused substantial increases in all parameters. Although HR and CO increased more than in control experiments, a larger rise in NIVC also occurred, and therefore the resultant pressor response was significantly attenuated.

α1 + β-Blockade (n = 6). Resting CO was decreased compared with control. From rest to exercise, MAP remained
unchanged and CO, HR, and NIVC significantly increased, although MAP and CO were still significantly lower than in control. Muscle metaboreflex activation led to an increase in MAP while CO, HR, and NIVC remained similar to exercise levels and significantly lower than in control.

**DISCUSSION**

Our principal new finding is that activation of the muscle metaboreflex during mild dynamic exercise evokes increases in plasma epinephrine levels and subsequent $\beta_2$-mediated vasodilation, which opposes $\alpha$-adrenergic vasoconstriction. Since $\beta_2$-receptors are richly expressed in skeletal muscle, epinephrine release may partially restrain $\alpha$-adrenergic vasoconstriction of active skeletal muscle during metaboreflex activation, thereby improving blood flow to the ischemic active skeletal muscle.

Muscle metaboreflex activation during dynamic exercise causes significant increases in MAP, HR, CO, and ventricular contractility (4, 8–11, 16, 24, 36–38, 47). However, the peripheral vascular responses have varied in previous studies. While significant vasoconstriction has been seen in the renal and forelimb vascular beds (4, 16, 21, 28, 29) when taken as a whole, the total vascular conductance of all vascular beds except the hindlimbs (termed NIVC) usually remains unchanged or slightly increases (4, 8, 21, 36, 44). Inasmuch as some individual vascular beds do constrict, the observation of no change or slight increase in NIVC indicates that some area(s) vasodilate. What causes this vasodilation has been unknown. Reflex increases in sympathetic nerve activity can lead to increased preganglionic sympathetic activity to the adrenal glands, which can cause substantial increases in epinephrine release (5, 22, 25, 26). In addition, postganglionic sympathetic nerve terminals have also been shown to release epinephrine (5, 22, 25, 26). Epinephrine is a powerful systemic vasodilator especially in skeletal muscle, which is richly endowed with $\beta_2$-adrenergic receptors (13, 23, 27, 45). In the present study we saw that metaboreflex activation caused substantial increases in arterial plasma epinephrine levels. A small but statistically significant peripheral vasodilation occurred that was abolished by $\beta$-blockade and accentuated by $\alpha_1$-adrenergic blockade. These results support our hypothesis that muscle metaboreflex activation causes increases in circulating epinephrine, which opposes $\alpha$-mediated vasoconstriction via $\beta_2$-mediated vasodilation. Therefore, this mechanism can possibly explain the observed significant increase in NIVC. Metaboreflex activation after combined $\alpha_1 + \beta$-blockade resulted in peripheral vasoconstriction, which may be due to...
increased vasopressin release. We previously demonstrated that the arterial baroreflex markedly suppresses metaboreflex-induced vasopressin release (31). With \( \alpha_1 + \beta \)-blockade the rise in arterial pressure with metaboreflex activation was lower, thereby likely causing less baroreflex buffering of vasopressin release, similar to that previously observed in our laboratory after ganglionic blockade (31).

What vascular beds are responsible for the small systemic vasodilation often seen with metaboreflex activation during dynamic exercise are not known. Inasmuch as about 50% of total vascular conductance in dogs even at rest is skeletal muscle (15), it is likely that some muscle beds participate in these vasodilatory responses. Furthermore, skeletal muscle has a greater density of \( \beta_2 \)-adrenergic receptors than other beds such as the renal or mesenteric circulations. It is also possible that the workload of some muscle groups is increased with metaboreflex activation. Respiratory muscles are potential candidates as metaboreflex activation may increase ventilation (2, 24, 34). Furthermore, with ischemia of the hindlimbs, there may also be an increase in work done by lumbar/gluteal muscles perfused by aortic branches not isolated in our preparation. The coronary vasculature is also a likely target bed for the increased plasma epinephrine with muscle metaboreflex activation. However, since the measurement of CO in our model does not include the coronary circulation (the coronary arteries arise from the ascending aorta below the CO blood flow transducer), \( \beta_2 \)-mediated coronary vasodilation did not contribute to the significant increase in NIVC. During exercise, the conductance of active skeletal muscle increases, and therefore skeletal muscle is also a likely target for \( \beta_2 \)-mediated vasodilation. Our calculation of NIVC does not include the ischemic hindlimb vasculature, and therefore this vascular bed does not play a role in the observed vasodilation during metaboreflex activation in our model. To what extent muscle metaboreflex activation (and potentially epinephrine) influences blood flow and vascular conductance to the ischemic active muscle is unknown.

**Limitations.** The plasma epinephrine levels were not measured during the experiments employing pharmacological approaches, and it is possible that epinephrine levels were different in these settings than in the control experiments. Nor-epinephrine released from sympathetic nerves could potentially also activate \( \beta \)-adrenergic receptors (as well as epinephrine activate \( \alpha \)-adrenergic receptors). However, a previous study...
demonstrated that norepinephrine infusion always caused vasoconstriction (17). It is possible that after α-adrenergic blockade norepinephrine released from sympathetic nerves caused some dilation via activation of β-receptors. The muscle metaboreflex is buffered by the arterial baroreflex (21, 39) as well as by cardiac afferents (7) (and likely by other reflexes as well). It is possible that the interaction between the metaboreflex and other reflexes may be affected by the pharmacological perturbations employed in the present study.

Perspectives and significance. Skeletal muscle is richly endowed with small group III/IV afferents that respond to changes in the mechanical and chemical environment, with many receptors being polymodal, e.g., some primarily mechanoreceptors increase activity with ischemia (19, 20). Activation of skeletal muscle afferents by metabolite accumulation during ischemic exercise leads to increased sympathetic nerve activity eliciting a powerful pressor response. The mechanisms mediating this pressor response may vary depending on the experimental paradigm. When the metaboreflex is elicited during submaximal dynamic exercise via reductions in blood flow to the active skeletal muscle, the rise in MAP is virtually solely driven by increases in CO in both canines and humans (12, 18, 35, 37). In human studies the metaboreflex is often activated via the technique of postexercise circulatory occlusion either after static or dynamic limb exercise. The data are mixed as to the relative roles of increases in CO vs. peripheral vasoconstriction and as we discussed in a previous study, this may be dependent on the intensity of contraction, type of exercise, and the muscle mass involved (44). When increases in CO are limited (e.g., maximal exercise, heart failure, etc.), metaboreflex activation leads to peripheral vasoconstriction (4, 9, 16, 18). Since active skeletal muscle constitutes a progressively higher proportion of total vascular conductance with increasing exercise intensity, it becomes an increasingly more likely target for vasoconstriction to raise MAP. This vasoconstriction would further reduce blood flow and induce a larger degree of ischemia in the active muscle causing heightened activation of the muscle metaboreflex, which in turn would cause a positive feedback cycle by further increasing the sympathetic tone. In such situations, release of epinephrine from adrenal glands and/or postganglionic sympathetic nerve terminals causing β2-mediated vasoconstriction could act to protect perfusion of the active skeletal muscle.

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

AUTHOR CONTRIBUTIONS

REFERENCES


