Role of cardiac output versus peripheral vasoconstriction in mediating muscle metaboreflex pressor responses: dynamic exercise versus postexercise muscle ischemia

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Spranger MD, Sala-Mercado JA, Coutsos M, Kaur J, Stayer D, Augustyniak RA, O’Leary DS. Role of cardiac output versus peripheral vasoconstriction in mediating muscle metaboreflex pressor responses: dynamic exercise versus postexercise muscle ischemia. Am J Physiol Regul Integr Comp Physiol 304: R657–R663, 2013. First published February 20, 2013; doi:10.1152/ajpregu.00601.2012.—Muscle metaboreflex activation (MMA) during submaximal dynamic exercise in normal individuals increases mean arterial pressure (MAP) via increases in cardiac output (CO) with little peripheral vasoconstriction. The rise in CO occurs primarily via increases in heart rate (HR) with maintained or slightly increased stroke volume. When the reflex is sustained during recovery (postexercise muscle ischemia, PEMI), HR declines yet MAP remains elevated. The role of CO in mediating thepressor response during PEMI is controversial. In seven chronically instrumented canines, steady-state values with MMA during mild exercise (3.2 km/h) were observed by reducing hindlimb blood flow by ~60% for 3.5 min. MMA during exercise was followed by 60 s of PEMI. Control experiments consisted of normal exercise and recovery. MMA during exercise increased MAP, HR, and CO by 55.3 ± 4.9 mmHg, 42.5 ± 6.9 beats/min, and 2.5 ± 0.4 l/min, respectively. During sustained MMA via PEMI, MAP remained elevated and CO remained well above the normal recovery levels. Neither MMA during dynamic exercise nor during PEMI significantly affected peripheral vascular conductance. We conclude that the sustained increase in MAP during PEMI is driven by a sustained increase in CO not peripheral vasoconstriction.

When the reflex is activated during sustained submaximal dynamic exercise in normal subjects, the pressor response occurs primarily via increased cardiac output (CO), which is driven by increased heart rate (HR) coupled with sustained or slightly increased stroke volume (SV) (17, 28, 53, 57, 65). SV is maintained despite this tachycardia due to both enhanced ventricular contractility (14, 16, 45, 53) and maintained or increased ventricular filling pressure via substantial central blood volume mobilization (54). In contrast, during PEMI, whereas mean arterial pressure (MAP) remains elevated for as long as the ischemia is maintained, HR precipitously declines toward resting levels with a time course similar to normal recovery, which led some to speculate that the muscle metaboreflex has little control over the heart (2, 23, 38, 51, 52, 61–63). With HR on the decline during the recovery from exercise with or without PEMI, the role of CO in mediating the pressor response during PEMI remains controversial. Indeed previous studies both support (11, 16–18, 48, 50, 57) and refute (9, 18, 48) any role for CO in mediating the pressor response during PEMI. In the present study, we elicited the muscle metaboreflex during dynamic exercise and sustained this activation during PEMI to compare the mechanisms underlying the muscle metaboreflex-mediated pressor responses in these two distinct settings.

METHODS

Experimental subjects. Seven adult mongrel canines were selected for the study. All animals were healthy, 20–25 kg body wt, of either sex (4 females; 3 males), well adapted to the laboratory environment, and willing to run on a motor-driven treadmill. During experimenta-
tion, all animals exercised voluntarily and no negative reinforcement techniques were utilized. The protocols developed and employed in the present study were reviewed and approved by the Institutional Animal Care and Use Committee of Wayne State University and complied with the National Institutes of Health Guide to the Care and Use of Laboratory Animals.

Surgical procedures. Each animal was completely instrumented with chronic, indwelling cardiovascular devices following two sterile surgical procedures: left thoracotomy and left flank retroperitoneal surgery in that order. The animals recovered a minimum of 10 days before the second surgery and a minimum of 7 days before the first experiment. During preoperative care, the animals were initially sedated with acepromazine (0.4–0.5 mg/kg im). After adequate se-
dation, the animals were anesthetized with a combined treatment of ketamine and diazepam (5.0 and 0.22 mg/kg iv, respectively). Anesthesia was maintained with isoflurane gas (1–3%) after endotracheal intubation. In addition, the animals received preoperative administration of cefazolin (antibiotic; 30 mg/kg iv), carprofen (analgesic; 4.0 mg/kg iv), buprenorphine (analgesic; 0.01 mg/kg im), and fentanyl [analgesic; 125–175 μg/h, (72h) TDD]. Before the left thoracotomy,
animals received selective intercostal nerve blockade with bupivacaine HCl (2.0 mg/kg sq). After each surgical procedure, animals received cefazolin (30 mg/kg iv) and prophylactic cephalixin [antibiotic; 30 mg/kg (bid) po] therapy for the term of the experimental protocol. During the 12-h postoperative period, animals were closely monitored and received buprenorphine and acepromazine (0.05 and 0.5 mg/kg iv, respectively) as needed. For the following 10 days, animals received carprofen [4 mg/kg (qd) po].

In the first surgical procedure, the thoracic cavity was opened via a left thoracotomy (4th intercostal space) approach. The pericardium was cut and reflected to expose the heart. An ultrasonic perivascular flow probe (20PAU, Transonic Systems) was positioned around the terminal aorta for measuring hindlimb blood flow (HLBF). All arterial side branches between the common iliacs and the flow probe were ligated and severed. In addition, two perivascular hydraulic occluders (8–10 mm, DocXS Biomedical Products) were positioned around the terminal aorta (distal to the flow probe) to provide the means to incrementally reduce HLBF. A 19-gauge polyvinyl catheter (S54-HL, Tygon, Norton) was advanced through a ligated lumbar artery and secured into the terminal aorta (cranial to the probe) to provide the means to incrementally reduce HLBF. A transmitter (TA11 PA-D70, Data Sciences International) was tethered subcutaneously. The catheter of the transmitter was tunneled into the chest, the catheter of the transmitter was tunneled subcutaneously and exteriorized between the scapulae, and the chest was closed in layers.

In the second surgical procedure, an incision was made in the left flank cranial to the iliac crest. The abdominal aorta was exposed and an ultrasonic perivascular flow probe (10PAA, Transonic Systems) was positioned around the descending aorta to measure CO. Approximately 10 cm caudal to the thoracotomy incision, an implantable telemetry blood pressure transmitter (TA11 PA-D70, Data Sciences International) was tethered subcutaneously. The catheter of the transmitter was tunneled into the thoracic cavity through the seventh intercostal space, and the tip was inserted and secured inside the left ventricle for measuring left ventricular pressure (LVP). For studies unrelated to the present investigation a blood flow transducer was also placed on the left circumflex artery. The pericardium was loosely reapproximated, the cables were tunneled subcutaneously and exteriorized between the scapulae, and the chest was closed in layers.

After complete postoperative recovery, each animal was brought into the laboratory and allowed to roam freely and acclimate for ~15–20 min. The animal was then directed onto the treadmill where the instrumentation was connected to the data acquisition system (TS420, Transonic Systems Blood Flow Meter, Gould; amplifiers, Data Science International, Telemetry System, LabScribe, iWorx).

Experimental procedures. All animals performed both control and experimental procedures on separate days, therefore, each animal served as its own control. The experiments began with the animal standing unrestrained on the treadmill until all hemodynamic data were observed to be stable (typically 5–10 min). The treadmill was turned on and the speed was gradually increased to 3.2 km/h at 0% grade [a mild workload for a canine (27)]. Steady state was generally reached within 3–5 min. In the control experiment, after all variables had reached steady state during exercise, the treadmill was abruptly stopped and postexercise (without ischemia) hemodynamic data were collected for 60 s while the animal was standing still. In a separate experiment, the muscle metaboreflex was engaged via partial reductions in HLBF during mild exercise. Once the reflex was strongly engaged, steady-state data were collected for 60 s. The treadmill was then abruptly stopped and the occlusion was sustained for an additional 60 s (PEMI).

Data analysis. CO, HLBF, LVP, HR, and MAP data were continuously recorded during each experiment. Other hemodynamic parameters were calculated off-line [e.g., SV, dP/dt max, dP/dt min, and non-ischemic vascular conductance (NIVC)]. NIVC was calculated as (CO – HLBF)/MAP and reflects vascular conductance of all vascular beds except the hindlimbs. Because of technical difficulties, we were only able to obtain LVPs from six animals. One-minute averages of steady-state data were calculated at rest, during exercise, and during metaboreflex activation. Five-second averages were computed for both 60-s postexercise conditions: postexercise (without ischemia) and PEMI. These mean values were then averaged across all animals to obtain the mean values for the entire population of the study. Finally, the last 10 s of recovery were averaged.

Statistical analysis. Averaged responses for each animal were analyzed via two-way repeated measures ANOVA to compare hemodynamic data for time and/or condition effects. In the event of a significant time-condition interaction, a C-matrix test for simple effects was performed. Data are reported as means ± SE, and statistical significance was ascribed as P < 0.05.

RESULTS

Figure 1 shows the responses in MAP, CO, HR, SV, and HLBF from a control and experimental protocol in one animal. In both protocols, with the transition from rest to exercise, there was a minimal increase in MAP and modest increases in CO and HR concomitant with a substantial rise in HLBF. During the normal recovery from exercise, MAP remained unchanged while CO and HR gradually fell toward resting levels. In the experimental protocol, the muscle metaboreflex was elicited immediately following steady-state exercise and sustained during the postexercise recovery period (PEMI). Muscle metaboreflex activation during the experimental protocol led to marked increases in MAP, CO, and HR and little change in SV. During PEMI, the rise in MAP was sustained. Although CO and HR initially fell during the onset of PEMI, both subsequently plateaued well above their normal recovery levels.

Figure 2 shows the average values of MAP, HR, CO, and NIVC during control and experimental procedures. The 60 s of postexercise recovery (PEMI) are plotted as 5-s averages. The changes in MAP, HR, CO, and NIVC from rest to exercise were not significantly different between the control and experimental protocols. With muscle metaboreflex activation during exercise, MAP, HR, and CO increased substantially.

During PEMI, the ~60 mmHg rise in MAP, which was observed with muscle metaboreflex activation, was sustained during the entire 60 s, as every data point during this maneuver was significantly different from control. NIVC fell during the PEMI period with a pattern not significantly different from during the normal recovery from exercise. HR and CO decreased abruptly during the first 15 s of PEMI; however, both of these variables subsequently plateaued significantly above normal recovery levels.

Figure 3 shows the mean hemodynamic responses during exercise and the final 10 s of recovery with and without muscle metaboreflex activation. During the last 10 s of ischemic exercise, there were significant increases in MAP, CO, HR, dP/dt max, and dP/dt min compared with the data during exercise without metaboreflex activation.

During the last 10 s of the recovery from ischemic exercise, MAP, CO, HR, dP/dt max, and dP/dt min were all significantly elevated compared with the values during the normal recovery from exercise, whereas there were no significant changes in NIVC or SV.

DISCUSSION

Our major finding is that the mechanisms mediating muscle metaboreflex-induced increases in arterial pressure are similar
both when the reflex is activated during dynamic exercise and when the reflex is sustained during recovery from exercise. In both settings, the pressor response is primarily due to a substantial elevation in CO with little, if any, peripheral vasoconstriction. Moreover, the elevation in CO is driven via an increased HR with a sustained SV. Inasmuch as tachycardia itself can lead to decreases in SV (32, 64), increases in ventricular contractility likely contribute to the sustained SV. The elevated dP/dt max supports this conclusion. Faster left ventricular relaxation (dP/dt min) may also aid in the maintenance of SV by increasing filling time. Therefore, our data demonstrate marked muscle metaboreflex-induced chronotropic, inotropic, and lusitropic responses both when the reflex is activated during dynamic exercise and when the reflex is sustained during recovery from exercise.

Muscle metaboreflex activation during exercise: CO versus vasoconstriction. Muscle metaboreflex activation during exercise evokes large increases in MAP, HR, and CO (2, 3, 21, 40, 44, 45, 65). However, the relative roles of CO versus peripheral vasoconstriction in this pressor response have been unclear. We observed a 45% increase in CO with muscle metaboreflex activation during exercise with no significant peripheral vasoconstriction. We determined the extent of peripheral vasoconstriction by calculating the conductance of all vascular beds with the exception of the hindlimbs (NIVC) (7). Changes in hindlimb conductance must be excluded due to the mechanical effects of the occlusion.

Previous studies have demonstrated a “switch” in the mechanisms of the muscle metaboreflex from a flow-mediated rise in MAP to a vasoconstriction-mediated pressor response when the reflex increase in CO is attenuated. For example, Sheriff et al. (54) and Ichinose et al. (28) demonstrated substantial peripheral vasoconstriction when the metaboreflex-induced increase in CO was either pharmacologically or mechanically prevented. In addition, Augustyniak et al. (8) demonstrated a shift from CO to peripheral vasoconstriction when workload approached maximal levels and further increases in CO were limited. Similar results have been observed in subjects with congestive heart failure, a setting where substantial increases in CO are limited (6, 15, 26, 47). Thus whether or not increased CO or increased vasoconstriction is utilized as a means to raise MAP with metaboreflex activation during dynamic exercise appears dependent on the ability to increase CO.

Muscle metaboreflex activation during PEMI: CO versus vasoconstriction. Previous studies have come to markedly different conclusions regarding the relative roles of CO versus peripheral vasoconstriction in mediating the pressor response during PEMI [(11, 16–18, 48, 50, 57) versus (9, 18, 48)]. One potential explanation is that a CO response is often seen during imposed PEMI following more intense exercise, whereas PEMI following relatively lower exercise intensities tends to demonstrate little change in CO and the pressor response occurs via peripheral vasoconstriction. We observed a maintained elevation in CO.
(≈50% above the normal recovery level) during PEMI with little or no change in NIVC. The time course of the recovery of NIVC during PEMI and the normal recovery from exercise are virtually indistinguishable and not significantly different. It should be noted that NIVC contains not only conductance to inactive areas, but a substantial amount of NIVC is skeletal muscle outside of the hindlimbs (25), which also vasodilates in response to the exercise. Thus with exercise NIVC increases and during recovery NIVC falls. To what extent this fall in NIVC during recovery with or without PEMI is neurogenic versus passive vasoconstriction due to reduced metabolic vasodilation in skeletal muscle is not known. Previously, Sheriff et al. (55) has shown that after ganglionic blockade, skeletal muscle vasodilation during exercise markedly exceeds normal levels. Regardless of whether or not tonic sympathetic activity controls the speed of recovery of NIVC, it is clear that the pattern of change in NIVC was not different between the normal recovery and during PEMI.

Role of HR and SV in mediating metaboreflex-induced increases in CO during exercise versus PEMI. Previous studies in dogs and humans have concluded that HR and CO increase markedly when the muscle metaboreflex is activated during exercise (6–8, 14–17, 20, 26, 28, 45, 65); however, during PEMI the effects on HR are more variable. In general, PEMI elicited from the arm following moderate exercise evokes little sustained tachycardia and any CO response occurs via increased SV (3, 23, 48). In contrast, if PEMI follows leg exercise, HR remains above normal recovery values, and therefore the relative tachycardia contributes to an elevated CO (3, 23, 48). The observations in humans following leg exercise are similar to those we observed in the present study using canines. Collectively, these studies indicate that either the HR response during PEMI depends on which limb is used or that these differential responses are due to differences in muscle mass.

Fig. 2. Averaged time course of MAP, HR, CO, and nonischemic vascular conductance (NIVC) during control (open circles) and experimental procedures (filled circles) in 7 animals. Data at rest are the average values from both settings (half-filled circles). *P < 0.05.

Fig. 3. Mean hemodynamic responses during exercise and the final 10 s of recovery with (filled bars) and without (open bars) muscle metaboreflex activation. *P < 0.05 between normal exercise and ischemic exercise; †P < 0.05 between normal recovery from exercise (open bars) and PEMI (filled bars).
Previous studies from our laboratory and others have concluded that during PEMI there are sustained increases in sympathetic tone to the heart; however, there are concurrent increases in parasympathetic activity as well (29, 41, 43). This combined activation of both arms of the autonomic nervous system causes bradycardia but sustained increased ventricular contractility (see Fig. 3). The differences in the HR responses between PEMI and metaboreflex activation during dynamic exercise may reflect that during PEMI one is observing responses during the recovery from exercise rather than during exercise per se. Muscle metaboreflex-induced tachycardia occurs primarily via increased sympathetic activity to the heart (22, 43). With the cessation of exercise, parasympathetic activity rises abruptly, which masks the chronotropic effects of sustained sympathetic tone (note that the inotropic effect is well sustained during PEMI; see Fig. 3). The extent to which sympathetic nerve activity remains elevated during PEMI may be dependent on the intensity and type of exercise performed (e.g., isometric vs. isotonic). For example, in canines, the muscle metaboreflex is not tonically active during mild, free-flow treadmill exercise but becomes tonically active as workload increases and/or ventricular function declines (8, 27, 65). In addition, Fisher et al. (22) demonstrated in humans performing static handgrip exercise that “robust” muscle metaboreflex activation (achieved during high-intensity exercise) was required to sustain sympathetic nerve activity during PEMI to a degree such that the prevailing parasympathetic effects on HR were counteracted. However, Amann et al. (4) recently concluded that skeletal muscle afferents contribute to the cardio-respiratory responses during relatively mild exercise in humans.

The increases in CO with metaboreflex activation likely require increased contractility as well as central blood volume mobilization to raise or maintain SV with the elevated afterload and shortened ventricular filling time. Previous studies from our laboratory showed that the muscle metaboreflex can elicit marked increases in central blood volume mobilization (54) as well as ventricular contractility [as evidenced by increased left ventricular maximal elastance, preload recruitable stroke work, as well as dP/dmax (14, 45, 53)]. Moreover, Little et al. (34, 35) have shown that increases in left ventricular end-diastolic volume per se can lead to increases in dP/dmax. Thus it is possible that a portion of the rise in contractility with metaboreflex activation may be directly attributable to enhanced central blood volume mobilization.

In the present study, the increase in contractility was sustained during PEMI (Fig. 3). Shoemaker et al. (57) showed a substantial muscle metaboreflex-induced increase in SV during ischemic exercise, and Crisafulli et al. (16) also reported an increase in SV and ventricular contractility with muscle metaboreflex activation during PEMI. These reflex increases in SV were suggested by these authors to be the principal mechanisms behind the increase in CO in these two distinct settings as HR was not affected. We did not observe a significant increase in SV with muscle metaboreflex activation during exercise or PEMI in our studies, and it is possible that these different results are species dependent. For example, there are reports that canines have a limited end-diastolic reserve compared to humans (10). In contrast, other studies have shown that canines possess significant preload reserve (33, 37). Nonetheless, a maintenance or increase in SV during a hemodynamic period in which ventricular filling time is markedly reduced can likely only be achieved if there is an increase in the contractile state and/or an increase in preload.

**Perspectives and Significance**

Activation of the muscle metaboreflex both during submaximal exercise and during the recovery from exercise elicits substantial increases in ventricular function. The major mechanism of the muscle metaboreflex-mediated pressor response is increased CO with little, if any, peripheral vasoconstriction in both settings. Inasmuch as when workload rises, an increasingly smaller fraction of CO is directed to nonactive vascular beds (e.g., brain, kidneys, and splanchnic organs), even complete vasoconstriction of these beds would cause only a limited increase in arterial pressure (42). In contrast, a metaboreflex-mediated rise in CO generates substantial increases in arterial pressure. Thus, in these settings, the muscle metaboreflex is a flow-raising, pressure-raising reflex. The rise in total systemic blood flow increases systemic perfusion pressure which likely acts to partially restore blood flow to ischemic muscle (46).

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


