Heart failure alters the strength and mechanisms of the muscle metaboreflex

ROBERT L. HAMMOND, ROBERT A. AUGUSTYNIAK, NOREEN F. ROSSI, PAUL C. CHURCHILL, KAREN LAPANOWSKI, AND DONAL S. O'LEARY. Heart failure alters the strength and mechanisms of the muscle metaboreflex. Am. J. Physiol. Heart Circ. Physiol. 278: H818–H828, 2000.—We hypothesized that excessive sympathoactivation observed during strenuous exercise in subjects with heart failure (HF) may result from tonic activation of the muscle metaboreflex (MMR) via hypoperfusion of active skeletal muscle. We studied MMR responses in dogs during treadmill exercise by graded reduction of terminal aortic blood flow (TAQ) before and after induction of HF by rapid ventricular pacing. At a low workload, in both control and HF experiments, large decreases in TAQ were required to elicit the MMR pressor response. During control experiments, this pressor response resulted from increased cardiac output (CO), whereas in HF CO did not increase; thus the pressor response was solely due to peripheral vasoconstriction. In HF, MMR activation also induced higher plasma levels of vasopressin, norepinephrine (NE), and renin. At a higher workload, in control experiments any reduction of TAQ elicited MMR pressor responses. In HF, before any vascular occlusion, TAQ was already below MMR control threshold levels and reductions in TAQ again did not result in higher CO; thus SAP increased via peripheral vasoconstriction. NE rose markedly, indicating intense sympathetic activation. We conclude that in HF, the MMR is likely tonically active at moderate workloads and contributes to the tonic sympathoactivation.

dynamic exercise; hormones; dogs; Frank-Starling; rapid ventricular pacing

WHEN OXYGEN DELIVERY to active skeletal muscle is insufficient for the ongoing metabolic demands, metabolites accumulate and stimulate afferents within the active skeletal muscle that elicit a powerful pressor response known as the muscle metaboreflex. Activation of the muscle metaboreflex during exercise elicits increases in heart rate, cardiac output, systemic arterial pressure, ventricular performance, central blood volume mobilization, and vasoconstriction in the renal and nonschematic active skeletal muscle vasculatures (9, 11, 12, 14, 16, 17, 22, 23, 25, 33). In addition, metaboreflex activation can also increase the circulating levels of vasoactive hormones (13, 18). These marked cardiovascular responses are buffered by arterial (23) and cardiopulmonary (4) baroreflexes. Studies from our laboratory and from others (11, 12, 16–18, 22, 23, 33) have shown that in dogs during mild exercise a clear threshold exists for metaboreflex activation, i.e., initial reductions in blood flow to active skeletal muscle (i.e., hindlimbs) do not elicit any metaboreflex responses. Only when oxygen delivery is reduced below a threshold do substantial metaboreflex pressor responses occur (25). In contrast, during moderate workloads no clear threshold exists, indicating that the metaboreflex may be tonically active or that the prevailing level of blood flow approximates the threshold for the reflex, and any reduction in perfusion to the active skeletal muscle will engage the muscle metaboreflex (20, 22, 33).

The cardiovascular responses to dynamic exercise are markedly altered in subjects with ventricular dysfunction. Previous studies have shown in normal dogs that little if any reductions in blood flow occur in inactive areas even during severe exercise (10, 29). However, during exercise at or near maximal levels in dogs with cardiac impairment, substantial vasoconstriction occurs in the renal and mesenteric vascular beds, and even active skeletal muscle becomes relatively vasoconstricted (e.g., the rise in iliac blood flow with exercise is depressed) (10, 29). The mechanisms mediating these altered cardiovascular responses to dynamic exercise in subjects with ventricular dysfunction are unknown. Nonetheless, as blood flow to active skeletal muscle is lower in this setting, this relative underperfusion could potentially elicit exaggerated sympathoactivation via the muscle metaboreflex because at even moderate workloads in normal animals no clear threshold is discernible for the reflex.

Little is known regarding the effects of heart failure (HF) on the strength and mechanisms of the muscle metaboreflex. To our knowledge, only three studies have directly addressed this issue. Sterns et al. (28) used the technique of posthandgrip circulatory arrest (PHG-CA) to isolate the effects of the muscle metaboreflex in normal humans and subjects with HF (i.e., inflation of a blood pressure cuff on the upper arm to suprasystolic levels at the cessation of static handgrip, thereby entrapping metabolites within the formerly
active muscle). They observed that the normally sustained or even elevated levels of muscle sympathetic nerve activity (MSNA) in this setting were suppressed in the subjects with HF, indicating that muscle metaboreflex responses may be impaired in HF. These authors proposed that the attenuation of the muscle metaboreflex may be due to desensitization of the receptor afferents. In a subsequent study from the same laboratory, Silber et al. (27) showed that during fatiguing rhythmic handgrip MSNA increased sooner in subjects with congestive HF and that during PHG-CA MSNA remained elevated, whereas in normal humans MSNA decreased toward control levels. The authors also observed markedly elevated forearm acidosis as well as greater $2\text{HPO}_4^-$ accumulation (metabolites thought to be important in mediating metaboreflex responses) in the HF subjects during PHG-CA. Subsequently, Shoemaker et al. (26) observed similar differences between normal human and HF subjects in response to 5 min of partially ischemic rhythmic handgrip exercise, i.e., higher venous lactate and H$^+$ levels and a larger pressor response in the HF group. These data indicate that during fatiguing rhythmic handgrip the muscle metaboreflex may become tonically active in HF subjects (possibly due to lower skeletal muscle blood flow, although this was not measured in either study) and thereby contribute to the increased sympathetic activity. However, with the PHG-CA technique, the muscle metaboreflex responses are observed during the recovery from exercise rather than during exercise per se. This may be an important issue because the mechanisms mediating the pressor response may be quite different in the two settings. For example, many studies have shown that during PHG-CA heart rate declines toward resting levels with a time course similar to that during the normal recovery from static handgrip without circulatory arrest (30). In contrast, in normal dogs muscle ischemia during exercise results in a marked increase in heart rate, and the increase in cardiac output is the primary mechanism of the metaboreflex pressor response (16, 17, 22, 33).

The objective of this study was to investigate the effects of HF on the strength and mechanisms of the muscle metaboreflex in conscious, chronically instrumented dogs during dynamic exercise. We hypothesized that the cardiac component of the reflex would be attenuated in HF, thereby limiting the reflex pressor response. In addition, we hypothesized that in HF skeletal muscle blood flow would be decreased during exercise, which may elicit tonic activation of the muscle metaboreflex. Some of these data have been published previously in abstract form (7, 8).

**METHODS**

**Experimental Subjects**

Ten healthy adult mongrel dogs (18–25 kg) of either sex were selected for the study, based on their successful adaptation to the laboratory environment and treadmill exercise. All aspects of the study were approved by the institutional Animal Investigation Committee and complied with the University guidelines and National Institutes of Health Guide to the Care and Use of Laboratory Animals [US DHSS Publication No. (NIH) 85–23, Rev. 1985]. All dogs exercised voluntarily; no negative reinforcement techniques were used.

**Surgical Procedures**

Indwelling instrumentation was installed in the dogs during three sterile surgical procedures. The animals were anesthetized with intravenous Pentothal Sodium (25 mg/kg) and maintained with 1.5–2.5% isoflurane. Through a right thoracotomy, the heart and ascending aorta were exposed, and an 18- or 20-mm blood flow transducer (electromagnetic or transit-time ultrasonic) was placed around the aortic root to measure cardiac output (CO). Three stranded stainless steel sutures (0-Flexon, Ethicon) were placed on the ventricular apex for subsequent rapid ventricular pacing. The pericardium was closed loosely over the heart, and the cables and leads were exteriorized between the scapulae.

In the second procedure a retroperitoneal incision was made on the left flank just above the iliac crest, and an 8- or 10-mm ultrasonic or electromagnetic blood flow transducer was placed on the terminal aorta to measure blood flow to the hindlimbs (TAQ). All vessels arising from the terminal aorta between the flow transducer and the iliac arteries were ligated. A hydraulic occluder (InVivo Metric) was also placed around the aorta distal to the flow probe to provide the means to progressively occlude the aorta. A 20-gauge polyvinyl catheter (Tygon, S54-HL, Norton) was introduced into the midthoracic aorta to monitor systemic arterial pressure (SAP). A pulsed Doppler blood flow probe (Crystal Biotech) was implanted on the left renal artery to measure renal blood flow (RBF) in seven animals, and ultrasonic transit-time probes (Transonic Systems) were installed in three dogs. All leads and the catheter were tunneled subcutaneously to the interscapular exit site. In a final procedure, a 20-gauge catheter (Tygon, S54-HL, Norton) was passed into atriocaval junction via the right external jugular vein to measure right atrial pressure (RAP). Catheters were also inserted through small side branches of the femoral artery and the femoral vein for subsequent rapid ventricular pacing. The pericardium was closed loosely over the heart, and the cables and leads were exteriorized between the scapulae.

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After all surgical procedures, postoperative pain was controlled by parenteral administration of buprenorphine (0.02–0.04 mg·kg$^{-1}$·h$^{-1}$) and acepromazine maleate (0.1 mg·kg$^{-1}$·h$^{-1}$) as needed. Antibiotic therapy included prophylactic perioperative administration of cefazolin (500 mg iv) and postoperative treatment with cephalixin (30 mg/kg, per os, 3 times/day) for at least 1 wk.

**Data Collection**

After at least 1 wk of recovery, the dog was transported to the laboratory, allowed to acclimate by roaming freely for 10–20 min, and then placed on the treadmill. The catheters were aspirated, flushed, and connected to pressure transducers (Spectramed DTX) set at midheart level, and the blood flow transducers were connected to the appropriate flowmeter (Zepeda SWF-5RD, Transonic T206, or Crystal Biotech). Heart rate (HR) was computed by a cardiotachometer triggered from the CO signal. Data were recorded continuously on a Gould 3800 pen recorder and on computerized data acquisition systems as beat by beat averages and as raw waveforms for subsequent offline analysis.
Exercise Protocols

Each dog was studied at standing rest, at a low workload (3.2 km/h at 0% grade), and at a moderate workload (6.4 km/h at 10% grade) without a predetermined schedule. After measurement in the standing rest position, the treadmill was accelerated to the selected speed and grade, and the hemodynamic responses were measured under free-flow conditions, i.e., with no ischemia induced experimentally by inflation of the hydraulic occluder on the terminal aorta. The terminal aorta was then progressively occluded in a stepwise fashion, and each level of occlusion was maintained until all variables reached steady state. Each dog performed several control experiments at both workloads and at least one experiment after the induction of HF by rapid ventricular pacing at 225 beats/min for a group average of 30 ± 1 days. In a subset of experiments, at standing rest, free-flow exercise, and at each level of partial vascular occlusion, arterial blood samples were drawn for analysis of plasma levels of arginine vasopressin (AVP) and plasma renin activity (PRA) (3, 21). Plasma norepinephrine (NE) levels were measured by high-performance liquid chromatography with electrochemical detection (Waters, Milford, MA).

Data Reduction and Statistical Analysis

During exercise at each level of occlusion, data were selected from the record when a steady-state condition had been achieved, typically for the last 60 s of a 3- to 5-min period. The signals from the electromagnetic flow probes were adjusted for drift and loss of sensitivity due to extended implantation periods (1). To accommodate the difference in measurement techniques between the pulsed Doppler and transit-time flow probes placed on the renal artery, the data for each dog were normalized as a percentage of the average value observed at standing rest before the induction of HF. The same method was employed for normalization of renal vascular conductance (RVC) = RBF/(SAP – RAP). Systemic vascular conductance to all areas except the hindlimbs and terminal aortic conductances were calculated as SVC = (CO – TAQ)/(SAP – RAP) and TAC = TAQ/(SAP – RAP), respectively. The hemodynamic data were analyzed by plotting the response variable (e.g., SAP) versus TAQ during free-flow exercise and at each level of partial vascular occlusion. As described in detail previously (16), during mild exercise initial reductions in hindlimb perfusion do not elicit metaboreflex responses; however, once hindlimb perfusion is reduced below a threshold level, a pressor response occurs. The threshold was approximated as the intersection between two regression lines, the initial response line in which no reflex responses occurred with the initial reductions in hindlimb perfusion and the pressor response line in which further reductions in hindlimb perfusion elicit a reflex pressor response. During moderate exercise, often no apparent threshold exists and the initial reduction in hindlimb perfusion elicits reflex responses. If no threshold was apparent, then the threshold was ascribed as the free-flow value of hindlimb perfusion.

The response of each dog was characterized by combining the data analyzed for each run under the control or HF conditions, and then the individual averages for the free flow, threshold, and maximum occlusion points were combined to describe the overall averaged response of the group (means ± SE) as plotted in Fig. 3. Utilizing the averaged responses for each subject, further statistical analyses were performed on the data with Systat software (Version 5, SPSS). Statistical significance was defined as P < 0.05 (one-tailed). For comparison of control and HF states, further statistical analyses were performed on the data with statistical analysis of variance (ANOVA) and post hoc Tukey’s test. For nonparametric data analysis, the Friedman test was used.

RESULTS

Comparison of Control and HF States at Standing Rest

Table 2 shows the average data observed at standing rest for the control and HF states. In the HF state, the

Table 1. Numbers of subjects for each measurement in each condition

<table>
<thead>
<tr>
<th>Event</th>
<th>Condition</th>
<th>CO</th>
<th>HR</th>
<th>SAP</th>
<th>RAP</th>
<th>RBF</th>
<th>TAQ</th>
<th>NE</th>
<th>PRA</th>
<th>AVP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>Control</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>10</td>
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<td>10</td>
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<tr>
<td>HF</td>
<td>Control</td>
<td>9</td>
<td>10</td>
<td>10</td>
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<td>9</td>
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<td>3.2 km/h</td>
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<tr>
<td>6.4 km/h</td>
<td>Control</td>
<td>8</td>
<td>9</td>
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<td>7</td>
<td>8</td>
<td>6</td>
<td>6</td>
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</tr>
</tbody>
</table>

Table 2. Resting hemodynamic and neuroendocrine data

<table>
<thead>
<tr>
<th>CO, l/min</th>
<th>SAP, mmHg</th>
<th>RAP, mmHg</th>
<th>HR, beats/min</th>
<th>RBF, %RC</th>
<th>TAQ, l/min</th>
<th>TAC, l·min⁻¹·mmHg⁻¹</th>
<th>NE, pg/ml</th>
<th>PRA, ANGIT ng·ml⁻¹·h⁻¹</th>
<th>AVP, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>3.36</td>
<td>94.2</td>
<td>1.4</td>
<td>95</td>
<td>100</td>
<td>100</td>
<td>0.65</td>
<td>7.08</td>
<td>113</td>
</tr>
<tr>
<td>SE</td>
<td>0.16</td>
<td>1.6</td>
<td>0.4</td>
<td>3.81</td>
<td>100</td>
<td>100</td>
<td>0.07</td>
<td>0.62</td>
<td>25</td>
</tr>
<tr>
<td>Mean HF</td>
<td>2.80</td>
<td>84.8</td>
<td>7.2</td>
<td>123</td>
<td>58.8</td>
<td>69.5</td>
<td>0.45</td>
<td>5.89</td>
<td>315</td>
</tr>
<tr>
<td>SE</td>
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<td>2.1</td>
<td>0.8</td>
<td>5.92</td>
<td>5.7</td>
<td>6.7</td>
<td>0.05</td>
<td>0.56</td>
<td>93</td>
</tr>
<tr>
<td>%HF/Con</td>
<td>83</td>
<td>90</td>
<td>518</td>
<td>129</td>
<td>58</td>
<td>69</td>
<td>69</td>
<td>83</td>
<td>280</td>
</tr>
<tr>
<td>P</td>
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<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Comparison of control and HF states at standing rest. %RC, normalized as a percentage of averaged control standing rest values for each subject.
animals displayed a resting tachycardia and elevated RAP, whereas CO, SAP, RBF, RVC, and TAC were diminished. NE, PRA, and AVP were elevated compared with the control state. Other clinical signs of HF included lassitude and, in several animals, ascites.

Comparison of Standing Rest to Exercise at 3.2 km/h at 0% Grade

In transition from standing rest to exercise at low workload under free-flow conditions (Fig. 1, solid bars),
HR, SV, CO, TAQ, SVC, and TAC rose significantly in response to exercise. SAP, RAP, RBF, and RVC did not change in the control runs from standing rest to the low workload. PRA, AVP, or NE also did not change (Fig. 2, solid bars). In HF (Fig. 1, open bars), there was significant tachycardia with respect to control values and HR increased further from standing rest to mild exercise. RAP was markedly elevated at standing rest with respect to control, and in contrast to the control experiments, RAP increased significantly further with mild exercise. SV and CO rose with exercise but were depressed significantly with respect to control. SAP, RBF, and RVC, although depressed compared with control at both standing rest and exercise, did not change significantly with the transition from standing rest to low workload. SVC rose significantly from standing rest to low workload, but these values were not different from the respective control values. TAQ rose on exercise, although the values were depressed with respect to control at both standing rest and during exercise. TAC was depressed significantly at rest but, with the transition to the low workload, rose to levels that were not significantly different from control. PRA and AVP (Fig. 2, open bars) were elevated compared with control experiments at standing rest and low workload. However, they did not rise with transition from standing rest to low workload. NE was elevated at standing rest and at low workload with respect to control and rose significantly between standing rest and low workload.

Comparison of Standing Rest to Exercise at 6.4 km/h at 10% Grade

In control experiments at moderate workload under free-flow conditions (Fig. 1), CO and HR were approximately twice that observed at standing rest, and TAQ approximately tripled. SV was higher than standing rest but did not increase beyond the value observed at low workload. RAP rose significantly beyond standing rest and the low workload value. As expected, SVC and TAC rose also and were significantly higher than observed at low workload. RBF did not change significantly compared with standing rest, but RVC fell. PRA, AVP, and NE rose significantly from standing rest to moderate workload (Fig. 2).

In HF at moderate workload (Fig. 1), CO, SV, TAQ, RBF, RVC, and SVC were significantly depressed with respect to control; however, SAP was not significantly different from control. HR was elevated with respect to control, and CO and HR both rose significantly from standing rest at the onset of exercise. SV increased

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**Fig. 2.** Neurohumoral responses at standing rest, free-flow exercise, and muscle metaboreflex activation. Left panels, 3.2 km/h at 0% grade; right panels, 6.4 km/h at 10% grade; solid bars, control; open bars, heart failure. *P < 0.05 vs. control; †P < 0.05 vs. rest; §P < 0.05 vs. free flow.
significantly beyond standing rest and low workload. RAP was markedly elevated from standing rest and from control. SVC was greater at moderate workload than at standing rest but depressed with respect to control. In HF, PRA, AVP, and NE all rose significantly at moderate workload from standing rest and their respective control values (Fig. 2).

Circulatory Response to Metaboreflex Activation During Dynamic Exercise

In the control runs, the hemodynamic responses were similar to those observed previously in normal dogs (16, 22, 33). At low workload (Fig. 3, filled circles) HR and CO remained relatively constant over substantial reductions in hindlimb perfusion from the free-flow state and then rose significantly after the reflex threshold was surpassed. SAP rose slightly but significantly at subthreshold levels of TAQ due to the mechanical effects of partial aortic occlusion. RAP, SV, and SVC did not change significantly at any reduction of TAQ. RBF did not vary significantly, although RVC fell significantly after the reflex was elicited. At the maximal levels of aortic occlusion, NE rose significantly (Fig. 2). At the maximal levels of aortic occlusion, AVP and PRA were slightly but significantly elevated compared with the resting levels. However, these values were not statistically different from those observed during free-flow exercise.

In HF at the low workload (Fig. 3, open circles), striking and significant changes occurred in the responses to metaboreflex activation vs. the control experiments. During free flow and subthreshold reductions in

![Diagram of hemodynamic parameters](http://ajpheart.physiology.org/)
TAQ, HR and RAP were elevated significantly with respect to control, whereas SAP, CO, SV, and RBF were depressed. On elicitation of the metaboreflex, SAP, RAP, and HR rose significantly; however, no significant increase in CO occurred. In addition, with metaboreflex activation during mild exercise in HF a significant decrease in SV occurred. SVC was maintained comparable to control values until the metaboreflex was activated, and then it fell significantly. As in the control runs, RBF did not fall significantly with elicitation of the reflex. Under subthreshold conditions, RVC was depressed and comparable to the value observed during metaboreflex activation (i.e., at maximal occlusion) in the control runs but fell significantly on elicitation of the reflex. PRA (Fig. 2) was higher than control but did not increase significantly from the free-flow value on metaboreflex activation. Concentrations of AVP and NE rose significantly from free-flow levels with activation of the reflex.

At moderate workload under control conditions, HR, CO, SAP, and RAP were much higher than at low workload (Fig. 3, filled triangles). Generally, any reduction of TAQ resulted in increases in HR, CO, and SAP, however, RAP and SV did not increase significantly on elicitation of the metaboreflex. RBF dropped significantly at the maximal occlusion. RVC was significantly lower under free-flow conditions or during subthreshold aortic occlusions than at rest or low workload and fell further on reflex activation. Metaboreflex activation did not result in higher PRA but did significantly increase concentrations of AVP and NE (Fig. 2).

In HF during exercise at moderate workload, any reduction in TAQ resulted in increases in HR and SAP. With maximal aortic occlusion, SAP increased significantly but was substantially lower than the control value, and CO did not increase significantly. SV remained ~75% of control. RAP was strikingly elevated beyond the control values under free-flow conditions but rose insignificantly at maximal aortic occlusion. SVC was lower than control but tended to fall insignificantly with reductions of TAQ. Before any aortic occlusion, RBF was similar to that observed in HF at low workload and fell significantly at maximal occlusion. RVC was significantly lower than control values (comparable to the control value observed at maximal occlusion) and, on partial aortic occlusion, RVC dropped significantly more. Plasma NE, AVP, and PRA were greatly elevated under free-flow conditions with respect to control values and became markedly elevated at maximum occlusion.

**DISCUSSION**

The major new finding in this study is that, in dogs with moderate HF, activation of the muscle metaboreflex still produces a significant pressor response; however, the mechanisms of this pressor response are markedly altered compared with those observed in control experiments. Specifically, in HF, the CO component of this reflex is virtually abolished and the pressor response occurs via peripheral vasoconstriction by markedly exaggerated sympathoactivation and the substantial release of vasoactive hormones. Finally, during moderate exercise in HF, skeletal muscle blood flow is reduced below the metaboreflex threshold as established in control experiments; therefore the exaggerated sympathoactivation and higher levels of vasoactive hormones in this setting may stem from tonic activation of the metaboreflex.

**Effect of HF on Hemodynamic Responses to Metaboreflex Activation**

CO, SAP, and RAP. The metaboreflex responses in the control experiments were similar to those observed in previous studies in this and other laboratories (16, 17, 22, 33). Activation of the metaboreflex at the low workload required substantial reductions in TAQ (~50%) and, once beyond the threshold for metaboreflex activation, further reductions in TAQ resulted in pronounced increases in HR, CO, and SAP. In a recent report, O’Leary and Augustyniak (17) demonstrated that the metaboreflex pressor response includes enhancement of ventricular contractility that preserves SV despite the rising afterload and falling filling time (i.e., increased HR), thus enabling increases in HR to increase CO. They also concluded, given that RAP remained constant, that the Frank-Starling mechanism does not contribute importantly to the maintenance of SV in normal dogs during metaboreflex activation.

In control experiments under free-flow conditions from standing rest to the moderate workload, substantial increases in HR, SV, CO, and RAP occurred. As described previously (33) the metaboreflex may already be active at this workload under free-flow conditions in normal dogs, so positive inotropic stimulation mediated by the metaboreflex may already be engaged. Furthermore, the rise in RAP from standing rest to free-flow exercise, probably in part due to increased activity of the skeletal muscle pump (24), indicates that the rise in SV may result from both increased contractility as well as the Frank-Starling mechanism. During the stepwise partial aortic occlusions, HR increased progressively yet SV was maintained constant across the measured range from free flow to maximum occlusion despite a progressively increasing afterload and decreasing filling time. This sustained stroke volume coupled with increased heart rate caused substantial increases in CO. As observed previously (17, 22, 33), during both mild and moderate workloads, the pressor response under control conditions was mediated via the increase in CO inasmuch as little net decrease in systemic vascular conductance occurred.

The metaboreflex cardiac responses were markedly altered after induction of HF. CO was depressed during free-flow exercise at both workloads, and the increase in CO with metaboreflex activation was virtually abolished. Despite a sustained tachycardic response, little change in CO occurred due to a concomitant fall in stroke volume. Previously, White et al. (32) demonstrated that tachycardia in and of itself does not augment CO in normal dogs within the range of HR observed in this study, owing to a fall in SV due to
decreased filling times. O’Leary et al. (17) concluded that improvements in ventricular contractility must accompany increases in HR to augment CO. Thus it appears that, after induction of HF, metaboreflex-mediated increases in ventricular performance were markedly attenuated. Although still chronotropically competent, the hearts became relatively inotropically insensitive and likely afterload sensitive. With metaboreflex activation in HF, despite a significant increase in filling pressure, stroke volume could not be maintained with the increase in afterload and decreased ventricular filling time. However, CO was significantly higher during free-flow exercise at the moderate workload versus the low workload in HF. In this setting, stroke volume was higher, probably due to the increased filling pressure and relatively low afterload (compared with that during metaboreflex activation).

Despite the inability to increase CO, activation of the metaboreflex in HF did result in a significant increase in SAP. However, in contrast to the control conditions wherein the rise in SAP was virtually solely due to the increase in CO, after induction of HF this pressor response was almost solely due to peripheral vasoconstriction. Quantification of the strength of the metaboreflex based on the ability to increase SAP can be problematic in this model. As described previously by Wyss et al. (33), in this experimental model of metaboreflex activation, SAP will increase due to reflex activation and also due to the mechanical effects of the partial vascular occlusion, i.e., a substantial mechanical reduction in conductance to a vascular bed that receives a large fraction of the CO can cause increases in SAP (15). Because perfusion pressure is a function of CO and SVC, the effects of the observed changes in CO and SVC on SAP can be calculated. This calculated change in SAP would therefore reflect the pressor response generated via the metaboreflex-mediated cardiac and peripheral vasomotor changes, independent of the passive mechanical effects of the occluder. Based solely on the observed reflex changes in CO and SVC, in control experiments arterial pressure would increase by 35 and 28 mmHg at the low and moderate workloads, respectively, whereas the observed changes were 56 and 47 mmHg. This difference between the changes in SAP calculated from the changes in CO and SVC versus those actually observed can be attributed to the mechanical effects of the vascular occluder (33). In HF, the average predicted changes in SAP during metaboreflex activation based on the observed reflex changes in CO and SVC were only 25 and 14 mmHg at the low and moderate workloads, respectively. Thus, despite significant peripheral vasoconstriction with metaboreflex activation, after induction of HF the inability to increase CO likely limits the ability of the metaboreflex to increase SAP and therefore limits the ability of the reflex to partially restore blood flow to the ischemic active skeletal muscle (20).

In the control experiments, no significant increase in RAP occurred with metaboreflex activation at either workload, although at the higher workload there tended to be a small increase that was not statistically significant. As discussed by Sheriff et al. (22), the lack of a change in RAP with a large increase in CO provides indirect evidence for metaboreflex-mediated central mobilization of blood volume that maintains ventricular filling pressure despite a large increase in CO. These conclusions are supported by the data obtained after induction of HF. In this setting, little or no change in CO occurred with metaboreflex activation, and large increases in RAP were observed at the low workload. At the higher workload RAP tended to increase with metaboreflex activation, but this change was not statistically significant, although it should be noted that RAP was already markedly elevated at this workload and further central volume mobilization via the metaboreflex may be limited.

Systemic vascular responses. Although SVC did not change appreciably with metaboreflex activation, this does not imply that regional circulations were unaffected (9, 11, 22). As shown previously, metaboreflex activation causes significant reductions in RVC (12, 22), and Mittelstat et al. (11) have shown that metaboreflex activation also decreases vascular conductance in nonschismic active skeletal muscle. In HF, although the absolute values of RVC and RBF were much lower with respect to the values observed at standing rest in the control experiments, the patterns of the responses were similar to those observed in the control experiments during both free-flow exercise and partial aortic occlusion. During low workload under free-flow conditions, RVC did not vary significantly from standing rest in HF. With partial aortic occlusion during the low workload a large decrease in RVC occurred. During moderate workload in HF, RBF under free-flow conditions was decreased ~10% from the values observed at standing rest; this approached statistical significance (P = 0.06, one-tailed). Previously, Mittelstadt et al. (12) used a similar model to study the responses to metaboreflex activation of paired normal and denervated kidneys at an intermediate workload of 10 km/h at 0% grade in otherwise normal dogs. Their results were similar to those obtained in the present investigation during control experiments with mild exercise (i.e., no change in RBF but decreases in RVC). They concluded that the renal vascular responses of the both normal and denervated kidneys in dogs likely reflect an auto-regulatory response to increased aortic pressure or a humorally mediated response, rather than increased renal sympathetic nerve activity. In contrast, in this study, with metaboreflex activation during moderate exercise after the induction of HF, significant decreases in both RBF and RVC occurred. This is not likely to be due solely to autoregulation. Millard et al. (10) also studied renal vascular responses in paired normal and denervated kidneys during severe exercise in groups of dogs with either intact or impaired hearts and with or without alpha receptor blockade. They demonstrated that the changes in RVC were not dependent on renal innervation or circulating catecholamines in normal dogs. However, in HF, blood flow to the innervated kidneys was depressed at rest and fell drastically
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Norepinephrine. In this study, the dogs were well socialized to the laboratory environment, so plasma NE, PRA, and AVP values observed at standing rest in the control experiments probably represent basal levels. During metaboreflex activation, the rise in sympathetic activity is clearly indicated by the threefold increase of plasma NE with low workload and the near tenfold increase with moderate workload. At these concentrations, plasma NE could possibly have direct influence on the heart and thus contribute to the metaboreflex response.

In HF, plasma NE tripled at standing rest, approximating the maximum response observed with metaboreflex activation during low workload. However, NE did not rise further with free-flow exercise at low workload but only upon metaboreflex activation. This, coupled with the demonstrable thresholds for HR and SAP responses, supports the concept that the metaboreflex is not active during mild exercise in HF, and the plasma NE concentrations observed at standing rest and free flow at low workload are due to some other mechanism. During moderate workload under free-flow conditions, the fivefold increase in plasma NE indicates intense sympathetic activation. On partial aortic occlusion, the plasma NE response became extreme, indicating massive sympathetic activation.

PRA. A number of studies have indicated that PRA rises with exercise workload under free-flow conditions in a number of species (5, 31), but few studies have directly addressed the role of the metaboreflex with respect to recruitment of the renin-angiotensin system. In a recent article, Nishiyasu et al. (13) demonstrated that metaboreflex activation stimulates renin and AVP release. Neither mild free-flow exercise nor metaboreflex activation during mild free-flow exercise elicits any increase of PRA in normal dogs (18). Similar results during control experiments at the low workload were observed in this study, but at the moderate workload under free-flow conditions, PRA was significantly elevated compared with standing rest, yet it did not increase further with definitive metaboreflex activation by partial aortic occlusion. Upon the induction of HF, PRA was elevated with respect to control at standing rest and during exercise at both workloads. As in the control experiments, no metaboreflex-induced release of renin occurred at the low workload, although sympathetic activation was evident at standing rest and increased with exercise and reflex activation. Apparently, the increased sympathetic activity mediated by the metaboreflex was insufficient to induce renin secretion at this workload. During moderate free-flow exercise in HF, the PRA increased dramatically (approximately twice that of standing rest) and, on partial occlusion of the terminal aorta, PRA rose further. Thus at this workload the renin response to metaboreflex activation likely contributed to the systemic vasoconstriction under free-flow conditions as well as during experimental metaboreflex activation.

AVP. As in the secretion of renin, previous studies have demonstrated that AVP secretion occurs during exercise, but relatively little is known about the relationship between the metaboreflex and AVP secretion. Yamashita et al. (34) have shown that stimulation of the group III and IV nerve afferents (the same classes of muscle afferents that mediate the muscle metaboreflex) increases the activity of AVP-secretory neurons located within the supraoptic nucleus. As noted above, Nishiyasu et al. (13) concluded that in humans metaboreflex activation elicits significant AVP secretion. There are two previous reports from this laboratory dealing with this subject. In the first, O’Leary et al. (18) demonstrated that the metaboreflex activation can stimulate AVP secretion during mild exercise, but only after ganglionic blockade of the large arterial pressor response. They attributed this effect to an inhibitory influence of the arterial baroreflex. In the second study, examining responses to graded exercise under free-flow conditions (19), heavy exercise induced AVP secretion during β-blockade, but the authors could not discriminate whether this response was mediated by the baroreflex (in response to relative hypotension) or by the metaboreflex (in response to decreased hindlimb blood flow).

In this study, we demonstrated in control experiments a very small but statistically significant increase of plasma AVP in response to metaboreflex activation at the low workload and, under free-flow conditions, at
moderate workload. On metaboreflex activation at moderate workload, a larger response occurred that may have directly induced vasoconstriction (6). In HF at standing rest and under free-flow conditions at low workload, although the values are significantly higher than respective controls, they probably do not have appreciable vasoconstrictive effects (6). However, in HF, metaboreflex activation induced increases of plasma AVP at the low workload that approach vasoactive concentrations (6). Furthermore, in HF at moderate workload, under free-flow conditions, AVP levels are substantially higher than observed under normal circumstances and rise dramatically on partial aortic occlusion. In this circumstance, plasma AVP probably produces direct vasoconstrictive effects (6). In all, these data further support the concept that the muscle metaboreflex is inactive during mild exercise in HF, is likely active during moderate exercise in HF, and on experimentally induced muscle ischemia (in normal or HF states) is clearly capable of recruiting potent humoral mechanisms to support or increase blood pressure.

Perspectives: Muscle Metaboreflex in HF

As observed in the present and previous studies, a reduction in cardiac performance during dynamic exercise causes decreases in skeletal muscle blood flow (19, 29). Inasmuch as at workloads above moderate levels no apparent threshold exists for the muscle metaboreflex in normal dogs, this reflex may be tonically active, and any reduction in skeletal muscle blood flow during moderate exercise or higher workloads likely further stimulates this reflex. Thus, during free-flow exercise at the moderate workload after induction of HF, the higher levels of vasoactive hormones, heart rate, renal vasoconstriction, and sympathetic nerve activity may stem in part from tonic activation of the muscle metaboreflex. In this setting, with the inability to further enhance CO, the metaboreflex elicited significant peripheral vasoconstriction. Previously, we observed that when CO was maintained constant (by combining β-adrenergic blockade with constant high HR via a pacemaker), a smaller but substantial pressor response still occurred with metaboreflex activation, but the mechanism of the pressor response shifted from primarily an increase in CO to solely peripheral vasoconstriction (22). That study, coupled with the present investigation, indicates that when the primary mechanism of the muscle metaboreflex to improve perfusion pressure is impaired (i.e., increased CO), this reflex can still evoke significant increases in SAP via peripheral vasoconstriction.

We do not know whether similar reductions in hind-limb blood flow elicit the same extent of metaboreceptor activation in HF. Inasmuch as the free-flow levels of TAQ are depressed in this setting and the metaboreflex may indeed be already tonically active at the moderate workload, it is possible that similar reductions in TAQ elicit larger increases in metabolite concentrations and thereby higher levels of metaboreceptor activation, as evidenced by the often markedly higher levels of metaboreflex-mediated increases in circulating vasoactive hormones and occasionally extreme renal vasoconstriction. In the present study the experiments were performed at the same absolute level of exercise before and after the induction of HF, and these workloads likely reflected different relative levels of exercise intensity (e.g., % maximal O2 consumption). Theoretically, the strength of the muscle metaboreflex in normal dogs may become less as workload increases toward the maximal levels of CO. In this setting, further increases in CO via the muscle metaboreflex would become progressively limited. In this study, the failure of the muscle metaboreflex to elicit any significant increase in CO at the low workload is not likely due to such limitations because, during the moderate workload, the free-flow level of CO increased substantially above that at the lower workload. To what extent the maximal CO is limited in this model has not been established, and this may be reflected in the limited ability to increase output further at the moderate workload. Finally, the metaboreflex is buffered by the arterial and cardiovacular baroreflexes (4, 23) and the gains of both of these baroreflexes are reduced in HF (2) such that the same level of metaboreceptor activation may yield larger reflex responses due to reduced baroreflex buffering. The extent to which HF alters the competing relationship between baroreflexes and the metaboreflex is unknown.

In summary, in normal dogs the metaboreflex is inactive at low workloads and possibly somewhat active at moderate workloads. On engagement, it normally operates by increasing cardiac performance, with adjustments to the peripheral vasculature that direct blood flow to the active muscles and support RAP. Humoral mechanisms are recruited only with strong metaboreflex activation, and therefore usually serve as secondary response mechanisms. However, with failure of the cardiac pump, during moderate and heavier exercise the metaboreflex is likely tonically activated, and the intense sympathetic stimulation plus the recruitment of potent vasoactive hormones become the primary mechanisms to support and increase blood pressure by peripheral vasoconstriction.

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