Altered muscle metaboreflex control of coronary blood flow and ventricular function in heart failure

Eric J. Ansorge,1 Robert A. Augustyniak,1 Mariana L. Perinot,1 Robert L. Hammond,1,2 Jong-Kyung Kim,1 Javier A. Sala-Mercado,1 Jaime Rodriguez,1 Noreen F. Rossi,3 and Donal S. O’Leary1

1Department of Physiology and 2Department of Surgery, Wayne State University School of Medicine, and 3Department of Internal Medicine, Wayne State University School of Medicine and John D. Dingell Veterans Administration Medical Center, Detroit, Michigan

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Address for reprint requests and other correspondence: D. S. O’Leary, Dept. of Physiology, Wayne State Univ. School of Medicine, 540 East Canfield Ave., Detroit, MI 48201 (E-mail: doleary@med.wayne.edu).

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When exercising skeletal muscle does not receive sufficient blood flow to meet the metabolic demands, metabolites (e.g., lactic acid, H+; diprotonated phosphate) accumulate and stimulate group III and group IV afferent neurons within the active skeletal muscle, which evokes a reflex increase in sympathetic nerve activity (SNA) and arterial pressure, known as the muscle metaboreflex (1–4, 7, 9, 11, 12, 21–24, 30–32, 35). In such instances, the metaboreflex acts to correct the blood flow deficit by increasing perfusion pressure, which is achieved via increases in cardiac output (CO) and vasoconstriction within nonischemic vascular beds (16, 17, 22, 25).

Prior studies in normal dogs have demonstrated that during mild and moderate workloads the major mechanism utilized by the muscle metaboreflex to improve blood flow to the ischemic muscle is to raise CO (4, 36). This increase in CO with muscle metaboreflex activation is likely the result of increases in heart rate (HR), ventricular performance, and central blood volume mobilization (21–24, 28). Previously, Stebbins (33) observed significant increases in left ventricular +dP/dt with electrically induced static muscle contraction in anesthetized cats, indicating that increases in ventricular contractility can be elicited via activation of reflexes arising from skeletal muscle afferents. However, because static muscle contraction can activate both mechanosensitive and metabosensitive afferents, it is unclear to what extent each subpopulation of skeletal muscle afferents mediated the observed responses. Using conscious dogs, O’Leary and Augustyniak (22) demonstrated that the normal rise in CO with muscle metaboreflex activation is predominately due to an increase in HR with little change in stroke volume (SV). When the animals were paced at constant HR and the muscle metaboreflex was activated, they observed a significant increase in SV, such that the rise in CO was similar to control experiments. These data allude to an increase in ventricular performance with muscle metaboreflex activation, inasmuch as there was no significant change in central venous pressure (CVP); thus the increase in SV was unlikely due to a Frank-Starling mechanism (22). We recently observed that this increase in SV with metaboreflex activation at constant HR was abolished after induction of heart failure (HF), indirectly indicating that the ability of the reflex to increase ventricular function is impaired in HF (24). To our knowledge, there are no prior studies that have used more specific indexes of ventricular function with muscle metaboreflex activation during dynamic exercise in normal subjects or in HF.

In dogs with HF, cardiac dysfunction likely prevents the normal elevation in CO observed with muscle metaboreflex activation (7, 24). In this setting, the mechanism by which the pressor response is generated with muscle metaboreflex activation during submaximal exercise is shifted from increases in CO to increases in peripheral vasoconstriction (7). In this pathological condition, skeletal muscle blood flow is lower and this attenuated perfusion during exercise may tonically activate the muscle metaboreflex (7, 8, 29).
Recently, we reported that the rise in cardiac SNA with muscle metaboreflex activation is capable of eliciting vasoconstriction within the coronary vasculature (2). Increases in cardiac SNA can oppose metabolic coronary vasodilatation via α-adrenergic receptor-mediated vasoconstriction (5, 6). Previously, Hammond et al. (7) demonstrated that muscle metaboreflex activation in dogs with HF elicited markedly greater sympathetic activation than in normal animals. This much greater rise in SNA could induce greater coronary vasoconstriction, which could thereby limit increases in ventricular function (5, 6). Thus the present study was designed to determine the effect of the muscle metaboreflex on coronary blood flow (CBF) and coronary vascular conductance (CVC), as well as changes in regional left ventricular performance before and after the induction of HF. We hypothesized that metaboreflex activation in normal animals would cause increases in regional myocardial performance and that in HF activation of this reflex would induce coronary vasoconstriction and little improvement in ventricular function.

METHODS

We performed all experiments using nine conscious dogs (21–26 kg, 7 male, 2 female) selected for their ability and willingness to run on a motor-driven treadmill. All procedures were reviewed and approved by the institutional Animal Care Committee and were in agreement with National Institutes of Health guidelines.

Surgical preparation. Each animal was instrumented via three surgical sessions with at least 1 wk between surgeries and between the last surgery and first experiment. For all surgical events, anesthesia was induced with Pentothal Sodium and maintained with isoflurane. Cefazolin (500 mg iv) was given both before and at the conclusion of the surgery, and then cephalaxin (30 mg/kg by mouth, 2 times/day) was given to avoid postoperative infection. Throughout the surgical preparation, buprenorphine (0.015 mg/kg iv) and acepromazine (0.1 mg/kg im) were administered for postoperative analgesia and sedation, whenever deemed necessary.

The first surgical procedure involved a left thoracotomy at the fourth intercostal space, where a blood-flow transducer (Transonic Systems, A series, 20 mm) was placed around the ascending aorta to measure CO. A second blood-flow transducer was placed around the left circumflex coronary artery (S series, 3 mm) to measure CBF. Two sonomicrometry crystals (Sonometrics) were implanted in the epicardium of the anterior free wall of the left ventricle (~1.5-2.0 cm apart for measurement of myocardial segment length. Stainless steel electrodes (O Flexon) were sutured to the apex of the left ventricle for ventricular pacing. The edges of the pericardium were reaproximated, and the chest was closed in layers.

The second surgical procedure employed either a midventral abdominal or left retroperitoneal approach, and blood-flow transducers (Transonic Systems, A Series, 10 mm) were placed around the terminal aorta to measure hindlimb blood flow (HLBF); another blood-flow transducer was also placed on the left renal artery (S Series, 3 mm) for studies unrelated to the present investigation. A vascular occluder (In Vivo Metrics, 10 mm) was placed around the terminal aorta distal to the flow probe. All side branches between the iliac arteries and the flow probe were ligated and cut. A catheter was inserted into a side branch of the aorta proximal to the flow probe and occluder for measurement of mean arterial pressure (MAP).

In the final surgical session, a fluid-filled catheter was inserted into the right jugular vein and advanced through the vein to the atrial-caval junction to measure CVP. All cables, occluder tubes, and catheters were tunneled subcutaneously and exited through the skin, between the scapulae.

Experimental procedures. All experiments were conducted after the animals had fully recovered from surgery and were active and had regained presurgery eating habits. Each animal was brought to the laboratory and allowed to roam freely for 15–30 min, thereby acclimating to its surroundings. The animal was then directed to the treadmill, the blood flow transducers were connected to the flowmeters (model T206, Transonic Systems), and the MAP and CVP catheters were connected to pressure transducers (Transpac IV, Abbott Laboratories). All flow and pressure transducers were coupled to a Gould recording system (model RS 3800). HR was measured via a cardiotachometer triggered by the CO signal. All data were sampled by a laboratory computer at 1,000 Hz, and mean values for each cardiac cycle were saved on the laboratory computer for future analysis.

As previously described, we activated the muscle metaboreflex during mild or moderate treadmill exercise (3.2 km/h, 0% grade and 6.4 km/h, 10% grade) via stepwise reductions in HLBF achieved by partially inflating the vascular occluder implanted on the terminal aorta (2, 4, 7, 21–23). The treadmill was started; after 3–5 min, all hemodynamic variables reached steady state, at which time the hindlimb occluder was then partially inflated, resulting in graded reductions in hindlimb perfusion. The occlusion was held constant until all cardiovascular variables reached steady state, ~3–5 min.

After control experiments were completed, the animals were paced at 225–250 beats/min for 41 ± 6 days until clinical signs of congestive HF were evident (i.e., reduced arterial blood pressure, elevated CVP, resting tachycardia, and depressed CO; see Table 1). Once the animals were in a moderate level of HF, muscle metaboreflex experiments were repeated at mild and moderate workloads. Before the start of the experiment, the pacemaker was disconnected and reconnected at the conclusion of each experiment.

Statistical analysis. Each dog served as its own control. The data were analyzed, as previously described by Wyss et al. (36) to ascertain 1) control values measured during free-flow exercise (no imposed hindlimb occlusion), 2) values measured at metaboreflex threshold (initial onset of muscle metaboreflex activation), and 3) the values measured during the largest reduction in HLBF. One-minute averages of all cardiovascular hemodynamic variables were measured during steady state and at each level of hindlimb vascular occlusion. During mild dynamic exercise, initial reductions of HLBF do not elicit metaboreflex responses, (i.e., changes in MAP, HR, and CO) as previously described (2, 4, 7). Once HLBF was reduced below a threshold level, additional reductions in HLBF elicit marked increases in MAP, HR, and CO. During mild exercise, when measured output variables, such as MAP and HR, are plotted vs. HLBF, the responses resemble a “dog leg” pattern. As a result, the data were approximated to two linear regressions, an initial response line, where no substantial change in MAP or HR occurred with initial reductions in hindlimb perfusion, and a pressor response line when additional reductions in

Table 1. Average hemodynamic values at rest before (Control) and after the induction of heart failure

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>CO, 1/min</th>
<th>HR, beats/min</th>
<th>SV, ml</th>
<th>HLBF, 1/min</th>
<th>CVP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>102.6 ± 2.8</td>
<td>5.52 ± 0.70</td>
<td>102.7 ± 6.5</td>
<td>54.2 ± 6.1</td>
<td>0.98 ± 0.14</td>
<td>3.8 ± 0.6</td>
</tr>
<tr>
<td>Heart failure</td>
<td>85.4 ± 4.8*</td>
<td>3.70 ± 0.25*</td>
<td>120.0 ± 6.6*</td>
<td>31.3 ± 2.3*</td>
<td>0.61 ± 0.07*</td>
<td>11.8 ± 0.8*</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; CO, cardiac output; HR, heart rate; SV, stroke volume; HLBF, hindlimb blood flow; CVP, central venous pressure. *P < 0.05 vs. control.
Hindlimb occlusion can increase MAP by two mechanisms: 1) via the metaboreflex activation and 2) through the passive, mechanical effects of the occluder. The rise in MAP resulting from the passive, mechanical effects of the occluder was calculated as previously described by Augustynia et al. (4). The rise in MAP due solely to the metaboreflex-mediated increases in CO and peripheral vasoconstriction (MAPactive) is the difference between the observed increase in MAP and that due solely to the mechanical effects of the occluder.

Regional myocardial performance was assessed by measuring the first derivative (i.e., rate of change) of myocardial segment length. Systolic function was measured by calculating the minimum first derivative of myocardial shortening (−dldtmax), and diastolic function was measured by calculating the maximum first derivative of myocardial relaxation (+dldtmax) during ventricular contraction and relaxation, respectively. Maximal values of hemodynamic data observed during muscle metaboreflex activation were compared with the values during exercise before hindlimb occlusion by paired Student’s t-test. Hemodynamic values at rest before and after induction of HF were also compared by Student’s t-test. The effects of exercise and metaboreflex activation on systolic and diastolic function (−dldtmin and +dldtmax, respectively) were tested using ANOVA for repeated measures. Comparisons of individual means were made with the “Test for Simple Effects” (Systat version 8.0 and 11.0 software). All data are presented as means ± SE. Because of technical problems, not all data could be obtained in all animals.

RESULTS

Table 1 shows the average hemodynamic values at rest during control experiments and in the same animals after the induction of HF (n = 9). HF caused significant decreases in MAP, CO, HLBF, and SV; whereas HR and CVP were significantly elevated, indicating that the animals were in a moderate stage of HF.

Figure 1 shows a representative tracing of CO, myocardial segment length, −dl/dtmin and +dl/dtmax during mild exercise, represented as a function of time.

Figure 2 shows the average hemodynamic responses to muscle metaboreflex activation during mild exercise in control experiments and after induction of HF (n = 8). As observed previously (2), with muscle metaboreflex activation in control experiments, MAPactive, CO, HR, SV, and CBF were significantly elevated with no significant change in CVC. In HF, CO during exercise was depressed; with muscle metaboreflex activation, the increases in MAPactive and CBF were attenuated. Although HR increased, SV decreased, resulting in no significant change in CO. Muscle metaboreflex activation in HF caused significant vasoconstriction within the coronary vasculature, as indicated by the reduction in CVC.

Figure 3 shows average hemodynamic data as a function of HLBF at moderate exercise in control studies and after the induction of HF (n = 5). In control experiments, with muscle metaboreflex activation, MAPactive, CO, HR, and CBF were significantly elevated, although there was no significant change in SV or CVC. In contrast, muscle metaboreflex activation in HF resulted in an attenuated, yet significant, rise in MAPactive; the same attenuated response patterns were also observed for CO and CBF. Activation of the muscle metaboreflex in HF elicited a significant reduction in CVC, indicating vasoconstriction within the coronary vasculature.

Figure 4 shows average −dl/dtmin and +dl/dtmax data during mild (n = 6) and moderate (n = 6) exercise in control experiments. When the muscle metaboreflex was activated during mild exercise, there was no significant change in −dl/dtmin. However, there was a significant increase in +dl/dtmax between free-flow exercise and maximum muscle metaboreflex activation. During moderate exercise, muscle metaboreflex activation elicited significant increases in both systolic (−dl/dtmin) and diastolic (+dl/dtmax) function compared with free-flow exercise.

Figure 5 shows average −dl/dtmin and +dl/dtmax data during mild exercise (n = 5) after induction of HF. When the muscle metaboreflex was activated during mild exercise in HF, there was no significant change in either −dl/dtmin or +dl/dtmax between free-flow exercise and maximum metaboreflex activation, indicative of the compromised ability to increase ventricular function.

DISCUSSION

The major new findings in this study are that, during HF, activation of the muscle metaboreflex induces vasoconstriction...
in the coronary vasculature during both mild and moderate exercise. In addition, activation of the muscle metaboreflex elicits significant increases in regional left ventricular performance in normal subjects, whereas, after the induction of HF, this reflex increase in ventricular function is abolished.

Previous studies from our laboratory and others have shown that muscle metaboreflex activation increases SNA to the peripheral vasculature and the heart, which elicits regional vasoconstriction and increased HR, ventricular performance, and CO (2, 3, 7, 16, 21, 22, 24, 28, 30, 35, 36). Thus, with muscle metaboreflex activation, there is a substantial increase in myocardial work (e.g., significantly increased CO pumped against a markedly higher arterial pressure). This rise in myocardial work with muscle metaboreflex activation is a strong stimulus for metabolic coronary vasodilation (20). However, we have previously shown in normal animals that, when activated, the muscle metaboreflex-induced rise in SNA to the heart functionally vasoconstricts the coronary vasculature, thereby attenuating the rise in CBF (2). In that study, muscle metaboreflex activation during mild exercise before (control) and after induction of HF. During mild exercise, active MAP, CO, HR, SV, and CBF increased with metaboreflex activation with no significant change in CVC. However, during heart failure, no increases in CO occurred, the rise in CBF was smaller, and significant decreases in SV and CVC occurred. *P < 0.05 vs. free-flow exercise; NS, no significant change from free-flow exercise.

**Fig. 2.** Average levels of the observed mean arterial pressure (MAP) (● and solid line), passive MAP (that due to the mechanical effects of the occluder; ▲ and dotted line), and active MAP (observed MAP minus passive MAP; ■ and dashed line); CO, heart rate (HR), stroke volume (SV), coronary blood flow (CBF), and coronary vascular conductance (CVC) plotted as a function of hindlimb blood flow (HLBF) during mild exercise before (control) and after induction of heart failure. During mild exercise, active MAP, CO, HR, SV, and CBF increased with metaboreflex activation with no significant change in CVC. However, during heart failure, no increases in CO occurred, the rise in CBF was smaller, and significant decreases in SV and CVC occurred. *P < 0.05 vs. free-flow exercise; NS, no significant change from free-flow exercise.
ventricular pacing and β-adrenergic blockade), a significant reduction in CVC was observed in response to hindlimb ischemia. Thus, in normal dogs, it appears that a balance exists with muscle metaboreflex-induced increases in SNA to the heart between vasodilator (via increased myocardial work) and vasoconstrictor (via vascular β-adrenergic receptor activation) effects.

There appears to be a paradox between β-adrenergic coronary vasoconstriction and its effect on cardiac function in varying conditions, inasmuch as coronary vasoconstriction may be helpful in normal animals and deleterious in those with HF. In normal dogs, we demonstrated that metabolic coronary vasodilation was counteracted by β-adrenergic-mediated vasoconstriction (2). The observed vasoconstrictor effect likely aids in the maintenance of ventricular transmural blood flow (10). However, it is likely that the heightened SNA-mediated α-adrenergic coronary vasoconstriction attenuates the rise in CBF during exercise in HF, thereby limiting myocardial perfusion and potentially attenuating ventricular performance (5, 6, 13).

Despite a functional vasoconstriction within the coronary vasculature in control experiments (e.g., no increases in CVC despite marked increases in myocardial O2 demand), significant increases in ventricular function occurred. Both systolic (−dl/dt_{min}) and diastolic (+dl/dt_{max}) performances were increased with muscle metaboreflex activation during moderate exercise, and diastolic function was increased during mild exercise, supporting the hypothesis that the muscle metaboreflex activation does indeed augment ventricular performance (22, 24).

The shift in the coronary vascular responses with muscle metaboreflex activation toward vasoconstriction in HF may be
due to lower vasodilatory drive (e.g., less increase in myocardial O$_2$ demand) and/or increased $\alpha$-adrenergic receptor activation (e.g., higher levels of SNA). These two factors may also be interrelated. With muscle metaboreflex activation in HF, little increase in CO occurred; thus the normal rise in myocardial O$_2$ demand is likely reduced (although still elevated from the free-flow exercise levels due to the increased HR and afterload). In addition, we have previously shown that arterial plasma levels of norepinephrine with muscle metaboreflex activation are enormously elevated in HF, indicating massive sympathetic activation (indeed in that study, renal blood flow was reduced to undetectable levels in some animals) (7). Leier et al. (14) discussed the effect of elevated plasma norepinephrine acting on $\alpha_1$-adrenergic receptors, resulting in enhanced coronary vasoconstriction in HF patients. Thus it is likely that, with muscle metaboreflex activation in HF, SNA to the heart is substantially higher than that shown in control experiments. However, this markedly elevated SNA elicits little increase in ventricular performance and thus a smaller metabolic vasodilatory drive to counteract the vasoconstrictor effects of the concomitant coronary vascular $\alpha$-adrenergic receptor activation. To what extent the decline in ventricular performance can be attributed to coronary vasoconstriction during metaboreflex activation in HF has yet to be determined. The increased functional coronary vasoconstriction observed with muscle metaboreflex activation in HF may, in turn, act to limit increases in ventricular function (5, 6, 13). The attenuated rise in CBF and significant coronary vasoconstriction observed in HF with muscle metaboreflex activation may be a contributing factor into the reduced ability to raise CO (i.e., reduced contractile and pump function). There was no significant change in $-\frac{d}{dt}$min or $+\frac{d}{dt}$max between free-flow

Fig. 4. Average minimum 1st derivative of myocardial shortening ($-\frac{d}{dt}$min) and maximum 1st derivative of myocardial lengthening ($+\frac{d}{dt}$max) data during rest, free-flow, and maximum metaboreflex (MMR) activation at mild and moderate workloads from control experiments before the induction of heart failure. *$P < 0.05$ vs. free-flow exercise; NS, no significant change from free-flow exercise.

Fig. 5. Average $-\frac{d}{dt}$min and $+\frac{d}{dt}$max data during rest, free-flow, and maximum MMR activation during mild exercise after the induction of heart failure. NS, no significant change from exercise before partial hindlimb occlusion.
exercise and maximal muscle metaboreflex activation during mild exercise in HF, indicative of systolic and diastolic dysfunction. Previous studies have focused on systolic function as the prime mediator in reduced contractile ability in HF (26, 34). However, there is a growing body of evidence indicating that the inability to increase CO during HF is related to the shortened period of time spent in diastole (18, 19). As a result of an attenuated diastole (15), SV is lessened with each contraction, thereby reducing CO; as a consequence, myocardial perfusion is also attenuated, likely contributing to systolic complications. Shannon et al. (27) concluded that the impairment in contractile function in HF animals was directly related to attenuated myocardial perfusion, especially coronary flow reserve, inasmuch as CBF and vasodilator reserve were substantially reduced in HF.

**Limitations.** There are several limitations in this study. We did not measure total or myocardial O2 consumption. In several previous studies, we have used these workloads as “mild” and “moderate” levels, as indicated by the clear threshold level of HLBF for metaboreflex activation during mild exercise and the lack of a clear threshold level of HLBF for metaboreflex activation during the moderate workload (2, 4, 7, 23). With maximal HR of ~270 beats/min observed in our laboratory during maximal levels of volitional exercise (2, 4), these workloads reflect ~45% and 70% of maximal HR in control conditions. After the induction of HF, the experiments were performed at the same absolute levels of exercise, which likely reflect a larger percentage of maximal O2 consumption in this model of HF (which may be different from other models such as ischemic or diabetic cardiomyopathy, etc.). The lack of any increase in CO with metaboreflex activation in HF is not due to the inability to further increase CO inasmuch as we have previously shown that dogs with this model of HF are capable of substantially higher levels of exercise at which higher levels of CO are observed (8). We used rates of change of regional workloads reflect substantial increases in SNA to the heart, which increases myocar-
dial work and would be expected to cause significant cor-
ronal vasodilation. However, in HF, SNA is markedly elevated, which likely gives rise to α-adrenergic-mediated vasoconstriction within the coronary vasculature. When coupled with already attenuated coronary flow reserve, this limits the ability to increase myocardial perfusion, and, as a result, myocardial contractile function is likely diminished. Thus muscle metaboreflex activation during HF increases coronary vasoconstriction, thereby attenuating increases in CBP and possibly myocardial function.

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