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Muscle Metaboreflex And Arterial Baroreflex: Action, Interaction And Altered Control In Heart Failure

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MUSCLE METABOREFLEX AND ARTERIAL BAROREFLEX: ACTION, INTERACTION AND ALTERED CONTROL IN HEART FAILURE

by

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Advisor

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DEDICATION

I would like to dedicate this dissertation to my parents, Sikander Singh and Surinder Kaur, who value education above all and gave me endless love and support during my pursuit of higher education.

To my brother Rajdeep Singh, who is always there to cheer me up and encourage me.

To my dearest friends: It would have been impossible without you all.

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
BCO	bilateral carotid occlusion
BID	twice a day
CO	cardiac output
dP/dt_{max}	maximal rate of the rise in left-ventricular pressure
dP/dt_{min}	maximal rate of the fall in left-ventricular pressure
EX	(mild, dynamic) exercise
FAP	femoral arterial pressure
HF	heart failure
HLBF	hindlimb blood flow
HR	heart rate
HVC	hindlimb vascular conductance
IM	intramuscular
IV	intravenous
LVP	left-ventricular pressure
MAP	mean arterial pressure
MMA	muscle metaboreflex activation
NIVC	non-ischemic vascular conductance
PO	by mouth
RBF	renal blood flow
RVC	renal vascular conductance
SAD	sinoaortic denervation
SE	standard error
SNA	sympathetic nerve activity
SUM	sum of individual MMA and BCO responses during exercise
TVC	total vascular conductance

CHAPTER 1 - INTRODUCTION

Cardiovascular responses during dynamic exercise are a result of a complex interaction between at least three mechanisms: central command, the arterial baroreflex and the muscle metaboreflex. Central command primarily controls parasympathetic activity to the heart and has little control over sympathetic nerve activity (SNA) (89). The arterial baroreflex is a negative feedback reflex which maintains arterial pressure primarily by modulating peripheral vascular conductance. An increase in arterial pressure causes baroreflex-induced peripheral vasodilation and bradycardia while a decrease in arterial pressure evokes peripheral vasoconstriction and tachycardia, restoring arterial pressure back to normal in both scenarios. During exercise, the arterial baroreflex is reset to a higher level but without any change in the gain of reflex (20, 53, 62). The muscle metaboreflex is activated when insufficient oxygen delivery to the exercising skeletal muscle results in accumulation of metabolites (e.g., hydrogen ions, adenosine, diprotonated phosphate, etc.) which stimulate the group III/IV muscle afferents eliciting a large pressor response, termed the muscle metaboreflex (1, 9, 65, 76, 77).

Muscle metaboreflex activation (MMA) during dynamic exercise causes significant increases in mean arterial pressure (MAP), heart rate (HR), cardiac output (CO) and ventricular contractility which along with enhanced central blood volume mobilization maintains or slightly increases stroke volume (6, 18, 43, 70, 71, 95). With stroke volume being maintained, the rise in HR elicits substantial increases in CO. However, the peripheral vascular responses have varied in previous studies. While significant vasoconstriction has been observed in the renal, coronary and forelimb vascular beds (6, 13, 48, 49) when taken as a whole, the total vascular conductance of

all vascular beds except the hindlimbs, termed non-ischemic vascular conductance (NIVC), usually remains unchanged or slightly increases (6, 13, 40, 69, 78). Inasmuch as some individual vascular beds do vasoconstrict, the observation of no change or slight increase in NIVC indicates that some vascular bed(s) must vasodilate. What causes this vasodilation is unknown. Metaboreflex-mediated increases in SNA can lead to elevated pre-ganglionic sympathetic activity to the adrenal glands which can substantially increase epinephrine release (8, 41, 44, 46) resulting in β_2-mediated vasodilation, especially in the skeletal muscle which is richly endowed with β_2-adrenergic receptors. In addition, post-ganglionic sympathetic nerve terminals have also been shown to release epinephrine (8, 41, 44, 46). However, whether epinephrine release contributes to muscle metaboreflex-induced peripheral vascular responses is unknown.

Muscle metaboreflex is generally considered as a flow-mediated, flow-raising reflex which is activated by insufficient blood flow and oxygen delivery to the active skeletal muscle and raises total systemic blood flow (i.e., CO) to improve blood flow and oxygen delivery to the ischemic vasculature. MMA during submaximal exercise increases MAP, HR, CO and ventricular contractility along with some regional vasoconstriction (6, 13, 48, 49). The metaboreflex-induced increase in MAP is primarily caused by an increase in CO, as little if any net peripheral vasoconstriction occurs (18, 32, 71, 74). Several studies have suggested that the metaboreflex-induced increase in CO partially restores blood flow to the ischemic active skeletal muscle (16, 56, 59, 68). Whether the increased SNA during MMA activation vasoconstricts the ischemic active muscle from which the reflex originates is unknown. Should this occur, then the ability of the muscle metaboreflex to correct the deficits in blood flow to the ischemic muscle

would be restrained.

Heart failure (HF) is characterized by low CO and high SNA during rest and exercise. With only moderate exercise, massive sympathetic activation is often observed in HF, resulting in an exaggerated metaboreflex activation (29). With the normal metaboreflex-induced increases in CO being substantially attenuated in HF, the mechanisms of metaboreflex shift from flow-mediated to vasoconstriction-mediated pressor responses in HF (16, 29, 58). The ability of vasoconstriction in a given vascular bed to raise MAP is directly related to the fraction of CO received by that bed. During exercise most of the CO is directed to the active skeletal muscle. Vasoconstriction of inactive vascular beds plays a very small role in any reflex pressor response as blood flow to the inactive beds constitutes only a small proportion of CO and thereby, total vascular conductance (TVC). With a large proportion of CO going to the exercising skeletal muscle, it is this vascular bed which is the likely target vasculature for metaboreflex-induced vasoconstriction in HF. With exaggerated SNA in HF, metaboreflex activation could lead to heightened vasoconstriction of active muscle, possibly including the ischemic muscle itself. In this case, vasoconstriction of the already ischemic muscle would lead to further ischemia in the muscle that would potentiate the reflex activation causing a positive feedback loop scenario. Whether MMA in HF induces an exaggerated vasoconstriction of ischemic active muscle itself is unknown.

Another key regulator of arterial blood pressure is the arterial baroreflex. During dynamic exercise in normal individuals, arterial baroreflex is reset to a higher arterial pressure although its strength to regulate pressure remains unaltered (21, 53, 62, 67). Sheriff et. al. (72) showed that during MMA, baroreflex buffers up to 50% of the

metaboreflex-mediated increases in MAP. Kim et. al. (40) demonstrated that this buffering occurs by restraining metaboreflex-induced peripheral vasoconstriction. After sinoaortic denervation (SAD) in the same animals, metaboreflex-induced pressor response was twice as large and resulted from a combination of peripheral vasoconstriction and increase in CO. Thus, the arterial baroreflex alters the strength and mechanisms of the muscle metaboreflex during dynamic exercise by inhibiting metaboreflex-mediated peripheral vasoconstriction. When carotid receptors are unloaded via bilateral carotid occlusion (BCO), the substantial increase in MAP occurs primarily due to peripheral vasoconstriction (11). Therefore, muscle metaboreflex and arterial baroreflex have distinct mechanisms for regulating MAP and no studies have yet investigated the interaction between the two when activated concurrently. The interaction of the arterial baroreflex and the muscle metaboreflex has not been well established, especially when these reflexes are coactivated. Furthermore, previous studies from our laboratory have concluded that active skeletal muscle is a primary target for baroreflex-induced peripheral vasoconstriction (11). To what extent ischemic active muscle may vasoconstrict is controversial.

HF alters the strength of arterial baroreflex both at rest (61) and during exercise (38). This impairment would lessen the ability of baroreflex to attenuate peripheral vasoconstriction induced by muscle metaboreflex, leading to a larger increase in vascular sympathetic tone compared to normal subjects. Inasmuch as the strength of the baroreflex and baroreflex buffering of metaboreflex-induced pressor response are impaired in HF, there would be a smaller pressor response with metaboreflex and baroreflex coactivation. To what extent HF alters the interaction between the baroreflex and muscle metaboreflex is unknown. Therefore, my studies are designed to address

three tightly coupled Specific Aims. My approach is to use a complex chronically instrumented canine model. All studies are longitudinal in nature. The animals are instrumented and responses are observed on different days and in different settings (e.g. agonist/antagonist infusions, rest vs. exercise, etc.), then heart failure is induced and the experiments are repeated in the same animals. Thus, each animal serves as its own control.

Specific Aim (I): **To determine the mechanism(s) mediating muscle metaboreflex control of blood flow to the non-ischemic active vasculature during dynamic exercise.**

Muscle metaboreflex-induced pressor response is caused virtually solely by an increase in cardiac output, as little, if any, increase in total vascular conductance occurs. In several studies, we observed that muscle metaboreflex activation caused a significant increase in vascular conductance of all vascular beds except the hindlimbs (termed non-ischemic vascular conductance; NIVC) (6, 40, 69). I hypothesized that this increase in NIVC may stem from a metaboreflex-induced release of epinephrine resulting in β_2-adrenergic mediated vasodilation in the skeletal muscle.

Specific Aim (II): **To determine the mechanism(s) mediating muscle metaboreflex control of blood flow to the ischemic active vasculature during submaximal dynamic exercise in normal animals and in the same animals after induction of heart failure.**

We and others have shown that MMA elicits vasoconstriction of the coronary, renal and forelimb vasculatures. Whether the metaboreflex vasoconstricts the ischemic muscle from which it originates has been controversial. HF is characterized by markedly elevated SNA both at rest and during exercise. When metaboreflex-induced increase in

cardiac output becomes limited in HF, MMA causes large peripheral vasoconstriction. Whether MMA in HF causes exaggerated vasoconstriction of the ischemic active muscle is unknown. I hypothesized that MMA elicits vasoconstriction of the active skeletal muscle in normal subjects and this vasoconstriction is exacerbated in HF.

***Specific Aim (III)*: To investigate the interaction between the muscle metaboreflex and the arterial baroreflex before and after induction of heart failure.**

Activation of both reflexes would cause an exaggerated pressor response, a resultant effect of both, an increase in CO and peripheral vasoconstriction. I tested whether the interaction between the two reflexes is additive, occlusive or facilitative. Since carotid baroreceptor unloading induces peripheral vasoconstriction, to what extent the ischemic muscle vasoconstricts during coactivation of the baroreflex and the muscle metaboreflex is unknown. The strength of arterial baroreflex is impaired in HF and coactivation of metaboreflex and baroreflex in HF would cause a smaller pressor response compared to normal individuals.

CHAPTER 2 - MUSCLE METABOREFLEX ACTIVATION DURING DYNAMIC EXERCISE EVOKES EPINEPHRINE RELEASE RESULTING IN β_2-MEDIATED VASODILATION

(This Chapter contains previously published material. See Appendix B)

Abstract

Muscle metaboreflex-induced increases in mean arterial pressure (MAP) during submaximal dynamic exercise are mediated principally by increases in cardiac output. To what extent, if any, the peripheral vasculature contributes to this rise in MAP is debatable. In several studies, we observed that in response to muscle metaboreflex activation (MMA; induced by partial hindlimb ischemia) a small but significant increase in vascular conductance occurred within the non-ischemic areas (calculated as cardiac output minus hindlimb blood flow and termed non-ischemic vascular conductance; NIVC). We hypothesized that these increases in NIVC may stem from a metaboreflex-induced release of epinephrine resulting in β_2-mediated dilation. We measured NIVC and arterial plasma epinephrine levels in chronically instrumented dogs during rest, mild exercise (3.2 kph) and MMA before and after β-adrenergic blockade (propranolol; 2 mg/kg), α_1-adrenergic blockade (prazosin; 50 μg/kg) and α_1+β-blockade. Both epinephrine and NIVC increased significantly from exercise to MMA: 81.9 ± 18.6 to 141.3 ± 22.8 pg/ml and 33.8 ± 1.5 to 37.6 ± 1.6 ml/min/mmHg, respectively. These metaboreflex-induced increases in NIVC were abolished after β-blockade (27.6 ± 1.8 to 27.5 ± 1.7 ml/min/mmHg) and potentiated after α_1-blockade (36.6 ± 2.0 to 49.7 ± 2.9 ml/min/mmHg), while α_1+β-blockade also abolished any vasodilation (33.7 ± 2.9 to 30.4 ± 1.9 ml/min/mmHg). We conclude that MMA during mild dynamic exercise induces epinephrine release causing β_2-mediated vasodilation.

Introduction

Insufficient oxygen delivery to active skeletal muscle during dynamic exercise causes accumulation of metabolites (e.g., hydrogen ions, lactate, diprotonated phosphate, etc.) which activate group III/IV chemosensitive afferents eliciting a reflex increase in arterial blood pressure and sympathetic outflow, known as the muscle metaboreflex (1, 9, 43, 65, 66, 75-77, 95). The metaboreflex-mediated pressor response during submaximal dynamic exercise occurs virtually solely via increases in cardiac output (CO) as no net peripheral vasoconstriction occurs (18, 32, 70, 71, 74, 78). The rise in CO is supported by substantial tachycardia, improved ventricular contractility which along with enhanced central blood volume mobilization facilitates a maintained or slightly elevated stroke volume (13, 16-18, 26, 69-71, 74).

Several studies have suggested that the muscle metaboreflex improves blood flow to the ischemic working muscle (16, 56, 59, 68, 95). However, very few studies have investigated the effect of muscle metaboreflex activation on blood flow to non-ischemic active muscle and the results are equivocal. Using the model originally developed by Wyss et al. (95) to investigate the metaboreflex (graded reductions in hindlimb blood flow), Mittelstadt et al. (48) showed that metaboreflex activation during moderate exercise in canines causes forelimb (a non-ischemic active tissue) vasoconstriction, however forelimb blood flow still increased due to the large pressor response (the rise in arterial pressure was greater than the fall in forelimb vascular conductance, therefore forelimb blood flow increased). In contrast, Augustyniak et al. (5) did not find a significant decrease in forelimb conductance with metaboreflex activation during moderate exercise.

In previous studies in normal animals, we often observed a small but statistically

significant metaboreflex-mediated increase in the total non-ischemic systemic vascular conductance (NIVC) (conductance to all vascular beds excluding the hindlimbs) during submaximal dynamic exercise (6, 40, 69). In contrast, after induction of heart failure this small vasodilation is reversed to a substantial metaboreflex-mediated peripheral vasoconstriction due to much greater reflex increase in sympathetic activity (29). Therefore there appears to be both vasodilator as well as vasoconstrictor processes elicited by metaboreflex activation. Whereas many studies have shown that activation of the muscle metaboreflex can elicit increases in sympathetic activity and peripheral vasoconstriction (6, 29, 87), the mechanisms mediating vasodilation are unknown. Increases in sympathetic activity to the adrenal gland increases epinephrine release which can elicit substantial vasodilation, especially so in the skeletal muscle (8, 41, 45, 46). However, whether epinephrine release contributes to muscle metaboreflex-induced peripheral vascular responses is unknown. In the present study we tested the hypothesis that during metaboreflex activation epinephrine is released and causes β_2-mediated peripheral vasodilation.

Methods

Experimental subjects

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

Surgical procedures

For each of the two surgical procedures, the animals were sedated with acepromazine (0.4-0.5 mg/kg IM) and received preoperative analgesics [carprofen (2.0 mg/kg IV), buprenorphine (0.01 mg/kg IM) and fentanyl (100-175 µg/h (72h) transdermal delivery)]. Anesthesia was induced with ketamine (5.0 mg/kg IV) and diazepam (0.22 mg/kg IV) and maintained with isoflurane gas (1-3%). For postoperative care, animals were closely monitored and given buprenorphine and acepromazine (0.05 and 0.5 mg/kg IV, respectively) as needed. To avoid acute postoperative infections, cefazolin (antibiotic, 30 mg/kg IV) was administered pre- and postoperatively. Cephalexin (antibiotic, 30 mg/kg PO BID) was administered prophylactically for the entire term of the experimental protocol.

In the first surgical procedure, the thoracic cavity was opened via a left thoracotomy (3rd/4th intercostal space) approach and the pericardium was cut to expose the heart. A perivascular flow probe (20PAU, Transonic Systems) was placed around the ascending aorta to measure CO. For studies unrelated to the present investigation, in some animals (n=6) a telemetry blood pressure transmitter (TA11 PA-D70, DSI) was placed in the left ventricle, a flow probe was placed on the left circumflex artery (n=5) and pacing wires were secured to the free wall of the right ventricle (n=6) as described previously (29). The pericardium was reapproximated, the wires were tunneled subcutaneously and exteriorized between the scapulae. The chest was closed in layers.

In the second surgical procedure, an incision was made on the left flank cranial to the iliac crest to expose the abdominal aorta and left renal artery. A perivascular flow probe (10PAU, Transonic Systems) was positioned around the terminal aorta to

measure hindlimb blood flow (HLBF). For studies unrelated to the present investigation, a blood flow transducer was also placed on the left renal artery. All side branches of the terminal aorta between the iliac arteries and the aortic flow probe were ligated and severed. Two hydraulic occluders (8-10 mm, DocXS Biomedical Products) were placed around the terminal aorta just distal to flow probe and, for unrelated studies, a hydraulic occluder was also placed on the left renal artery. A 19-gauge polyvinyl catheter (Tygon, S54-HL, Norton) was inserted into a side branch of the aorta cranial to the flow probe to measure systemic arterial pressure. All instrumentation was tunneled subcutaneously and exteriorized between the scapulae and the abdomen was closed in layers. Lastly, in some animals (n=6), a midline neck incision was made to expose and catheterize the right jugular vein for unrelated studies.

Data acquisition

Each animal was brought into the laboratory and allowed to roam freely and acclimate for ~10-20 minutes, following which it was directed onto the treadmill. The flow probe cables were connected to flow meters (TS420, Transonic Systems). The arterial catheter was aspirated, flushed and connected to a pressure transducer (Transpac IV, ICU Medical). All hemodynamic variables were monitored as real-time waveforms by a data acquisition system (LabScribe, iWorx) and recorded for subsequent off-line analysis.

Experimental procedures

All experiments were performed after the animals had fully recovered from surgery (i.e., were active, afebrile and of good appetite). Each experiment began with the animal standing still on the treadmill until all resting hemodynamic data were stable (typically 5-10 min). The treadmill was turned on and the speed was gradually

increased to 3.2 kph at 0% grade. The muscle metaboreflex was then engaged via graded reductions in HLBF via partial inflation of terminal aortic occluders. Free-flow exercise and each level of vascular occlusion were maintained until all parameters reached steady state (typically 3-5 min). In a subset of experiments in some animals (n=5), arterial blood samples were drawn for analysis of arterial epinephrine levels at rest, during free-flow exercise and at peak metaboreflex activation. Epinephrine levels were measured by high-performance liquid chromatography with electrochemical detection (Waters, Milford, MA). Control experiments (n=11) were repeated in the same animals after β-adrenergic blockade (2 mg/kg intra-arterial propranolol; n=7), α_1-adrenergic blockade (50 μg/kg intra-arterial prazosin; n=6) and α_1+β blockade (n=6) on separate days. The drugs were administered 30 minutes before each experiment and subsequent experiments were not performed for at least 48 hours.

Data analysis

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

Statistical analysis

All hemodynamic data are reported as means ± SE. An α-level of $P<0.05$ was used to determine statistical significance. Averaged responses for each animal were

analyzed with Systat software (Systat 11.0). A two-way ANOVA with repeated measures was used to compare hemodynamic data for time and/or conditional effects. In the event of a significant time-condition interaction, individual means were compared using the Test for Simple Effects.

Results

Figure 2.1 shows two minutes of steady state levels of MAP, CO, NIVC and HLBF during rest, mild (3.2 kph) exercise and muscle metaboreflex activation in a control experiment. From rest to exercise, there is negligible change in MAP but a moderate increase in CO, NIVC and HLBF. Muscle metaboreflex activation during exercise resulted in large increases in MAP and CO with a small increase in NIVC.

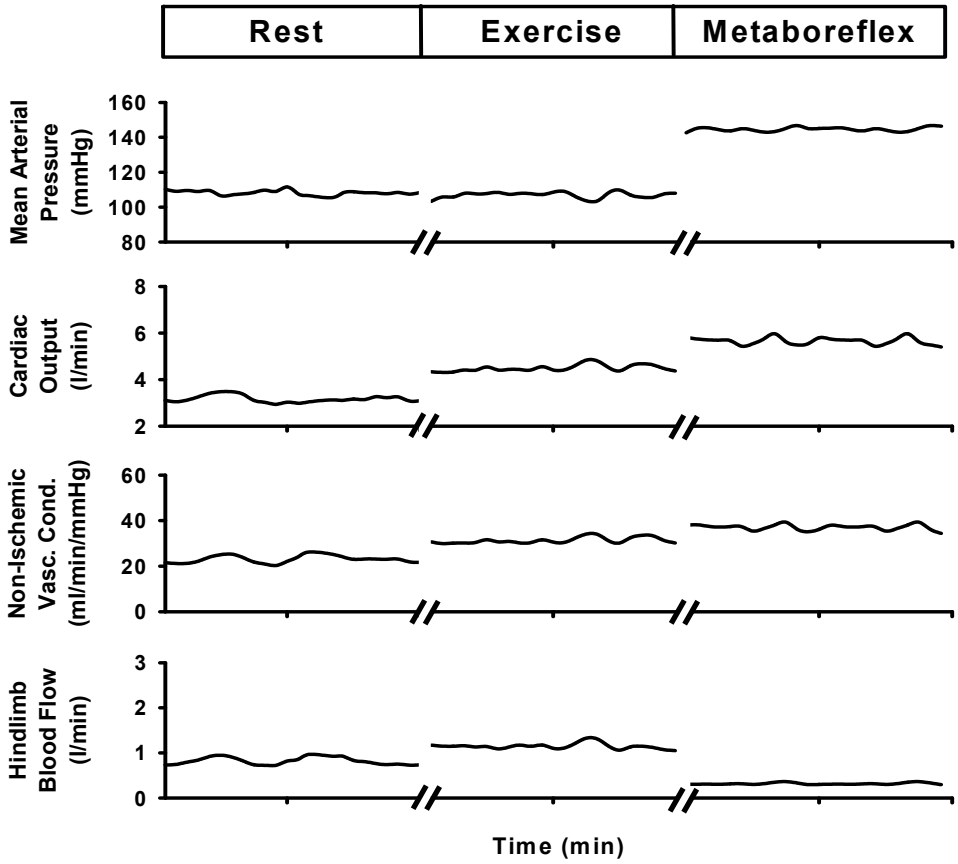


Figure 2.1. Data from one control experiment showing mean arterial pressure (MAP), cardiac output (CO), non-ischemic vascular conductance (NIVC) and hindlimb blood flow (HLBF) during rest, exercise and metaboreflex activation.

Figure 2.2 shows the average arterial epinephrine levels during rest, mild exercise and metaboreflex activation in control experiments. There was no change in epinephrine levels from rest to exercise, however with metaboreflex activation, arterial epinephrine levels increased by ~75%.

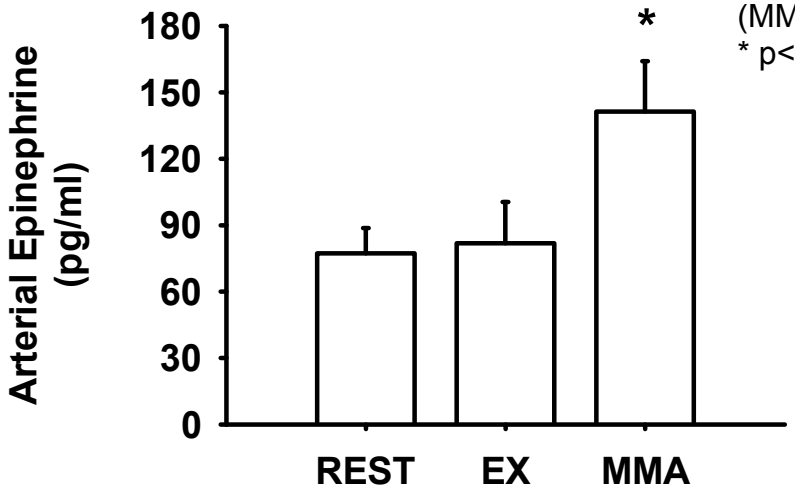


Figure 2.2. Arterial epinephrine levels during rest, exercise (EX) and muscle metaboreflex activation (MMA) in control experiments (n=5). * p<0.05 vs. previous setting.

Figure 2.3 shows mean values of MAP, CO, HR and NIVC during rest, mild exercise and metaboreflex activation in control and after β-blockade, α_1-blockade and α_1+β-blockade.

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

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

responses during metaboreflex activation.

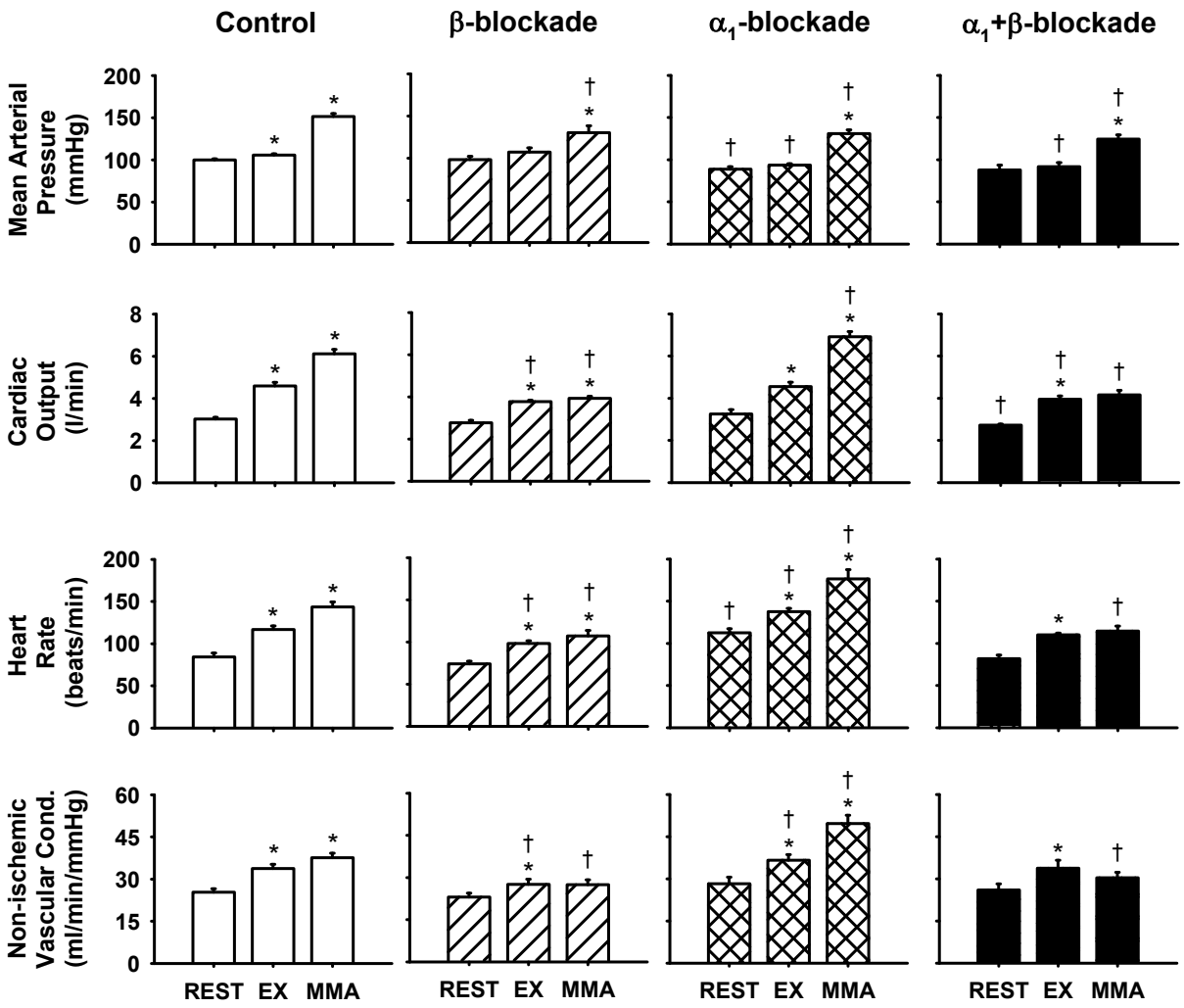


Figure 2.3. Average values of MAP, CO, HR and NIVC during rest, exercise (EX) and muscle metaboreflex activation (MMA) in control experiments (open bars; n=11), after β-adrenergic blockade (hatched bars; n=7), α_1-adrenergic blockade (crosshatched bars; n=6) and α_1+β-blockade (filled bars; n=6). * $p<0.05$ vs. previous setting; † $p<0.05$ vs. control.

α_1-adrenergic blockade (n=6). Resting MAP was significantly lower while HR was significantly higher when compared to control. CO, HR and NIVC significantly increased from rest to exercise while there was no change in MAP. Muscle metaboreflex activation caused substantial increases in all parameters. Although HR and CO increased more than in control experiments, a larger rise in NIVC also occurred

and therefore the resultant pressor response was significantly attenuated.

α_1+β-blockade (n=6). Resting CO was decreased when compared to control. From rest to exercise, MAP remained unchanged and CO, HR and NIVC significantly increased, although MAP and CO were still significantly lower than in control. Muscle metaboreflex activation led to an increase in MAP while CO, HR and NIVC remained similar to exercise levels and significantly lower than in control.

Discussion

Our principal new finding is that activation of the muscle metaboreflex during mild dynamic exercise evokes increases in plasma epinephrine levels and subsequent β_2-mediated vasodilation which opposes α-adrenergic vasoconstriction. Since β_2-receptors are richly expressed in skeletal muscle, epinephrine release may partially restrain α-adrenergic vasoconstriction of active skeletal muscle during metaboreflex activation, thereby improving blood flow to the ischemic active skeletal muscle.

Muscle metaboreflex activation during dynamic exercise causes significant increases in MAP, HR, CO and ventricular contractility (6, 13, 16-18, 29, 43, 69-71, 95). However, the peripheral vascular responses have varied in previous studies. While significant vasoconstriction has been seen in the renal and forelimb vascular beds (6, 29, 40, 48, 49) when taken as a whole, the total vascular conductance of all vascular beds except the hindlimbs (termed non-ischemic vascular conductance; NIVC) usually remains unchanged or slightly increases (6, 13, 40, 69, 78). Inasmuch as some individual vascular beds do constrict, the observation of no change or slight increase in NIVC indicates that some area(s) vasodilate. What causes this vasodilation has been unknown. Reflex increases in sympathetic nerve activity can lead to increased pre-ganglionic sympathetic activity to the adrenal glands which can cause substantial

increases in epinephrine release (8, 41, 45, 46). In addition, post-ganglionic sympathetic nerve terminals have also been shown to release epinephrine (8, 41, 45, 46). Epinephrine is a powerful systemic vasodilator especially in skeletal muscle which is richly endowed with β_2-adrenergic receptors (25, 42, 47, 86). In the present study we saw that metaboreflex activation caused substantial increases in arterial plasma epinephrine levels. A small, but statistically significant peripheral vasodilation occurred which was abolished by β-adrenergic blockade and accentuated by α_1-adrenergic blockade. These results support our hypothesis that muscle metaboreflex activation causes increases in circulating epinephrine which opposes α-mediated vasoconstriction via β_2-mediated vasodilation. Therefore, this mechanism can possibly explain the observed significant increase in NIVC. Metaboreflex activation after combined α_1+β-blockade resulted in peripheral vasoconstriction which may be due to increased vasopressin release. We previously demonstrated that the arterial baroreflex markedly suppresses metaboreflex-induced vasopressin release (57). With α_1+β-blockade the rise in arterial pressure with metaboreflex activation was lower thereby likely causing less baroreflex buffering of vasopressin release; similar to that previously observed in our laboratory after ganglionic blockade (57).

What vascular beds are responsible for the small systemic vasodilation often seen with metaboreflex activation during dynamic exercise are not known. Inasmuch as about 50% of total vascular conductance in dogs even at rest is skeletal muscle (28), it is likely that some muscle beds participate in these vasodilatory responses. Furthermore, skeletal muscle has a greater density of β_2-adrenergic receptors than other beds such as the renal or mesenteric circulations. It is also possible that the workload of some muscle groups is increased with metaboreflex activation. Respiratory

muscles are potential candidates as metaboreflex activation may increase ventilation (4, 43, 66). Furthermore, with ischemia of the hindlimbs, there may also be an increase in work done by lumbar/gluteal muscles perfused by aortic branches not isolated in our preparation. The coronary vasculature is also a likely target bed for the increased plasma epinephrine with muscle metaboreflex activation. However, since the measurement of CO in our model does not include the coronary circulation (the coronary arteries arise from the ascending aorta below the CO blood flow transducer), β_2-mediated coronary vasodilation did not contribute to the significant increase in NIVC. During exercise, the conductance of active skeletal muscle increases, and therefore skeletal muscle is also a likely target for β_2-mediated vasodilation. Our calculation of NIVC does not include the ischemic hindlimb vasculature, and therefore this vascular bed does not play a role in the observed vasodilation during metaboreflex activation in our model. To what extent muscle metaboreflex activation (and potentially epinephrine) influences blood flow and vascular conductance to the ischemic active muscle is unknown.

Limitations

The plasma epinephrine levels were not measured during the experiments employing pharmacological approaches and it is possible that epinephrine levels were different in these settings than in the control experiments. Norepinephrine released from sympathetic nerves could potentially also activate β-adrenergic receptors (as well as epinephrine activates α-adrenergic receptors). However, a previous study demonstrated that norepinephrine infusion always caused vasoconstriction (31). It is possible that after α-adrenergic blockade norepinephrine released from sympathetic nerves caused some dilation via activation of β-receptors. The muscle metaboreflex is

buffered by the arterial baroreflex (40, 72) as well as by cardiac afferents (12) (and likely by other reflexes as well). It is possible that the interaction between the metaboreflex and other reflexes may be affected by the pharmacological perturbations employed in the present study.

Perspectives and significance

Skeletal muscle is richly endowed with small group III/IV afferents which respond to changes in the mechanical and chemical environment, with many receptors being polymodal, e.g. some primarily mechano-receptors increase activity with ischemia (34, 35). Activation of skeletal muscle afferents by metabolite accumulation during ischemic exercise leads to increased sympathetic nerve activity eliciting a powerful pressor response. The mechanisms mediating this pressor response may vary depending on the experimental paradigm. When the metaboreflex is elicited during submaximal dynamic exercise via reductions in blood flow to the active skeletal muscle, the rise in MAP is virtually solely driven by increases in CO in both canines and humans (24, 32, 68, 70). In human studies the metaboreflex is often activated via the technique of post-exercise circulatory occlusion after either static or dynamic limb exercise. The data are mixed as to the relative roles of increases in CO vs. peripheral vasoconstriction and as we discussed in a previous study, this may be dependent on the intensity of contraction, type of exercise and the muscle mass involved (78). When increases in CO are limited (e.g., maximal exercise, heart failure, etc.), metaboreflex activation leads to peripheral vasoconstriction (6, 16, 29, 32). Since active skeletal muscle constitutes a progressively higher proportion of total vascular conductance with increasing exercise intensity, it becomes an increasingly more likely target for vasoconstriction in order to raise MAP. This vasoconstriction would further reduce blood flow and induce a larger degree of

ischemia in the active muscle causing heightened activation of the muscle metaboreflex, which in turn would cause a positive feedback cycle by further increasing the sympathetic tone. In such situations, release of epinephrine from adrenal glands and/or post-ganglionic sympathetic nerve terminals causing β_2-mediated vasodilation could act to protect perfusion of the active skeletal muscle.

CHAPTER 3 - MUSCLE METABOREFLEX ACTIVATION DURING DYNAMIC EXERCISE VASOCONSTRICTS THE ISCHEMIC ACTIVE SKELETAL MUSCLE

(This Chapter contains previously published material. See Appendix C.)

Abstract

Metabolite accumulation due to ischemia of active skeletal muscle stimulates group III/IV chemosensitive afferents eliciting reflex increases in arterial blood pressure and sympathetic activity, termed the muscle metaboreflex. We and others have previously demonstrated sympathetically-mediated vasoconstriction of coronary, renal and forelimb vasculatures with muscle metaboreflex activation (MMA). Whether MMA elicits vasoconstriction of the ischemic muscle from which it originates is unknown. We hypothesized that the vasodilation in the active skeletal muscle with imposed ischemia becomes progressively restrained by the increasing sympathetic vasoconstriction during MMA. We activated the metaboreflex during mild dynamic exercise in chronically instrumented canines via graded reductions in hindlimb blood flow (HLBF) before and after α_1-adrenergic blockade (prazosin; 50 μg/kg), β-adrenergic blockade (propranolol; 2 mg/kg) and α_1+β-blockade. Hindlimb resistance was calculated as femoral arterial pressure/HLBF. During mild exercise, HLBF must be reduced below a threshold level before the reflex is activated. With initial reductions in HLBF, vasodilation occurred with the imposed ischemia. Once the muscle metaboreflex was elicited, hindlimb resistance increased. This increase in hindlimb resistance was abolished by α_1-adrenergic blockade and exacerbated after β-adrenergic blockade. We conclude that metaboreflex activation during submaximal dynamic exercise causes sympathetically-mediated α-adrenergic vasoconstriction in the ischemic skeletal muscle. This limits the ability of the reflex to improve blood flow to the muscle.

Introduction

When O_2 delivery to the active muscle is insufficient to meet the O_2 demands, metabolites (e.g., hydrogen ions, adenosine, lactic acid, etc.) accumulate and activate chemosensitive group III and IV afferents (1, 9, 35, 43, 65, 75-77). Activation of these sensory nerves elicits reflex increases in sympathetic outflow, heart rate (HR), cardiac output (CO), ventricular contractility and arterial blood pressure, termed the muscle metaboreflex (17, 32, 70, 76, 78, 88, 95).

When the muscle metaboreflex is engaged in normal subjects during submaximal dynamic exercise, the reflex raises arterial blood pressure primarily by increasing CO (17, 32, 71, 74, 78). This increase in CO raises the total blood flow available for tissue perfusion and thereby improves blood flow to the ischemic muscle (24, 56, 59, 68). O'Leary and Sheriff (59) concluded that metaboreflex-mediated increases in CO and arterial pressure restore about 50% of the blood flow deficit induced by imposed partial reductions in muscle blood flow during dynamic exercise in canines. Similarly, Rowell et al. (68) concluded that metaboreflex activation during dynamic leg exercise in humans partially restores blood flow to the ischemic active muscle. Sheriff et al. (73) concluded that the substances responsible for initiating the reflex accumulate due to reduced oxygen delivery rather than a failure of adequate washout. As blood flow, and thereby the oxygen delivery, to the active muscle becomes insufficient, muscle afferent activity rises and the metaboreflex elicits increases in CO which partially restores the muscle blood flow. Therefore, the muscle metaboreflex is often regarded as a flow-sensitive, flow-raising reflex: engaged by suboptimal muscle blood flow and acting to increase total body blood flow (i.e., cardiac output) which partially restores blood flow to the underperfused active skeletal muscle (24, 56, 59, 68). Recently, our laboratory

concluded that muscle metaboreflex activation induces epinephrine release causing β_2-mediated vasodilation within skeletal muscle (37). This may be another potential mechanism which improves blood flow to the ischemic muscle during exercise.

We and others have shown that metaboreflex activation elicits vasoconstriction of coronary, renal and forelimb vasculatures (3, 6, 13, 48, 49). Further, Joyner (33) suggested that the muscle metaboreflex may vasoconstrict the ischemic active muscle as venous O_2 saturation significantly declined with metaboreflex activation during rhythmic forearm exercise in humans. Whether the muscle metaboreflex vasoconstricts the ischemic active muscle from which it originates is debatable (54). The reflex increases in CO and MAP partially restore blood flow and oxygen delivery to the ischemic muscle (24, 56, 59, 68). However, this restoration could be attenuated by vasoconstriction within the ischemic muscle. To address this controversy, we investigated the changes in vascular tone within ischemic muscle during metaboreflex activation. We hypothesized that with imposed reductions in skeletal muscle blood flow during exercise, metabolic vasodilation in the ischemic skeletal muscle would be progressively restrained by metaboreflex-mediated increase in sympathetic activity. This vasoconstriction would be attenuated after α-adrenergic blockade and potentiated after β-adrenergic blockade.

Methods

Experimental subjects

Six adult mongrel canines (~19-24 kg) of either sex were selected for the study. All animals were acclimatized to the laboratory surroundings and willing to run on a motor-driven treadmill. All methods and procedures employed in the study were approved by the Institutional Animal Care and Use Committee (IACUC) of Wayne State

University and complied with the National Institutes of Health *Guide to the Care and Use of Laboratory Animals*. All animals exercised voluntarily during experimentation; no negative reinforcement techniques were utilized.

Surgical procedures

For each of the three surgical procedures, the animals were sedated with acepromazine (0.4-0.5 mg/kg IM) and received preoperative analgesics [carprofen (2.0 mg/kg IV), buprenorphine (0.01 mg/kg IM) and fentanyl (75–125 μg/h (72h) transdermal delivery)]. Anesthesia was induced with ketamine (5.0 mg/kg IV) and diazepam (0.22 mg/kg IV) and maintained with isoflurane gas (1–3%). For postoperative care, animals were closely monitored and given acepromazine (0.5 mg/kg IV) and buprenorphine (0.05 mg/kg IV) as needed. To avoid acute postoperative infections, cefazolin (antibiotic, 30 mg/kg IV) was administered pre- and post-operatively. Cephalexin (antibiotic, 30 mg/kg PO BID) was administered prophylactically for the entire term of the experimental protocol. The animals recovered for two weeks after each surgery.

In the first surgical procedure, the thoracic cavity was opened via a left thoracotomy (3rd/4th intercostal space) approach and the pericardium was cut to expose the heart. A perivascular flow probe (20PAU, Transonic Systems) was placed around the ascending aorta to measure CO. Due to the anatomical limitation for the aortic flow probe placement, coronary circulation is not included in the CO measurements. In dogs at rest myocardial blood flow is approximately 6% of the total CO and this fraction remains relatively unchanged across workloads (27, 50). A telemetry blood pressure transmitter (TA11 PA-D70, DSI) was tethered subcutaneously at the height of the left ventricular apex and two intercostal spaces caudal to the thoracotomy incision. The tip of the pressure transducer catheter was inserted and

secured inside the left ventricle to measure left ventricular pressure. Three pacing electrodes were secured to the right ventricular free wall for studies unrelated to the present investigation. All wires were tunneled subcutaneously and exteriorized between the scapulae. The pericardium was reapproximated and the chest was closed in layers.

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

Data acquisition

Each animal was brought into the laboratory and allowed to roam freely and acclimate for ~10-20 minutes, following which it was directed onto the treadmill. The

flow probe cables were connected to flow meters (TS420, Transonic Systems). The two arterial catheters were aspirated, flushed and connected to pressure transducers (Transpac IV, ICU Medical). All hemodynamic variables were monitored as real-time waveforms by a data acquisition system (LabScribe2, iWorx) and recorded for subsequent off-line analysis.

Experimental procedures

All experiments were performed after the animals had fully recovered from surgery (i.e., were active and of good appetite). Each experiment began with the animal standing still on the treadmill until all resting hemodynamic data were stable (typically 5-10 min). The treadmill was turned on and the speed was gradually increased to 3.2 kph at 0% grade. To activate the muscle metaboreflex, HLBF was reduced to ~40% of free-flow exercise levels via graded reductions (by partial inflation of terminal aortic occluders). Free-flow exercise and each level of vascular occlusion were maintained until all hemodynamic parameters reached steady state (typically 3-5 min). Control experiments were repeated in the same animals after α_1-adrenergic blockade (prazosin 50 μg/kg intra-arterial), β-adrenergic blockade (propranolol 2 mg/kg intra-arterial) and α_1+β blockade on separate days. The drugs were administered 20-30 minutes before the experiment and subsequent experiments were not performed for at least 48 h. The large vasoconstrictor effect of phenylephrine was completely abolished with 50 μg/kg dose of prazosin. Preliminary studies showed that 2 mg/kg dose of propranolol completely abolished the vasodilatory response caused by isoproterenol (0.5 μg/kg). Animals performed the same exercise workload after each blockade as during control experiments.

Data analysis

Mean arterial pressure (MAP), femoral arterial pressure (FAP), HR, CO and HLBF were continuously recorded during each experimental procedure. Hindlimb vascular resistance was calculated as FAP/HLBF. Vascular conductance to all vascular beds with the exception of the hindlimb (termed non-ischemic vascular conductance, NIVC) was calculated as NIVC = (CO-HLBF)/MAP. One minute averages of all variables were taken during steady-state at rest, free-flow exercise and each graded reduction in HLBF. The data were analyzed as initially described by Wyss et al. (95). Previous studies have shown that during mild exercise in canines HLBF must be reduced below a clear threshold before the muscle metaboreflex is activated (6, 73, 95). Initial reductions in HLBF did not evoke metaboreflex responses while reductions in HLBF below the threshold resulted in a significant pressor response. Therefore, the data were approximated to two linear regression lines: an initial slope line that includes free-flow exercise and each reduction in blood flow that did not elicit reflex response, and a pressor slope line that includes reductions in HLBF that resulted in a large pressor response (Figure 3.2). The threshold for metaboreflex activation was approximated as the intersection of the initial and pressor slope lines. Mean values were averaged across all animals to obtain the sample mean of the study. Each animal served as its own control.

Statistical analysis

All hemodynamic data are reported as means ± SE. An α-level of $P<0.05$ was used to determine statistical significance. Averaged responses for each animal were analyzed with Systat software (Systat 11.0). A two-way ANOVA with repeated measures was used to compare hemodynamic data for time and/or condition effects. In

the event of a significant time-condition interaction, individual means were compared using the Test for Simple Effects.

Results

Figure 3.1 shows 5 second averages of MAP, CO, HLBF and hindlimb resistance in a control experiment during rest, mild (3.2 kph) exercise and graded reductions in HLBF. From rest to exercise, hindlimb resistance decreased and HLBF increased along with the rise in CO. Initial graded reductions in HLBF did not evoke changes in MAP or CO, but caused further decreases in hindlimb resistance. When reductions in HLBF elicited the metaboreflex, marked increases in MAP and CO occurred and despite the continued progressive hindlimb ischemia, hindlimb vascular resistance rose indicating that vasoconstriction occurred.

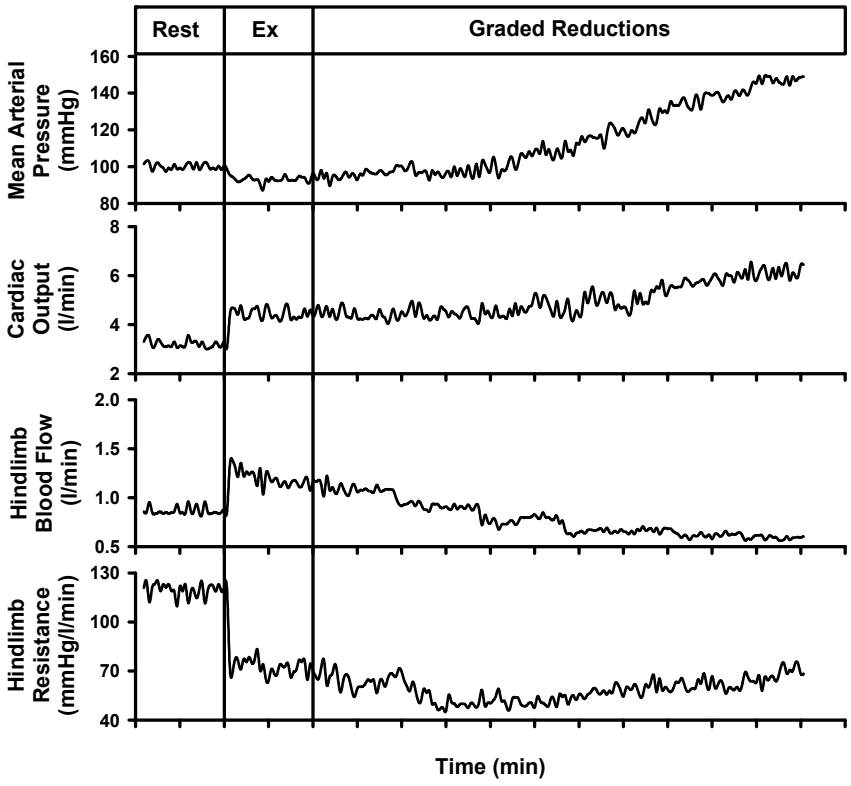


Figure 3.1. Data from one control experiment showing mean arterial pressure (MAP), cardiac output (CO), hindlimb blood flow (HLBF), and hindlimb resistance during rest, exercise (Ex) and graded reductions in HLBF eliciting the muscle metaboreflex.

Figure 3.2. The initial and pressor slopes of mean arterial pressure (*top panel*) and hindlimb resistance (*bottom panel*) obtained by dual linear regression model from a control experiment.

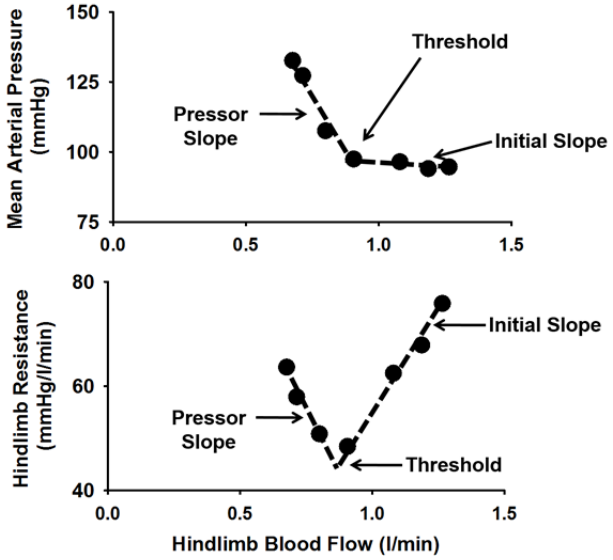


Figure 3.2 shows the relationships between MAP vs. HLBF (*top*) and hindlimb resistance vs. HLBF (*bottom*) from a control experiment. Figure 3.3 shows average hindlimb resistance responses as a function of HLBF in control, after α_1-adrenergic blockade, β-adrenergic blockade and α_1+β-blockade.

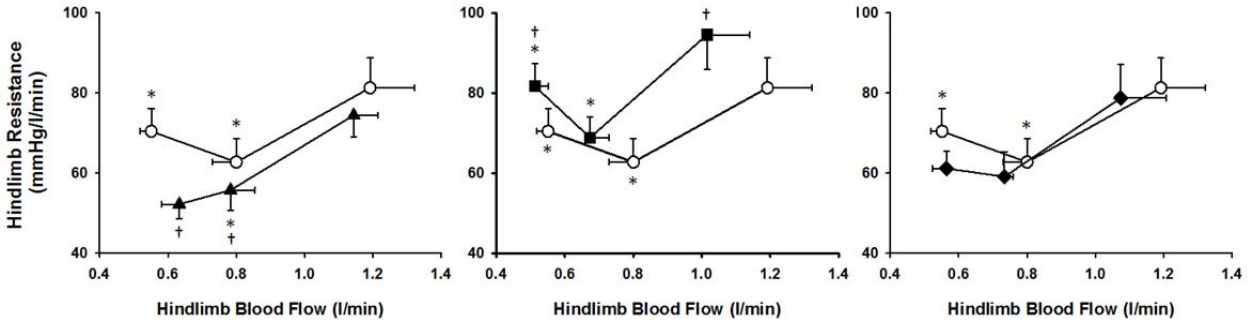


Figure 3.3. Hindlimb resistance responses during free-flow exercise, metaboreflex threshold and maximal metaboreflex activation (n=6) for control (open circles) vs. α_1-blockade (filled triangles) (left), control vs. β-blockade (filled squares) (center) and control vs. α_1+β-blockade (filled diamonds) (right). * $p<0.05$ vs. previous setting in same experiment; † $p<0.05$ vs. control setting.

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

α1-adrenergic blockade. Initial HLBF reductions above the threshold caused a significant decrease in hindlimb resistance as in control. However, when HLBF was reduced below threshold, the increase in resistance observed in control was abolished revealing a continued decrease in resistance. The magnitude of hindlimb resistance during maximal metaboreflex activation was significantly lower than control.

β-adrenergic blockade. During free-flow exercise, hindlimb resistance was significantly higher than control. With initial reductions in HLBF, hindlimb resistance significantly decreased to a level not different from control. Following metaboreflex activation, there was a significant increase in hindlimb resistance. The magnitude of resistance at maximal metaboreflex activation was significantly larger than in control.

α1+β-blockade. With initial reductions in HLBF, hindlimb resistance decreased to a level not different from control. Following metaboreflex activation, there was a small increase in hindlimb resistance which was not different from control.

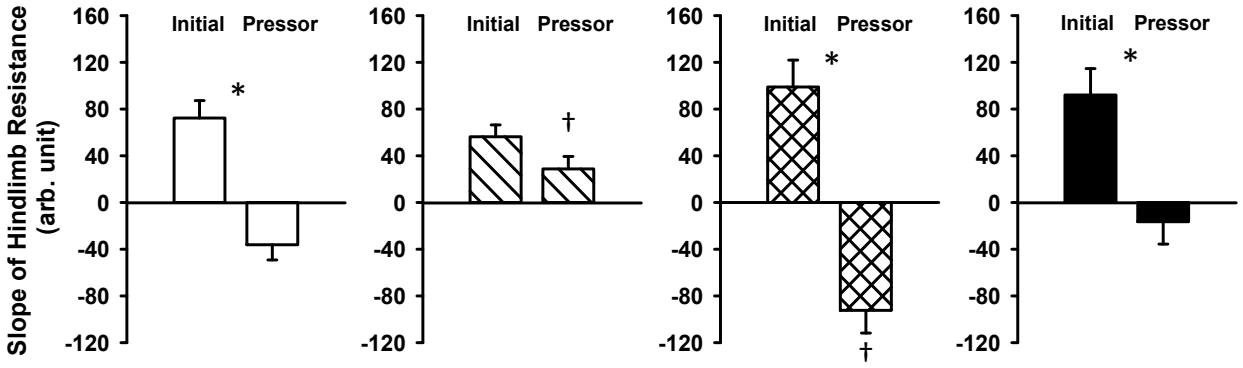


Figure 3.4. Initial and pressor slope responses of hindlimb vascular resistance during muscle metaboreflex activation (n=6) in control (open bars), after α_1-adrenergic blockade (hatched bars), β-adrenergic blockade (cross-hatched bars), and α_1+β-blockade (filled bars). * $p<0.05$ vs. initial slope; † $p<0.05$ vs. control setting.

Figure 3.4 shows average slopes of the relationship between hindlimb vascular resistance vs. hindlimb blood flow during the initial and pressor responses in control and following α_1-blockade, β-blockade and α_1+β-blockade. In control, the initial slope was

significantly different from the pressor slope. After α_1-adrenergic blockade, the initial and pressor slopes were not significantly different from each other. The initial slope after α_1-blockade was similar to that in control while the pressor slope was reversed and significantly different from control. After β-blockade, the initial and pressor slopes were significantly different from each other and the pressor slope was markedly larger (more negative) than in control. After α_1+β-blockade, the two slopes were significantly different from each other but not from control.

Table 3.1. Mean hemodynamic values during rest, mild exercise (EX) and muscle metaboreflex activation (MMA) in control and after α_1-blockade, β-blockade and α_1+β-blockade in normal animals.

		Control	α_1-blockade	β-blockade	α_1+β-blockade
MAP	Rest	88.4 ± 1.9	81.4 ± 1.4 †	84.4 ± 2.2	76.9 ± 2.0 †
(mmHg)	Ex	92.1 ± 1.2 *	83.5 ± 1.6 *†	90.3 ± 3.5 *	81.5 ± 1.9 †
	MMA	143.6 ± 1.6 *	119.5 ± 3.4 *†	114.4 ± 5.4 *†	113.7 ± 4.8 *†
CO	Rest	2.75 ± 0.15	2.87 ± 0.18	2.59 ± 0.10	2.70 ± 0.16
(l/min)	Ex	4.32 ± 0.23 *	4.48 ± 0.21 *	3.71 ± 0.25 *†	4.03 ± 0.24 *
	MMA	6.50 ± 0.27 *	6.66 ± 0.21 *	4.11 ± 0.23 *†	4.71 ± 0.32 *†
HR	Rest	69.9 ± 4.2	83.2 ± 5.5 †	70.4 ± 3.1	80.3 ± 4.4
(bpm)	Ex	101.0 ± 2.8 *	112.9 ± 4.8 *†	95.3 ± 1.0 *†	106.6 ± 4.7 *
	MMA	141.0 ± 3.6 *	159.4 ± 8.6 *†	104.3 ± 4.3 †	119.6 ± 6.7 *†
NIVC	Rest	24.3 ± 1.3	28.1 ± 2.2 †	24.6 ± 1.2	27.9 ± 1.8 †
(ml/min/mmHg)	Ex	34.2 ± 1.7 *	39.6 ± 2.6 *†	29.7 ± 1.3 *†	36.3 ± 1.7 *
	MMA	41.4 ± 1.7 *	50.8 ± 1.7 *†	31.5 ± 1.3 †	36.4 ± 1.9 †
HLBF	Rest	0.60 ± 0.07	0.60 ± 0.04	0.52 ± 0.05	0.55 ± 0.04
(l/min)	Ex	1.18 ± 0.13 *	1.20 ± 0.09 *	1.02 ± 0.12 *†	1.07 ± 0.13 *
	MMA	0.55 ± 0.04	0.64 ± 0.05 †	0.52 ± 0.04	0.56 ± 0.04

Mean hemodynamic values during rest, free-flow exercise and maximal metaboreflex activation during control experiments and following α_1-blockade, β-blockade and α_1+β-blockade in six animals, represented as means ± SE. MAP, mean arterial pressure; CO, cardiac output; HR, heart rate; NIVC, non-ischemic vascular conductance; HLBF, hindlimb blood flow; *P<0.05 vs. previous setting in same experiment; †P<0.05 vs. control.

Table 3.1 shows mean hemodynamic data during rest, mild exercise and muscle metaboreflex activation in control experiments and following α_1-blockade, β-blockade and α_1+β-blockade. From rest to exercise in control, there were significant increases in MAP, CO, HR, NIVC and HLBF. With muscle metaboreflex activation, there were

further significant increases in MAP, CO, HR and NIVC. Following α_1-adrenergic blockade, resting MAP was significantly lower while HR and NIVC were significantly higher than in control. With mild exercise there was a significant increase in all variables and MAP, HR and NIVC were significantly higher than control exercise levels. Muscle metaboreflex activation following α_1-blockade resulted in substantial increases in MAP, CO, HR and NIVC with a significantly attenuated pressor response compared to control. Following β-adrenergic blockade, all the parameters increased with exercise but the levels of CO, HR, NIVC and HLBF were significantly lower than those observed during control experiments. Muscle metaboreflex activation significantly increased MAP, CO, and HR while NIVC was unchanged and all parameters were significantly attenuated compared with metaboreflex responses in control. Following α_1+β-blockade, resting MAP and NIVC was lower than in control. From rest to exercise, CO, HR, NIVC and HLBF significantly increased while MAP remained unchanged and was significantly lower than in control. Muscle metaboreflex activation led to an increase in MAP, CO and HR but all responses were significantly lower than in control.

Discussion

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

The muscle metaboreflex is activated when oxygen supply to the muscle is unable to meet the metabolic demands of the active muscle (73, 95). During mild

exercise in canines, initial reductions in HLBF did not cause any marked increases in MAP, CO or HR, indicating adequate oxygen supply to the working muscle. With further reductions in HLBF oxygen supply becomes insufficient to meet the oxygen demand of the active muscle leading to accumulation of metabolites (H^+, lactic acid, diprotonated phosphate, etc.) (9, 65, 75-77). As a result, the muscle metaboreflex is activated and marked elevations in MAP, CO, HR and ventricular contractility are observed (13, 16, 17, 70, 78, 95). Thus, during mild dynamic exercise in canines there is a clear threshold before the muscle metaboreflex is activated (Figure 3.2).

In control, initial reductions in HLBF resulted in metabolic vasodilation as indicated by a decrease in hindlimb vascular resistance (Figure 3.3). When reductions in HLBF below the threshold elicited the metaboreflex, metabolic vasodilation was opposed by vasoconstriction as indicated by an increase in vascular resistance within the ischemic muscle. Following α_1-blockade, the vasoconstriction observed during metaboreflex activation in control was abolished, revealing a continued vasodilation in the ischemic vasculature with further reductions in hindlimb blood flow. These findings indicate that muscle metaboreflex activation induces α_1-mediated sympathetic vasoconstriction within the ischemic skeletal muscle. After β-blockade, metaboreflex activation caused a significantly larger vasoconstriction in the hindlimb vasculature than in control. These findings are in agreement with our previous work demonstrating metaboreflex-induced β_2-mediated vasodilation in ischemic skeletal muscle (37). Following α_1+β-blockade, metaboreflex activation resulted in a vasoconstriction not significantly different from control. This could be due to the contribution of other hemodynamic factors such as vasopressin, endothelin, neuropeptide Y etc., and/or a potential α_2-mediated vasoconstriction (7, 10, 19, 30, 57). Therefore, vasomotor tone of

the ischemic muscle with muscle metaboreflex activation during submaximal exercise is a combined result of a complex interplay between metabolic vasodilation and neurogenic and circulating vasoconstrictor factors as well as possible local factors released from the endothelium. In the control experiments, the prevailing neurogenic vasoconstriction results in frank vasoconstriction within the ischemic vasculature.

We and others have shown that metaboreflex activation elicits vasoconstriction of coronary, renal and forelimb vasculatures (3, 6, 13, 48, 49). Whether the muscle metaboreflex vasoconstricts the ischemic muscle itself has been controversial (33, 54, 59). Muscle metaboreflex activation during dynamic exercise in canines markedly increases MAP and CO which restores about half of the blood flow deficit to the ischemic skeletal muscle (59). In humans, Rowell et al. (68) showed that metaboreflex activation in normal subjects partially restores blood flow to ischemic muscle. Eiken and Bjurstedt (24) reached similar conclusions whereas Joyner (33) suggested that a reflex-induced sympathetic vasoconstriction in the ischemic muscle limits the restoration of blood flow. Several factors could contribute to these conflicting findings: 1) the studies performed in humans did not directly measure blood flow, blood pressure or sympathetic nerve activity within the active skeletal muscle, 2) the exercise intensity, muscle mass and muscle type involved in the exercise were different and 3) species differences may exist in the extent of metaboreflex activation (2, 6, 68, 95). This is the first study to directly measure arterial pressure and blood flow within the ischemic active muscle. We show that metaboreflex activation induces α_1-mediated sympathetic vasoconstriction within the ischemic active muscle. If no sympathetic vasoconstriction occurred with metaboreflex activation, the amount of blood flow restoration to the ischemic vasculature would have been larger than 50%. Therefore, muscle

metaboreflex-induced neurogenic vasoconstriction limits the ability of the reflex to restore blood flow to the muscle.

Vasoconstriction within the ischemic muscle would result in a decrease in muscle blood flow which would lead to exaggerated metaboreflex activation. Therefore, this becomes a positive feedback loop wherein vasoconstriction decreases the blood flow to the already ischemic muscle, further activating muscle afferents and causing a larger vasoconstriction. However, the positive feedback could either become run-away positive feedback, where each cycle would lead to exaggerated metaboreflex activation and a larger vasoconstriction, or become limiting positive feedback, where the stimulus and the response progressively become smaller and reach a plateau. Whether this positive feedback loop becomes a run-away cycle of continuing amplified responses or serves as an amplifier of the initial responses which reaches a plateau depends on the magnitude of the change in sympathetic activity engendered by the reflex and the efficacy of this change in sympathetic activity on blood flow to the ischemic muscle. That is, if the rise in sympathetic activity elicited by a one unit decrease in muscle blood flow evokes vasoconstriction in the muscle which causes a less than one unit fall in flow, then the positive feedback will cycle with ever decreasing amplitude and eventually plateau at a heightened response. If however, the fall in blood flow caused by the rise in sympathetic activity is greater than the change in flow which caused the rise in sympathetic activity itself, then this becomes a “run-away” positive feedback with ever increasing responses eventually leading to complete vasoconstriction. Using the average results in our study, the pressor slope for the relationship between the imposed decrease in HLBF and the observed increase in hindlimb vascular resistance was -36 units which indicates that for every l/min decrease in flow, there would be a 36

mmHg/l/min increase in resistance. This 36 mmHg/l/min increase in resistance caused by metaboreflex activation would itself cause a decrease in flow. At the threshold level of HLBF, an increase in hindlimb resistance by 36 mmHg/l/min would cause a 0.54 l/min further decrease in flow if pressure remained constant. This decrease in flow would additionally increase resistance by 19.7 mmHg/l/min which in turn would decrease flow by 0.15 l/min and so forth. With each cycle, the increase in the stimulus and the resultant reflex response becomes progressively smaller and the positive feedback will eventually reach a steady state. Whereas a variety of factors can affect the hindlimb resistance response, our results indicate that this is a limiting positive feedback which is a stable system resulting in amplified gain rather than run-away instability.

In canines, nearly half of the CO at rest perfuses skeletal muscle (28) and during exercise all of the rise in CO is directed to the active skeletal muscle (50). When further increases in CO become limited (e.g., during severe exercise, heart failure, following β-blockade, etc.), peripheral vasoconstriction is the only mechanism available to increase arterial pressure. Since the active skeletal muscle constitutes a large proportion of total vascular conductance during severe exercise, the ischemic active muscle is the likely target for progressively larger vasoconstriction. Several studies indicate that metabolic by-products in skeletal muscle reduce neurogenic vasoconstriction, termed functional sympatholysis (63, 85). Although to some extent conclusions may also be dependent on methods of data analysis (52). In patho-physiological states functional sympatholysis may be reduced (85, 90) which may thereby heighten vasoconstrictor responses in the ischemic muscle in these settings. This would further limit O_2 delivery to the ischemic muscle leading to an exaggerated activation of the muscle metaboreflex, stimulating a dangerous positive feedback loop. To what extent this

metaboreflex positive feedback approaches run-away levels in pathophysiological states is unknown. Fundamentally, this feedback could impair the ability to exercise and contribute to exercise intolerance.

Limitations

The arterial baroreflex buffers about one-half of the muscle metaboreflex-induced pressor response and this occurs primarily by inhibition of peripheral vasoconstriction (40). In our study, MAP during muscle metaboreflex activation after α_1- and β-blockade was significantly smaller than in control. This lower arterial pressure could potentially result in a reduced buffering by the baroreflex and cause larger metaboreflex-induced peripheral vasoconstriction. A larger vasoconstrictor drive could limit the hindlimb vasodilation we observed with metaboreflex activation after α_1-blockade and could exaggerate the vasoconstriction we observed after β-blockade. Our observation of continued hindlimb vasodilation after α_1-blockade despite possible larger sympathetic activation would argue in favor of our conclusion that sympathetic vasoconstriction occurs in the ischemic hindlimb during metaboreflex activation. The larger vasoconstriction seen after β-blockade may have been exaggerated due to the lower arterial pressure and less baroreflex buffering of the metaboreflex responses.

CHAPTER 4 - MUSCLE METABOREFLEX-INDUCED VASOCONSTRICTION OF THE ISCHEMIC ACTIVE MUSCLE IS EXAGGERATED IN HEART FAILURE

Abstract

When oxygen delivery to active muscle is insufficient to meet metabolic demands, metabolites accumulate and stimulate skeletal muscle afferents inducing a reflex rise in blood pressure, termed the muscle metaboreflex. Muscle metaboreflex activation (MMA) during submaximal dynamic exercise increases arterial pressure virtually solely via an increase in cardiac output (CO), as no net peripheral vasoconstriction occurs. In heart failure (HF), increases in CO are limited and metaboreflex-induced pressor responses occur via peripheral vasoconstriction. We recently demonstrated that MMA induces sympathetic vasoconstriction in the ischemic active muscle, limiting the ability of the metaboreflex to restore blood flow. In the present study we tested the hypothesis that vasoconstriction in the ischemic muscle is exaggerated in HF. Changes in hindlimb vascular resistance (femoral arterial pressure/hindlimb blood flow (HLBF)) were observed during MMA induced via graded reductions in HLBF during mild exercise with and without α_1-adrenergic blockade (prazosin; 50 μg/kg) before and after the induction of HF. During mild exercise, HLBF must be reduced below a clear threshold before MMA occurs. In normal animals, initial HLBF reductions caused metabolic vasodilation while reductions below the MMA threshold elicited reflex vasoconstriction in the ischemic muscle which was abolished following α_1-adrenergic blockade. MMA-induced vasoconstriction of the ischemic muscle was exaggerated after induction of HF. This heightened vasoconstriction impairs the ability of the metaboreflex to restore blood flow to ischemic muscle in HF and may contribute to exercise intolerance observed in these patients. We conclude

that sympathetically-mediated vasoconstriction of the ischemic skeletal muscle during muscle metaboreflex activation is exaggerated in HF.

Introduction

Ischemia in the exercising skeletal muscle causes accumulation of metabolites (e.g., protons, diprotonated phosphate, lactic acid, etc.) which activate the chemosensitive muscle afferents eliciting a reflex pressor response, termed the muscle metaboreflex (1, 9, 35, 77). Muscle metaboreflex activation in healthy individuals increases heart rate (HR), cardiac output (CO), mean arterial pressure (MAP), ventricular contractility and right atrial pressure (17, 70, 71, 78, 88) along with some regional vasoconstriction (6, 13, 48, 49).

During submaximal dynamic exercise in normal subjects, muscle metaboreflex-induced pressor response, primarily driven by an increase in CO (6, 17, 32), partially restores blood flow to ischemic active muscle (24, 59, 70). Muscle metaboreflex-induced epinephrine release resulting in β_2-mediated vasodilation within the skeletal muscle (37) is another viable mechanism for blood flow restoration to ischemic active muscle. We recently showed however, that the very muscle from which the muscle metaboreflex originates is itself a target vasculature for sympathetically-mediated vasoconstriction during muscle metaboreflex activation (36). This functionally limits the ability of metaboreflex to restore blood flow to the ischemic muscle.

The strength and mechanisms of metaboreflex activation are altered in heart failure (HF) (14, 29, 51, 81). HF is characterized by low resting CO and heightened sympathetic nerve activity (SNA). Inasmuch as metaboreflex-induced increases in CO and ventricular contractility are substantially attenuated in HF, the pressor response occurs due to enhanced vasoconstriction of the peripheral vasculature (14, 16, 29, 58).

Whether the ischemic muscle itself undergoes exaggerated reflex vasoconstriction is unknown. Therefore, in this study, we investigated whether the vasoconstriction in the ischemic active skeletal muscle is exaggerated in HF.

Methods

Experimental subjects

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

Surgical procedures

For each surgical procedure, anesthesia was induced with ketamine (5 mg/kg IV) and diazepam (0.2-0.3 mg/kg IV) and maintained with isoflurane gas (1–3%). The animals received preoperative analgesics [carprofen (4.4 mg/kg IV), buprenorphine (0.01-0.03 mg/kg IM) and fentanyl (75–125 μg/h (72h) transdermal delivery)]. Acepromazine (0.2-0.3 mg/kg IV) and buprenorphine (0.01-0.03 mg/kg IM) was given during postoperative care. To avoid acute postoperative infections, cefazolin (antibiotic, 30 mg/kg IV) was administered pre- and post-operatively. Cephalexin (antibiotic, 30 mg/kg PO BID) was administered prophylactically for the entire term of the experimental protocol.

In the first surgical procedure, the heart was exposed via a left thoracotomy (3rd/4th intercostal space) approach. A 20 mm blood flow transducer (Transonic

Systems) was placed around the ascending aorta to measure CO. A telemetry pressure transmitter (TA11 PA-D70, DSI) was tethered subcutaneously and its tip was secured inside the left ventricle to measure left ventricular pressure. Three stainless steel pacing electrodes (0-Flexon) were secured to the right ventricle. All wires were tunneled subcutaneously and exteriorized between the scapulae. The chest was closed in layers after reapproximating the pericardium.

In the second surgical procedure, the abdominal aorta was exposed via a left flank incision cranial to the iliac crest. A 10 mm blood flow transducer (Transonic Systems) was placed around the terminal aorta to measure hindlimb blood flow (HLBF) and two hydraulic occluders (8-10 mm, DocXS Biomedical Products) were placed just distal to it. All side branches of the terminal aorta between the iliac arteries and the aortic flow probe were ligated. A 19-gauge polyvinyl catheter (Tygon, S54-HL, Norton) was inserted into a side branch of the aorta to measure systemic arterial pressure. A second 19-gauge polyvinyl catheter was inserted into a side branch of the aorta caudal to the occluders to measure arterial pressure below the occluders. When the catheterization of a caudal aortic branch was not possible in this surgery, a catheter was placed into a side branch of the femoral artery in a separate procedure. All instrumentation was tunneled subcutaneously and exteriorized between the scapulae. The abdomen was closed in layers. For experiments unrelated to this study, a 4 mm flow probe was placed around the left renal artery during retroperitoneal surgery and hydraulic occluders were placed around common carotid arteries in a separate procedure.

Data acquisition

Before each experiment, the animal was brought into the laboratory and allowed

to roam freely and acclimate for ~10-20 minutes. Then, the animal was led to the treadmill, arterial catheters were connected to pressure transducers, left ventricular telemetry implant was turned on and flow probes were connected to flow meters (Transonic Systems). All hemodynamic variables were monitored as real-time waveforms by a data acquisition system (LabScribe2, iWorx) and recorded for subsequent off-line analysis.

Experimental procedures

Animals recovered for at least two weeks after each surgery. Experiments were performed after the animals had fully recovered from surgery (i.e., were active and of good appetite). During each experiment, resting steady-state hemodynamic parameters were recorded with the animal standing on the treadmill followed by mild exercise (3.2 kph at 0% grade). The muscle metaboreflex was activated via graded reductions in HLBF (by partial inflation of terminal aortic occluders) during mild exercise. Free-flow exercise and each reduction in HLBF were maintained until all hemodynamic parameters reached steady state (~3-5 min). Control experiments were repeated in the animals after α_1-adrenergic blockade (prazosin 50 μg/kg intra-arterial). The drug was administered 20-30 minutes before the experiment and no subsequent experiments were performed for next 48 hours. In each experiment, the dose of prazosin was sufficient to abolish the large vasoconstrictor effect of phenylephrine (4 μg/kg). After the completion of experiments in normal animals, heart failure was induced via rapid ventricular pacing (220-240 beats/min) for a group average of 32 ± 4 days. The development of HF was established by resting tachycardia and decreases in resting mean arterial pressure (MAP), CO, maximal rate of the rise in left-ventricular pressure (dP/dt_{max}) and maximal rate of fall in left-ventricular pressure (dP/dt_{min}) (Table 4.1).

Data analysis

MAP, femoral arterial pressure (FAP), HR, CO, left ventricular pressure and HLBF were continuously recorded during each experimental procedure. Hindlimb vascular resistance was calculated as FAP/HLBF. Vascular conductance to all vascular beds except the hindlimb (termed non-ischemic vascular conductance, NIVC) was calculated as NIVC = (CO-HLBF)/MAP. One minute averages of all variables were taken during steady-state at rest, free-flow exercise and each graded reduction in HLBF. Inasmuch as the HLBF during mild exercise in canines must be reduced below a clear threshold before the muscle metaboreflex is activated (6, 73, 95), initial reductions in HLBF did not evoke any metaboreflex responses while reductions in HLBF below the threshold resulted in a significant pressor response. Therefore, the data were approximated to two linear regression lines: an initial slope line that includes free-flow exercise and each reduction in blood flow that did not elicit reflex response, and a pressor slope line that includes reductions in HLBF that resulted in large pressor response (Figure 4.2). The threshold for metaboreflex activation was approximated as the intersection of the initial and pressor slope lines. Mean values were averaged across all animals to obtain the sample mean of the study. Each animal served as its own control.

Statistical analysis

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

using the Test for Simple Effects.

Results

Figure 4.1 MAP, CO and HLBF in a control experiment during rest, mild exercise and muscle metaboreflex activation induced by graded reductions in HLBF (data are averaged over every 5 seconds for clarity). From rest to exercise, MAP remained unchanged while CO and HLBF increased. With initial graded reductions in HLBF, there was a small increase in both MAP and CO. When further reductions in HLBF elicited the muscle metaboreflex, marked increases in MAP and CO occurred. Figure 4.2 shows the relationships between MAP vs. HLBF and hindlimb resistance vs. HLBF during the control experiment. Initial reductions in HLBF caused a small increase in MAP and a decrease in hindlimb resistance. Once metaboreflex threshold is reached, further reductions in HLBF caused large reflex increase in MAP and hindlimb resistance.

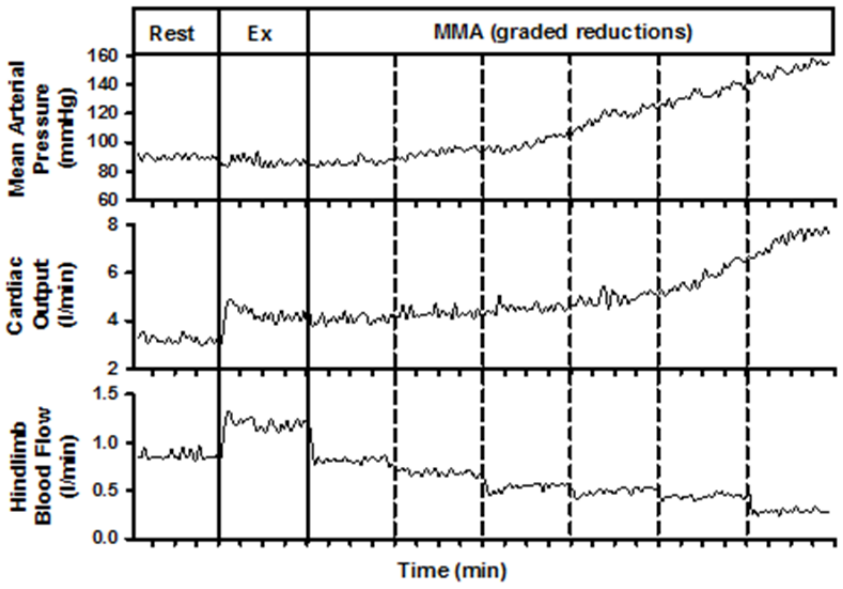


Figure 4.1. Data from one control experiment showing mean arterial pressure, cardiac output and hindlimb blood flow during rest, mild free-flow exercise (Ex) and muscle metaboreflex activation (MMA) induced via graded reductions in hindlimb blood flow.

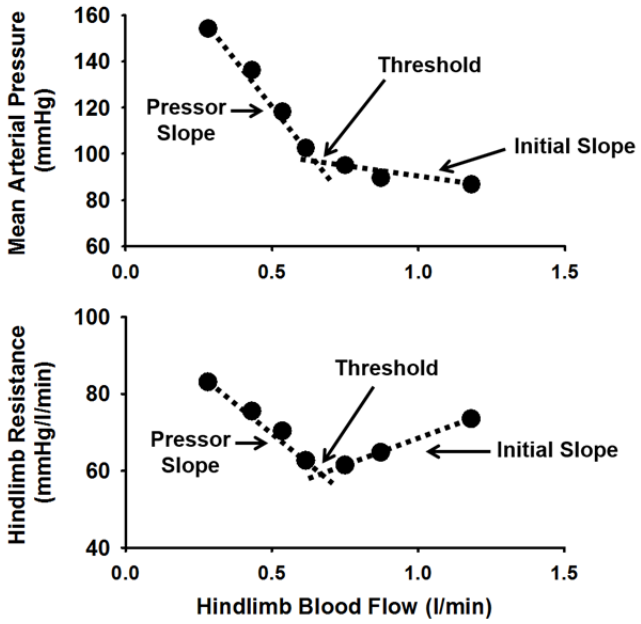


Figure 4.2. Initial and pressor slopes of mean arterial pressure (top) and hindlimb resistance (bottom) obtained by using dual linear regression model in a control experiment.

Figure 4.3 shows average hindlimb resistance responses as a function of HLBF in control and α_1-adrenergic blockade experiments before and after induction of HF. During control experiments in healthy animals, initial reductions in HLBF caused a significant decrease in hindlimb vascular resistance, indicating vasodilation in the hindlimb skeletal muscle. Once HLBF was reduced below threshold, there was a significant increase in hindlimb resistance indicating vasoconstriction of the ischemic skeletal muscle. Following α_1-adrenergic blockade, initial HLBF reductions caused a similar decrease in hindlimb resistance as in control. However, once HLBF was reduced below threshold, the vasoconstriction observed in control was completely abolished, hindlimb resistance continued to fall with further reductions in HLBF and at maximal metaboreflex activation hindlimb resistance was significantly lower than control. After induction of HF in the same animals, hindlimb resistance during free-flow exercise was significantly higher than in healthy subjects and HLBF was significantly lower (Table 4.1). Hindlimb resistance decreased with initial reductions in HLBF and markedly increased in response to metaboreflex activation.

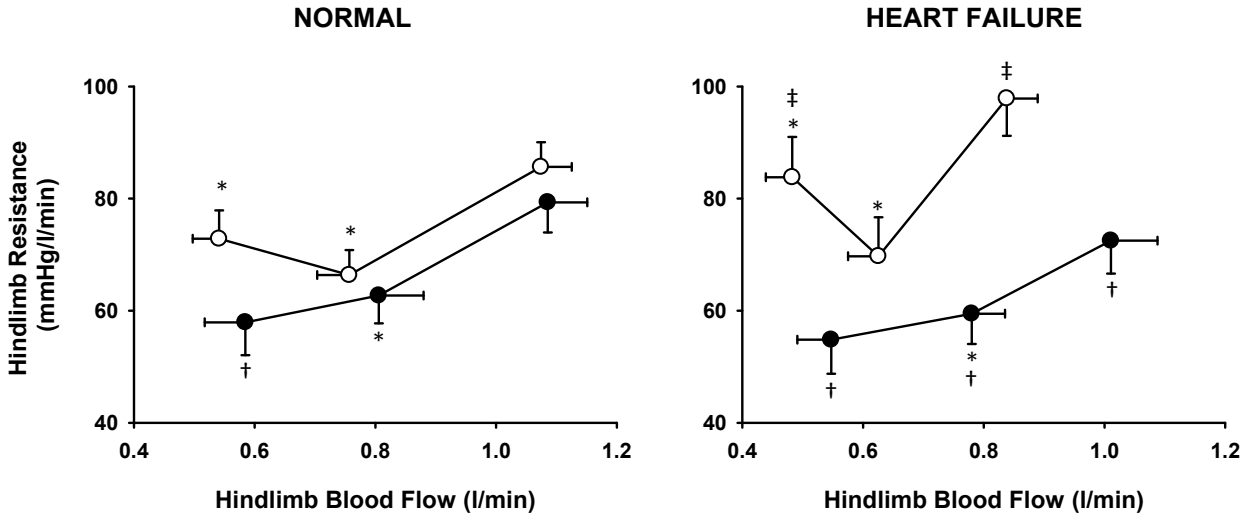


Figure 4.3. Average hindlimb resistance responses during free-flow exercise, metaboreflex threshold and maximal metaboreflex activation during control (open circles) and α_1-blockade (filled circles) experiments in normal (*left panel*) and heart failure (*right panel*). *p<0.05 vs. previous setting in same experiment; † p<0.05 vs. control; ‡ p<0.05 vs. normal.

Hindlimb resistance at maximal metaboreflex activation was significantly higher than in healthy subjects. Following α_1-blockade in HF animals, hindlimb resistance during free-flow exercise was significantly lower and HLBF was markedly higher than during control experiment in HF animals. Initial reductions in HLBF led to a decrease in hindlimb resistance, indicating vasodilation. Following muscle metaboreflex activation, the large vasoconstriction observed in control experiments in HF was completely abolished.

Figure 4.4 shows average slopes of the relationship between hindlimb vascular resistance and HLBF during control and α_1-blockade experiments before and after induction of HF. During control experiments in healthy subjects, the initial slope was significantly different from the pressor slope. After α_1-adrenergic blockade, the initial and pressor slopes were not significantly different from each other. The initial slope after α_1-blockade was similar to that in control while the pressor slope was reversed and

significantly different from control. After induction of HF, the initial and pressor slopes were significantly different from each other and markedly larger than in normal subjects. After α_1-adrenergic blockade in HF animals, the two slopes were significantly different from each other and from the control experiments in HF.

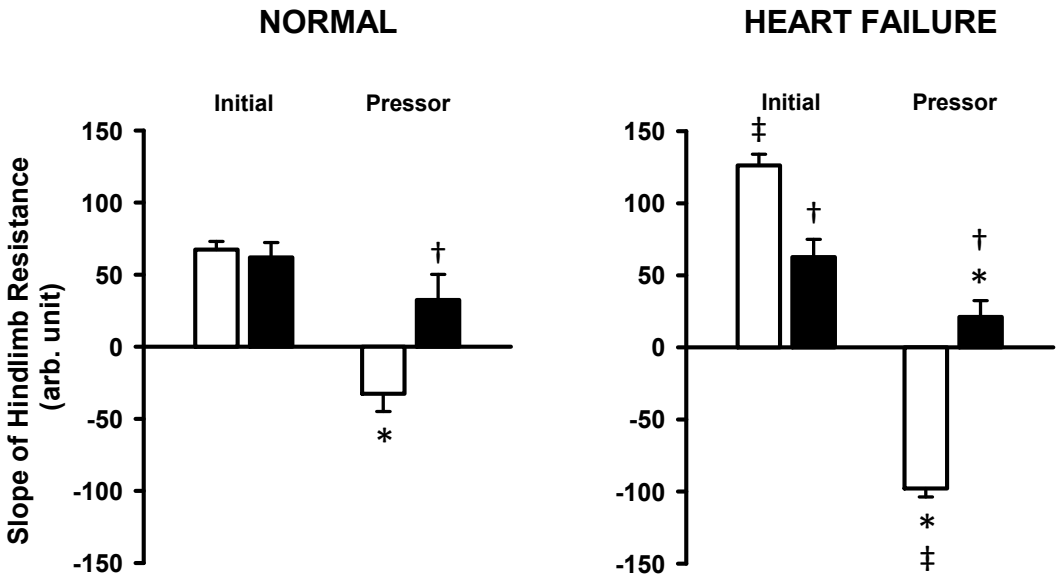


Figure 4.4. Average initial and pressor slope values of hindlimb resistance during control (open bars) and α_1-blockade (filled bars) experiments in normal (*left panel*) and heart failure (*right panel*) animals. *p<0.05 vs. initial slope; † p<0.05 vs. control; ‡ p<0.05 vs. normal.

Table 1 shows mean hemodynamic data during rest, mild exercise and muscle metaboreflex activation in control and α_1-blockade experiments in normal and HF animals. From rest to exercise in control, there were significant increases in MAP, CO, HR, NIVC, HLBF and ventricular contractility. With muscle metaboreflex activation, there were further significant increases in MAP, CO, HR, NIVC, and indices of ventricular inotropic and lusitropic state. Following α_1-adrenergic blockade in normal animals, resting MAP was significantly lower while NIVC and contractility were significantly higher than in control. With mild dynamic exercise there was a significant increase in all variables and MAP, CO, NIVC, and dP/dt_{max} were markedly higher than

control exercise levels. Muscle metaboreflex activation following α_1-blockade resulted in substantial increases in MAP, CO, HR, NIVC dP/dt_{max} and dP/dt_{min}. The reflex-induced pressor response following α_1-blockade was significantly attenuated compared to control while CO, NIVC and ventricular contractility during maximal metaboreflex activation was substantially higher that during control.

Table 4.1. Mean hemodynamic values during rest, mild exercise (EX) and muscle metaboreflex activation (MMA) before and after α_1-blockade in normal and heart failure animals.

		NORMAL		HEART FAILURE	
		Control	α_1-blockade	Control	α_1-blockade
MAP	Rest	89.1±1.8	80.9±1.5 †	77.0±0.6 ‡	71.1±1.6
(mmHg)	Ex	91.5±1.9 *	83.8±1.4 *†	79.6±2.0 ‡	72.3±1.0
	MMA	142.6±1.5 *	117.5±2.4 *†	111.2±3.8 ‡	105.3±3.8
CO	Rest	2.78±0.16	2.96±0.21	2.26±0.11 ‡	2.53±0.13 †
(l/min)	Ex	4.31±0.22 *	4.48±0.22 *†	3.38±0.14 *‡	4.00±0.17 *†
	MMA	6.16±0.13 *	6.37±0.19 *†	3.84±0.22 ‡	5.57±0.17 *†
HR	Rest	75.2±4.1	89.8±5.3	105.0±5.8	102.0±4.0
(bpm)	Ex	105.4±3.4 *	119.6±3.4 *	137.2±5.8 *	139.6±6.0 *
	MMA	140.3±3.2 *	159.9±10.1 *	165.5±6.6 *	173.6±5.0 *†
NIVC	Rest	24.9±1.7	29.5±2.4 †	24.5±1.6	29.2±2.1 †
(ml/min/mmHg)	Ex	35.4±2.2 *	40.6±2.6 *†	33.1±1.3 *	41.0±2.6 *†
	MMA	40.2±1.2 *	49.6±1.7 *†	29.9±1.2 *‡	48.0±2.2 *†
HLBF	Rest	0.57±0.05	0.58±0.04	0.40±0.02 ‡	0.49±0.02 †
(l/min)	Ex	1.06±0.05 *	1.08±0.07 *	0.85±0.05 *‡	1.03±0.08 *†
	MMA	0.54±0.05 *	0.58±0.07 *	0.48±0.04 *‡	0.57±0.06 *†
dP/dt_{max}	Rest	1854±61	1827±69 †	792±20 ‡	882±39
(mmHg/s)	Ex	2030±93 *	2120±71 *†	911±57 *‡	1064±53 *
	MMA	2892±104 *	3211±145 *†	1266±24 *‡	1640±136 *†
dP/dt_{min}	Rest	-1593±83	-1435±42	-786±31 ‡	-882±56
(mmHg/s)	Ex	-1653±84	-1513±30 *	-863±52 ‡	-1033±32 †
	MMA	-2585±89 *	-2219±128 *	-1200±9 *‡	-1521±89 *†

Values are means ± SE. MAP, mean arterial pressure; CO, cardiac output; HR, heart rate; NIVC, non-ischemic vascular conductance; HLBF, hindlimb blood flow; dP/dt_{max}, maximal rate of left ventricular pressure increase; dP/dt_{min} maximal rate of left ventricular relaxation. *p<0.05 vs. previous setting in same experiment; † p<0.05 vs. control; ‡ p<0.05 vs. normal.

After induction of HF in the same animals, resting levels of MAP, CO, HLBF, dP/dt_{max} and dP/dt_{min} were significantly lower. Although CO, HR, NIVC, HLBF and

ventricular contractility increased from rest to exercise, they were significantly lower than normal exercising levels with the exception of HR. Following metaboreflex activation in HF animals, increases in MAP, CO, dP/dt_{max} and dP/dt_{min} were significantly attenuated and there was vasoconstriction in the non-ischemic vasculature. Following α_1-adrenergic blockade, resting CO, HLBF and NIVC increased. From rest to exercise, significant increases in CO, HR, NIVC, HLBF and ventricular contractility were observed. Muscle metaboreflex activation following α_1-blockade in HF animals significantly increased CO, HR, NIVC, dP/dt_{max} and dP/dt_{min} and these parameters were significantly improved compared to metaboreflex responses in HF.

Discussion

The major new finding of this study is that muscle metaboreflex-induced vasoconstriction of the ischemic active skeletal muscle is exaggerated in heart failure. Previous studies have shown that metaboreflex-induced increase in MAP partially restores blood flow to ischemic active muscle in healthy subjects. Whether the muscle metaboreflex-induced increase in sympathetic activity causes vasoconstriction the ischemic active muscle has been controversial (33, 54, 59). We recently showed that metaboreflex activation during submaximal exercise induces α-mediated sympathetic vasoconstriction within the ischemic active muscle, limiting the capability of metaboreflex to restore blood flow to the muscle (36). In HF, the ability of the reflex to increase CO is markedly limited and the metaboreflex-induced pressor response occurs primarily via peripheral vasoconstriction. Inability to increase CO in HF limits restoration of blood flow to the ischemic muscle and exaggerated vasoconstriction of ischemic active muscle would further limit blood supply and enhance muscle ischemia. This generates a positive feedback loop that potentially contributes to exercise intolerance in

HF.

Metaboreflex responses in HF

Inasmuch as heightened coronary vasoconstriction limits the cardiac function (15) and little increase in CO occurs in HF, mechanisms of muscle metaboreflex shift from CO-mediated to peripheral vasoconstriction induced pressor response (16, 29, 58). The limited CO increase in HF attenuates the rise in MAP and consequently, limits the ability of metaboreflex to partially restore blood flow to the ischemic muscle. Vasoconstriction of inactive beds plays a very small role in generating the pressor response as blood flow to inactive beds constitutes only a small proportion of CO and thereby, total vascular conductance (52). With active skeletal muscle comprising the largest fraction of total vascular conductance, it is the only target vasculature for vasoconstriction during metaboreflex activation capable of generating large pressor responses. This is the first study to show that metaboreflex-induced hindlimb vasoconstriction is exaggerated in HF. The vasoconstriction is abolished after α_1-blockade, indicating the sympathetic origin of the vasoconstriction. In addition, α_1-blockade also markedly improved HLBF during free-flow exercise, revealing a potential therapeutic target for exercise intolerance in HF.

If muscle metaboreflex activation induces sympathetically-mediated constriction of the ischemic muscle from which it originates, active ischemic muscle is both the origin and the target of the reflex. On the afferent side, metabolic accumulation in the muscle stimulates group III/IV nerves and elicits the muscle metaboreflex which in turn increases CO, redistributes blood flow and causes vasoconstriction of the ischemic muscle on the efferent side. Vasoconstriction of the already ischemic muscle would further limit the oxygen delivery to the muscle causing more ischemia and heightened

metaboreflex activation. We previously showed that in normal subjects, metaboreflex-induced vasoconstriction of the ischemic active muscle acts as a limiting positive feedback where afferent and efferent responses progressively become smaller and plateau at an amplified response level (36). The slope of the relationship between hindlimb resistance and HLBF in normal animals is -32.6 units compared to -97.9 units after induction of HF. The slope value indicates that for every liter/min decrease in HLBF, there is a 97.9 unit increase in hindlimb resistance. At the threshold level of HLBF, a 97.9 mmHg/l/min increase in hindlimb resistance would cause 0.84 l/min fall in HLBF if pressure remained constant. This decrease in HLBF would cause further ischemia and increase hindlimb resistance by 82.1 mmHg/l/min, which in turn would reduce HLBF by 0.2 l/min and so forth. Thus, the stimulus and the response become smaller with each cycle of positive feedback and will eventually reach a plateau. Therefore, muscle metaboreflex activation in HF is still a limiting positive feedback reflex albeit with a markedly amplified slope than in normal subjects. Previous studies have shown a larger vasoconstriction of the peripheral vasculature with heavier workloads (6, 30). With increase in workload, the metaboreflex could easily convert into a run-away feedback loop where the stimulus and the response get larger with each cycle, rapidly reaching infinite resistance. Indeed, the large vasoconstriction we observed in HF is perilously close to a level in which the increase in resistance would elicit a fall in flow greater than that which engaged the reflex. The result would be a rapidly escalating response leading to total vasoconstriction.

Mechanisms for heightened SNA in HF

Heightened SNA and impaired muscle blood flow in HF could result from various different pathways and vascular factors. The arterial baroreflex buffers about one-half

of the metaboreflex-induced pressor response in normal animals by restraining peripheral vasoconstriction (40). In HF, the strength of baroreflex is impaired at both rest (61) and during exercise (38). The reduced baroreflex buffering of the metaboreflex potentially contributes to elevated SNA and peripheral vasoconstriction in HF. Respiratory muscles play an important role in muscle metaboreflex activation during dynamic exercise (64). Low CO in HF causes hypoperfusion of all tissues including respiratory muscles. Increased exertion of the respiratory muscles during exercise in HF likely causes enhanced afferent activation augmenting the metaboreflex-induced responses. A recent study also implicated aberrant activation of cardiac sympathetic afferents in HF which contributes to elevated SNA at rest (92). Enhanced arterial chemoreflex activation has also been shown in HF (83). Neurogenic vasoconstriction of the active skeletal muscle is attenuated by the metabolic by-products in the muscle, termed functional sympatholysis (63). However, functional sympatholysis is impaired in pathophysiological states such as HF, hypertension, etc. (85, 90). Moreover, impaired endothelium-mediated vasodilation and elevated circulating levels of catecholamines, vasopressin, angiotensin II, etc. could further increase the peripheral vasoconstriction in HF subjects (22, 30). Some studies have shown reduced oxidative type I fibers and increased glycolytic type II b fibers in HF subjects which could reduce their exercise capacity and lead to exercise intolerance (23, 82).

Perspectives

The muscle metaboreflex is not tonically active at mild workload in canines as HLBF must be reduced below threshold flow before any metaboreflex responses are observed. Due to lower perfusion in HF, metaboreflex threshold during mild exercise is achieved earlier and likely contributes to heightened vasoconstriction with metaboreflex

activation in HF as workload increase and tonic activity of the reflex ensues. (6). To what extent the muscle metaboreflex approaches run-away positive feedback levels with higher intensity exercise is unknown. Furthermore, there is evidence that reflexes arising from skeletal muscle afferents are tonically active in humans during even relatively mild exercise (2, 68). The exaggerated vasoconstriction within the ischemic active muscle together with limited metaboreflex restoration of the blood flow to the ischemic muscle likely impairs the ability to exercise and contributes to exercise intolerance in HF.

CHAPTER 5 - MUSCLE METABOREFLEX AND ARTERIAL BAROREFLEX: ACTION, INTERACTION AND CONTROL OF BLOOD FLOW TO ACTIVE MUSCLE

Abstract

The muscle metaboreflex and arterial baroreflex regulate arterial pressure through distinct mechanisms. Muscle metaboreflex activation (MMA) elicits a pressor response virtually solely by increasing cardiac output (CO) while baroreceptor unloading increases mean arterial pressure (MAP) primarily through peripheral vasoconstriction. We activated the muscle metaboreflex in chronically instrumented canines during dynamic exercise (via graded reductions in hindlimb blood flow; HLBF) followed by simultaneous baroreceptor unloading (via bilateral carotid occlusion; BCO). We hypothesized that simultaneous activation of both reflexes would result in an exacerbated pressor response owing to both an increase in CO and vasoconstriction. We observed that MMA caused a significant increase in MAP, heart rate (HR), CO, and non-ischemic vascular conductance (NIVC; conductance of all vascular beds except the hindlimb vasculature). When followed by BCO during MMA, there were further increases in MAP and HR however, NIVC decreased by ~25% indicating substantial peripheral vasoconstriction. Although there was significant vasoconstriction within the ischemic muscle itself, the remaining vasculature vasoconstricted to a greater extent, thereby redirecting blood flow to the ischemic muscle. We conclude that baroreceptor unloading during MMA induces preferential peripheral vasoconstriction to improve blood flow to the ischemic active skeletal muscle.

Introduction

Insufficient oxygen delivery to the active skeletal muscle during exercise causes accumulation of metabolites (e.g., hydrogen ions, adenosine, lactic acid, etc.) which

stimulate the group III and IV muscle afferents eliciting a reflex pressor response, termed the muscle metaboreflex (1, 9, 35, 43, 65, 77). Muscle metaboreflex activation during dynamic exercise significantly increases mean arterial pressure (MAP), cardiac output (CO), heart rate (HR) and ventricular contractility which combined with enhanced right atrial pressure maintains or slightly increases stroke volume (16, 32, 70, 71, 78, 88, 95). Although some regional vasoconstriction occurs with metaboreflex activation during submaximal exercise (e.g., coronary, renal, forelimbs (3, 6, 13, 48, 49)), total vascular conductance (TVC) is relatively unchanged (6, 17, 18, 29, 74). Thus, muscle metaboreflex-induced pressor response during dynamic exercise occurs virtually solely due to an increase in CO (6, 29, 55, 71, 78, 95).

At rest and during exercise the arterial baroreflex is the primary short-term negative feedback reflex which maintains arterial pressure by regulating TVC and CO. The arterial baroreflex primarily modulates TVC as little change in CO occurs during steady state (11, 60, 79, 80, 91). Therefore, the muscle metaboreflex and the arterial baroreflex regulate arterial pressure via two distinct mechanisms. Muscle metaboreflex activation increases MAP primarily by increasing CO whereas arterial baroreflex activation increases MAP primarily through peripheral vasoconstriction. As workload increases, active skeletal muscle vasculature becomes an increasingly important target for baroreflex-induced peripheral vasoconstriction (11). We recently showed that muscle metaboreflex activation induces sympathetic vasoconstriction within the ischemic muscle thereby limiting the ability of the metaboreflex to improve muscle blood flow (36). The interaction of the muscle metaboreflex and the arterial baroreflex has not been well established, especially when these reflexes are coactivated. In this study, we quantified the contribution of CO and TVC to the pressor response elicited by carotid

baroreceptor unloading (via bilateral carotid occlusion; BCO) at rest, during mild exercise and during muscle metaboreflex activation. We investigated whether concurrent activation of the two reflexes undergoes an additive, occlusive or facilitative interaction. Since carotid baroreceptor unloading induces peripheral vasoconstriction, to what extent the ischemic muscle vasoconstricts during concurrent activation of the baroreflex and the muscle metaboreflex is unknown. Previous studies hypothesized that increases in metabolic by-products in skeletal muscle during exercise reduces the efficacy of sympathetic nerves to elicit vasoconstriction (63, 84). We hypothesized that baroreceptor unloading during metaboreflex activation would still elicit substantial systemic peripheral vasoconstriction even within the ischemic hindlimb. However, if non-ischemic beds are more susceptible to neurogenic vasoconstriction, then there may be redistribution of the available CO towards the ischemic muscle.

Methods

Experimental subjects

Eight adult mongrel canines (5 females, 3 males; 20-25 kg) were selected for the study. All the procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Wayne State University and complied with the National Institutes of Health *Guide to the Care and Use of Laboratory Animals*. All animals were acclimatized to the laboratory surroundings and willing to run on a motor-driven treadmill; no negative reinforcement techniques were utilized.

Surgical procedures

For each of the three surgical procedures, the animals were sedated with acepromazine (0.2-0.5 mg/kg IM) and received preoperative analgesics [carprofen (4.4 mg/kg IV), buprenorphine (0.01-0.03 mg/kg IM) and fentanyl (50–125 μg/h (72h)

transdermal delivery)]. Anesthesia was induced with ketamine and diazepam (5 mg/kg IV and 0.2-0.3 mg/kg IV, respectively) and maintained with isoflurane gas (1–3%). Cefazolin (antibiotic, 30 mg/kg IV) was administered pre- and postoperatively to avoid acute postoperative infections. Animals were closely monitored postoperatively and given buprenorphine (0.01-0.03 mg/kg IM) and acepromazine (0.2-0.3 mg/kg IV) as needed. Cephalexin (antibiotic, 30 mg/kg (BID) PO) was administered prophylactically for the entire term of the experimental protocol. The animals recovered for two weeks after each surgery.

In the first surgical procedure, the thoracic cavity was opened via a left thoracotomy (3rd/4th intercostal space) approach and the pericardium was cut to expose the heart. A telemetry pressure transmitter (TA11 PA-D70, DSI) was tethered subcutaneously at the height of the left ventricular apex and two intercostal spaces caudal to the thoracotomy incision (n=5). The tip of the pressure transducer catheter was inserted into the left ventricle and secured. A perivascular flow probe (20PAU, Transonic Systems) was placed around the ascending aorta to measure CO. Pacing wires were secured to the free wall of the right ventricle for studies unrelated to the present study. The pericardium was reapproximated and all wires were tunneled subcutaneously and exteriorized between the scapulae. The chest was closed in layers.

In the second surgical procedure, the abdominal aorta and left renal artery were exposed through a left retroperitoneal approach. Perivascular flow probes (Transonic Systems) were positioned around the terminal aorta (10PAU) and the left renal artery (4PSB) to measure hindlimb blood flow (HLBF) and renal blood flow (RBF), respectively. All side branches of the terminal aorta between the iliac arteries and the

aortic flow probe were ligated and severed. Two hydraulic occluders (8-10 mm, DocXS Biomedical Products) were placed around the terminal aorta distal to the flow probe. A 19-gauge polyvinyl catheter (Tygon, S54-HL, Norton) was inserted into a side branch of the aorta cranial to the flow probe to measure systemic arterial pressure. A second catheter was inserted into a side branch of the aorta caudal to the occluders to measure arterial pressure below the occluders (femoral arterial pressure; FAP). When the catheterization of a caudal aortic branch was not possible, a side branch of the femoral artery was catheterized in the third procedure. All instrumentation was tunneled subcutaneously and exteriorized between the scapulae and the abdomen was closed in layers.

In the third surgical procedure, a hydraulic occluder (4-6 mm, DocXS Biomedical Products) was placed around each common carotid artery via a midline neck incision in order to perform bilateral carotid occlusion (BCO). The right jugular vein was catheterized (n=3) for studies unrelated to the present study. Instrumentation was tunneled subcutaneously and exteriorized between the scapulae and the neck was closed in layers.

Data acquisition

Before each experiment, animals were brought into the laboratory and allowed to roam freely and acclimate for ~10-20 minutes, before being directed onto the treadmill. The CO, RBF, and HLBF flow probe cables were connected to flow meters (TS420, Transonic Systems) and the left ventricular implant transmitter was turned on to collect data via telemetry (DSI). The arterial catheters were aspirated, flushed and connected to pressure transducers (Transpac IV, ICU Medical). All hemodynamic variables, in addition to MAP (calculated) and HR (triggered by the CO signal), were monitored as

real-time waveforms by a data acquisition system (LabScribe, iWorx) and recorded for subsequent off-line analysis.

Experimental procedures

All experiments were performed after the animals had fully recovered from surgery (i.e., were active and of good appetite). Each experiment began with the animal standing still on the treadmill until all resting hemodynamic data were observed to be stable (~ 5-10 min). Three different experimental protocols were used: BCO at rest, BCO during exercise and BCO with muscle metaboreflex activation. In the first experimental protocol, steady state data collection at rest was followed by BCO performed by inflating the carotid occluders for 2 min while the animals were standing on the treadmill. BCO during exercise: After collecting resting steady state data, the treadmill was turned on and gradually increased to a speed of 3.2 kph at 0% grade (mild exercise). The animals exercised for 5-10 min followed by BCO for 2 min during the same workload. BCO during metaboreflex activation: After collecting steady state data during rest and mild exercise, muscle metaboreflex was engaged via graded reductions in HLBF (via partial inflation of terminal aortic occluders) during mild exercise. Each level of vascular occlusion was maintained until all parameters had reached steady state (~ 3-5 min). After the steady state for maximal metaboreflex activation, BCO was performed for 2 min. The large increase in MAP with BC during metaboreflex activation would force more flow through the hindlimb occluders which would lessen metaboreflex activation, therefore the occluder resistance was increased to keep HLBF constant.

Data analysis

MAP, FAP, CO, HR, RBF, left ventricular pressure and HLBF were continuously recorded during each experimental procedure. Other hemodynamic parameters were

calculated during off-line data analysis (e.g., dP/dt_{max}, dP/dt_{min}, total vascular conductance (TVC), renal vascular conductance (RVC), hindlimb vascular conductance (HVC) and conductance of all vascular beds except the hindlimbs (non-ischemic vascular conductance; NIVC). TVC, RVC, HVC and NIVC were calculated as CO/MAP, RBF/MAP, HLBF/FAP and (CO-HLBF)/MAP, respectively. Due to technical difficulties, dP/dt_{max} and dP/dt_{min} values were only calculated in five animals. One minute averages of all variables were taken during steady-state at rest, free-flow exercise, muscle metaboreflex activation and BCO. Mean values were averaged across all animals to obtain the sample mean of the study.

Partial reductions in HLBF by using the hydraulic occluders cause MAP to increase passively ($MAP_{passive}$) (6, 95). Thus, the pressor response observed with metaboreflex activation in our experiments is a combination of a reflex increase in MAP (MAP_{active}) and the passive effect of the occluder. $MAP_{passive}$ was calculated as $CO_{EX}/(NIVC_{EX} + HVC_{Observed})$, where the subscript EX indicates free-flow exercise levels. MAP_{active}, the increase in MAP due to reflex activation, was calculated by subtracting $MAP_{passive}$ from the observed MAP values.

The percent contributions of CO and TVC to the baroreflex-induced pressor response were calculated as follows:

$$\Delta MAP_{TVC} = \frac{CO_{Pre}}{NIVC_{BCO} + HVC_{BCO}} - \frac{CO_{Pre}}{NIVC_{Pre} + HVC_{Pre}}$$

$$\% \text{ contribution of TVC} = \frac{\Delta MAP_{TVC}}{\Delta MAP_{Observed}} \times 100$$

$$\Delta MAP_{CO} = \frac{(CO_{BCO} - CO_{Pre})}{NIVC_{Pre} + HVC_{Pre}}$$

$$\% \text{ contribution of CO} = \frac{\Delta MAP_{CO}}{\Delta MAP_{Observed}} \times 100$$

where the subscripts BCO and Pre indicate BCO and pre-BCO levels, respectively.

Since all of the pressor response to BCO during muscle metaboreflex activation was due to peripheral vasoconstriction, the percent contribution of TVC was calculated as 100 – percent contribution of CO. BCO during metaboreflex activation causes a large pressor response which leads to an increase in HLBF which would in turn change the stimulus for muscle metaboreflex. Thus, HLBF was kept constant by increasing the occluder resistance. We calculated the predicted rise in HLBF, if there was no increase in occluder resistance, as (MAP during BCO)/(hindlimb resistance during BCO + occluder resistance during metaboreflex activation).

Statistical analysis

All hemodynamic data are reported as means ± SE. An α-level of $P < 0.05$ was used to determine statistical significance. Paired *t*-tests were performed to compare the responses between rest and BCO during rest. Averaged responses for each animal were analyzed with Systat software (Systat 11.0). A one-way ANOVA with repeated measures was used to compare hemodynamic data for time effect. In the event of a significant time effect, a C-matrix test for simple effects was performed.

Results

Figure 5.1 shows representative hemodynamic responses evoked by BCO during rest, mild exercise and muscle metaboreflex activation in one animal. BCO at rest resulted in a large pressor response with little change in CO and a large decrease in NIVC, indicating that the pressor response was mediated predominately by peripheral vasoconstriction. From rest to exercise, there was moderate increase in CO, NIVC and HLBF. BCO during mild exercise increased MAP again predominately due to a large decrease in NIVC. Small increases in CO and HLBF also occurred. With metaboreflex activation during mild exercise, the large pressor response is occurred via a large

increase in CO as there was a modest increase in NIVC. Baroreceptor unloading during muscle metaboreflex activation caused a further large increase in MAP. This pressor response was mediated via peripheral vasoconstriction inasmuch as a large decrease in NIVC as well as a small decrease in CO occurred. In order to keep the metaboreflex stimulus constant during concurrent activation of baroreflex and metaboreflex, HLBF was maintained constant by manually increasing the resistance of the hydraulic occluders on the terminal aorta.

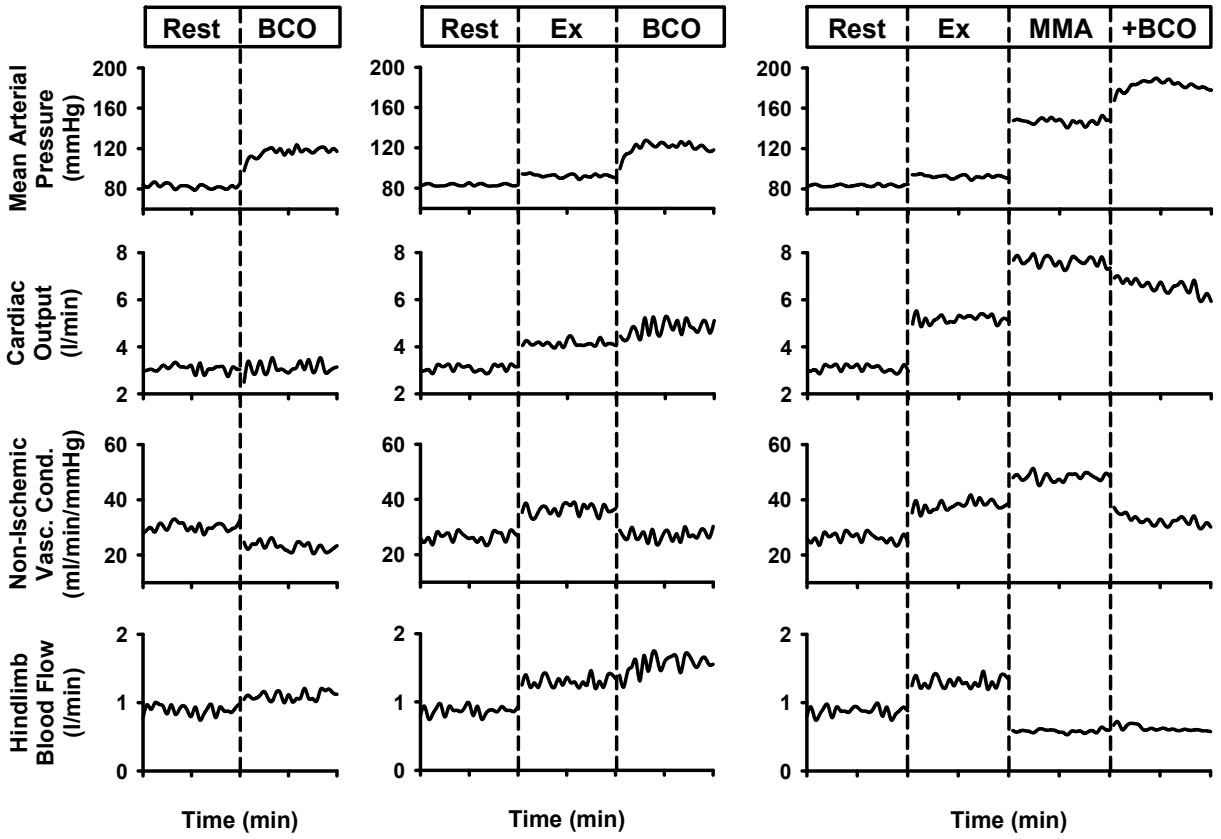


Figure 5.1. Data from one animal showing mean arterial pressure, cardiac output, non-ischemic vascular conductance and hindlimb blood flow in response to bilateral carotid occlusion (BCO) during rest (*left panel*), exercise (Ex; *middle panel*) and muscle metaboreflex activation (MMA; *right panel*).

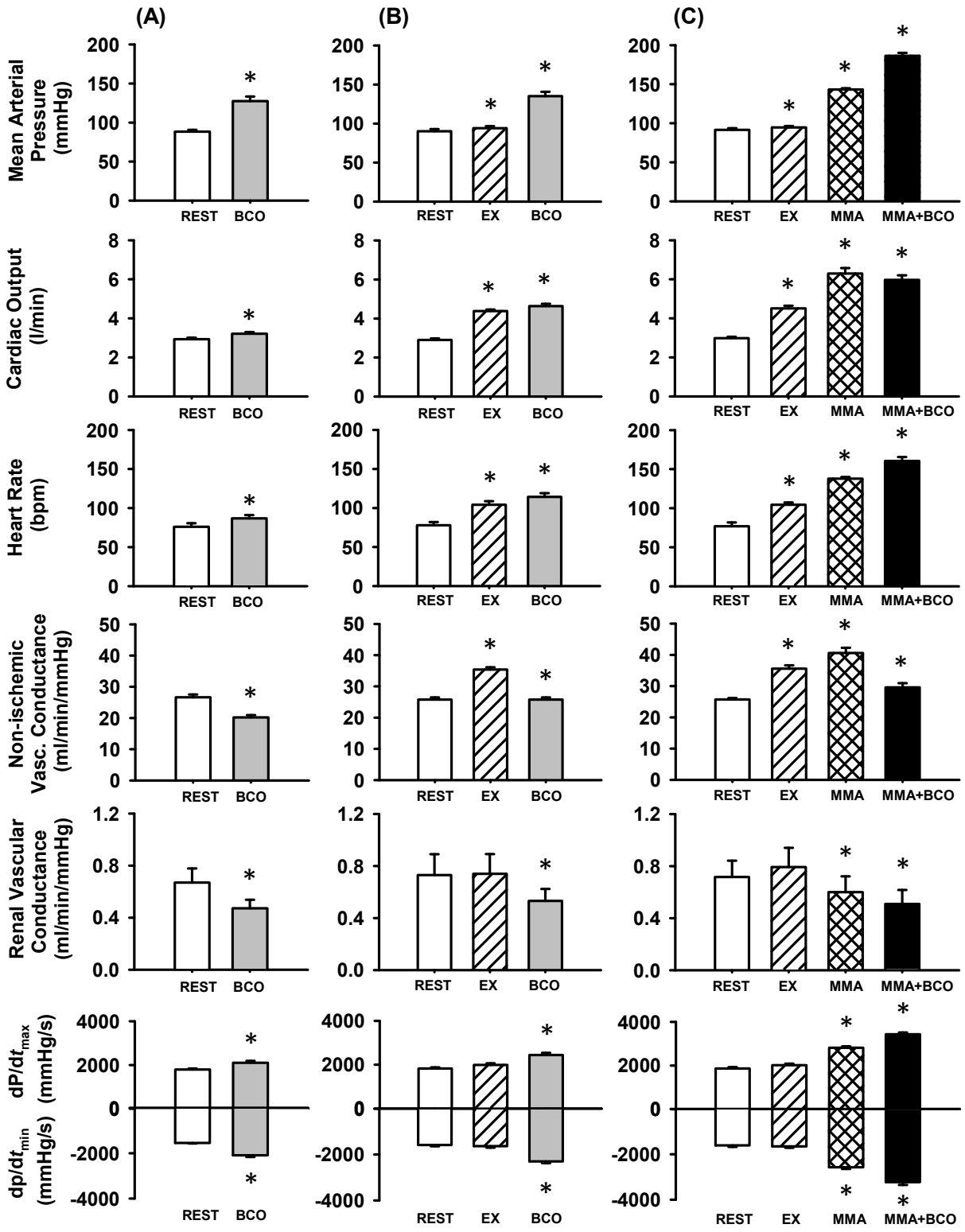


Figure 5.2. Average hemodynamic data during (A) rest (open bars) and bilateral carotid occlusion (BCO) at rest (grey bars); (B) rest, mild exercise (Ex; hatched bars) and BCO during Ex (grey bars); (C) rest, Ex, muscle metaboreflex activation (MMA; cross-hatched bars) and BCO during MMA (solid bars). * $p<0.05$ vs. previous setting.

Figure 5.2 shows average values of MAP, CO, HR, NIVC, RVC, dP/dt_{max} and dP/dt_{min} during BCO at rest, during mild exercise and metaboreflex activation.

BCO at rest. Carotid baroreceptor unloading at rest resulted in a significant increase in MAP with small but significant increases in CO, HR, dP/dt_{max} and dP/dt_{min}. Large significant decreases in NIVC and RVC indicates that the pressor response was primarily caused by peripheral vasoconstriction.

BCO during exercise. From rest to exercise, there were a significant increases in NIVC, CO, HLBF, and HR. BCO during exercise resulted in significant increases in MAP, CO, HR, dP/dt_{max} and dP/dt_{min} and significant decreases in NIVC and RVC.

BCO during metaboreflex activation. NIVC, CO, and HR increased significantly from rest to exercise. Muscle metaboreflex activation (via partial reduction in HLBF) caused significant increases in MAP, NIVC, CO, HR, dP/dt_{max} and dP/dt_{min} while RVC decreased significantly. Coactivation of baroreflex and muscle metaboreflex resulted in a further significant increases in MAP, HR, dP/dt_{max} and dP/dt_{min} and significant decreases in NIVC, CO and RVC.

Figure 5.3 shows the hemodynamic changes in hindlimb vascular conductance and HLBF during BCO at rest, during mild exercise and muscle metaboreflex activation. During BCO at rest, HVC significantly decreased indicating vasoconstriction in the hindlimb vasculature. HLBF increased despite the vasoconstriction due to the increase in MAP. From rest to exercise, both HVC and HLBF increased significantly. In response to BCO during exercise, there was a large decrease in HVC and HLBF again increased somewhat HLBF. Muscle metaboreflex was activated during mild exercise by reducing HLBF to ~40% of the value observed during free-flow exercise. We have recently shown that initial reductions in HLBF cause vasodilation in the hindlimb, but

when the metaboreflex is engaged, vasoconstriction ensues which brings HVC back towards the levels observed prior to hindlimb ischemia (37). With BCO during muscle metaboreflex activation, there is a significant decrease in HVC indicating vasoconstriction. The HLBF during the co-activation of both reflexes was kept constant by increasing the hindlimb occluder resistance. If there was no change in the occluder resistance, HLBF would have increased significantly (predicted HLBF).

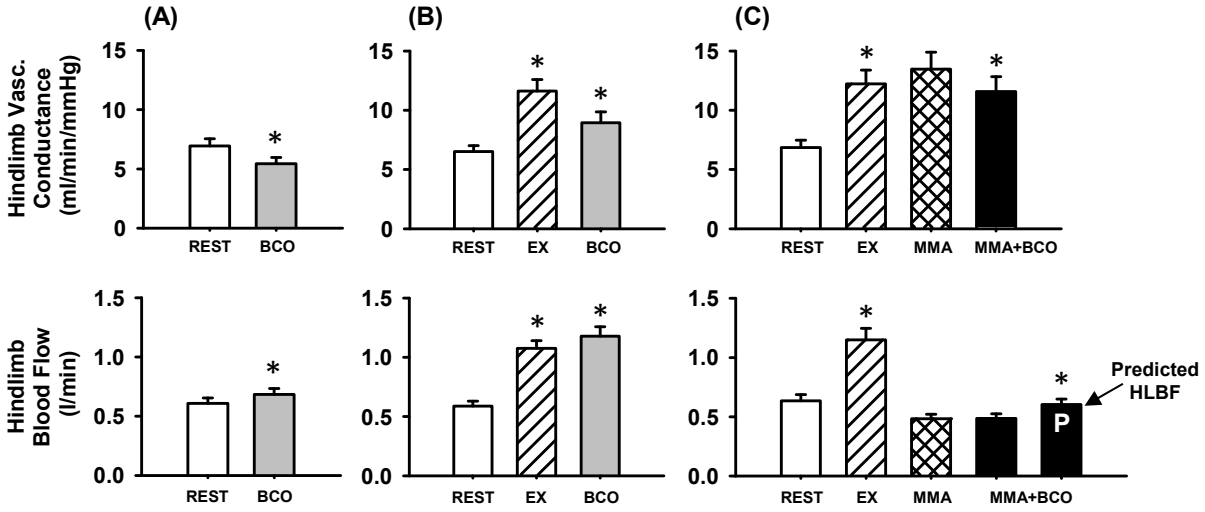


Figure 5.3. Average hindlimb vascular conductance and hindlimb blood flow responses during (A) rest (open bars) and bilateral carotid occlusion (BCO) at rest (grey bars); (B) rest, mild exercise (Ex; hatched bars) and BCO during Ex (grey bars); (C) rest, Ex, muscle metaboreflex activation (MMA; cross-hatched bars) and BCO during MMA (solid bars). The bar inscribed with P indicates the predicted HLBF during coactivation of two reflexes.* $p<0.05$ vs. previous setting.

Figure 5.4 shows the absolute changes in the cardiovascular parameters evoked by BCO at rest, during mild exercise and during metaboreflex activation in eight normal animals. The pressor responses evoked by BCO at rest, during exercise and during metaboreflex activation were not different from each other. Changes in CO, HR, dP/dt_{max}, dP/dt_{min}, RVC and HLBF were similar during BCO at rest and BCO during mild exercise. However, decreases in NIVC and HVC were significantly larger with BCO

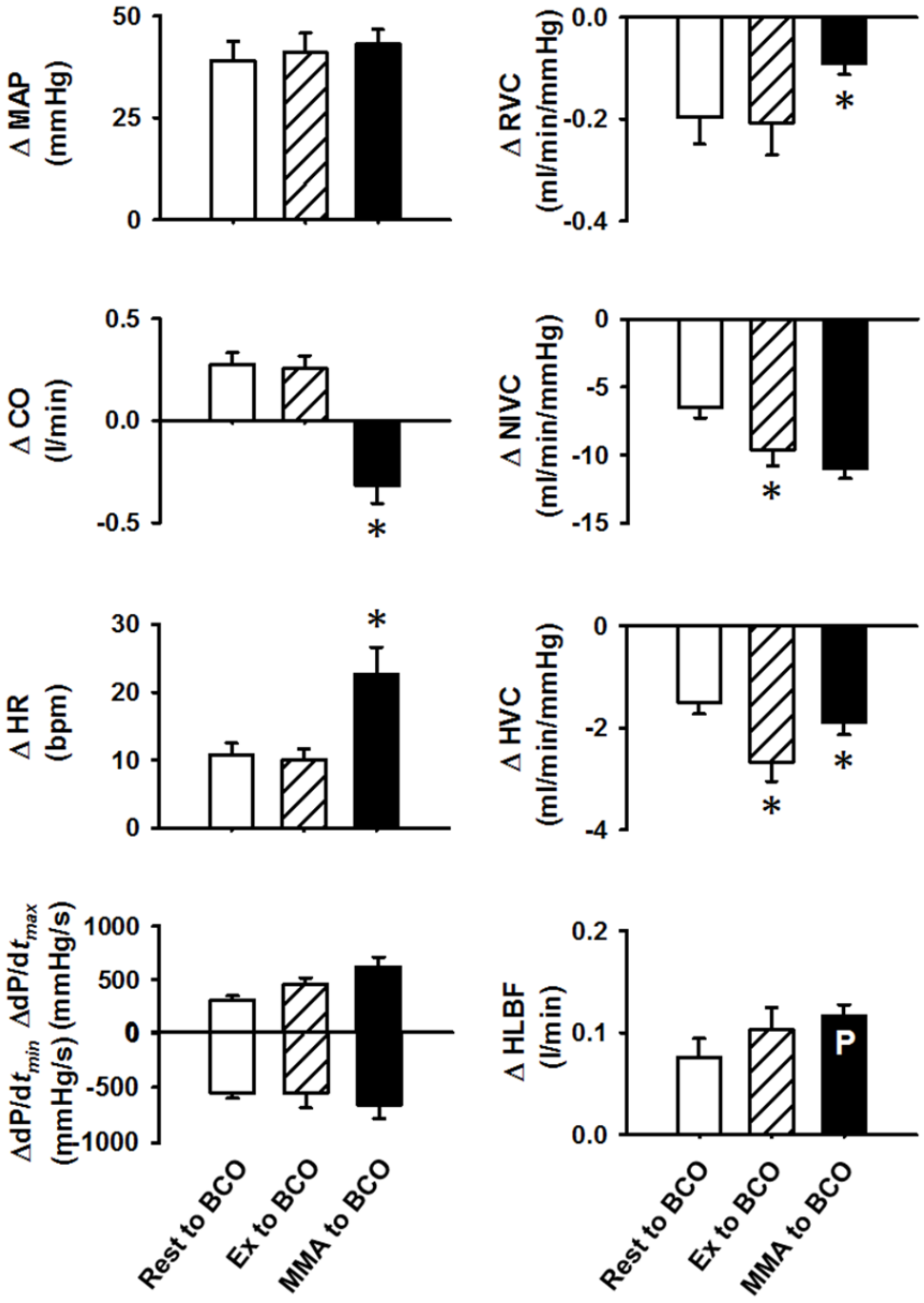


Figure 5.4. The absolute changes (Δ) in hemodynamic values from rest to bilateral carotid occlusion (BCO) during rest (open bars), from exercise (Ex) to BCO during Ex (hatched bars) and from muscle metaboreflex activation (MMA) to BCO during MMA (filled bars). * $p<0.05$ vs. previous setting. MAP, mean arterial pressure; CO, cardiac output; HR, heart rate; dP/dt_{max}, maximal rate of change in left ventricular pressure; dP/dt_{min}, minimal rate of change in left ventricular pressure; RVC, renal vascular conductance; NIVC, non-ischemic vascular conductance; HVC, hindlimb vascular conductance; HLBF, hindlimb blood flow.

during exercise than BCO at rest. With coactivation of baroreflex and metaboreflex, there was a large increase in HR with further improvement in contractility and lusitropy, however, CO decreased slightly. While the vasoconstriction of non-ischemic vascular beds in response to coactivation of reflexes was similar to that during BCO with exercise, the reduction in RVC and HVC was significantly smaller than with BCO during exercise. The predicted increase in HLBF during coactivation of reflexes was similar to the increase in HLBF seen in response to BCO during mild exercise.

To investigate the mode of interaction between arterial baroreflex and muscle metaboreflex (i.e., additive, facilitative or occlusive interaction), the observed responses during coactivation of baroreflex and metaboreflex activation were compared to the sum of metaboreflex-induced and BCO-induced responses during exercise. Figure 5.5 shows absolute changes in hemodynamic parameters in response to BCO during exercise, muscle metaboreflex activation, the sum of muscle metaboreflex-induced and BCO-induced responses during exercise and the observed hemodynamic changes in response to BCO during metaboreflex activation. The sum of individual reflex responses and the observed responses during coactivation were not different for MAP, NIVC and ventricular function, indicating an additive interaction. However, the observed responses during coactivation of the two reflexes were smaller for CO and RVC indicating that for these parameters the arterial baroreflex and the muscle metaboreflex summate as occlusive interaction. On the contrary, coactivation responses were significantly higher than the sum of individual responses for HR, indicating a facilitative interaction.

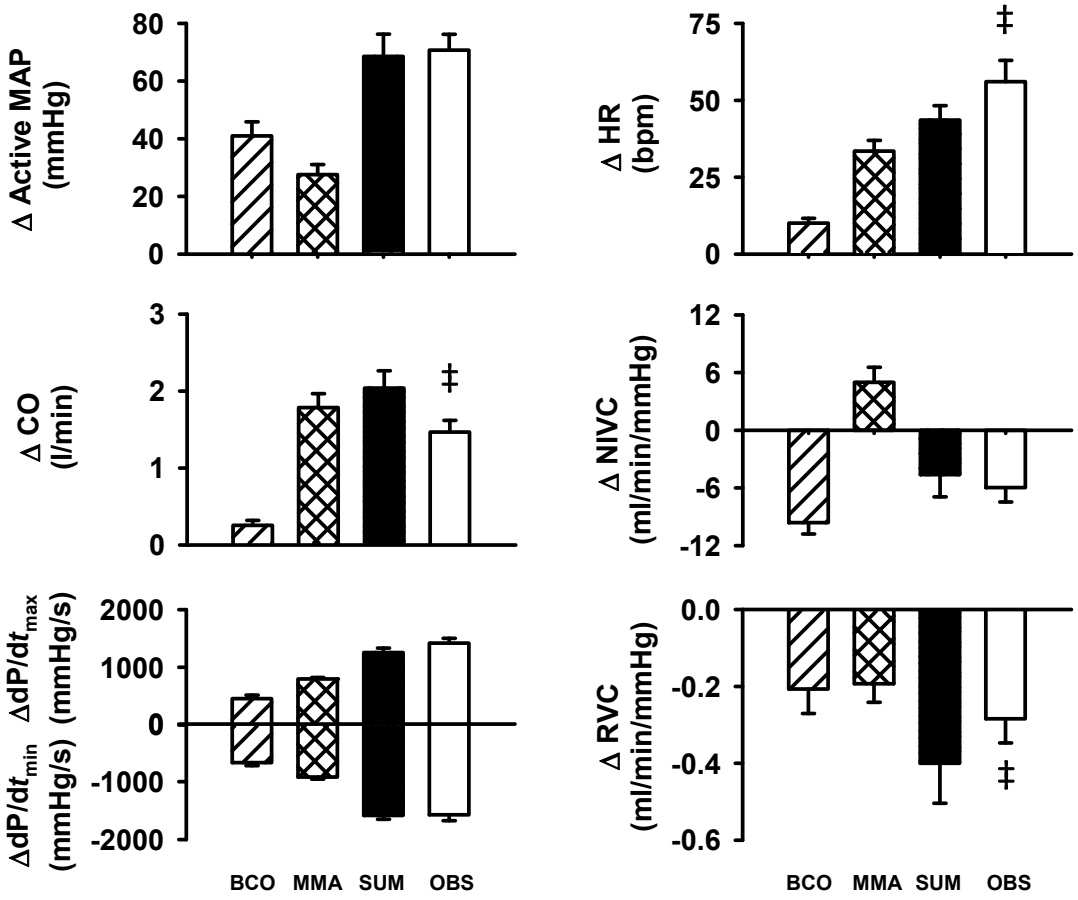


Figure 5.5. Absolute changes (Δ) in cardiovascular responses evoked by bilateral carotid occlusion (BCO) during mild exercise (hatched bars), muscle metaboreflex activation (MMA; cross-hatched bars), sum of individual MMA and BCO responses during exercise (SUM; filled bars) and observed combined effect of the MMA and BCO during MMA (OBS; open bars). ‡ $p<0.05$ vs. calculated sum of reflex-induced response during MMA and BCO during exercise.

Figure 5.6 shows the relative contribution of CO and TVC in mediating the rise in MAP in response to BCO during rest, BCO during exercise, muscle metaboreflex activation and BCO during metaboreflex activation. Pressor response to BCO during rest and exercise was primarily due to peripheral vasoconstriction as there is only a small contribution of CO. Muscle metaboreflex-induced pressor response was virtually solely driven by increase in CO as there is negative contribution of TVC. Pressor response with BCO during metaboreflex activation was solely due to the increase in peripheral vasoconstriction.

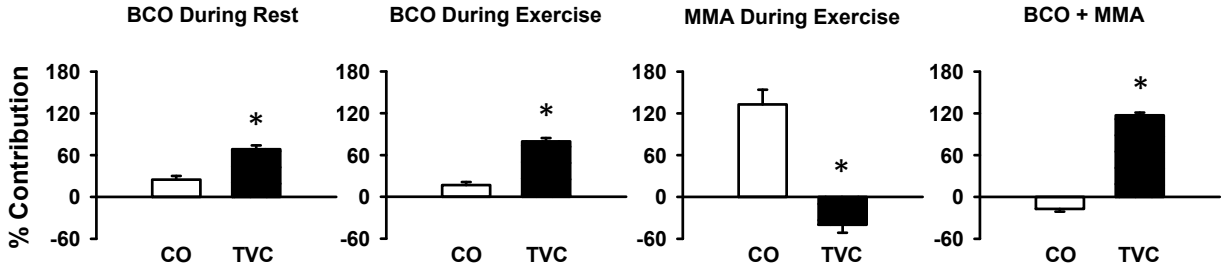


Figure 5.6. Percent contribution of cardiac output (CO; open bars) and total vascular conductance (TVC; filled bars) to pressor response evoked by bilateral carotid occlusion (BCO) at rest, BCO during exercise, muscle metaboreflex activation (MMA) and BCO during MMA. * $p<0.05$ vs. contribution of CO.

Discussion

This is the first study to investigate the strength and mechanisms of the muscle metaboreflex and arterial baroreflex when activated separately and concurrently, and the effect of this interaction on ventricular function, cardiac output and the distribution of total systemic peripheral blood flow. We found that the pressor responses to baroreceptor unloading and metaboreflex activation were additive, however the mechanisms mediating each pressor response were diametrically opposite: arterial baroreflex activation primarily induces peripheral vasoconstriction whereas metaboreflex activation primarily raises CO. When activated concurrently, profound pressor responses occur due to substantial vasoconstriction coupled with increased CO. Further, we found that baroreceptor unloading during metaboreflex activation causes vasoconstriction even within the ischemic active skeletal muscle. However, the vasoconstriction in all other vascular beds combined is greater than that in the ischemic muscle which thereby causes redistribution of cardiac output towards the ischemic beds. This shift of total systemic blood flow towards the ischemic muscle during combined baroreflex and metaboreflex activation acts to attenuate the level of metaboreflex stimulation.

Muscle metaboreflex and arterial baroreflex interaction

Reflexes interact in three different ways: additive interaction, where the coactivation responses equal the sum of reflex responses when activated individually; facilitative interaction, where responses during coactivation of two reflexes are larger than the sum of individual reflex responses; occlusive interaction, where the coactivation responses are smaller than the sum of individual reflex responses. We observed that the pressor response evoked by BCO during metaboreflex activation was similar to the sum of metaboreflex-induced and BCO-induced pressor response during exercise, indicating additive interaction between the two reflexes. The same conclusion holds for ventricular function and NIVC responses. However, CO and RVC responses exhibit occlusive interaction and HR responses undergo facilitative interaction upon coactivation of the two reflexes. Therefore, the interaction between arterial baroreflex and muscle metaboreflex when activated simultaneously is intimately dependent on the hemodynamic parameter being investigated.

Regulation of skeletal muscle blood flow

During progressive dynamic exercise the skeletal muscle vasculature becomes an increasingly important target vascular bed mediating the rise in arterial pressure during in response to baroreceptor unloading (11, 60). We recently showed that muscle metaboreflex activation induces sympathetic vasoconstriction within ischemic active muscle thereby, limiting the ability of the metaboreflex to improve muscle blood flow (36). BCO during metaboreflex activation causes further vasoconstriction within the ischemic hindlimb vasculature, although the decrease in HVC during coactivation of two reflexes is significantly smaller than that observed in response to BCO during normal exercise. One of the plausible causes for this smaller vasoconstriction in the hindlimb

vasculature could be functional sympatholysis, a phenomenon described as attenuated sympathetic vasoconstriction of the active skeletal muscle due to metabolic accumulation in the muscle (63, 84). The large increase in MAP in response to BCO during metaboreflex activation increases HLBF despite the vasoconstriction of hindlimb vasculature. Therefore, in order to maintain the same metaboreflex stimulus during coactivation of the reflexes, HLBF was kept constant by increasing the occluder resistance. If there was no change in the occluder resistance, the HLBF (predicted HLBF) would have increased significantly with the rise in arterial pressure driving more flow through the occluder resistance as well as the hindlimb vascular resistance. With a decrease in CO and vasoconstriction in the hindlimb vasculature during coactivation of the reflexes, the only way HLBF could have increased is by a larger vasoconstriction of the non-ischemic vasculature than the ischemic hindlimb vasculature. This preferential vasoconstriction in the non-ischemic vasculature redirects blood flow to the hindlimbs which thereby increases HLBF. This rise in HLBF would act to actually attenuate the metaboreflex activation. Interestingly, we calculated that the predicted HLBF during coactivation of the two reflexes would have virtually restored HLBF almost back to the metaboreflex threshold level of HLBF (Figure 5.7).

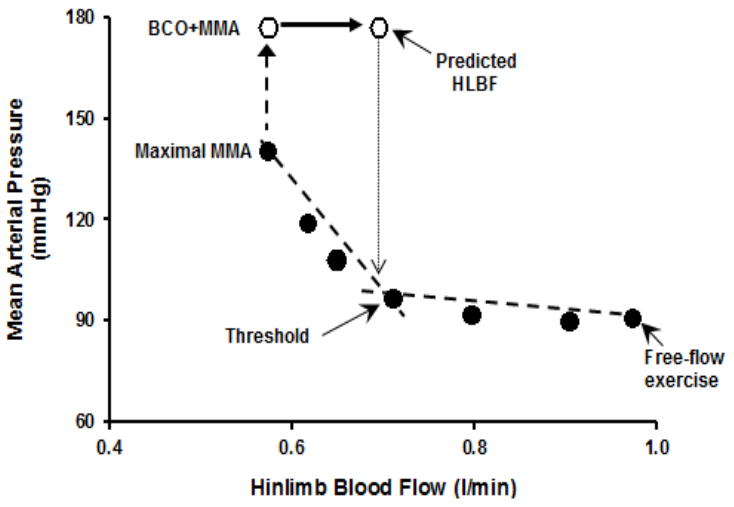


Figure 5.7. Mean arterial pressure and hindlimb blood flow during muscle metaboreflex activation (MMA; filled circles) followed by bilateral carotid occlusion (BCO) during MMA (open circles) in an experiment.

Where does vasoconstriction occur?

Previous studies have shown that active skeletal muscle becomes the primary target bed for baroreflex-induced peripheral vasoconstriction with increasing exercise intensity (11). We observed that although BCO during metaboreflex activation caused vasoconstriction within ischemic active muscle, blood flow to the ischemic muscle would still have improved (given that occluder resistance was not changed) due to a larger vasoconstriction in other vascular beds. Since active skeletal muscle constitutes the largest proportion of TVC during exercise, the preferential vasoconstriction during coactivation of reflexes most likely occurs in active skeletal muscle other than the hindlimb. Although there is some vasoconstriction of inactive beds such as renal vasculature during coactivation of baroreflex and muscle metaboreflex, inactive beds do not have much potential to increase MAP or redirect blood flow to other vasculatures (11, 52). In the present study, the RVC responses underwent occlusive interaction upon coactivation of baroreflex and muscle metaboreflex.

Perspectives and significance

In normal individuals, metaboreflex activation causes large increases in CO and MAP which in turn increase blood flow and oxygen delivery to the active vascular beds. The arterial baroreflex buffers the metaboreflex pressor response by about 50% by attenuating peripheral vasoconstriction (40). In conditions such as heart failure, where increases in CO become limited, the only mechanism to increase MAP is via peripheral vasoconstriction. Inasmuch as the strength of arterial baroreflex is impaired in heart failure (38, 61), this would lessen the ability of arterial baroreflex to attenuate peripheral vasoconstriction induced by metaboreflex activation, leading to markedly large increases in vascular sympathetic tone, particularly the active ischemic muscle.

Moreover, baroreceptor unloading during metaboreflex activation leads to larger vasoconstriction of the non-ischemic vasculature causing improvement in HLBF. In heart failure, this increase in HLBF could be attenuated, abolished, or possibly even reduce HLBF which could elicit a rampant positive feedback spiral where the baroreflex further engages the metaboreflex leading to a veritable sympathetic storm. It is unknown whether these interactions contribute to the often massive peripheral vasoconstriction which occurs during exercise in heart failure and likely contribute to exercise intolerance in these patients.

CHAPTER 6 - INTERACTION BETWEEN ARTERIAL BAROREFLEX AND MUSCLE METABOREFLEX IS ALTERED IN HEART FAILURE

Abstract

Previous studies have shown that heart failure (HF) alters the strength and mechanisms of muscle metaboreflex and arterial baroreflex during dynamic exercise. Muscle metaboreflex activation (MMA) in normal individuals increases mean arterial pressure (MAP) virtually solely by an increase in cardiac output (CO) whereas in HF, metaboreflex-induced pressor response occurs via peripheral vasoconstriction. The pressor response evoked by baroreceptor unloading in normal subjects occurs primarily through peripheral vasoconstriction and this pressor response is blunted in HF. However, the interaction between the two reflexes when activated simultaneously during dynamic exercise in HF is unknown. We activated the muscle metaboreflex in chronically instrumented canines during mild exercise (via graded reductions in hindlimb blood flow; HLBF) followed by baroreceptor unloading (via bilateral carotid occlusion; BCO) before and after induction of HF. We hypothesized that coactivation of both reflexes in HF would cause a smaller increase in MAP and a larger vasoconstriction of the ischemic hindlimb vasculature which would result in attenuated restoration of blood flow to the ischemic muscle observed in normal. We observed that BCO during MMA increases MAP by substantial vasoconstriction of all vascular beds including the ischemic active muscle. All cardiovascular responses with the exception of ventricular function exhibit occlusive interaction in response to baroreceptor unloading during MMA in HF. We conclude that vasoconstriction of ischemic active skeletal muscle during coactivation of both reflexes attenuates the restoration of HLBF.

Introduction

Stimulation of skeletal muscle afferents by metabolites, such as hydrogen ions, lactic acid, diprotonated phosphate, etc., that accumulate in the exercising muscle elicits a large pressor response, termed the muscle metaboreflex (1, 9, 35, 65, 77). Muscle metaboreflex activation significantly increases mean arterial pressure (MAP), cardiac output (CO), heart rate (HR), ventricular contractility and right atrial pressure (16, 32, 43, 70, 71, 88, 95). During submaximal exercise, muscle metaboreflex-induced increase in MAP occurs virtually solely due to an increase in CO as there is little, if any, peripheral vasoconstriction (6, 17, 18, 29, 71, 74, 78). Another blood pressure regulating reflex is the arterial baroreflex, a negative feedback reflex that maintains arterial pressure primarily by modulating peripheral vascular tone (11, 60, 79, 80, 91). Thus, the muscle metaboreflex and the arterial baroreflex regulate arterial pressure via two distinct mechanisms; muscle metaboreflex activation has a stronger control over cardiac sympathetic activity and increases MAP by increasing CO whereas arterial baroreflex activation has a stronger control over peripheral sympathetic activity and increases MAP primarily through peripheral vasoconstriction.

In heart failure (HF), the strength and mechanisms of both muscle metaboreflex and arterial baroreflex are altered (29, 38, 39, 51, 81). Impaired baroreflex sensitivity in HF results in a smaller increase in MAP in response to carotid hypotension at rest (61, 93, 94) and during exercise (38, 39). With limited CO reserve in HF, muscle metaboreflex activation elicits a pressor response primarily via peripheral vasoconstriction (14, 16, 29). In normal subjects, baroreceptor unloading during muscle metaboreflex activation showed diverse interactions between different variables; MAP, NIVC and ventricular function were additive; CO and RVC were occlusive and HR

responses were facilitative. Moreover, a larger preferential vasoconstriction of the non-ischemic vascular beds redirected blood flow to the ischemic muscle increasing HLBF. In this study we investigate whether the interaction between the two reflexes when activated simultaneously is altered in HF. We further studied the effect of coactivation of reflexes on ischemic active muscle in HF and whether there is any restoration of blood flow to the ischemic muscle.

Methods

Experimental subjects

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Wayne State University and complied with the National Institutes of Health *Guide to the Care and Use of Laboratory Animals*. Five adult mongrel canines (20-25 kg; 1 male, 4 females) were selected for the study. The animals were acclimatized to the laboratory surroundings and exercised voluntarily during experimentation; no negative reinforcement techniques were utilized.

Surgical procedures

The surgical procedures and medications used have been previously described (36). Briefly, a 20-mm flow transducer was placed around the aortic root to measure cardiac output (CO). A telemetry pressure transmitter was tethered subcutaneously and its tip was inserted into the left ventricle to measure left ventricular pressure. 3-4 stainless steel pacing wires (0-Flexon) were secured to the right ventricular free wall. Blood flow transducers was placed around the terminal aorta and the left renal artery to measure hindlimb blood flow (HLBF) and renal blood flow (RBF), respectively. Two hydraulic occluders were placed around the terminal aorta distal to the flow probe to reduce HLBF. Arterial catheters were placed to measure systemic arterial pressure and

femoral arterial pressure (FAP). Hydraulic occluders were also placed around common carotid arteries to perform bilateral carotid occlusion (BCO). The animals recovered for at least two weeks after each surgery.

Data acquisition

Before each experiment, the animals acclimated to the laboratory for ~10-20 minutes, following which they were directed onto the treadmill. The arterial catheters were aspirated, flushed and connected to pressure transducers (Transpac IV, ICU Medical), the flow probe cables were connected to flow meters (TS420, Transonic Systems) and the left ventricular telemetry implant was turned on. All hemodynamic variables were monitored as real-time waveforms by LabScribe2 data acquisition system (iWorx) and recorded for off-line analysis.

Experimental procedures

Three different experimental protocols were used: BCO at rest, BCO during exercise and BCO during muscle metaboreflex activation. BCO at rest: Steady state data at rest were collected while the animal stood still on the treadmill. This was followed by BCO performed by inflating the carotid occluders for 2 minutes. BCO during exercise: After collecting resting steady state data, the treadmill speed was gradually increased to 3.2 kph at 0% grade (mild exercise). The animals exercised until all hemodynamic data were stable (~5-10 minutes) followed by BCO for 2 minutes during the same workload. BCO during muscle metaboreflex activation: After collecting steady state data at rest and during mild exercise, HLBF was gradually reduced to ~40% of free-flow exercising levels (via partial inflations of the terminal aortic occluders) to activate the muscle metaboreflex. Once steady state for muscle metaboreflex activation was achieved and data were recorded, BCO was performed for 2 minutes. The large

increase in MAP with BCO during metaboreflex activation caused an increase in HLBF which in turn alters the metaboreflex stimulus. Thus, HLBF was kept constant by increasing the occluder resistance. After completion of experiments in normal animals, rapid ventricular pacing at the rate of 220-240 beats/min was used to induce HF in the same animals. All three experimental protocols were repeated in HF.

Data analysis

MAP, FAP, CO, HLBF, RBF, heart rate (HR; triggered from CO signal) and left ventricular pressure (LVP) were continuously recorded during each experiment. Other hemodynamic parameters were calculated during off-line data analysis (e.g., dP/dt_{max}, dP/dt_{min}, total vascular conductance (TVC), renal vascular conductance (RVC), hindlimb vascular conductance (HVC) and conductance of all vascular beds except the hindlimbs (non-ischemic vascular conductance; NIVC). dP/dt_{max} and dP/dt_{min} were calculated as the maximal rate of rise and fall in LVP, respectively. Due to technical difficulties, these values were only calculated in four animals. Vascular conductance for different vascular beds were calculated as follows: TVC = CO/MAP, RVC = RBF/MAP, HVC = HLBF/FAP and NIVC = (CO-HLBF)/MAP. One minute averages of all variables were taken during steady-state at rest, BCO at rest, mild exercise, BCO during mild exercise, muscle metaboreflex activation and BCO during metaboreflex activation. Mean values were averaged across all animals to obtain the sample mean of the study. The pressor response observed with metaboreflex activation in our experiments is a combination of a reflex increase in MAP due to metaboreflex activation (MAP_{Active}) and the passive increase in MAP solely due to the mechanical effect of terminal aortic occluders ($MAP_{Passive}$). $MAP_{Passive}$ was calculated as $CO_{EX}/(NIVC_{EX} + HVC_{Observed})$, where the subscript EX indicates free-flow exercise level (6, 95). MAP_{Active} was calculated by

subtracting the passive increase in MAP from the observed MAP values at maximal metaboreflex activation. HLBF during coactivation of the two reflexes was kept constant by increasing the occluder resistance. We calculated the HLBF if there was no increase in occluder resistance (predicted HLBF) as (MAP during BCO)/(hindlimb vascular resistance during BCO + occluder resistance during metaboreflex activation).

Statistical analysis

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

Results

Figure 6.1 shows average hemodynamic responses evoked by BCO at rest before and after induction after HF. Carotid baroreceptor unloading at rest caused a significant increase in MAP, CO and HR. After induction of HF, resting MAP and CO were significantly lower and resting HR was significantly higher. BCO at rest elicited large increases in MAP and HR in HF, however the magnitude of pressor response was significantly lower and HR response was markedly higher than in normal. The small but significant increase in CO observed in normal was abolished in HF. For NIVC, RVC, HVC, dP/dt_{max} and dP/dt_{min}, there was a significant effect of BCO and HF.

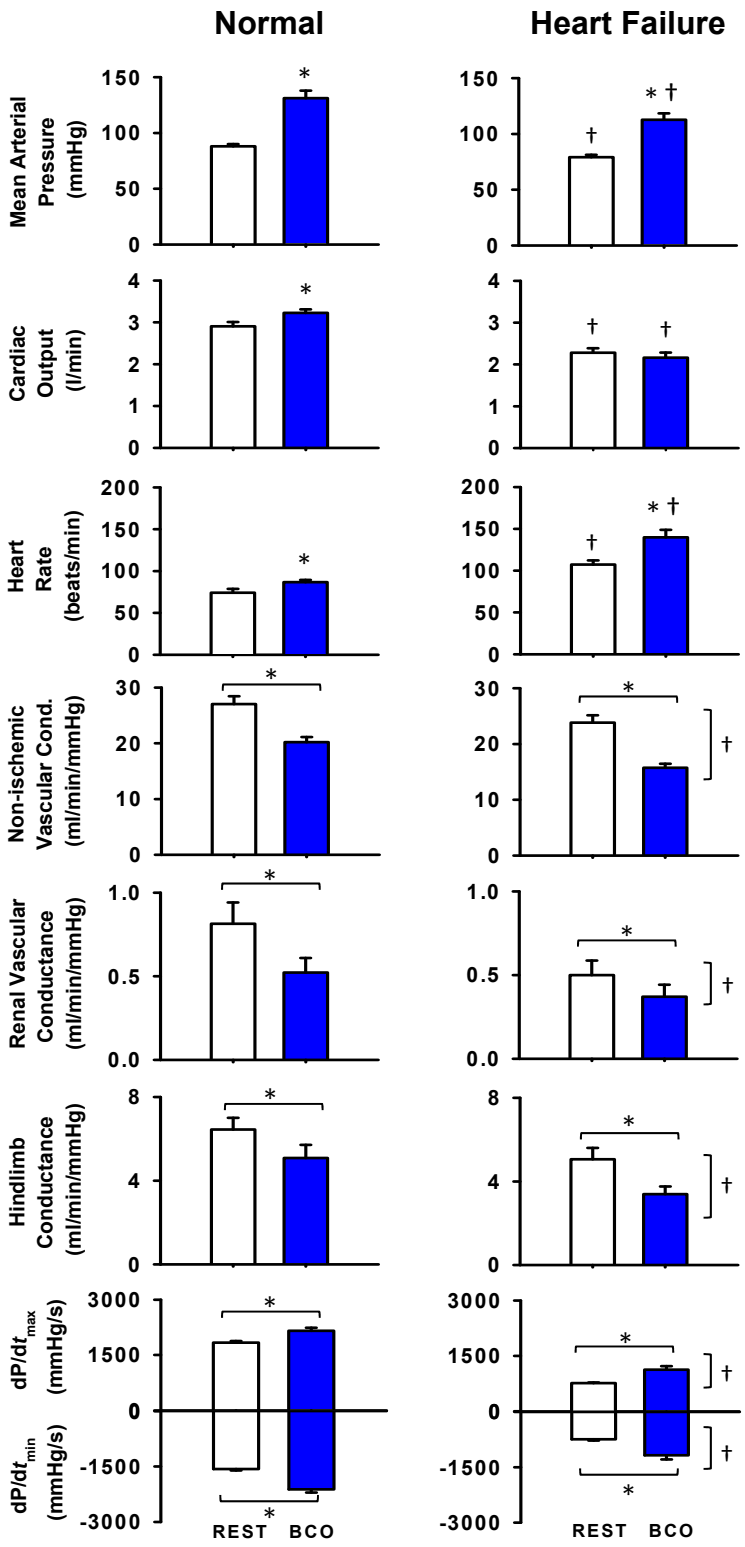
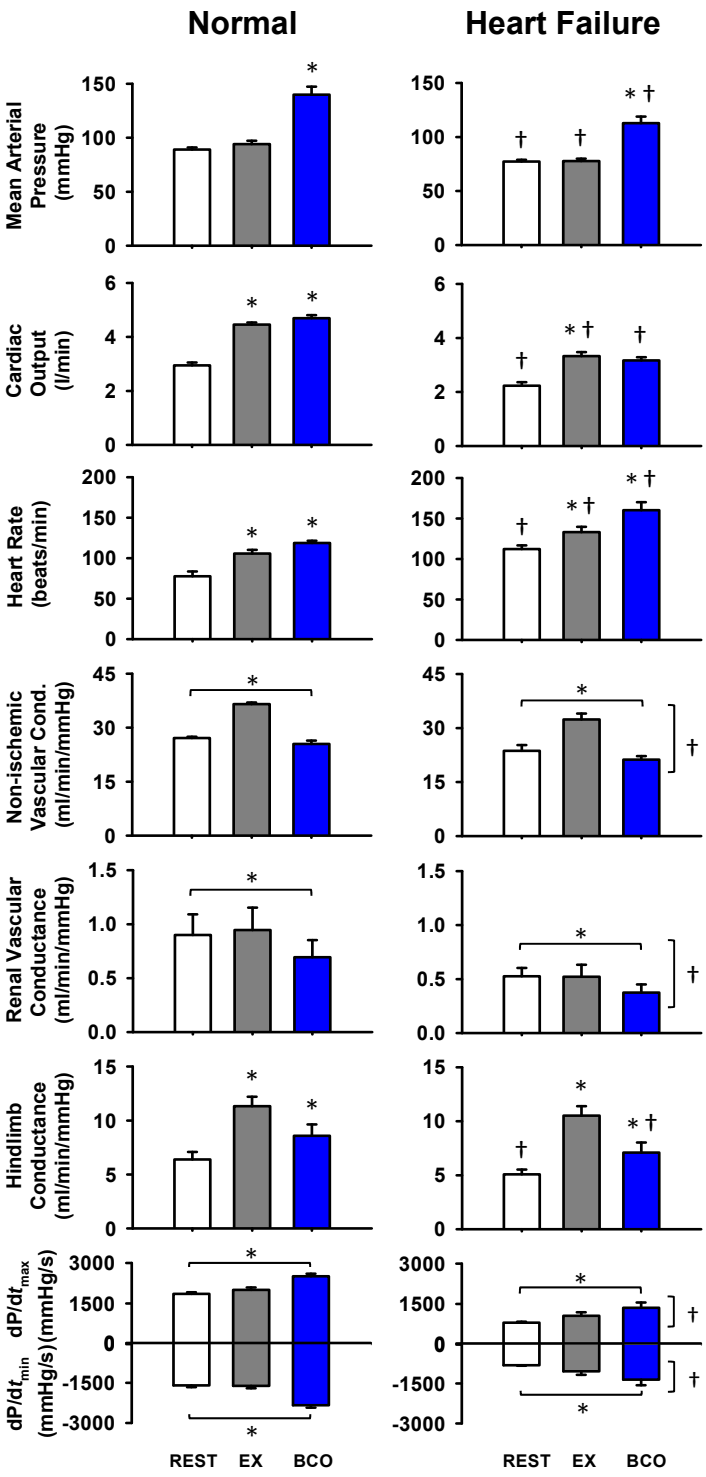


Figure 6.1. Average values for cardiovascular variables during rest (open bars) and bilateral carotid occlusion (BCO) at rest (blue bars) in normal (*left panel*) and after induction of heart failure (*right panel*). * p<0.05 vs. rest, † p<0.05 vs. normal. Horizontal brackets reflect a significant effect of BCO and vertical brackets reflect a significant effect of heart failure.

Figure 6.2. Average hemodynamic data during rest (open bars), mild exercise (Ex; grey bars) and bilateral carotid occlusion (BCO) during exercise (blue bars) in normal (left panel) and after induction of heart failure (right panel). * p<0.05 vs. previous setting, † p<0.05 vs. normal. Horizontal brackets reflect a significant settings effect and vertical brackets reflect a significant effect of heart failure.



Average cardiovascular responses to BCO during exercise before and after induction of HF are shown in Figure 6.2. From rest to mild exercise, there was a moderate increase in CO, HR, NIVC and HVC. BCO during mild exercise resulted in large increases in MAP, CO, HR and a decrease in HVC. After induction of HF, responses to mild exercise were similar to those in normal animals but with attenuated resting and exercise levels of MAP, CO and HVC. With BCO during exercise in HF, there was a significant increase in MAP and HR with no change in CO and a decrease in HVC. There was a significant settings and HF effect on NIVC, RVC, dP/dt_{max} and dP/dt_{min} responses.

Figure 6.3 shows the average hemodynamic changes in response to BCO during muscle metaboreflex activation before and after induction of HF. Muscle metaboreflex activation during mild exercise elicited significant increases in MAP, CO, NIVC, dP/dt_{max} and dP/dt_{min}. With BCO during metaboreflex activation, there was further large increase in MAP and ventricular function with significant decreases in CO and NIVC. Statistical analysis showed a significant settings effect on HR, RVC and HVC in normal. After induction of HF, muscle metaboreflex activation caused substantial increases in MAP and ventricular function but the responses are significantly attenuated compared to normal. The increase in CO observed in normal animals was abolished after induction of HF and there was a significant vasoconstriction of non-ischemic vasculature. BCO during metaboreflex activation resulted in a large pressor response, vasoconstriction of non-ischemic vascular beds, improvement in ventricular function, however, these responses were markedly lower than in normal. There was a significant effect of HF and settings effect on HR, RVC and HVC responses.

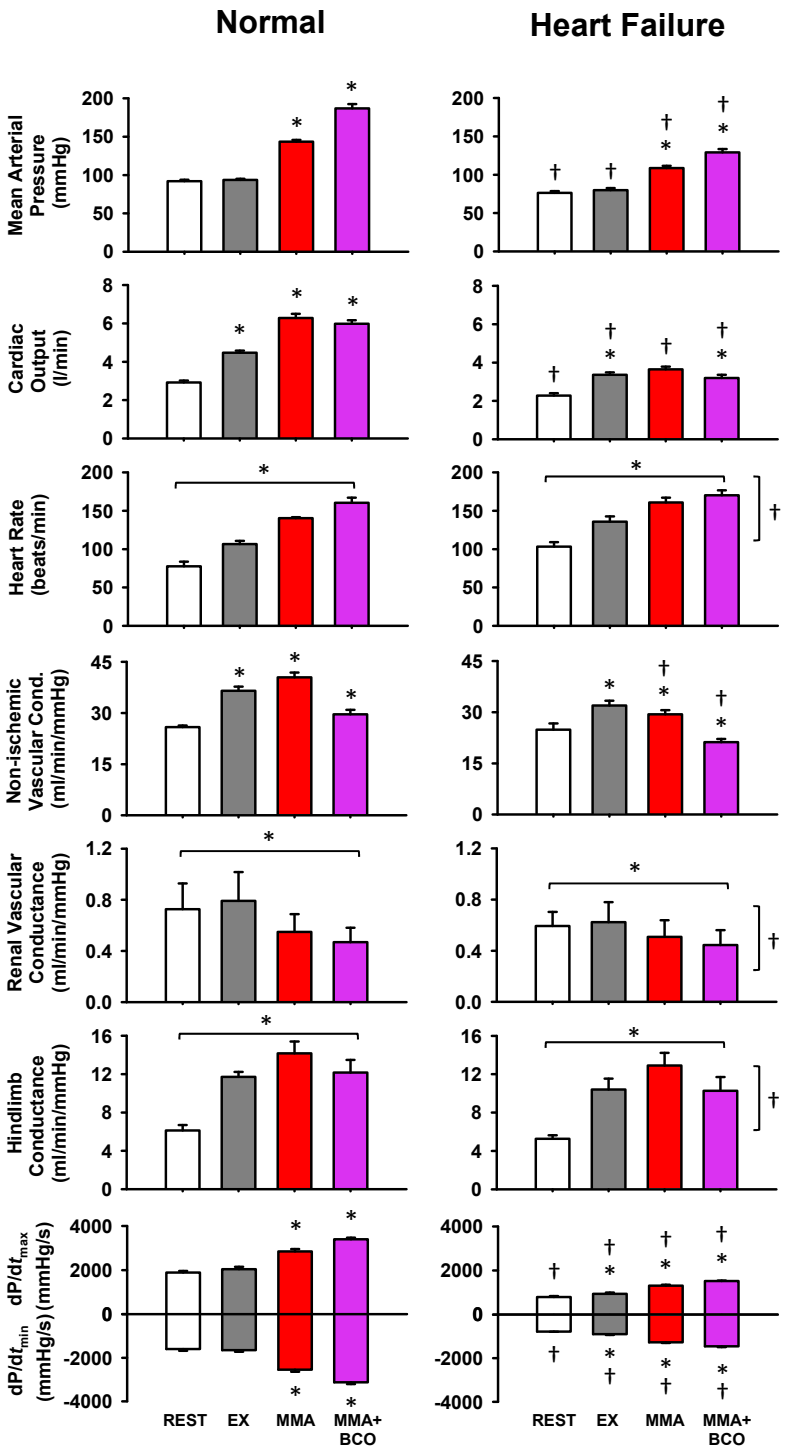


Figure 6.3. Average hemodynamic data during rest (open bars), mild exercise (Ex; grey bars), muscle metaboreflex activation (MMA; red bars) and bilateral carotid occlusion (BCO) during MMA (purple bars) in normal (*left panel*) and after induction of heart failure (*right panel*). * p<0.05 vs. previous setting, † p<0.05 vs. normal. Horizontal brackets reflect a significant settings effect and vertical brackets reflect a significant effect of heart failure.

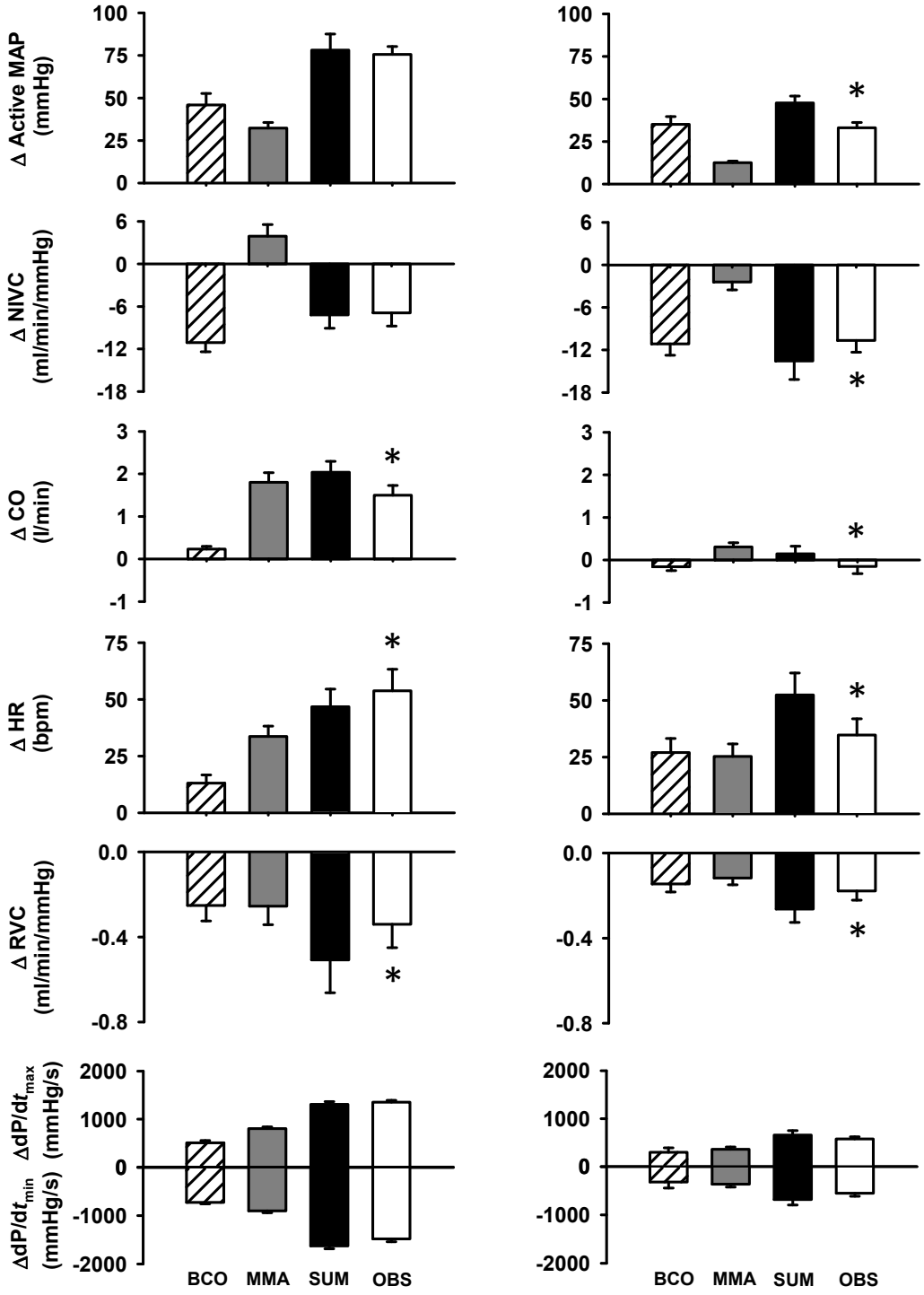


Figure 6.4. Absolute changes (Δ) in cardiovascular responses evoked by bilateral carotid occlusion (BCO) during mild exercise (hatched bars), muscle metaboreflex activation (MMA; grey bars), sum of MMA and BCO responses during exercise (SUM; filled bars) and observed effect of coactivation of MMA and BCO during MMA (OBS; open bars). *p<0.05 vs. calculated sum of reflex-induced response during MMA and BCO during exercise.

Figure 6.4 shows average cardiovascular responses when the arterial baroreflex and the muscle metaboreflex were activated separately and concurrently. To investigate whether the interaction between the two reflexes is altered in HF, the observed responses during coactivation of both reflexes in HF were compared to the sum of metaboreflex-induced and BCO-induced responses during exercise in HF. In healthy animals, MAP, NIVC and ventricular function responses displayed an additive interaction, HR responses exhibited a facilitative interaction while CO and RVC responses demonstrate an occlusive interaction. After induction of HF, all cardiovascular parameters exhibited occlusive interactions with the exception of ventricular function responses, which displayed an additive interaction.

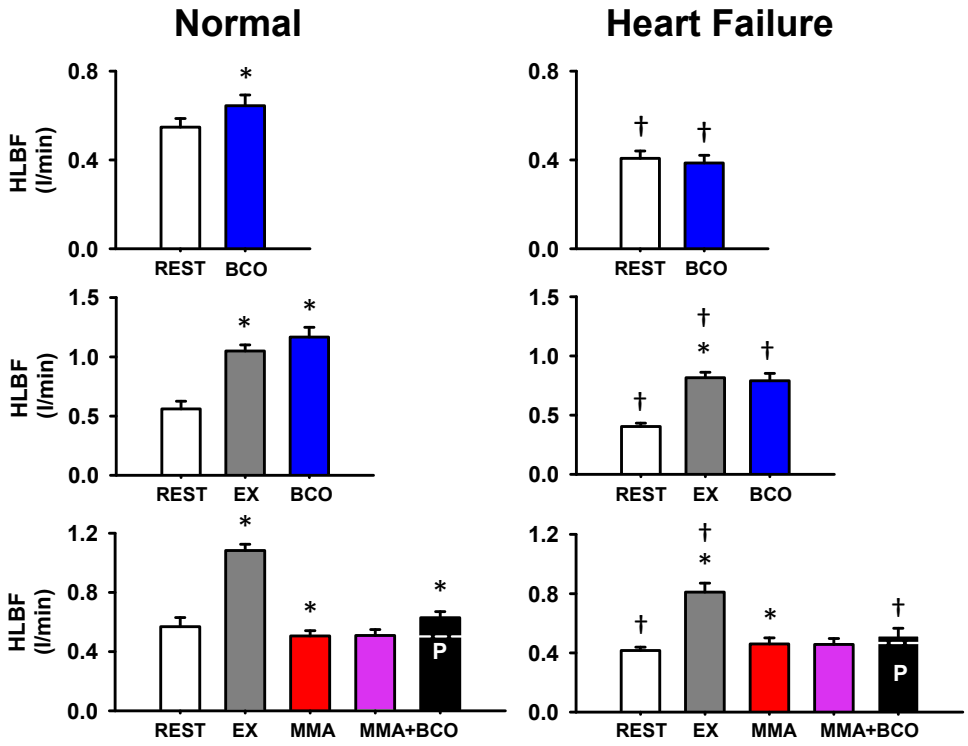


Figure 6.5. Average hindlimb blood flow (HLBF) during rest (open bars), bilateral carotid occlusion (BCO) during rest (blue bars), mild exercise (Ex; grey bars), BCO during mild exercise (blue bars), muscle metaboreflex activation (MMA; red bars) and BCO during MMA (purple bars). The bars inscribed with P indicate the predicted HLBF during coactivation of two reflexes.* $p<0.05$ vs. previous setting, † $p<0.05$ vs. normal.

Figure 6.5 shows HLBF responses during BCO at rest, during mild exercise and muscle metaboreflex activation in normal animals and in same animals after induction of HF. In normal animals, there was a significant increase in HLBF with BCO at rest and during exercise which was abolished after induction of HF. Although BCO during muscle metaboreflex activation in normal individuals caused hindlimb vasoconstriction, other vascular beds vasoconstricted more thereby, redirecting blood flow to ischemic hindlimb vasculature. This significant increase in HLBF (predicted) during coactivation of the two reflexes was substantially attenuated in HF.

Discussion

This is the first study to investigate the effect of HF on the interaction between the muscle metaboreflex and the arterial baroreflex when activated concurrently. We found that although baroreceptor unloading during metaboreflex activation in HF causes a large pressor response via peripheral vasoconstriction, the rise in MAP is significantly attenuated compared to normal. Peripheral vasoconstriction occurs in all vascular beds including the ischemic active skeletal muscle. In normal animals, vasoconstriction in non-ischemic vasculature is greater than that in the ischemic muscle, partially restoring blood flow to the ischemic beds. This restoration of blood flow to the ischemic muscle is significantly attenuated in HF.

Muscle metaboreflex and arterial baroreflex interaction

As previously shown, the interaction between arterial baroreflex and muscle metaboreflex when activated simultaneously is dependent on the hemodynamic parameter being investigated. Our results agree with the previous study as MAP, NIVC and ventricular function responses during coactivation of two reflexes exhibit additive interactions, CO and RVC responses were occlusive and HR responses exhibited

facilitative interaction. However, all the hemodynamic responses except ventricular function converted to occlusive interactions after induction of HF. Although significantly attenuated in HF, dP/dt_{max} and dP/dt_{min} responses maintained an additive interaction upon coactivation on the two reflexes. Therefore, the interaction between muscle metaboreflex and arterial baroreflex when activated simultaneously in HF is occlusive and the responses are significantly depressed compared to normal.

Regulation of skeletal muscle blood flow

Baroreceptor unloading during muscle metaboreflex activation in healthy subjects causes vasoconstriction in all vascular beds including the ischemic hindlimb vasculature but preferential vasoconstriction of the non-ischemic vasculature redistributes CO towards the ischemic active muscle increasing HLBF. This increase in HLBF attenuates muscle metaboreflex activation. After induction of HF, BCO during metaboreflex activation increases MAP via peripheral vasoconstriction, including vasoconstriction of the ischemic skeletal muscle. In contrast to normal animals, there is a very small restoration of HLBF with coactivation of both reflexes in HF. Smaller vasoconstriction of ischemic active muscle in normal animals could be a result of functional sympatholysis; attenuated sympathetic vasoconstriction of active muscle due to metabolic accumulation in the muscle (63). Since functional sympatholysis is impaired in HF (85), the unopposed sympathetic vasoconstriction in the ischemic vascular bed prevents any significant increase in HLBF in HF.

Perspectives and significance

In normal individuals, the arterial baroreflex buffers about one-half of the metaboreflex-induced pressor response by attenuating peripheral vasoconstriction (40). In heart failure, mechanisms of muscle metaboreflex activation switch from CO-

mediated to peripheral vasoconstriction-mediated pressor response (29, 51, 81). Impaired baroreflex buffering of the metaboreflex in HF would markedly increase sympathetic vasoconstriction of all vascular beds, especially to the active ischemic muscle.

APPENDIX A

IACUC Protocol Approval Letter

WAYNE STATE UNIVERSITY

INSTITUTIONAL ANIMAL
CARE AND USE COMMITTEE
87 E. Canfield, Second Floor
Detroit, MI 48201-2011
Telephone: (313) 577-1629
Fax Number: (313) 577-1941

ANIMAL WELFARE ASSURANCE # A3310-01

PROTOCOL # A 01-06-14

Protocol Effective Period: February 28, 2014 – January 31, 2017

TO: Dr. Donal S. O'Leary
Department of Physiology
1206 Elliman Clinical Research Building

FROM: Lisa Anne Polin, Ph.D. Lisa Anne Polin
Chairperson
Institutional Animal Care and Use Committee

SUBJECT: Approval of Protocol # A 01-06-14
"Blood pressure control during exercise in heart failure"

DATE: February 28, 2014

Your animal research protocol has been reviewed by the Wayne State University Institutional Animal Care and Use Committee, and given final approval for the period effective **February 28, 2014** through **January 31, 2017**. The listed source of funding for the protocol is **NIH**. The species and number of animals approved for the duration of this protocol are listed below.

Species	Strain	Qty.	USDA Cat.
DOGS	Class A mongrel dog of either sex, btw. 20-25kg	61	D
*To be purchased			
DOGS	hound mixes, both sexes, 20-25kg	3	D
**To be transferred from WSU protocol #A 02-01-11			

Be advised that this protocol must be reviewed by the IACUC on an annual basis to remain active. Any change in procedures, change in lab personnel, change in species, or additional numbers of animals requires prior approval by the IACUC. Any animal work on this research protocol beyond the expiration date will require the submission of a new IACUC protocol form and full committee review.

The Guide for the Care and Use of Laboratory Animals is the primary reference used for standards of animal care at Wayne State University. The University has submitted an appropriate assurance statement to the Office for Laboratory Animal Welfare (OLAW) of the National Institutes of Health. The animal care program at Wayne State University is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

APPENDIX B

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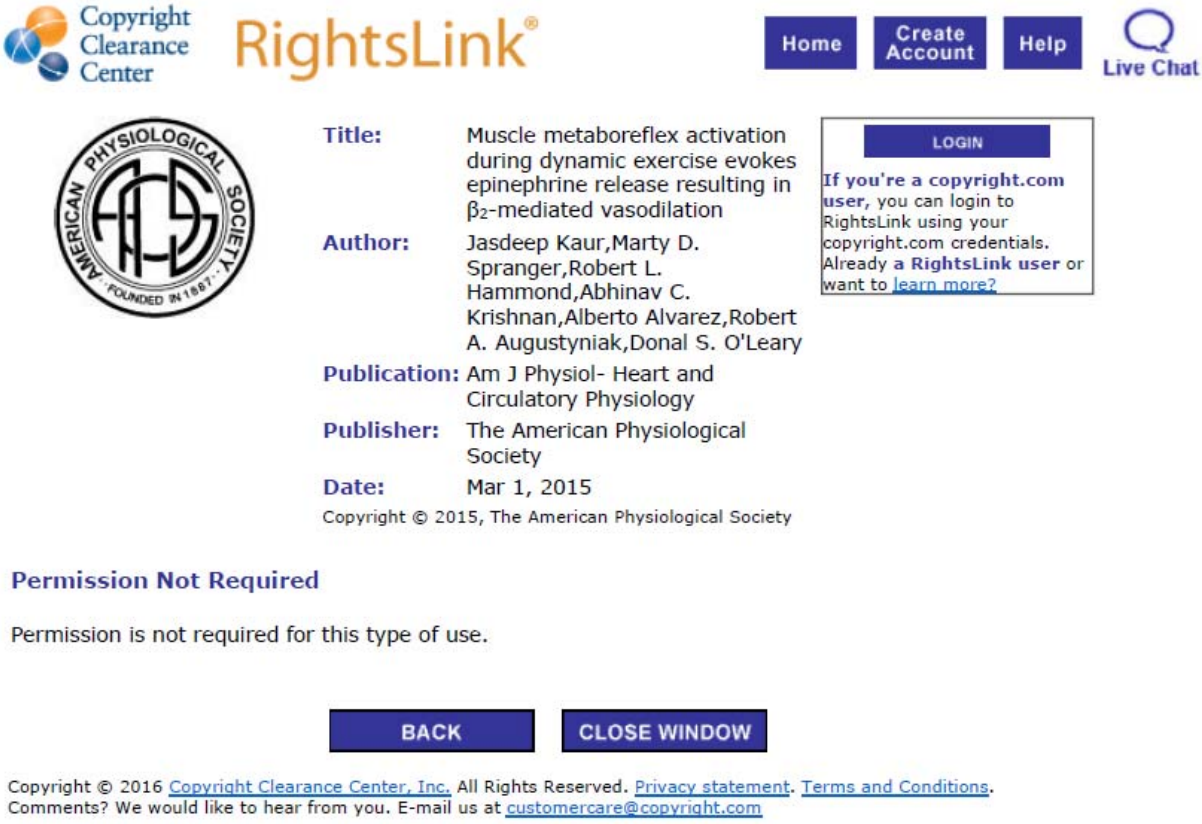

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Title: Muscle metaboreflex activation during dynamic exercise evokes epinephrine release resulting in β_2-mediated vasodilation

Author: Jasdeep Kaur,Marty D. Spranger,Robert L. Hammond,Abhinav C. Krishnan,Alberto Alvarez,Robert A. Augustyniak,Donal S. O'Leary

Publication: Am J Physiol- Heart and Circulatory Physiology

Publisher: The American Physiological Society

Date: Mar 1, 2015

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APPENDIX C

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Home | Create Account | Help | Live Chat

Title: Muscle metaboreflex activation during dynamic exercise vasoconstricts ischemic active skeletal muscle

Author: Jasdeep Kaur,Tiago M. Machado,Alberto Alvarez,Abhinav C. Krishnan,Hanna W. Hanna,Yasir H. Altamimi,Danielle Senador,Marty D. Spranger,Donal S. O'Leary

Publication: Am J Physiol- Heart and Circulatory Physiology

Publisher: The American Physiological Society

Date: Dec 15, 2015

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REFERENCES

1. Alam M and Smirk FH. Observations in man upon a blood pressure raising reflex arising from the voluntary muscles. *J Physiol* 89: 372-383, 1937.
2. Amann M, Blain GM, Proctor LT, Sebranek JJ, Pegelow DF and Dempsey JA. Group III and IV muscle afferents contribute to ventilatory and cardiovascular response to rhythmic exercise in humans. *J Appl Physiol* 109: 966-976, 2010.
3. Ansorge EJ, Shah SH, Augustyniak R, Rossi NF, Collins HL and O'Leary DS. Muscle metaboreflex control of coronary blood flow. *Am J Physiol Heart Circ Physiol* 283: H526-H532, 2002.
4. Asmussen E and Nielsen M. Experiments on nervous factors controlling respiration and circulation during exercise employing blocking of the blood flow. *Acta Physiol Scand* 60: 103-111, 1964.
5. Augustyniak RA, Ansorge EJ and O'Leary DS. Muscle metaboreflex control of cardiac output and peripheral vasoconstriction exhibit differential latencies. *Am J Physiol* 278: H530-H537, 2000.
6. Augustyniak RA, Collins HL, Ansorge EJ, Rossi NF and O'Leary DS. Severe exercise alters the strength and mechanisms of the muscle metaboreflex. *Am J Physiol Heart Circ Physiol* 280: H1645-H1652, 2001.
7. Barrett-O'Keefe Z, Ives SJ, Trinity JD, Morgan G, Rossman MJ, Donato AJ, Runnels S, Morgan DE, Gmelch BS, Bledsoe AD, Richardson RS and Wray DW. Taming the "sleeping giant": the role of endothelin-1 in the regulation of skeletal muscle blood flow and arterial blood pressure during exercise. *Am J Physiol Heart Circ Physiol* 304: H162-H169, 2013.
8. Berecek KH and Brody MJ. Evidence for a neurotransmitter role for epinephrine

derived from the adrenal medulla. *Am J Physiol* 242: H593-H601, 1982.

9. Boushel R, Madsen P, Nielsen HB, Quistorff B and Secher NH. Contribution of pH, diprotonated phosphate and potassium for the reflex increase in blood pressure during handgrip. *Acta Physiol Scand* 164: 269-275, 1998.
10. Buckwalter JB, Hamann JJ and Clifford PS. Neuropeptide Y1 receptor vasoconstriction in exercising canine skeletal muscles. *J Appl Physiol (1985)* 99: 2115-2120, 2005.
11. Collins HL, Augustyniak RA, Ansorge EJ and O'Leary DS. Carotid baroreflex pressor responses at rest and during exercise:cardiac output vs. regional vasoconstriction. *Am J Physiol Heart Circ Physiol* 280: H642-H648, 2001.
12. Collins HL and Dicarlo SE. Cardiac afferents attenuate the muscle metaboreflex in the rat. *J Appl Physiol* 75: 114-120, 1993.
13. Coutsos M, Sala-Mercado JA, Ichinose M, Li Z, Dawe EJ and O'Leary DS. Muscle metaboreflex-induced coronary vasoconstriction functionally limits increases in ventricular contractility. *J Appl Physiol* 109: 271-278, 2010.
14. Coutsos M, Sala-Mercado JA, Ichinose M, Li Z, Dawe EJ and O'Leary DS. Muscle metaboreflex-induced coronary vasoconstriction limits ventricular contractility during dynamic exercise in heart failure. *Am J Physiol Heart Circ Physiol* 304: H1029-H1037, 2013.
15. Coutsos M, Sala-Mercado JA, Ichinose M, Li Z and O'Leary DS. Muscle Metaboreflex Control of Coronary Blood Flow and Ventricular Contractility During Dynamic Exercise in Heart Failure. *FASEB J* 2009.
16. Crisafulli A, Salis E, Tocco F, Melis F, Milia R, Pittau G, Caria MA, Solinas R, Meloni L, Pagliaro P and Concu A. Impaired central hemodynamic response and

exaggerated vasoconstriction during muscle metaboreflex activation in heart failure patients. *Am J Physiol Heart Circ Physiol* 292: H2988-H2996, 2007.

17. Crisafulli A, Scott AC, Wensel R, Davos CH, Francis DP, Pagliaro P, Coats AJS, Concu A and Piepoli MF. Muscle metaboreflex-induced increases in stroke volume. *Medicine and Science in Sports and Exercise* 35: 221-228, 2003.
18. Crisafulli A, Piras F, Filippi M, Piredda C, Chiappori P, Melis F, Milia R, Tocco F and Concu A. Role of heart rate and stroke volume during muscle metaboreflex-induced cardiac output increase: differences between activation during and after exercise. *The Journal of Physiological Sciences* 61: 385-394, 2011.
19. Delorey DS, Hamann JJ, Valic Z, Kluess HA, Clifford PS and Buckwalter JB. Alpha-Adrenergic receptor responsiveness is preserved during prolonged exercise. *Am J Physiol Heart Circ Physiol* 292: H392-H398, 2007.
20. Dicarlo SE and Bishop VS. Onset of exercise shifts operating point of arterial baroreflex to higher pressures. *Am J Physiol* 262: H303-H307, 1992.
21. Dicarlo SE, Chen CY and Collins HL. Onset of exercise increases lumbar sympathetic nerve activity in rats. *Med Sci Sports Exerc* 28: 677-684, 1996.
22. Drexler H, Hayoz D, Munzel T, Hornig B, Just H, Brunner HR and Zelis R. Endothelial function in chronic congestive heart failure. *Am J Cardiol* 69: 1596-1601, 1992.
23. Drexler H, Riede U, Munzel T, Konig H, Funke E and Just H. Alterations of skeletal muscle in chronic heart failure. *Circulation* 85: 1751-1759, 1992.
24. Eiken O and Bjurstedt H. Dynamic exercise in man as influenced by experimental restriction of blood flow in the working muscles. *Acta Physiol Scand* 131: 339-345, 1987.

25. Elfellah MS, Dalling R, Kantola IM and Reid JL. Beta-adrenoceptors and human skeletal muscle characterisation of receptor subtype and effect of age. *Br J Clin Pharmacol* 27: 31-38, 1989.

26. Fisher JP, Seifert T, Hartwich D, Young CN, Secher NH and Fadel PJ. Autonomic control of heart rate by metabolically sensitive skeletal muscle afferents in humans. *J Physiol* 588: 1117-1127, 2010.

27. Hales JR, Rowell LB and King RB. Regional distribution of blood flow in awake heat-stressed baboons. *Am J Physiol* 237: H705-H712, 1979.

28. Hales JRS and Dampney RAL. The redistribution of cardiac output in the dog during heat stress. *J Thermal Biol* 1: 29-34, 1975.

29. Hammond RL, Augustyniak RA, Rossi NF, Churchill PC, Lapanowski K and O'Leary DS. Heart failure alters the strength and mechanisms of the muscle metaboreflex. *Am J Physiol* 278: H818-H828, 2000.

30. Hammond RL, Augustyniak RA, Rossi NF, Lapanowski K, Dunbar JC and O'Leary DS. Alteration of humoral and peripheral vascular responses during graded exercise in heart failure. *J Appl Physiol* 90: 55-61, 2001.

31. Hjemdahl P, Belfrage E and Daleskog M. Vascular and metabolic effects of circulating epinephrine and norepinephrine. Concentration-effect study in dogs. *J Clin Invest* 64: 1221-1228, 1979.

32. Ichinose MJ, Sala-Mercado JA, Coutsos M, Li Z, Ichinose TK, Dawe E and O'Leary DS. Modulation of cardiac output alters the mechanisms of the muscle metaboreflex pressor response. *Am J Physiol Heart Circ Physiol* 298: H245-H250, 2010.

33. Joyner MJ. Does the pressor response to ischemic exercise improve blood flow

to contracting muscles in humans? *J Appl Physiol* 71: 1496-1501, 1991.

34. Kaufman MP and Rybicki KJ. Discharge properties of group III and IV muscle afferents: their responses to mechanical and metabolic stimuli. *Circ Res* 61: I60-I65, 1987.

35. Kaufman MP, Rybicki KJ, Waldrop TG and Ordway GA. Effect of ischemia on responses of group III and IV afferents to contraction. *J Appl Physiol* 57: 644-650, 1984.

36. Kaur J, Machado TM, Alvarez A, Krishnan AC, Hanna HW, Altamimi YH, Senador D, Spranger MD and O'Leary DS. Muscle metaboreflex activation during dynamic exercise vasoconstricts ischemic active skeletal muscle. *Am J Physiol Heart Circ Physiol* 309: H2145-H2151, 2015.

37. Kaur J, Spranger MD, Hammond RL, Krishnan AC, Alvarez A, Augustyniak RA and O'Leary DS. Muscle metaboreflex activation during dynamic exercise evokes epinephrine release resulting in beta2-mediated vasodilation. *Am J Physiol Heart Circ Physiol* 308: H524-H529, 2015.

38. Kim JK, Augustyniak RA, Sala-Mercado JA, Hammond RL, Ansorge EJ and O'Leary DS. Heart failure alters the strength and mechanisms of arterial baroreflex pressor responses during dynamic exercise. *Am J Physiol Heart Circ Physiol* 287: H1682-H1688, 2004.

39. Kim JK, Sala-Mercado JA, Hammond RL, Rodriguez J, Scislo TJ and O'Leary DS. Attenuated arterial baroreflex buffering of muscle metaboreflex in heart failure. *Am J Physiol Heart Circ Physiol* 289: H2416-H2423, 2005.

40. Kim JK, Sala-Mercado JA, Rodriguez J, Scislo TJ and O'Leary DS. Arterial baroreflex alters strength and mechanisms of muscle metaboreflex during

dynamic exercise. *Am J Physiol Heart Circ Physiol* 288: H1374-H1380, 2005.

41. Kitchen AM, Scislo TJ and O'Leary DS. NTS purinoceptor activation elicits hindlimb vasodilation primarily via a beta-adrenergic mechanism. *Am J Physiol* 278: H1775-H1782, 2000.

42. Lynch GS and Ryall JG. Role of beta-adrenoceptor signaling in skeletal muscle: implications for muscle wasting and disease. *Physiol Rev* 88: 729-767, 2008.

43. McCloskey DL and Mitchell JH. Reflex cardiovascular and respiratory responses originating exercising muscle. *J Physiol* 224: 173-186, 1972.

44. McClure JM, O'Leary DS and Scislo TJ. Stimulation of NTS A1 adenosine receptors evokes counteracting effects on hindlimb vasculature. *Am J Physiol Heart Circ Physiol* 289: H2536-H2542, 2005.

45. McClure JM, O'Leary DS and Scislo TJ. Stimulation of NTS A1 adenosine receptors evokes counteracting effects on hindlimb vasculature 2. *Am J Physiol Heart Circ Physiol* 289: H2536-H2542, 2005.

46. McClure JM, O'Leary DS and Scislo TJ. Neural and humoral control of regional vascular beds via A1 adenosine receptors located in the nucleus tractus solitarii. *Am J Physiol Regul Integr Comp Physiol* 2010.

47. Minneman KP, Hedberg A and Molinoff PB. Comparison of beta adrenergic receptor subtypes in mammalian tissues. *J Pharmacol Exp Ther* 211: 502-508, 1979.

48. Mittelstadt SW, Bell LB, O'Hagan KP and Clifford PS. Muscle chemoreflex alters vascular conductance in nonischemic exercising skeletal muscle. *J Appl Physiol* 77: 2761-2766, 1994.

49. Mittelstadt SW, Bell LB, O'Hagan KP, Sulentic JE and Clifford PS. Muscle

chemoreflex causes renal vascular constriction. *Am J Physiol* 270: H951-H956, 1996.

50. Musch TI, Friedman DB, Pitetti KH, Haidet GC, Stray-Gundersen J, Mitchell JH and Ordway GA. Regional distribution of blood flow of dogs during graded dynamic exercise. *J Appl Physiol* 63: 2269-2277, 1987.

51. Notarius CF, Atchison DJ and Floras JS. Impact of heart failure and exercise capacity on sympathetic response to handgrip exercise. *American Journal of Physiology-Heart and Circulatory Physiology* 280: H969-H976, 2001.

52. O'Leary DS. Regional vascular resistance vs. conductance: which index for baroreflex responses? *Am J Physiol* 260: H632-H637, 1991.

53. O'Leary DS. Heart rate control during exercise by baroreceptors and skeletal muscle afferents. *Med Sci Sports Exerc* 28: 210-217, 1996.

54. O'Leary DS. Point: the muscle metaboreflex does restore blood flow to contracting muscles. *J Appl Physiol* 100: 357-358, 2006.

55. O'Leary DS and Augustyniak RA. Muscle metaboreflex increases ventricular performance in conscious dogs. *Am J Physiol* 275: H220-H224, 1998.

56. O'Leary DS, Augustyniak RA, Ansorge EJ and Collins HL. Muscle metaboreflex improves O2 delivery to ischemic active skeletal muscle. *Am J Physiol* 276: H1399-H1403, 1999.

57. O'Leary DS, Rossi NF and Churchill PC. Muscle metaboreflex control of vasopressin and renin release. *Am J Physiol* 264: H1422-H1427, 1993.

58. O'Leary DS, Sala-Mercado JA, Augustyniak RA, Hammond RL, Rossi NF and Ansorge EJ. Impaired muscle metaboreflex-induced increases in ventricular function in heart failure. *Am J Physiol Heart Circ Physiol* 287: H2612-H2618,

2004.

59. O'Leary DS and Sheriff DD. Is the muscle metaboreflex important in control of blood flow to ischemic active skeletal muscle in dogs? *Am J Physiol* 268: H980-H986, 1995.

60. Ogoh S, Fadel PJ, Nissen P, Jans O, Selmer C, Secher NH and Raven PB. Baroreflex-mediated changes in cardiac output and vascular conductance in response to alterations in carotid sinus pressure during exercise in humans. *Journal of Physiology-London* 550: 317-324, 2003.

61. Olivier NB and Stephenson RB. Characterization of baroreflex impairment in conscious dogs with pacing-induced heart failure. *Am J Physiol* 265: R1132-R1140, 1993.

62. Potts JT and Mitchell JH. Rapid resetting of carotid baroreceptor reflex by afferent input from skeletal muscle receptors. *American Journal of Physiology-Heart and Circulatory Physiology* 44: H2000-H2008, 1998.

63. Remensnyder JP, Mitchell JH and Sarnoff SJ. Functional sympatholysis during muscular activity. Observations on influence of carotid sinus on oxygen uptake. *Circ Res* 11: 370-380, 1962.

64. Rodman JR, Henderson KS, Smith CA and Dempsey JA. Cardiovascular effects of the respiratory muscle metaboreflexes in dogs: rest and exercise. *J Appl Physiol* 95: 1159-1169, 2003.

65. Rotto DM and Kaufman MP. Effect of metabolic products of muscular contraction on discharge of group III and IV afferents. *J Appl Physiol* 64: 2306-2313, 1988.

66. Rowell LB, Hermansen L and Blackmon JR. Human cardiovascular and respiratory responses to graded muscle ischemia. *J Appl Physiol* 41: 693-701,

1976.

67. Rowell LB and O'Leary DS. Reflex control of the circulation during exercise: chemoreflexes and mechanoreflexes. *J Appl Physiol* 69: 407-418, 1990.

68. Rowell LB, Savage MV, Chambers J and Blackmon JR. Cardiovascular responses to graded reductions in leg perfusion in exercising humans. *Am J Physiol* 261: H1545-H1553, 1991.

69. Sala-Mercado JA, Spranger MD, Abu-Hamdah R, Kaur J, Coutsos M, Stayer D, Augustyniak RA and O'Leary DS. Attenuated muscle metaboreflex-induced increases in cardiac function in hypertension. *Am J Physiol Heart Circ Physiol* 305: H1548-H1554, 2013.

70. Sala-Mercado JA, Hammond RL, Kim JK, Rossi NF, Stephenson LW and O'Leary DS. Muscle metaboreflex control of ventricular contractility during dynamic exercise. *Am J Physiol Heart Circ Physiol* 290: H751-H757, 2006.

71. Sheriff DD, Augustyniak RA and O'Leary DS. Muscle chemoreflex-induced increases in right atrial pressure. *Am J Physiol* 275: H767-H775, 1998.

72. Sheriff DD, O'Leary DS, Scher AM and Rowell LB. Baroreflex attenuates pressor response to graded muscle ischemia in exercising dogs. *Am J Physiol* 258: H305-H310, 1990.

73. Sheriff DD, Wyss CR, Rowell LB and Scher AM. Does inadequate oxygen delivery trigger pressor response to muscle hypoperfusion during exercise? *Am J Physiol* 253: H1199-H1207, 1987.

74. Shoemaker JK, Mattar L, Kerbeci P, Trotter S, Arbeille P and Hughson RL. WISE 2005: stroke volume changes contribute to the pressor response during ischemic handgrip exercise in women. *J Appl Physiol* 103: 228-233, 2007.

75. Sinoway LI, Rea RF, Mosher TJ, Smith MB and Mark AL. Hydrogen ion concentration is not the sole determinant of muscle metaboreceptor responses in humans. *J Clin Invest* 89: 1875-1884, 1992.

76. Sinoway LI, Smith MB, Enders B, Leuenberger U, Dzwonczyk T, Gray K, Whisler S and Moore RL. Role of diprotonated phosphate in evoking muscle reflex responses in cats and humans. *Am J Physiol* 267: H770-H778, 1994.

77. Sinoway LI, Wroblewski KJ, Prophet SA, Ettinger SM, Gray KS, Whisler SK, Miller G and Moore RL. Glycogen depletion-induced lactate reductions attenuate reflex responses in exercising humans. *Am J Physiol* 263: H1499-H1505, 1992.

78. Spranger MD, Sala-Mercado JA, Coutsos M, Kaur J, Stayer D, Augustyniak RA and 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.

79. Stephenson RB and Donald DE. Reflexes from isolated carotid sinuses of intact and vagotomized conscious dogs. *Am J Physiol* 238: H815-H822, 1980.

80. Stephenson RB and Donald DE. Reversible vascular isolation of carotid sinuses in conscious dogs. *Am J Physiol* 238: H809-H814, 1980.

81. Sterns DA, Ettinger SM, Gray KS, Whisler SK, Mosher TJ, Smith MB and Sinoway LI. Skeletal muscle metaboreceptor exercise responses are attenuated in heart failure. *Circulation* 84: 2034-2039, 1991.

82. Sullivan MJ, Green HJ and Cobb FR. Skeletal muscle biochemistry and histology in ambulatory patients with long-term heart failure. *Circulation* 81: 518-527, 1990.

83. Sun SY, Wang W, Zucker IH and Schultz HD. Enhanced peripheral chemoreflex

function in conscious rabbits with pacing-induced heart failure. *J Appl Physiol (1985)* 86: 1264-1272, 1999.

84. Thomas GD and Segal SS. Neural control of muscle blood flow during exercise. *J Appl Physiol (1985)* 97: 731-738, 2004.

85. Thomas GD, Zhang W and Victor RG. Impaired modulation of sympathetic vasoconstriction in contracting skeletal muscle of rats with chronic myocardial infarctions: role of oxidative stress. *Circ Res* 88: 816-823, 2001.

86. Vanhoutte B. Heterogeneity in vascular smooth muscle. In: Microcirculation, edited by Kaley G and Altura BM. Baltimore: University Park Press, 1978, p. 181-310.

87. Victor RG, Bertocci LA, Pryor SL and Nunnally RL. Sympathetic nerve discharge is coupled to muscle cell pH during exercise in humans. *J Clin Invest* 82: 1301-1305, 1988.

88. Victor RG and Seals DR. Reflex stimulation of sympathetic outflow during rhythmic exercise in humans. *Am J Physiol* 257: H2017-H2024, 1989.

89. Victor RG, Seals DR and Mark AL. Differential control of heart rate and sympathetic nerve activity during dynamic exercise. Insight from intraneural recordings in humans. *J Clin Invest* 79: 508-516, 1987.

90. Vongpatanasin W, Wang Z, Arbique D, Arbique G, Adams-Huet B, Mitchell JH, Victor RG and Thomas GD. Functional sympatholysis is impaired in hypertensive humans. *J Physiol* 589: 1209-1220, 2011.

91. Walgenbach SC and Donald DE. Inhibition by carotid baroreflex of exercise-induced increases in arterial pressure. *Circ Res* 52: 253-262, 1983.

92. Wang HJ, Wang W, Cornish KG, Rozanski GJ and Zucker IH. Cardiac

sympathetic afferent denervation attenuates cardiac remodeling and improves cardiovascular dysfunction in rats with heart failure. *Hypertension* 64: 745-755, 2014.

93. Wang W, Chen JS and Zucker IH. Carotid sinus baroreceptor sensitivity in experimental heart failure. *Circulation* 81: 1959-1966, 1990.

94. Wilson JR, Lanoce V, Frey MJ and Ferraro N. Arterial baroreceptor control of peripheral vascular resistance in experimental heart failure. *Am Heart J* 119: 1122-1130, 1990.

95. Wyss CR, Ardell JL, Scher AM and Rowell LB. Cardiovascular responses to graded reductions in hindlimb perfusion in exercising dogs. *Am J Physiol* 245: H481-H486, 1983.

ABSTRACT

MUSCLE METABOREFLEX AND ARTERIAL BAROREFLEX: ACTION, INTERACTION AND ALTERED CONTROL IN HEART FAILURE

by

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Advisor: Dr. Donal S. O'Leary

Major: Physiology

Degree: Doctor of Philosophy

Stimulation of skeletal muscle afferents by metabolites that accumulate in the exercising muscle elicits a large pressor response, termed the muscle metaboreflex. Muscle metaboreflex activation during submaximal exercise induces large increases in arterial pressure, cardiac output, heart rate and ventricular contractility however, the vascular responses have varied in previous studies. We addressed three specific questions: 1) what are the mechanism(s) regulating the non-ischemic vasculature during muscle metaboreflex activation in normal subjects, 2) whether muscle metaboreflex activation vasoconstricts the ischemic active muscle from which this reflex originates and if this vasoconstriction is exaggerated in heart failure and 3) how do the arterial baroreflex and muscle metaboreflex interact with each other when activated simultaneously in normal and heart failure animals. Using chronically instrumented canine model, data was collected before and after the induction of heart failure. We found that: 1) muscle metaboreflex activation induces epinephrine release causing β_2-mediated vasodilation in skeletal muscle, 2) muscle metaboreflex activation elicits vasoconstriction of ischemic active muscle which is exaggerated after induction of heart

failure and 3) interaction between baroreflex and muscle metaboreflex in normal animals depends on the parameter being investigated; however in heart failure, most cardiovascular variables exhibit occlusive interactions. Preferential vasoconstriction of non-ischemic vasculature redirects cardiac output and increases hindlimb blood flow in normal animals but this increase is significantly attenuated in heart failure. The exaggerated vasoconstriction and limited increase in blood flow to the ischemic muscle would impair the ability to exercise and contribute to exercise intolerance in heart failure patients.

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Publications

1. **Kaur J**, Machado TM, Alvarez A, Krishnan AC, Hanna HW, Altamimi YH, Senador D, Spranger MD, O'Leary DS. Muscle metaboreflex activation during dynamic exercise vasoconstricts the ischemic active skeletal muscle. *Am J Physiol Heart Circ Physiol* 309(12): H2145-51, 2015.
2. Spranger MD, **Kaur J**, Sala-Mercado JA, Machado TM, Krishnan AC, Alvarez A, O'Leary DS. Attenuated muscle metaboreflex-induced pressor response during post-exercise muscle ischemia in renovascular hypertension. *Am J Physiol Reg Integ Comp Physiol* 308 (7): R650-R658, 2015.
3. **Kaur J**, Spranger MD, Hammond RL, Krishnan AC, Alvarez A, Augustyniak RA and O'Leary DS. Muscle metaboreflex activation during dynamic exercise evokes epinephrine release resulting in β2-mediated vasodilation. *Am J Physiol Heart Circ Physiol* 308 (5): H524-H529, 2015.
4. Spranger MD, Sala-Mercado JA, Coutsos M, **Kaur J**, Stayer D, Augustyniak RA and O'Leary DS. Role of cardiac output versus peripheral vasoconstriction in mediating muscle metaboreflex pressor responses: dynamic exercise versus post-exercise muscle ischemia. *Am J Physiol Reg Integ Comp Physiol* 304(8): R657-63, 2013.
5. Sala-Mercado JA, Spranger MD, Abu-Hamdah R, **Kaur J**, Coutsos M, Stayer D,Augustyniak RA and O'Leary DS. Attenuated muscle metaboreflex-induced increases in cardiac function in hypertension. *Am J Physiol Heart Circ Physiol* 305: H1548–H1554, 2013.

Abstracts

1. **Kaur J**, Spranger MD, Machado TM, Krishnan AC, Alvarez A and O'Leary DS. Baroreflex sympatho-activation during muscle metaboreflex activation improves blood flow to the ischemic muscle. FASEB J 29: 648.8, 2015.
2. **Kaur J**, Spranger MD, Machado TM, Krishnan AC, Alvarez A, Hanna HW, Altamimi YH and O'Leary DS. Muscle metaboreflex-induced vasoconstriction of ischemic active skeletal muscle. FASEB J 29: 1054.1, 2015.
3. **Kaur J**, Spranger MD, Hammond RL, Krishnan AC, Alvarez A, Augustyniak RA and O'Leary DS. Muscle metaboreflex activation during dynamic exercise evokes epinephrine release resulting in β2-mediated vasodilation. FASEB J 28:1172.2, 2014.
4. Spranger MD, **Kaur J**, Sala-Mercado JA, Krishnan AC, Alvarez A and O'Leary DS. Mechanisms mediating the muscle metaboreflex pressor response during post-exercise muscle ischemia are altered in hypertension. FASEB J 28:1170.2, 2014.
5. Augustyniak RA, Spranger MD, Abu-Hamdah R, **Kaur J**, Hammond RL, Sala-Mercado JA, Ichinose M and O'Leary DS. Hypertension impairs spontaneous baroreflex heart rate control during exercise and muscle metaboreflex activation. FASEB J 28:1132.9, 2014.
6. Spranger MD, Sala-Mercado JA, **Kaur J**, Abu-Hamdah R, Augustyniak RA and O'Leary DS. Muscle metaboreflex-induced increases in ventricular performance are limited in hypertension due to exaggerated coronary vasoconstriction. FASEB J 27:1118.16, 2013.