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Muscle Metaboreflex Control Of Cardiovascular Function In Hypertension

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MUSCLE METABOREFLEX CONTROL OF CARDIOVASCULAR FUNCTION IN HYPERTENSION

by

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DEDICATION

I would like to dedicate this dissertation to all of my family and friends that remained dedicated to me during my two-decade pursuit of higher education.

To my Father: Your predilection for nature was clearly a genetic gift, and I am infinitely grateful for that. However, in regards to the expression of that gene set, you left little to chance. You brought me into the field at a very young age and exposed me to what you had marveled over since the beginning of your existence, nature. You were a brilliant naturalist, and I admired and embraced your love and sense of wonder for nature. My love for nature drove me to become a biologist and my wonder for the science of nature drove me to become a physiologist. Thank you for everything you have done for me Father.

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LIST OF ABBREVIATIONS

2K1C	two-kidney one-clip
ANOVA	analysis of variance
ATP	adenosine triphosphate
AVP	arginine vasopressin
BID	twice a day
CBF	coronary blood flow
CNS	central nervous system
CO	cardiac output
CP	cardiac power
CVBM	central blood volume mobilization
CVC	coronary vascular conductance
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
HLBF	hindlimb blood flow
HR	heart rate
HTN	hypertension
IM	intramuscular
IV	intravenous
LVP	left-ventricular pressure
MAP	mean arterial pressure
MMA	muscle metaboreflex activation
MSNA	muscle sympathetic nerve activity
MVC	maximal voluntary contraction
NE	norepinephrine
NIVC	non-ischemic vascular conductance
OPD	once per day
PEMI	post-exercise muscle ischemia
PO	by mouth
POST-EX	post-exercise

PZ	prazosin
RAP	right atrial pressure
RBF	renal blood flow
SE	standard error
SNA	sympathetic nerve activity
SQ	subcutaneously
SV	stroke volume
TDD	transdermal drug delivery
TPR	total peripheral resistance

CHAPTER 1

Introduction

Muscle Metaboreflex: the Afferent Arm

Skeletal muscle is extensively innervated with afferent sensory nerve fibers which communicate information to the central nervous system (CNS) regarding its relative state or condition. Skeletal muscle afferents refer to signals emerging from encapsulated skeletal muscle structures such as muscle spindles and Golgi tendon organs (i.e., proprioceptors) which relay proprioceptive information (e.g., muscle position, tension and rate of length change) to the CNS by way of group I and II afferent fibers. In addition, skeletal muscle afferents relay “chemical” and “mechanical” information to the CNS regarding the current state of its interstitial chemical milieu and physical stress associated with muscle work (20; 79). These types of signals are collectively and aptly referred to as ergoceptive information emerging from encapsulated, free nerve endings within skeletal muscle (i.e., ergoceptors) and are carried by neurons classified as group III and IV afferent fibers (76).

In a classic series of human experiments by Alam and Smirk (4; 5), they discovered that exercise during complete circulatory occlusion (i.e., ischemic exercise) evoked marked elevations in blood pressure and heart rate (HR) that were sustained upon cessation of the exercise so long as the blood flow to the exercising muscle remained arrested. The absence of arterial inflow to and venous outflow from the muscle effectively eliminated the potential for endocrine communication between the muscle and the CNS. As a result, they concluded that the observed reflex response must be neurogenic and that the stimulus initiating and sustaining the reflex was the accumulation of metabolites within the muscle. Although the findings of their initial

experiments were purely serendipitous, their studies are regarded as the pioneering work demonstrating the presence of such a ‘muscle metabolite’ reflex. Following up on this work, Asmussen and Nielsen (11) produced similar results in dynamic exercising humans, however in addition to suggesting muscle chemoreceptors they also implicated muscle mechanoreceptors as the potential source of the reflex pressor response. Subsequent animal experiments by Coote and Perez-Gonzalez (25) and McCloskey and Mitchell (102) offered insights into the afferent arm of this reflex as they demonstrated the capacity of group III and IV ergoceptive fibers to elicit a reflex pressor response (i.e., increase in arterial blood pressure), while in addition they showed that the group I and II proprioceptive afferents played no role in evoking such a reflex. Further animal studies discovered multiple stimulus modalities for group III and IV afferents. In general, group III afferents were shown to be primarily responsive to the mechanical stresses generated during muscle contraction (e.g., increases in tissue pressure) (63; 77; 78; 125), while group IV afferents were found to be acutely sensitive to metabolites (e.g., hydrogen ion, lactate, potassium ion, diprotonated phosphate and ATP) which accumulate within underperfused active skeletal muscle (34; 61; 86; 95; 128; 145; 146; 157). In support of these findings, the anatomical location of ergoceptive afferents has been visualized via electron microscopy revealing, in general, that group III afferent nerve endings are intimately associated with the connective tissue elements of skeletal muscle, whereas group IV afferent nerve endings emanate from small blood vessels within the muscle (160). Adding a layer of complexity, many studies have demonstrated that “mechanoreceptors” can be sensitized by muscle metabolites developed during ischemia and that “metaboreceptors” are sensitive to mechanical stimulation (77; 85; 86; 127; 129). Nonetheless, as a result of these findings, group III

and IV afferents were classified as mechanoreceptors and metaboreceptors, respectively. It therefore became evident that the metabolically, chemo-sensitive reflex pressor response suggested by Alam and Smirk and other investigators, now referred to as the muscle metaboreflex, was indeed initiated by group III and group IV afferents.

Muscle Metaboreflex: the Efferent Arm

Following up on the early work elucidating the afferent structure and function of the muscle metaboreflex, a litany of experiments has been performed in humans and in animals with the intent of investigating its efferent arm. Early investigators not only observed reflex-induced pressor responses, reflex increases in other hemodynamic (e.g., HR and pulmonary (e.g., ventilation) parameters were additionally reported in their studies (5; 11; 24; 102). In order to study the efferent mechanisms of the muscle metaboreflex, focus was shifted to measuring and analyzing the hemodynamic outcomes of muscle metaboreflex-induced increases in sympathetic outflow from the CNS. Incidentally, the mechanisms of integration in the control center of this reflex (i.e., the brainstem) are not well known. A very common approach developed to elicit and study the reflex is the induction of skeletal muscle ischemia (by way of vascular occlusion) prior to or during a bout of static or dynamic exercise (3; 4; 11; 24; 26; 31; 74; 116; 144; 163). Data from these types of experiments not only supported the findings of earlier studies, but also expanded the list of muscle metaboreflex-induced efferent responses. Along with increases in mean arterial pressure (MAP), HR and cardiac output (CO), the muscle metaboreflex has additionally been shown to increase stroke volume (SV) (144), right atrial pressure (RAP) (140), ventricular performance (27; 34; 116; 138; 151; 152) and sympathetic nerve activity (SNA) (114; 158; 159). Even though there are innumerable observations of reflex-induced increases in these

cardiovascular parameters during exercise, most data suggest that the muscle metaboreflex only presents its negative feed-back influence over cardiovascular control when skeletal muscle is faced with a mismatch between oxygen supply and demand (i.e., during ischemia). As muscle metabolites normally accumulate as a result of a decrease in perfusion to the working muscle, the muscle metaboreflex has often been regarded as a flow-sensitive reflex (120). In fact, Wyss et al. (163) demonstrated the existence of a threshold for such a "flow error signal" while studying canines dynamically exercising at varying work intensities. In general, as the intensity of the workload increased hindlimb perfusion progressively fell and the margin for error in muscle blood flow approached zero. In contrast, data from Sheriff et al. (143) showed that it is not a reduction in flow *per se* that triggers the accumulation of muscle metabolites, rather it is a reduction in oxygen delivery to the muscle. In any event, there is strong evidence that the muscle metaboreflex is tonically active during higher intensity (moderate to severe) exercise in dynamically exercising canines (14; 120; 163). Furthermore, in disease states where perfusion to skeletal muscle is impaired, such as in heart failure or peripheral arterial stenosis, the muscle metaboreflex may indeed be active even during lower intensity (mild to moderate) dynamic exercise (59; 94). It is generally believed that cardiovascular responses to mild exercise are controlled by feed-forward CNS efferent commands (i.e., central command) and principally modulated by the negative feed-back influence of the arterial baroreflex. However, there are data implicating muscle metaboreflex influence over cardiovascular function during mild exercise as well (2; 6; 135). Very recently, Amann et al. (6) provided evidence in humans that increases in MAP, CO, HR and ventilatory rate during mild exercise might be, in part, controlled by a tonically active muscle metaboreflex.

Muscle Metaboreflex Activation During and Following the Cessation of Exercise

In an attempt to separate the previously reported efferent responses of the muscle metaboreflex from the influence of central command and the baroreflex, investigators sought to "isolate" the reflex during the recovery from exercise. This technique, first employed by Alam and Smirk, was designed to induce ischemia (by way of vascular occlusion) in the muscle immediately upon or before the cessation of the exercise and is commonly referred to as post-exercise muscle ischemia (PEMI) (16; 19; 31; 45; 102; 132). As expected, the cardiovascular parameters previously reported to be augmented by muscle metaboreflex activation during ischemic exercise are sustained during the recovery from exercise so long as the imposed circulatory occlusion is sustained, or PEMI (4; 19; 30; 45; 115; 133; 144). Curiously though, there is one notable exception. The vast majority of studies sustaining muscle metaboreflex activation during PEMI report a decline in HR during this maneuver while the pressor response is sustained (5; 16; 31; 45; 133; 144; 159). With HR precipitously declining towards resting levels during PEMI, with a time-course similar to normal recovery from exercise, muscle metaboreflex control over the chronotropic function of the heart was drawn into question (46; 133; 134; 159). Moreover, as CO is directly proportional to HR, questions immediately arose regarding the mechanism(s) sustaining the pressor response observed during PEMI in the absence of a sustained elevation in HR. In the absence of a CO component, the only other mechanism available to sustain an elevation in MAP during PEMI would be peripheral vasoconstriction. Indeed, previous studies both support (19; 30-32; 123; 132; 144) and refute (16; 32; 123) any role for CO in mediating the pressor response during PEMI. Clearly, the role of CO vs. peripheral vasoconstriction in mediating the pressor response during PEMI is debatable, and it

remains a controversial issue yet to be resolved.

In contrast to PEMI, it is well documented that during submaximal ischemic exercise, the pressor response occurs primarily via increased CO with little, if any, peripheral vasoconstriction (38; 59; 83; 116; 131; 140; 163). Interestingly, many studies have demonstrated a "switch" from a flow-mediated rise in MAP during exercise to a vasoconstriction-mediated pressor response, when the reflex increase in CO is attenuated. For example, Sheriff et al. (140) and Ichinose et al. (68) demonstrated substantial decreases in peripheral vascular conductance (i.e., increases in peripheral vasoconstriction) when they abolished the metaboreflex-induced increase in CO during mild, dynamic exercise via ventricular pacing with β_1-adrenergic blockade or mechanical occlusion of the vena cavae, respectively. In addition, Augustyniak et al. (14) demonstrated a shift from CO to peripheral vasoconstriction when workload approached maximal levels and further increases in CO were limited. Moreover, studies from O'Leary and colleagues (59) and others (29) have demonstrated this same shift from CO to peripheral vasoconstriction in heart failure, a condition in which CO is attenuated as a result of impaired inotropic and/or chronotropic function. Thus, it appears that whether or not increases in CO or peripheral vasoconstriction is utilized as a means to raise MAP with metaboreflex activation during dynamic exercise is dependent on the ability to increase CO.

Very recently, Sala-Mercado et al. (136) reported attenuated metaboreflex-induced chronotropic, inotropic and lusitropic responses in hypertension (HTN) during submaximal dynamic exercise. Inasmuch as increases in CO with metaboreflex activation were markedly reduced, the rise in MAP was attenuated as well. The pressor response likely resulted from a combination of the small increase in CO and enhanced

peripheral vasoconstriction. Other studies investigating muscle metaboreflex function in HTN are limited, and the overall findings are equivocal: exaggerated (35; 52; 89; 106; 107; 139; 148) vs. attenuated (126) metaboreflex responses. Moreover, the mechanisms sustaining the muscle metaboreflex-mediated pressor response during PEMI in HTN are virtually unknown.

Muscle Metaboreflex Activation and Ventricular Performance

It is well known that CO is impaired in heart failure and that exercise in this condition results in muscle metaboreflex-mediated pressor responses that are principally generated by increases in peripheral vasoconstriction, not CO. Hammond et al. (59) concluded that the observed peripheral vasoconstriction in heart failure was mediated by exaggerated SNA (as indexed by elevated plasma norepinephrine (NE) levels) and elevations in other vasoactive hormones such as arginine vasopressin (AVP) and renin. Though one of the hallmarks of heart failure is heightened sympathoactivation at rest, this study additionally reported significant increases in SNA (and vasoactive hormones) with muscle metaboreflex activation during exercise. The peripheral vasculature is not the sole efferent target of this reflex-induced elevation in SNA as there is marked sympathoactivation of the heart as well leading to increases in its chronotropic and inotropic functions as previously discussed. Inasmuch as vascular smooth muscle cells of coronary blood vessels express alpha adrenergic receptors, they too are potential efferent targets of this elevated sympathetic outflow. An increase in SNA to the heart can activate vascular smooth muscle α_1-adrenergic receptors resulting in vasoconstriction of the coronary arteries (27; 57; 108). On the other hand, an increase in SNA to the heart will also increase the work done by the heart which can in turn lead to the generation of local metabolites that induce a coronary vasodilation.

Another potential mechanism of coronary vasodilation is the feed-forward sympathetic activation of coronary β_2-adrenergic receptors (49). Overall, coronary vasomotor tone appears to be principally modulated by local metabolic vasodilation (i.e., active hyperemia) and sympathetically-mediated α_1-adrenergic vasoconstriction (37; 40; 156). The balance of these antagonistic mechanisms will ultimately determine the degree of perfusion of the heart, or coronary blood flow (CBF). At rest, the heart is an oxygen sink, as it extracts upwards of 80% of the oxygen in arterial blood (37; 40). An increase in workload on the heart (e.g., the onset of exercise) increases myocardial oxygen demand, however this poses a significant challenge to the heart as it is incapable of extracting a significantly greater fraction of oxygen from the very blood it strips 80% from at rest. Therefore, a coronary vasodilatory reserve is of paramount physiological importance to the heart, as an increase in CBF serves as the primary mechanism to increase the supply of oxygen available to the heart. An increase in perfusion pressure to the heart will lead to an increase in oxygen delivery but will also initiate a myogenic autoregulatory vasoconstriction. However, this negative feed-back mechanism is counteracted by a metabolic coronary vasodilation when the workload of the heart significantly increases (e.g., during moderate or severe exercise) permitting marked increases in CBF.

There are reports, however, of an α_1-adrenergic constrictor tone which reduces this coronary vasodilatory reserve during exercise. Gwirtz et al. (57) demonstrated that as workload increases, there is progressively greater α_1-adrenergic tone which restrains the metabolic coronary vasodilation. Ansorge et al. (10) examined the potential contribution of the muscle metaboreflex to this α-adrenergic-mediated coronary vasoconstriction phenomenon by measuring CBF and coronary vascular conductance

(CVC) during dynamic exercise in canines. During severe exercise, they reported an attenuated reflex increase in CBF along with a significant reduction in CVC due to accentuated coronary vasoconstriction with muscle metaboreflex activation. They concluded that the muscle metaboreflex-induced rise in SNA to the heart functionally vasoconstricts the coronary vasculature, thereby attenuating the rise in CBF. Thus, during strenuous exercise any coronary metabolic vasodilation induced by the increase in myocardial performance is counteracted by an increase in sympathetically-mediated coronary vasoconstriction. These findings, along with the data demonstrating heightened SNA in canines with heart failure at rest and during exercise with muscle metaboreflex activation, suggested a possible mechanism for the impaired CO responses observed in heart failure. Indeed, Ansorge et al. (9) demonstrated a significant reduction in CVC leading to an attenuated rise in CBF with muscle metaboreflex activation during mild and moderate exercise in animals with heart failure. They suggested that the marked coronary vasoconstriction observed in heart failure with muscle metaboreflex activation was as a potential mechanism responsible for the reduced ability to raise CO in this condition. O'Leary et al. (118) reported another potential mechanism for this impaired CO response with muscle metaboreflex activation in heart failure during exercise when they demonstrated a concomitant reduction in SV. A reduced SV with muscle metaboreflex activation in heart failure suggested impaired ventricular function and/or preload and, in fact, subsequent studies from O'Leary and colleagues (137) reported impaired ventricular contractility with muscle metaboreflex activation in heart failure during exercise. Furthermore, Gwirtz et al. (57) and O'Leary et al. (119) additionally reported an increase cardiac performance and CO, respectively, as a result of an abolition of coronary vasoconstriction following α-adrenergic blockade

during exercise. Coutsos et al. recently demonstrated that during mild, dynamic exercise sympathetically-mediated restraint of coronary vasodilation (the extent determined by α-adrenergic blockade) during muscle metaboreflex activation impairs increases in left ventricular contractility in normal (27) and heart failure (28) animals. Collectively, these data suggest that a sympathetically-mediated restraint of metabolic coronary vasodilation during periods of increased myocardial metabolic demand can limit the rise in CBF and therefore further limit increases in ventricular performance and the rise in CO as seen in heart failure.

As with heart failure, HTN is a condition in which individuals present with exaggerated levels of SNA (8; 51; 54; 72; 97; 101; 105; 155). Moreover, resting coronary vasoconstriction and restrained coronary metabolic vasodilation during submaximal dynamic exercise (presumably due to heightened SNA) has been demonstrated in canines with renovascular HTN (55). Sala-Mercado et al. (136) reported attenuated metaboreflex-induced increases in ventricular function and CO in HTN (Goldblatt 2K1C (47; 48)) during submaximal dynamic exercise. The extent to which coronary vasoconstriction contributes to impaired ventricular function observed during muscle metaboreflex activation in HTN is unknown.

Summary/Dissertation Aims

Skeletal muscle ischemia during or immediately following exercise leads to the accumulation of metabolites (e.g., lactate and proton) which activate chemoreceptive afferents within the muscle leading to a reflex increase in sympathetic outflow generating substantial increases in MAP, CO and HR - termed the muscle metaboreflex. The muscle metaboreflex is generally regarded as a flow-sensitive reflex that is elicited when skeletal muscle is faced with a mismatch between oxygen supply

and demand, and thereby increases cardiac function in an attempt to increase perfusion to the ischemic muscle.

Two different approaches have been employed to study this reflex. Blood flow to the exercising muscle can be reduced prior to or during a bout of static or dynamic exercise thereby activating the reflex during ischemic exercise. In contrast, the reflex can be elicited by inducing ischemia in the muscle immediately before or upon cessation of the exercise – PEMI. When the reflex is activated during sustained submaximal dynamic exercise, the pressor response occurs primarily via increased CO. In contrast, during PEMI, whereas MAP remains elevated for as long as the ischemia is maintained, HR precipitously declines towards resting levels drawing into question the role of CO in mediating the pressor response during this period. Indeed, the role of CO in mediating the pressor response during PEMI remains debatable.

Specific Aim (I): **To determine the mechanism(s) mediating the muscle-metaboreflex-induced pressor response observed during PEMI in normal subjects.**

Many studies have demonstrated a “switch” from a flow-mediated rise in MAP during exercise to a vasoconstriction-mediated pressor response when the reflex increase in CO is attenuated (e.g., in heart failure). It appears that whether or not increases in CO or peripheral vasoconstriction is utilized as a means to raise MAP with metaboreflex activation during dynamic exercise or sustain it during PEMI is dependent on the ability to increase CO. In HTN, metaboreflex-mediated increases in CO are markedly attenuated during mild exercise.

***Specific Aim (II)*: To determine the mechanism(s) mediating the muscle-metaboreflex-induced pressor response observed during PEMI in hypertensive subjects.**

In heart failure, a sympathetically-mediated restraint of metabolic coronary vasodilation with metaboreflex activation during submaximal dynamic exercise limits the rise in CBF and therefore further limits increases in ventricular performance and the rise in CO. In HTN, metaboreflex-mediated increases in ventricular function and CO are markedly attenuated during mild exercise. As SNA is exaggerated in both heart failure and HTN, it is plausible that the mechanisms mediating the attenuation of CO in HTN with muscle metaboreflex activation are similar to those previously described in heart failure.

***Specific Aim (III)*: To determine if muscle metaboreflex activation during submaximal dynamic exercise in hypertensive animals induces a sympathetic restraint of metabolic coronary vasodilation that limits the rise in CBF and whether this restraint of CBF further limits increases in ventricular performance.**

CHAPTER 2

Role of Cardiac Output vs. Peripheral Vasoconstriction in Mediating Muscle Metaboreflex Pressor Responses: Dynamic Exercise vs. Post-Exercise Muscle Ischemia

Abstract

Muscle metaboreflex activation (MMA) during submaximal dynamic exercise in normal individuals increases mean arterial pressure (MAP) via increases in cardiac output (CO) with little peripheral vasoconstriction. The rise in CO occurs primarily via increases in heart rate (HR) with maintained or slightly increased stroke volume. When the reflex is sustained during recovery (post-exercise muscle ischemia - PEMI), HR declines yet MAP remains elevated. The role of CO in mediating the pressor response during PEMI is controversial. In seven chronically instrumented canines, steady-state values with MMA during mild exercise (3.2 km/h) were observed by reducing hindlimb blood flow by ~60% for 3-5 minutes. MMA during exercise was followed by 60 s of PEMI. Control experiments consisted of normal exercise and recovery. MMA during exercise increased MAP, HR, and CO by 55.3 ± 4.9 mmHg, 42.5 ± 6.9 beats/min and 2.5 ± 0.4 l/min, respectively. During sustained MMA via PEMI, MAP remained elevated and CO remained well above the normal recovery levels. Neither MMA during dynamic exercise nor during PEMI significantly affected peripheral vascular conductance. We conclude that the sustained increase in MAP during PEMI is driven by a sustained increase in CO not peripheral vasoconstriction.

Introduction

Skeletal muscle ischemia during or immediately after exercise leads to the accumulation of metabolic by-products that activate group III and IV chemosensitive

afferents within the muscle (21; 34; 79; 86; 95; 128; 143; 145; 146; 157). Activation of these skeletal muscle afferents causes a reflex increase in sympathetic outflow that generates substantial increases in arterial pressure termed the muscle metaboreflex (4; 11; 24; 46; 102; 114-116; 121; 132; 133; 163). Two different approaches have been employed to study this reflex. Blood flow to the exercising muscle can be reduced before or during a bout of static or dynamic exercise thereby activating the reflex during ischemic exercise (3; 4; 11; 24; 26; 31; 116; 144; 163). In contrast, the reflex can be elicited by inducing ischemia in the muscle immediately before or upon cessation of the exercise [a technique termed post-exercise muscle ischemia (PEMI)] (16; 19; 31; 45; 46; 102; 132; 133; 157; 161).

When the reflex is activated during sustained submaximal dynamic exercise in normal subjects, the pressor response occurs primarily via increased cardiac output (CO), which is driven by increased heart rate (HR) coupled with sustained or slightly increased stroke volume (SV) (31; 68; 138; 144; 163). SV is maintained despite this tachycardia due to both enhanced ventricular contractility (27; 30; 116; 138) and maintained or increased ventricular filling pressure via substantial central blood volume mobilization (140). In contrast, during PEMI, whereas mean arterial pressure (MAP) remains elevated for as long as the ischemia is maintained, HR precipitously declines toward resting levels with a time course similar to normal recovery, which led some to speculate that the muscle metaboreflex has little control over the heart (4; 45; 98; 133; 158; 159; 161). With HR on the decline during the recovery from exercise with or without PEMI, the role of CO in mediating the pressor response during PEMI remains controversial. Indeed previous studies both support (19; 30-32; 123; 132; 144) and refute (16; 32; 123) any role for CO in mediating the pressor response during PEMI. In

the present study, we elicited the muscle metaboreflex during dynamic exercise and sustained this activation during PEMI to compare the mechanisms underlying the muscle metaboreflex-mediated pressor responses in these two distinct settings.

Methods

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

Surgical procedures. Each animal was completely instrumented with chronic, indwelling cardiovascular devices following two sterile surgical procedures: left thoracotomy and left flank retroperitoneal surgery in that order. The animals recovered a minimum of 10 days before the second surgery and a minimum of 7 days before the first experiment. During preoperative care, the animals were initially sedated with acepromazine (0.4-0.5 mg/kg IM). After adequate sedation, the animals were anesthetized with a combined treatment of ketamine and diazepam (5.0 and 0.22 mg/kg IV, respectively). Anesthesia was maintained with isoflurane gas (1-3%) after endotracheal intubation. In addition, the animals received preoperative administration of cefazolin (antibiotic; 30 mg/kg IV), carprofen (analgesic; 4.0 mg/kg IV), buprenorphine (analgesic; 0.01 mg/kg IM) and fentanyl [analgesic; 125-175 μg/h, (72h) TDD]. Before the left thoracotomy, animals received selective intercostal nerve blockade with

bupivacaine HCl (2.0 mg/kg SQ). After each surgical procedure, animals received cefazolin (30 mg/kg IV) and prophylactic cephalexin [antibiotic; 30 mg/kg (BID) PO] therapy for the term of the experimental protocol. During the 12-hour post-operative period, animals were closely monitored and received buprenorphine and acepromazine (0.05 and 0.5 mg/kg IV, respectively) as needed. For the following 10 days, animals received carprofen [4 mg/kg (OPD) PO].

In the first surgical procedure, the thoracic cavity was opened via a left thoracotomy (4th intercostal space) approach. The pericardium was cut and reflected to expose the heart. An ultrasonic perivascular flow probe (20PAU, Transonic Systems) was positioned around the ascending aorta to measure CO. Approximately 10 cm caudal to the thoracotomy incision, an implantable telemetry blood pressure transmitter (TA11 PA-D70, Data Sciences International) was tethered subcutaneously. The catheter of the transmitter was tunneled into the thoracic cavity through the 7th intercostal space, and the tip was inserted and secured inside the left ventricle for measuring left ventricular pressure (LVP). For studies unrelated to the present investigation a blood flow transducer was also placed on the left circumflex artery. The pericardium was loosely re-approximated, the cables were tunneled subcutaneously and exteriorized between the scapulae and the chest was closed in layers.

In the second surgical procedure, an incision was made in the left flank cranial to the iliac crest. The abdominal aorta was exposed and an ultrasonic perivascular flow probe (10PAA, Transonic Systems) was positioned around the terminal aorta for measuring hindlimb blood flow (HLBF). All arterial side branches between the common iliacs and the flow probe were ligated and severed. In addition, two perivascular hydraulic occluders (8-10 mm, DocXS Biomedical Products) were positioned around the

terminal aorta (distal to the flow probe) to provide the means to incrementally reduce HLBF. A 19-gauge polyvinyl catheter (S54-HL, Tygon, Norton) was advanced through a ligated lumbar artery and secured into the terminal aorta cranial to the probe and occluders to measure arterial pressure. For studies unrelated to the current investigation a blood flow transducer and vascular occluder were placed on the left renal artery. The cables and vascular occluder tubing were tunneled subcutaneously and exteriorized between the scapulae.

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

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

60 s. The treadmill was then abruptly stopped and the occlusion was sustained for an additional 60 s (PEMI).

Data analysis. CO, HLBF, LVP, HR and MAP data were continuously recorded during each experiment. Other hemodynamic parameters were calculated off-line [e.g., SV, dP/dt_{max}, dP/dt_{min} and non-ischemic vascular conductance (NIVC)]. NIVC was calculated as (CO - HLBF)/MAP and reflects vascular conductance of all vascular beds except the hindlimbs. Because of technical difficulties, we were only able to obtain LVPs from six animals. One-minute averages of steady-state data were calculated at rest, during exercise and during metaboreflex activation. 5 s averages were computed for both 60 s post-exercise conditions: post-exercise (without ischemia) and PEMI. These mean values were then averaged across all animals to obtain the mean values for the entire population of the study. Finally, the last 10 s of recovery were averaged.

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

Results

Figure 2.1 shows the responses in MAP, CO, HR, SV and HLBF from a control and experimental protocol in one animal. In both protocols, with the transition from rest to exercise, there was a minimal increase in MAP and modest increases in CO and HR concomitant with a substantial rise in HLBF. During the normal recovery from exercise, MAP remained unchanged while CO and HR gradually fell toward resting levels. In the experimental protocol, the muscle metaboreflex was elicited immediately following

steady-state exercise and sustained during the post-exercise recovery period (PEMI). Muscle metaboreflex activation during the experimental protocol led to marked increases in MAP, CO and HR and little change in SV. During PEMI, the rise in MAP was sustained. Although CO and HR initially fell during the onset of PEMI, both subsequently plateaued well above their normal recovery levels.

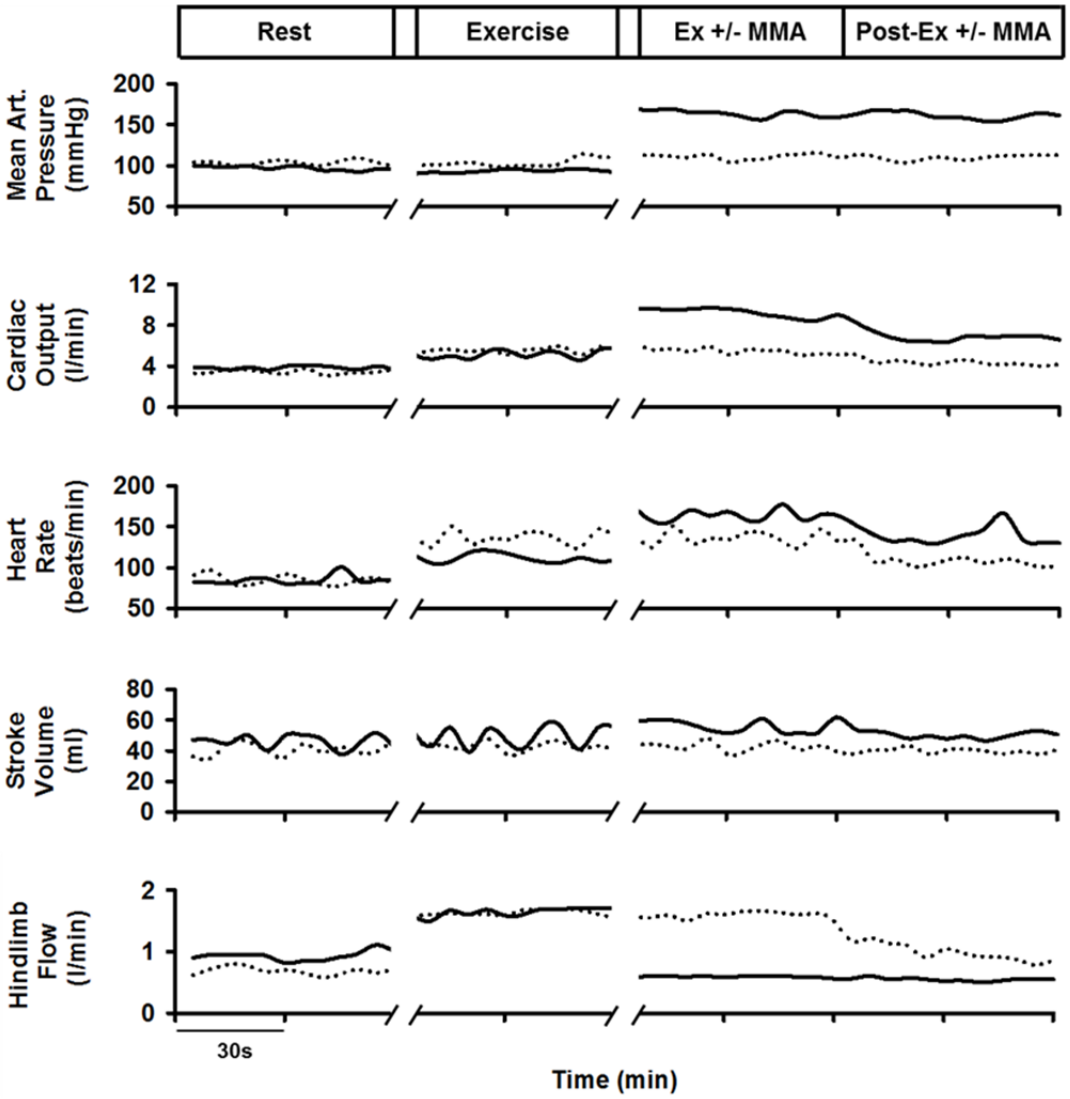

Figure 2.1. Time-course of mean arterial pressure (MAP), cardiac output (CO), heart rate (HR), stroke volume (SV) and hindlimb blood flow (HLBF) during control (dotted lines) and experimental (solid lines) protocols in one animal. MMA, metaboreflex activation.

Figure 2.2 shows the average values of MAP, HR, CO and NIVC during control and experimental procedures. The 60 s of post-exercise recovery are plotted as 5 s averages. The changes in MAP, HR, CO and NIVC from rest to exercise were not significantly different between the control and experimental protocols. With muscle metaboreflex activation during exercise, MAP, HR and CO increased substantially.

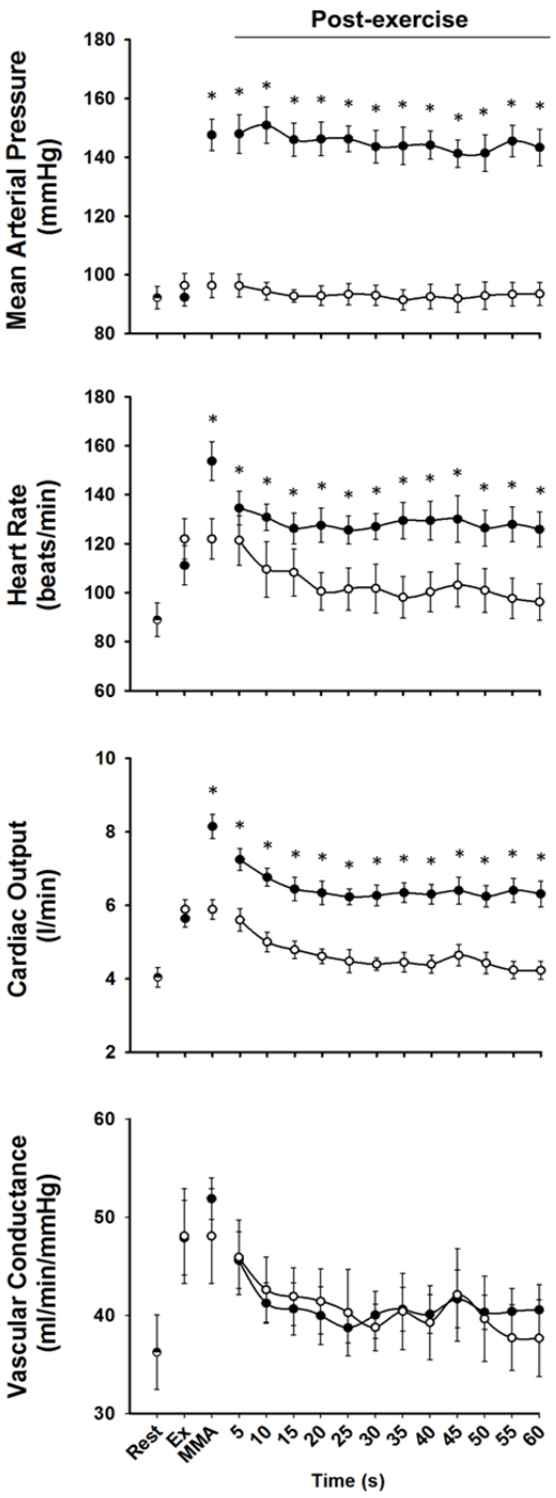

Figure 2.2. Averaged time-course of MAP, HR, CO and non-ischemic vascular conductance (NIVC) during control (open circles) and experimental procedures (filled circles) in 7 animals. Data at rest are the average values from both settings (half-filled circles). * - $p<0.05$.

During PEMI, the ~60 mmHg rise in MAP, which was observed with muscle metaboreflex activation, was sustained during the entire 60 s, as every data point during this maneuver was significantly different from control. NIVC fell during the PEMI period

with a pattern not significantly different from during the normal recovery from exercise. HR and CO decreased abruptly during the first 15 s of PEMI; however, both of these variables subsequently plateaued significantly above normal recovery levels.

Figure 2.3 shows the mean hemodynamic responses during exercise and the final 10 s of recovery with and without muscle metaboreflex activation. During the last 10 s of ischemic exercise, there were significant increases in MAP, CO, HR, dP/dt_{max} and dP/dt_{min} compared with the data during exercise without metaboreflex activation.

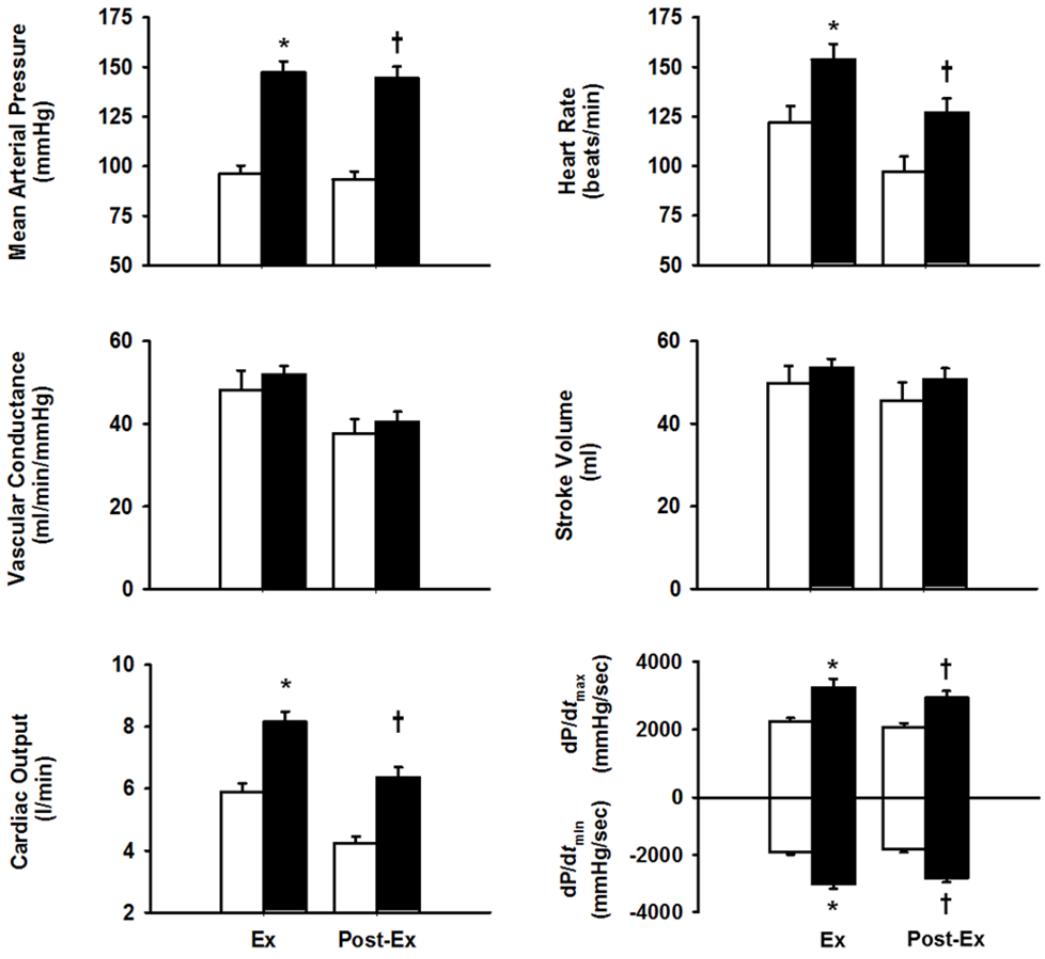

Figure 2.3. Mean hemodynamic responses during exercise and the final 10 seconds of recovery with (filled bars) and without (open bars) muscle metaboreflex activation. * - $p<0.05$ between normal exercise and ischemic exercise, † - $p<0.05$ between normal recovery from exercise (open bars) and post-exercise muscle ischemia (PEMI) (filled bars).

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

Discussion

Our major finding is that the mechanisms mediating muscle metaboreflex-induced increases in arterial pressure are similar both when the reflex is activated during dynamic exercise and when the reflex is sustained during the recovery from exercise. In both settings, the pressor response is primarily due to a substantial elevation in CO with little, if any, peripheral vasoconstriction. Moreover, the elevation in CO is driven via an increased HR with a sustained SV. Inasmuch as tachycardia itself can lead to decreases in SV (87; 162), increases in ventricular contractility likely contribute to the sustained SV. The elevated dP/dt_{max} supports this conclusion. Faster left ventricular relaxation (dP/dt_{min}) may also aid in the maintenance of SV by increasing filling time. Therefore, our data demonstrate marked muscle metaboreflex-induced chronotropic, inotropic and lusitropic responses both when the reflex is activated during dynamic exercise and when the reflex is sustained during recovery from exercise.

Muscle metaboreflex activation during exercise. CO versus vasoconstriction. Muscle metaboreflex activation during exercise evokes large increases in MAP, HR and CO (4; 5; 39; 104; 115; 116; 163). However, the relative roles of CO versus peripheral vasoconstriction in this pressor response have been unclear. We observed a 45% increase in CO with muscle metaboreflex activation during exercise with no significant peripheral vasoconstriction. We determined the extent of peripheral vasoconstriction by calculating the conductance of all vascular beds with the exception of the hindlimbs

(NIVC) (13). Changes in hindlimb conductance must be excluded due to the mechanical effects of the occlusion.

Previous studies have demonstrated a “switch” in the mechanisms of the muscle metaboreflex from a flow-mediated rise in MAP to a vasoconstriction-mediated pressor response when the reflex increase in CO is attenuated. For example, Sheriff et al. (140) and Ichinose et al. (68) demonstrated substantial peripheral vasoconstriction when the metaboreflex-induced increase in CO was either pharmacologically or mechanically prevented. In addition, Augustyniak et al. (14) demonstrated a shift from CO to peripheral vasoconstriction when workload approached maximal levels and further increases in CO were limited. Similar results have been observed in subjects with congestive heart failure, a setting where substantial increases in CO are limited (12; 29; 59; 121). Thus whether or not increased CO or increased vasoconstriction is utilized as a means to raise MAP with metaboreflex activation during dynamic exercise appears dependent on the ability to increase CO.

Muscle metaboreflex activation during PEMI. CO versus vasoconstriction. Previous studies have come to markedly different conclusions regarding the relative roles of CO versus peripheral vasoconstriction in mediating the pressor response during PEMI [(19; 30-32; 123; 132; 144) versus (16; 32; 123)]. One potential explanation is that a CO response is often seen during imposed PEMI following more intense exercise, whereas PEMI following relatively lower exercise intensities tends to demonstrate little change in CO and the pressor response occurs via peripheral vasoconstriction. We observed a maintained elevation in CO (~50% above the normal recovery level) during PEMI with little or no change in NIVC. The time course of the recovery of NIVC during PEMI and the normal recovery from exercise are virtually indistinguishable and not

significantly different. It should be noted that NIVC contains not only conductance to inactive areas, but a substantial amount of NIVC is skeletal muscle outside of the hindlimbs (58), which also vasodilates in response to the exercise. Thus with exercise NIVC increases and during recovery NIVC falls. To what extent this fall in NIVC during recovery with or without PEMI is neurogenic versus passive vasoconstriction due to reduced metabolic vasodilation in skeletal muscle is not known. Previously, Sheriff et al. (141) has shown that after ganglionic blockade, skeletal muscle vasodilation during exercise markedly exceeds normal levels. Regardless of whether or not tonic sympathetic activity controls the speed of recovery of NIVC, it is clear that the pattern of change in NIVC was not different between the normal recovery and during PEMI.

Role of HR and SV in mediating metaboreflex-induced increases in CO during exercise versus PEMI. Previous studies in dogs and humans have concluded that HR and CO increase markedly when the muscle metaboreflex is activated during exercise (12-14; 27; 29-31; 38; 59; 68; 116; 163); however, during PEMI the effects on HR are more variable. In general, PEMI elicited from the arm following moderate exercise evokes little sustained tachycardia and any CO response occurs via increased SV (5; 45; 123). In contrast, if PEMI follows leg exercise, HR remains above normal recovery values, and therefore the relative tachycardia contributes to an elevated CO (5; 45; 123). The observations in humans following leg exercise are similar to those we observed in the present study using canines. Collectively, these studies indicate that either the HR response during PEMI depends on which limb is used or that these differential responses are due to differences in muscle mass.

Previous studies from our laboratory and others have concluded that during PEMI there are sustained increases in sympathetic tone to the heart; however, there are

concurrent increases in parasympathetic activity as well (69; 111; 114). This combined activation of both arms of the autonomic nervous system causes bradycardia but sustained increased ventricular contractility (Figure 2.3). The differences in the HR responses between PEMI and metaboreflex activation during dynamic exercise may reflect that during PEMI one is observing responses during the recovery from exercise rather than during exercise *per se*. Muscle metaboreflex-induced tachycardia occurs primarily via increased sympathetic activity to the heart (44; 114). With the cessation of exercise, parasympathetic activity rises abruptly, which masks the chronotropic effects of sustained sympathetic tone (note that the inotropic effect is well sustained during PEMI; (Figure 2.3). The extent to which sympathetic nerve activity remains elevated during PEMI may be dependent on the intensity and type of exercise performed (e.g., isometric vs. isotonic). For example, in canines, the muscle metaboreflex is not tonically active during mild, free-flow treadmill exercise but becomes tonically active as workload increases and/or ventricular function declines (14; 60; 163). In addition, Fisher et al. (44) demonstrated in humans performing static handgrip exercise that “robust” muscle metaboreflex activation (achieved during high-intensity exercise) was required to sustain sympathetic nerve activity during PEMI to a degree such that the prevailing parasympathetic effects on HR were counteracted. However, Amann et al. (7) recently concluded that skeletal muscle afferents contribute to the cardiorespiratory responses during relatively mild exercise in humans.

The increases in CO with metaboreflex activation likely require increased contractility as well as central blood volume mobilization to raise or maintain SV with the elevated afterload and shortened ventricular filling time. Previous studies from our laboratory showed that the muscle metaboreflex can elicit marked increases in central

blood volume mobilization (140) as well as ventricular contractility [as evidenced by increased left ventricular maximal elastance, preload recruitable stroke work, as well as dP/dt_{max} (27; 116; 138)]. Moreover, Little et al. (92; 93) have shown that increases in left ventricular end-diastolic volume *per se* can lead to increases in dP/dt_{max}. Thus it is possible that a portion of the rise in contractility with metaboreflex activation may be directly attributable to enhanced central blood volume mobilization.

In the present study, the increase in contractility was sustained during PEMI (Figure 2.3). Shoemaker et al. (144) showed a substantial muscle metaboreflex-induced increase in SV during ischemic exercise, and Crisafulli et al. (30) also reported an increase in SV and ventricular contractility with muscle metaboreflex activation during PEMI. These reflex increases in SV were suggested by these authors to be the principal mechanisms behind the increase in CO in these two distinct settings as HR was not affected. We did not observe a significant increase in SV with muscle metaboreflex activation during exercise or PEMI in our studies, and it is possible that these different results are species dependent. For example, there are reports that canines have a limited end-diastolic reserve compared to humans (17). In contrast, other studies have shown that canines possess significant preload reserve (90; 96). Nonetheless, a maintenance or increase in SV during a hemodynamic period in which ventricular filling time is markedly reduced can likely only be achieved if there is an increase in the contractile state and/or an increase in preload.

Perspectives and Significance. Activation of the muscle metaboreflex both during submaximal exercise and during the recovery from exercise elicits substantial increases in ventricular function. The major mechanism of the muscle metaboreflex-mediated pressor response is increased CO with little, if any, peripheral

vasoconstriction in both settings. Inasmuch as when workload rises, an increasingly smaller fraction of CO is directed to non-active vascular beds (e.g., brain, kidneys and splanchnic organs), even complete vasoconstriction of these beds would cause only a limited increase in arterial pressure (113). In contrast, a metaboreflex-mediated rise in CO generates substantial increases in arterial pressure. Thus, in these settings, the muscle metaboreflex is a flow-raising, pressure-raising reflex. The rise in total systemic blood flow increases systemic perfusion pressure which likely acts to partially restore blood flow to ischemic muscle (120).

CHAPTER 3

Mechanisms Mediating the Muscle Metaboreflex Pressor Response during Post-Exercise Muscle Ischemia are altered in Hypertension

Abstract

During dynamic exercise, muscle metaboreflex activation (MMA; induced via partial hindlimb ischemia) markedly increases mean arterial pressure (MAP) and MAP is sustained when the ischemia is maintained following the cessation of exercise (post-exercise muscle ischemia – PEMI). We previously reported that the sustained pressor response during PEMI in normal individuals is driven by a sustained increase in cardiac output (CO) with no peripheral vasoconstriction. However, we have recently shown that the rise in CO with MMA is significantly blunted in hypertension (HTN). The mechanism(s) sustaining the pressor response during PEMI in HTN are virtually unknown. In six chronically instrumented canines, hemodynamic responses were observed during rest, mild exercise (3.2 km/h), MMA and PEMI in the same animals before and after the induction of HTN (Goldblatt 2K1C). In control, MAP, CO and HR increased with MMA (+52 ± 6 mmHg, +2.1 ± 0.3 l/min and +37 ± 7 bpm). After the induction of HTN, MAP at rest increased from 97 ± 3 to 130 ± 4 mmHg, and the metaboreflex responses were markedly attenuated (+32 ± 5 mmHg, +0.6 ± 0.2 l/min and +11 ± 3 bpm). During PEMI in HTN, HR and CO were not sustained and MAP fell to normal recovery levels. We conclude that the attenuated metaboreflex-induced HR, CO and MAP responses are not sustained during PEMI in HTN.

Introduction

The muscle metaboreflex is a very powerful blood pressure-raising reflex (4; 11; 24; 46; 116; 132; 136; 163). Metabolically-sensitive afferents of this reflex (group III/IV)

are stimulated by metabolites (e.g., protons, lactate, potassium and diprotonated phosphate) which accumulate within underperfused active skeletal muscle (21; 34; 79; 86; 95; 128; 130; 145-147; 157). Activation of the muscle metaboreflex results in a reflex increase in sympathetic outflow from the brainstem (20; 71; 75; 79; 98-100; 158). During submaximal dynamic exercise, metaboreflex activation markedly increases heart rate (HR), cardiac output (CO) and ventricular contractility (dP/dt_{max}) with no net effect on the peripheral vasculature (14; 30; 31; 59; 68; 144; 150; 163). Therefore, the substantial metaboreflex-mediated pressor response observed during mild dynamic exercise is virtually solely the result of the increase in CO.

In contrast, when metaboreflex activation is maintained following the cessation of submaximal dynamic exercise (post-exercise muscle ischemia - PEMI) in normal subjects, the pressor response is sustained despite several investigators reporting that HR falls towards resting levels (4; 5; 45; 123; 133; 134; 158; 159; 161). The fall of HR during PEMI led many investigators to question the role of CO in mediating the sustained pressor response during PEMI (45; 133; 134; 159). We recently demonstrated that the sustained pressor response during PEMI following submaximal dynamic exercise is principally driven by elevated CO, not peripheral vasoconstriction (150). The sustained elevation in CO during PEMI is driven by enhanced chronotropy (5; 31; 43; 67; 114; 138; 163) coupled with a sustained or slightly elevated stoke volume (SV) (27; 31; 116; 138; 144; 150) which is supported by enhanced inotropy (dP/dt_{max}) (27; 30; 68; 116; 138; 150), lusitropy (dP/dt_{min}) (68; 150) and central blood volume mobilization (140). Therefore, the mechanisms mediating muscle metaboreflex-induced increases in MAP are similar regardless of whether the reflex is activated during dynamic exercise or if it is sustained during the recovery from exercise (150).

Very recently, we reported attenuated metaboreflex-induced chronotropic, inotropic and lusitropic responses in hypertension (HTN) during submaximal dynamic exercise (136). Several studies from our group (12; 14; 59; 68; 121; 140) and others (29) have demonstrated a "switch" from a flow-mediated to a vasoconstriction-mediated pressor response during exercise when the reflex increase in CO is artificially attenuated (68; 140) or when there is a physiological inability to increase CO, such as during severe exercise (14) or in heart failure (12; 29; 59; 121). Thus, it appears that whether or not increases in CO or peripheral vasoconstriction is utilized as a means to raise MAP with metaboreflex activation during dynamic exercise is dependent on the ability to increase CO.

The mechanisms sustaining the pressor response during PEMI in HTN are virtually unknown. Inasmuch as metaboreflex-induced increases in HR, CO and dP/dt_{max} are markedly attenuated in HTN, we tested the hypothesis that the sustained pressor response during PEMI would be principally supported by increased peripheral vasoconstriction, not CO.

Methods

Experimental subjects. Six adult female mongrel canines were selected for the study. All animals were healthy, ~20-25 kg body weight, well adapted to the laboratory environment and willing to run on a motor-driven treadmill. During experimentation, all animals exercised voluntarily and no negative reinforcement techniques were utilized. The protocols developed and employed in the present study were reviewed and approved by the Institutional Animal Care and Use Committee of Wayne State University and complied with the National Institutes of Health *Guide to the Care and Use of Laboratory Animals*.

Surgical procedures. The animals were prepared as described previously (136). Briefly, in the first sterile surgical procedure (left thoracotomy), an ultrasonic perivascular flow probe was positioned around the ascending aorta to measure CO and the catheter of an implantable telemetry blood pressure transmitter was inserted and secured into the left ventricle to measure left ventricular pressure (LVP). In the second surgical procedure, ultrasonic perivascular flow probes were placed around the terminal aorta and left renal artery for measuring hindlimb blood flow (HLBF) and renal blood flow (RBF), respectively. Two perivascular hydraulic occluders were positioned around the terminal aorta (distal to the flow probe) to provide the means to incrementally reduce HLBF in order to engage the muscle metaboreflex. One perivascular hydraulic occluder was positioned around the left renal artery (distal to the flow probe) in order to reduce RBF to induce HTN (2K1C Goldblatt model). Lastly, a catheter was secured into the terminal aorta (cranial to the flow probe and occluders) to measure arterial pressure.

Following both surgical procedures, buprenorphine (0.05 mg/kg IV) and acepromazine (0.5 mg/kg IM) were administered for analgesia and sedation, respectively. The animals were treated with cefazolin (30 mg/kg IV) pre- and postoperatively and with cephalexin (30mg/kg PO, twice daily) prophylactically for the term of the experimental protocol. The animals were allowed a minimum of ten days for recovery between surgical procedures and a minimum of seven days before running control experiments.

Data acquisition. After complete postoperative recovery, each animal was brought into the laboratory and allowed to roam freely and acclimate for approximately 15-20 minutes. The animal was then directed onto the treadmill where the instrumentation was connected to the data acquisition system. CO, CBF, HLBF and

RBF flow probe cables were connected to transit-time perivascular flow meters (TS420, Transonic Systems). The LVP telemetry signal was collected by a receiver connected to a calibrated analog adapter and a barometric pressure reference device (RMC-1, R11CPA, APR-1, respectively; DSI). The arterial catheter was connected to a pressure transducer (Transpac IV, ICU Medical). All aforementioned hemodynamic variables, in addition to MAP (calculated) and HR (triggered by the CO signal), were monitored as beat-by-beat averages and real-time waveforms by a data acquisition system (LabScribe, iWorx) and recorded for subsequent off-line analysis.

Induction of hypertension. After completion of control experiments, HTN was induced via a Goldblatt 2K1C model (47; 48). Blood flow to the left kidney was reduced to a target level of ~30% of baseline via partial inflation of the renal vascular occluder. HTN gradually developed over the next several weeks. We defined HTN as systolic pressure ≥140 mmHg and diastolic pressure ≥90 mmHg. The experiments were repeated after 31.5 ± 3.4 days of sustained HTN.

Experimental design. Each animal performed two experimental protocols before (normal) and after induction of HTN, and therefore each animal served as its own control. On each day, the experimental protocol sequence was randomized to avoid any order effect. All experiments began with the animal standing unrestrained on the treadmill until all hemodynamic resting data were observed to be stable (~5-10 min). The treadmill was turned on and the speed was gradually increased to 3.2 km/h at 0% grade (mild exercise for a canine). Steady-state exercise was generally reached within 3-5 minutes. The following is the design of the two experimental protocols:

1) *Free-flow Exercise and Post-exercise Recovery:* Following the 3.2 km/h steady-state, the treadmill was abruptly stopped and post-exercise recovery (without

ischemia – i.e., free-flow) hemodynamic data were collected for 60 seconds while the animal was standing still.

2) *Muscle Metaboreflex Activation and Post-exercise Muscle Ischemia:* Following the 3.2 km/h steady-state, the metaboreflex was engaged during the exercise via partial reductions in HLBF and steady-state data were collected for 60 seconds. The treadmill was then abruptly stopped and the occlusion was sustained for an additional 60 seconds while the animal was standing still.

Data analysis. CO, HLBF, LVP, HR and MAP data were continuously recorded during each experiment. Other hemodynamic parameters were calculated off-line (e.g., SV, non-ischemic vascular conductance (NIVC), total peripheral resistance (TPR), dP/dt_{max} and dP/dt_{min}). Due to technical difficulties, we were only able to obtain dP/dt_{max} and dP/dt_{min} data for five animals. One minute averages of steady-state data were calculated at rest, during exercise and during metaboreflex activation. 5 second averages were computed for the 60 seconds of post-exercise recovery and PEMI. Responses for these two settings were taken as the last averaged 10 seconds. Mean values were averaged across all animals to obtain mean values for the entire population of the study.

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

Results

Following induction of HTN, MAP was significantly elevated at rest which was

due to an increase in TPR (Table 3.1).

Table 3.1. MAP, CO and TPR (mmHg, l/min and mmHg/l/min ± SE, respectively) during rest, exercise (EX), muscle metaboreflex activation (MMA) and the last 10 s of post-exercise muscle ischemia (PEMI) before (normal) and after induction of hypertension.

	Normal			Hypertension		
	MAP	CO	TPR	MAP	CO	TPR
REST	96.9 ± 2.9	3.45 ± 0.20	28.7 ± 2.2	129.5 ± 3.7*	3.56 ± 0.26	37.4 ± 3.1*
EX	101.8 ± 3.6	4.91 ± 0.32	21.4 ± 2.0	124.8 ± 4.4*	4.92 ± 0.25	25.8 ± 1.8*
MMA	153.5 ± 4.9	7.03 ± 0.60	22.6 ± 2.0	156.7 ± 6.5	5.49 ± 0.36*	29.0 ± 1.8*
PEMI	151.5 ± 5.0	5.19 ± 0.49	28.5 ± 2.1	142.6 ± 6.8	4.08 ± 0.38*	36.0 ± 2.6*

* - p<0.05 normal vs. hypertension

Figure 3.1 shows responses in MAP, CO, HR, SV and HLBF from one animal during rest, mild dynamic exercise, muscle metaboreflex activation and PEMI before (normal) and after induction of HTN. Resting MAP was markedly elevated following induction of HTN while CO, HR, SV and HLBF were similar to the levels observed prior

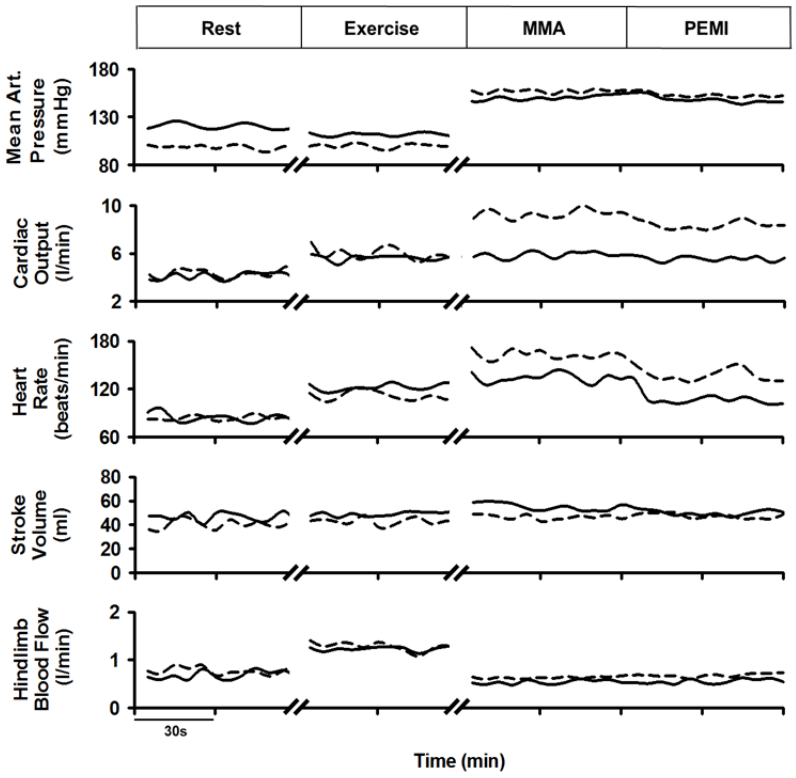

Figure 3.1. Time-course of mean arterial pressure (MAP), cardiac output (CO), heart rate (HR), stroke volume (SV) and hindlimb blood flow (HLBF) during rest, mild exercise, muscle metaboreflex activation (MMA) AND PEMI before (normal) (dotted black lines) and after induction of hypertension (solid black lines) in one animal. Data represented are the final 60 s of steady-state data during rest, exercise and MMA. 60 s of PEMI immediately followed the MMA steady-state.

to induction of HTN. From rest to exercise, CO, HR and HLBF markedly increased while MAP and SV remained similar to the values observed at rest. These cardiovascular responses were similar following induction of HTN. MAP, CO, HR and SV significantly increased with muscle metaboreflex activation, however these responses were attenuated following induction of HTN. During PEMI, MAP, CO and HR were sustained in control, but fell to normal recovery levels following induction of HTN.

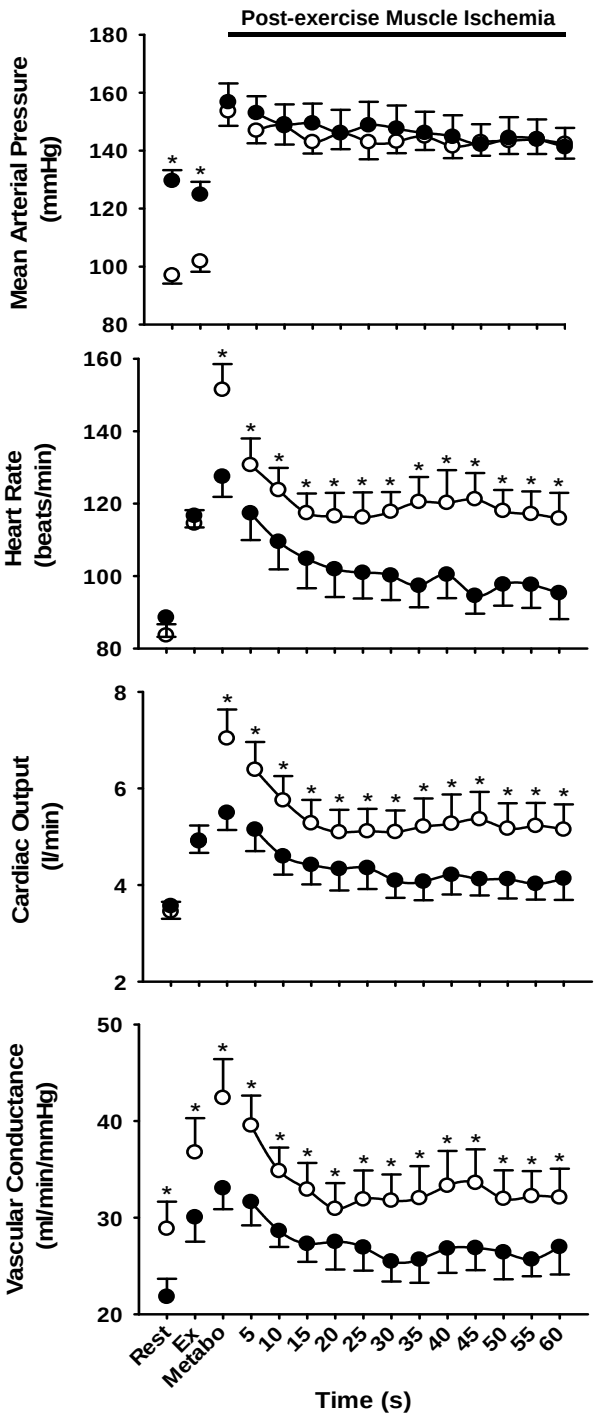

Figure 3.2 shows average values of MAP, HR, CO, and NIVC during rest, mild exercise, muscle metaboreflex activation and PEMI before and after induction of HTN. The 60 seconds of PEMI are plotted as 5 second averages. Following induction of HTN, MAP was significantly higher, HR and CO were unchanged and NIVC was significantly lower at rest and during mild exercise with respect to normal. HLBF was

Figure 3.2. Averaged time-course MAP, HR, CO and non-ischemic vascular conductance (NIVC) responses during rest, exercise, MMA (Metabo) and 60 s of PEMI before (open circles) and after induction of HTN (filled circles). * - $p<0.05$ between normal and HTN.

reduced to the same level to induce muscle metaboreflex activation and was similarly maintained during PEMI before and after induction of HTN. Metaboreflex-induced increases in MAP, HR, CO and NIVC were markedly attenuated following induction of HTN. During PEMI, HR, CO and NIVC were significantly lower than normal during the entire 60 seconds of PEMI following induction of HTN, while MAP was not any different.

Figure 3.3 shows mean hemodynamic responses in MAP, NIVC, CO, HR, SV, dP/dt_{max} and dP/dt_{min} during rest, mild exercise and the final averaged 10 seconds of post-exercise recovery before and after induction of HTN. Resting MAP and dP/dt_{max} were significantly elevated, NIVC significantly reduced and CO, HR, SV and dP/dt_{min}

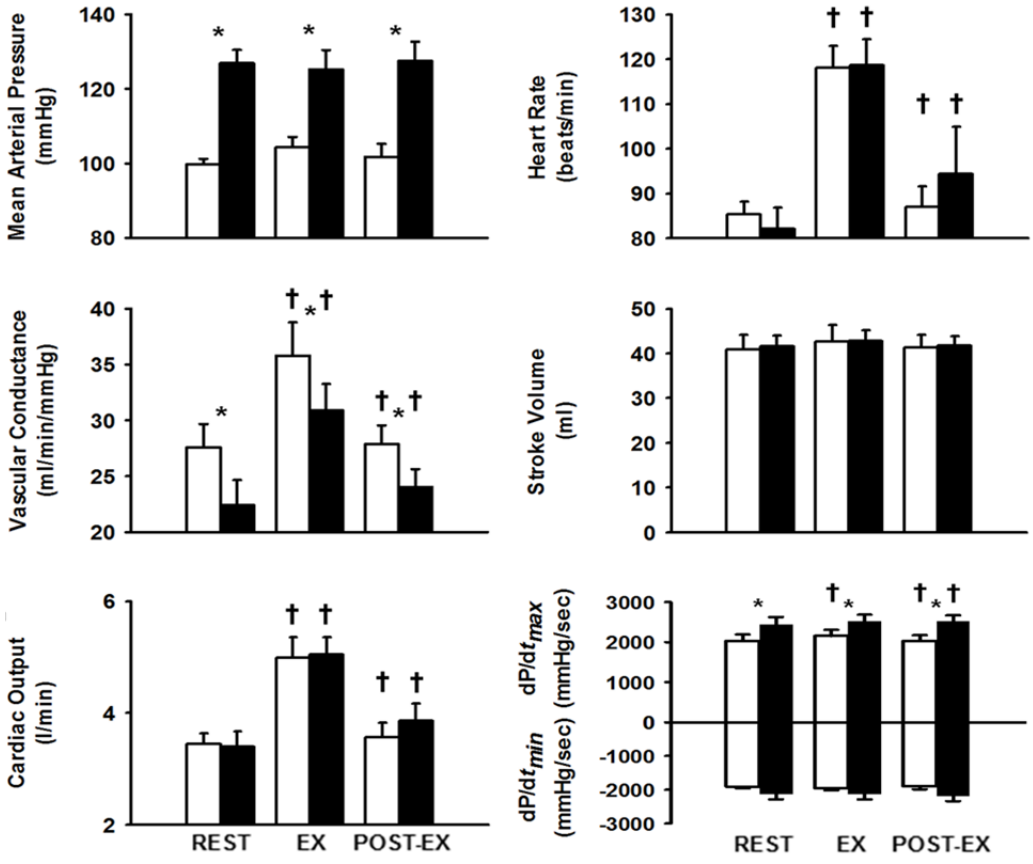

Figure 3.3. Mean hemodynamic responses in MAP, NIVC, CO, HR, SV, dP/dt_{max} and dP/dt_{min} during rest, mild exercise (EX) and post-exercise recovery (POST-EX) before (open bars) and after induction of HTN (filled bars). * - $p<0.05$ between normal and HTN; † - $p<0.05$ from previous setting

were similar to normal following induction of HTN. NIVC, CO, HR and dP/dt_{max} significantly increased from rest to mild exercise while MAP, SV and dP/dt_{min} were unchanged. Following induction of HTN, NIVC were reduced during exercise, CO and HR significantly increased as in normal, while MAP, SV and dP/dt_{max} and dP/dt_{min} were similar to rest. During the final averaged 10 seconds of post-exercise recovery, MAP, SV and dP/dt_{min} remained unchanged while NIVC, CO, HR and dP/dt_{max} fell to levels not significantly different from those observed at rest ($p>0.05$). Following induction of HTN, MAP, SV and dP/dt_{min} remained unchanged while CO and HR fell to rest as in normal. NIVC was significantly reduced and dP/dt_{max} significantly elevated with respect to normal and both parameters fell to rest ($p>0.05$; rest vs. post-exercise recovery).

Figure 3.4 shows mean hemodynamic responses in MAP, NIVC, CO, HR, SV, dP/dt_{max} and dP/dt_{min} during rest, mild exercise, muscle metaboreflex activation and the final averaged 10 seconds of PEMI before and after induction of HTN. All hemodynamic responses at rest and during mild exercise before and after induction of HTN were similar as shown in Figure 3.3. All cardiovascular parameters significantly increased with muscle metaboreflex activation, however following induction of HTN metaboreflex-induced increase in these parameters were markedly attenuated. During the final averaged 10 seconds of PEMI, all parameters fell significantly below metaboreflex activation levels and, with the exception of NIVC, all remained significantly elevated above post-exercise recovery levels. Following induction of HTN, SV, dP/dt_{max} and dP/dt_{min} remained at metaboreflex activation levels while MAP, NIVC, CO and HR fell to levels not significantly different from those observed during post-exercise recovery without PEMI.

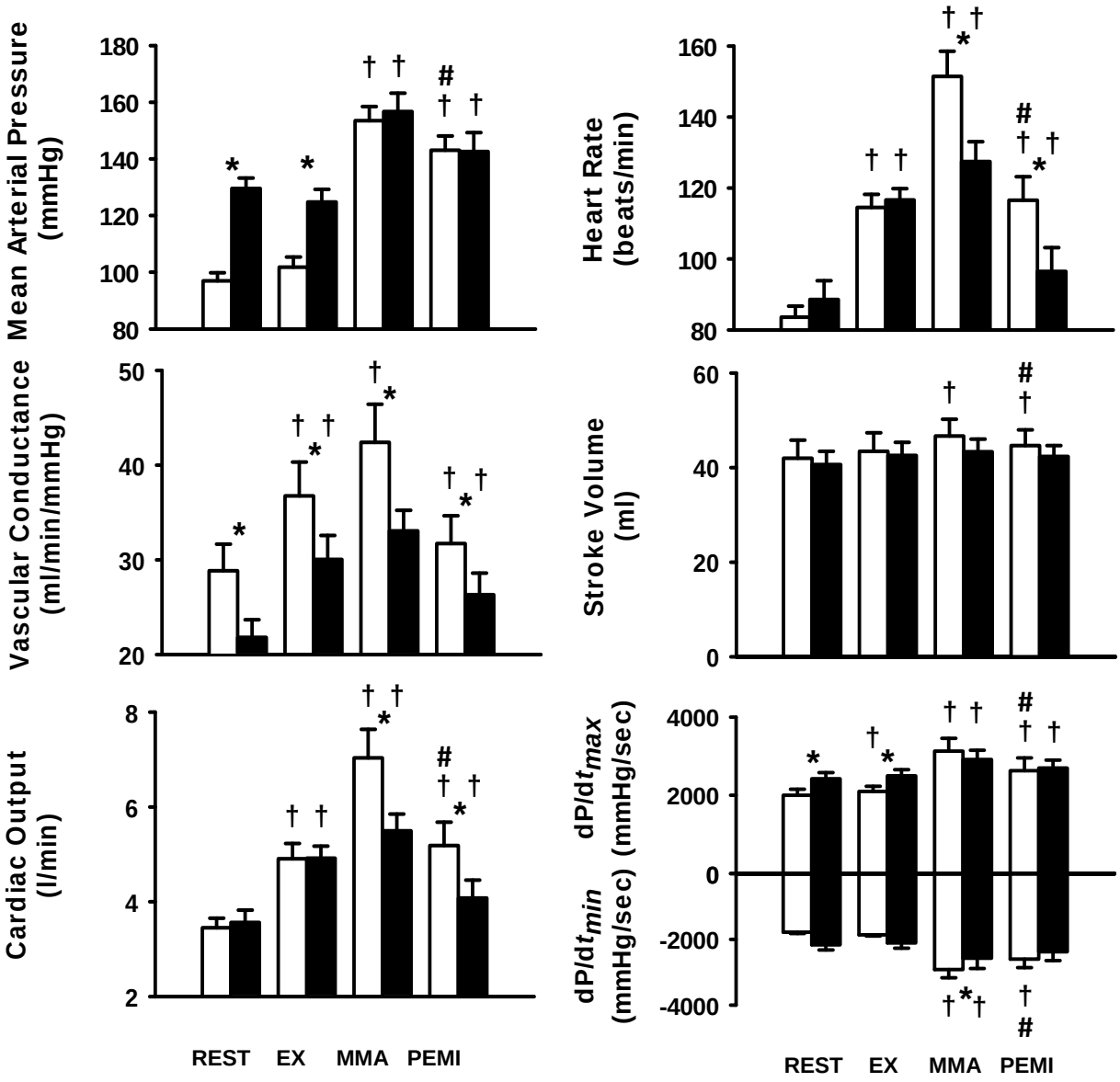

Figure 3.4. Mean hemodynamic responses in MAP, NIVC, CO, HR, SV, dP/dt_{max} and dP/dt_{min} during rest, mild exercise (EX), MMA and PEMI before (open bars) and after induction of HTN (filled bars). * - $p<0.05$ between normal and HTN; † - $p<0.05$ from previous setting; # - $p<0.05$ between POST-EX and PEMI.

Discussion

Our major new findings are that the attenuated muscle metaboreflex-induced pressor response in HTN is not sustained during PEMI and the mechanisms sustaining the pressor response during PEMI in normal subjects are altered in HTN.

Muscle metaboreflex activation during exercise in normal subjects elicits a

substantial pressor response which is sustained during PEMI (4; 16; 19; 30-32; 45; 46; 102; 123; 132; 144; 157; 161). We recently demonstrated that this sustained pressor response during PEMI is supported by a sustained increase in CO and not peripheral vasoconstriction (150). The sustained CO during PEMI was driven by enhanced chronotropy coupled with a sustained SV which was supported by enhanced inotropy and lusitropy. We further concluded that the mechanisms mediating metaboreflex-induced pressor responses are similar both when the reflex is activated during dynamic exercise and when the reflex is sustained during the recovery from exercise (PEMI). Several studies have demonstrated a "switch" from a flow-mediated to a vasoconstriction-mediated pressor response during exercise when the reflex increase in CO is attenuated (14; 29; 59; 68; 140). In the present study we observed substantially attenuated metaboreflex-induced CO and MAP responses in HTN as recently shown by Sala-Mercado et al. (136). However, the pressor response during PEMI was not sustained via enhanced peripheral vasoconstriction. During PEMI, CO, HR, dP/dt_{max} and dP/dt_{min} fell to normal recovery levels and, despite a downward baseline shift in NIVC during all settings, the recovery pattern of NIVC was similar to control indicating no increase in peripheral vasoconstriction. Therefore, the attenuated pressor response was not sustained during PEMI in HTN.

Few studies have investigated muscle metaboreflex function during PEMI in HTN and the findings are equivocal. Farquhar and colleagues recently demonstrated in elderly hypertensive humans that the pressor response to static handgrip exercise (30-40% MVC) is exaggerated in HTN and sustained during PEMI (35; 52). However, in a previous study from this group with similar subjects (139), the pressor response to rhythmic handgrip exercise (60% MVC) was not exaggerated in HTN, yet MAP was

sustained during PEMI. In contrast, Rondon et al. (126) did not find an exaggerated pressor response to static handgrip exercise (30% MVC) in humans with untreated HTN and blood pressure fell to baseline during PEMI. While the lack of a sustained pressor response during PEMI in the hypertensive groups is in agreement with the present study, the absence of a sustained pressor response during PEMI in the normotensive group is perplexing. The lack of congruency of the present and aforementioned studies may be due to several confounding factors including: differences in species, age, type and intensity of exercise performed and experimental methodology. We discussed several of these issues in a previous study (150).

Studies from Farquhar and colleagues have reported sustained elevations in muscle sympathetic nerve activity (MSNA) during PEMI in HTN indicating enhanced peripheral vasoconstriction (35; 52). However, as CO was not measured in these studies, to what extent CO contributed to the maintenance of MAP during PEMI is unknown. The conflicting results of the present study and those from Farquhar and colleagues is potentially due to differences in exercise intensity and the muscle group in which the reflex was evoked. For example, CO responses are often seen during PEMI following more intense exercise, whereas with PEMI following lower intensity exercise the pressor response occurs primarily via peripheral vasoconstriction (19; 30; 32; 144). In our model we robustly activated the metaboreflex, whereas Farquhar and colleagues elicited the reflex only during 30-40% MVC. Moreover, as opposed to evoking the reflex from one arm, we engage the reflex from both hindlimb muscles during full body dynamic exercise. In general, with PEMI following leg exercise, HR and CO remain elevated, while with PEMI following moderate arm exercise there is little HR or CO response (5; 45; 123). Therefore, it is possible that the experimental model of Farquhar

and colleagues is one that is driven by peripheral vasoconstriction and not CO. Indeed previous studies both support (19; 30-32; 123; 132; 144; 150) and refute (16; 32; 123) any role for CO in mediating the pressor response during PEMI. As such, we would not expect the pressor response to fall during PEMI in HTN as in our model.

Contrary to our hypothesis, we did not observe a vasoconstriction-mediated pressor response during PEMI in HTN. Following induction of HTN, there was a marked downward baseline shift in NIVC during all settings indicating enhanced peripheral vasoconstriction, however the recovery pattern of NIVC during PEMI was similar to control indicating no increase in peripheral vasoconstriction. In the present study, and a few previous studies (14; 27; 28; 83), we observed a small, but significant increase in NIVC with metaboreflex activation. We have concluded from preliminary data that this significant increase in peripheral vascular conductance is due to a metaboreflex-induced release of epinephrine from the adrenal glands mediating a β_2-mediated vasodilation (80). The rise in NIVC with metaboreflex activation after induction of HTN was blunted. Whether this is due to reduced epinephrine release, fewer beta receptors or enhanced sympathetic α_1-mediated vasoconstriction opposing the β_2-mediated vasodilation is unknown.

In summary, we previously demonstrated in normal subjects that CO sustains the pressor response during PEMI. Following induction of HTN, metaboreflex-induced CO and HR responses were markedly attenuated and the mechanisms sustaining the pressor response during PEMI were altered as CO and HR fell to normal recovery levels. Moreover, the recovery pattern of NIVC during PEMI was similar to control indicating no increase in peripheral vasoconstriction. Therefore, the attenuated pressor response was not sustained during PEMI in HTN.

CHAPTER 4

Exaggerated Coronary Vasoconstriction Limits Muscle Metaboreflex-Induced Increases in Ventricular Performance in Hypertension

Abstract

Increases in myocardial oxygen consumption mainly occur via increases in coronary blood flow (CBF) inasmuch as cardiac oxygen extraction is high even at rest. However, during exercise, sympathetically-mediated constrictor tone can limit increases in CBF. Increased sympathetic nerve activity (SNA) during exercise may stem from muscle metaboreflex activation (MMA). SNA is often elevated even at rest in subjects with hypertension (HTN). We tested whether HTN causes exaggerated coronary vasoconstriction during mild treadmill exercise with MMA (imposed via reducing hindlimb blood flow by ~60%) which thereby limits increases in CBF and ventricular performance. Experiments were repeated after α_1-adrenergic blockade (prazosin; 75 μg/kg) and in the same animals following induction of HTN (Goldblatt 2K1C model). HTN increased mean arterial pressure from 97.1 ± 2.6 to 132.1 ± 5.6 mmHg at rest and MMA-induced increases in CBF, left ventricular dP/dt_{max} and cardiac output were markedly reduced to only 32 ± 13%, 26 ± 11% and 28 ± 12% of the changes observed in control. In HTN, α_1-adrenergic blockade restored the coronary vasodilation and increases in ventricular function to the levels observed when normotensive. We conclude that in HTN, exaggerated MMA-induced increases in SNA functionally vasoconstrict the coronary vasculature impairing increases in CBF which limits oxygen delivery and ventricular performance.

Introduction

When oxygen demand of working skeletal muscle exceeds oxygen supply the

muscle releases metabolites (e.g., hydrogen ion (21; 157), potassium ion (95), diprotonated phosphate (21; 146), lactate (34; 86; 128; 143; 147) and ATP (62; 91; 153)) which stimulate metabolically-sensitive group III/IV afferents (3; 7; 24; 86; 102; 128). This reflexively increases sympathetic outflow from the brainstem – termed the muscle metaboreflex (20). When engaged during submaximal dynamic exercise, the metaboreflex increases heart rate (HR) and ventricular contractility resulting in large increases in cardiac output (CO) and mean arterial pressure (MAP) (4; 11; 31; 38; 94; 116; 132; 150; 163). The rise in CO is driven by marked increases in HR (5; 31; 43; 67; 114; 138; 163) coupled with a sustained or slight increase in stroke volume (SV) (27; 31; 116; 138; 144; 150) which is supported via enhanced ventricular contractility (dP/dt_{max}) (27; 30; 68; 116; 138), lusitropy (dP/dt_{min}) (68; 150) and central blood volume mobilization (CBVM) (140). This increase in CO is the primary mechanism mediating the rise in arterial blood pressure as little if any net peripheral vasoconstriction is observed (14; 30; 59; 68; 144; 150; 163). Therefore, the muscle metaboreflex has been described as a flow-sensitive, flow-raising reflex (120) that works to improve oxygen supply to the ischemic working muscle (117).

During exercise, the increased cardiac oxygen demand is met primarily via increases in CBF inasmuch as oxygen extraction is already ~80% even at rest (37; 40; 156). The rise in CBF is achieved via coronary metabolic and feed-forward, β_2-adrenergic vasodilation (37; 40; 156) which is restrained by the vasoconstrictor actions of the rise in cardiac SNA (57). Muscle metaboreflex activation increases CBF solely via the large increase in MAP; despite large increases in cardiac work (greater CO pumped against a higher afterload), no coronary vasodilation occurs due to enhanced α_1-adrenergic-mediated coronary vasoconstriction (10; 27; 119). Blockade of the

constrictor tone increases CBF during muscle metaboreflex activation resulting in improved ventricular performance and CO (27; 28; 119).

Resting SNA is heightened in hypertension (HTN) (51; 54; 72; 97; 101; 105; 155). Moreover, an enhanced sympathetically-mediated coronary constrictor tone and restrained coronary metabolic vasodilation during dynamic exercise has been demonstrated in HTN (55). Recent studies support (23; 35; 52; 106; 107; 139) and refute (126) accentuated muscle metaboreflex function in HTN. Very recently, we reported attenuated metaboreflex-induced chronotropic and inotropic responses in HTN (136). We hypothesized that enhanced coronary vasoconstriction contributes to the impaired ability to increase ventricular performance during muscle metaboreflex activation in HTN.

Methods

Experimental subjects. Six mongrel canines were designated for the study. All animals were healthy, adult, female, ~20-25 kg in body weight, acclimatized to the laboratory surroundings and willing to run on a motor-driven treadmill. During experimentation, all animals exercised voluntarily and no negative reinforcement techniques were utilized. The protocols developed and employed in the present 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*.

Surgical procedures. Each animal was completely instrumented with chronic, indwelling cardiovascular devices following two sterile surgical procedures: left thoracotomy followed by a left flank retroperitoneal surgery. The animals recovered a minimum of ten days prior to the second surgery and a minimum of seven days prior to

the first experiment. During preoperative care, the animals were initially sedated with acepromazine (0.4-0.5 mg/kg IM). Following adequate sedation, the animals were anesthetized with a combined treatment of ketamine and diazepam (5.0 and 0.22 mg/kg IV, respectively). Anesthesia was maintained with isoflurane gas (1-3%) following endotracheal intubation. In addition, the animals received preoperative administration of cefazolin (antibiotic, 30 mg/kg IV), carprofen (analgesic, 2.0 mg/kg IV), buprenorphine (analgesic, 0.01 mg/kg IM) and fentanyl (analgesic, 125–175 µg/h (72h) TDD). Prior to the left thoracotomy, animals received selective intercostal nerve blockade with bupivacaine HCL (2.0 mg/kg). Following each surgical procedure, animals received cefazolin (30 mg/kg IV) and prophylactic cephalexin (antibiotic, 30 mg/kg (BID) PO) therapy for the term of the experimental protocol. During the 12h postoperative period, animals were closely monitored and received buprenorphine and acepromazine (0.05 and 0.5 mg/kg IV, respectively) as needed to control any potential discomfort. For the following 10 days, animals received carprofen (4 mg/kg (OPD) PO).

In the first surgical procedure, the thoracic cavity was opened via a left thoracotomy (4th intercostal space) approach. The pericardium was cut and reflected to expose the heart. An ultrasonic perivascular flow probe (20PAU, Transonic Systems) was positioned around the ascending aorta to measure CO. Approximately 10 cm caudal to the thoracotomy incision, an implantable telemetry blood pressure transmitter (TA11 PA-D70, Data Sciences International) was tethered subcutaneously. The catheter of the transmitter was tunneled into the thoracic cavity through the 7th intercostal space and the tip was inserted and secured inside the left ventricle for measuring left ventricular pressure (LVP). Lastly, the left atrial appendage was reflected and the circumflex branch of the left coronary artery was exposed. An

ultrasonic perivascular flow probe (3PSB, Transonic Systems) was positioned around the circumflex artery to measure coronary blood flow (CBF). The pericardium was loosely re-approximated, the cables were tunneled subcutaneously and exteriorized between the scapulae and the chest was closed in layers.

In the second surgical procedure, an incision was made in the left flank cranial to the iliac crest. The abdominal aorta was exposed and an ultrasonic perivascular flow probe (10PAA, Transonic Systems) was positioned around it for measuring hindlimb blood flow (HLBF). All side branches of the terminal aorta, between the common iliacs and the flow probe, were ligated and severed. In addition, two perivascular hydraulic occluders (8-10 mm, DocXS Biomedical Products) were positioned around the terminal aorta (distal to the flow probe) to provide the means to incrementally reduce HLBF. A 19-gauge polyvinyl catheter (Tygon, S54-HL, Norton) was advanced through a ligated lumbar artery and secured into the terminal aorta cranial to the probe and occluders to measure arterial pressure. Lastly, the left renal artery was exposed and an ultrasonic perivascular flow probe (4PSB, Transonic Systems) was positioned around the vessel to measure renal blood flow (RBF). A perivascular hydraulic occluder (4-6 mm, DocXS Biomedical Products) was positioned around the renal artery (distal to the flow probe) to provide the means to reduce RBF. The cables and vascular occluder tubing were tunneled subcutaneously and exteriorized between the scapulae and the abdomen was closed in layers.

Data acquisition. After complete postoperative recovery, each animal was brought into the laboratory and allowed to roam freely and acclimate for approximately 15-20 minutes. Following this period, the animal was directed onto the treadmill where its instrument cables and tubing were unpacked and prepared for data collection. The

CO, RBF, CBF and HLBF flow probe cables were connected to transit-time perivascular flow meters (TS420, Transonic Systems). The LVP transmitter was turned on and the signal was collected via telemetry (Data Sciences International). The arterial catheter was aspirated, flushed and connected to a pressure transducer (Transpac IV, ICU Medical). All aforementioned hemodynamic variables, in addition to MAP (calculated) and HR (triggered by the CO signal), were monitored as beat-by-beat averages and real-time waveforms by a data acquisition system (LabScribe, iWorx) and recorded for subsequent off-line analysis.

Induction of hypertension. HTN was induced via a modified Goldblatt (2K1C) model (48). After competition of control experiments, blood flow to the left kidney was reduced to a target level of ~30% of baseline via partial inflation of the renal vascular occluder. The level of RBF was checked at least 2x/day and the vascular occluder adjusted until flow was stable. HTN gradually developed over the next several weeks. We defined HTN as a systolic pressure ≥ 140 mmHg and a diastolic pressure ≥ 90 mmHg. The experiments were repeated after 34.4 ± 1.6 days of sustained HTN. Thus, the experiments were longitudinal in nature as each animal served as its own control.

Experimental procedures. Both control and experimental procedures began with the animal standing unrestrained and still on the treadmill until all resting hemodynamic data were observed to be stable (typically 5-10 min). The treadmill was turned on and the speed was gradually increased to 3.2 km/h at 0% grade (mild exercise for a canine). Steady-state was generally reached within 3-5 minutes. The muscle metaboreflex was engaged via partial reductions in HLBF during mild, dynamic exercise.

Data analysis. CO, CBF, HLBF, RBF, LVP, HR and MAP data were continuously recorded during each experimental procedure. Other hemodynamic parameters were

calculated during off-line data analysis (e.g., stroke volume (SV), dP/dt_{max}, dP/dt_{min}, total peripheral resistance (TPR), non-ischemic vascular conductance (NIVC), coronary vascular conductance (CVC) and cardiac power (CP)). TPR, NIVC, CVC and CP were calculated as (MAP/CO), [(CO-HLBF)/MAP], CBF/MAP and [(MAPxCO)/451] (42), respectively. Due to technical difficulties, we were only able to obtain dP/dt_{max} data for five animals. One minute averages of steady-state data were calculated at rest, during exercise and during metaboreflex activation. Mean values were averaged across all animals to obtain the population mean of the study.

For slope analysis (Figure 4.5, left panel), all data points were fitted to two linear regression lines (initial response and metaboreflex-induced response lines) and three points were used to characterize the responses: free-flow exercise (mild exercise with no reduction in HLBF), threshold (intersection of the two linear regression lines) and max (maximal metaboreflex-induced response at lowest imposed level of HLBF). For Figure 4.7, a straight line was ascribed (via least squares linear regression analysis) to all data points (i.e., all graded reductions) between and including free-flow exercise and max (from left to right, respectively) for each of the four conditions. In Figure 4.8, the ratio of the changes in CVC and CP from free-flow exercise to max (i.e., ΔCVC:ΔCP) were calculated for each of the four conditions. For Figure 4.9, a straight line was ascribed (via least squares linear regression analysis) to all data points: rest, free-flow exercise and max (from left to right, respectively) for each of the four conditions.

Statistical analysis. Averaged responses for each animal were analyzed with Systat software (Systat 11.0). A $P < 0.05$ was set to determine statistical significance. A two-way repeated measures ANOVA was used to compare hemodynamic data for time and/or conditional effects. In the event of a significant time-condition interaction, a

C-matrix test for simple effects was performed. Data are reported as means ± SE.

Results

Resting MAP and TPR were significantly elevated while CO was unaffected following induction of HTN (Table 4.1).

Table 4.1. MAP, CO and TPR (mmHg, l/min and mmHg/l/min ± SE, respectively) at rest in the same animals before (normal) and after induction of hypertension.

	Normal			Hypertension		
	MAP	CO	TPR	MAP	CO	TPR
Rest	97.1 ± 2.6	3.44 ± 0.18	28.7 ± 1.9	132.1 ± 5.6*	3.56 ± 0.17	37.6 ± 2.4*

* - $p<0.05$ between normal and hypertension.

Average steady-state MAP, HR, SV, CO and NIVC (Figure 4.1) and CBF, CVC, dP/dt_{max} and CP (Figure 4.2) values are shown during rest, mild exercise and muscle metaboreflex activation in control and after α_1-adrenergic blockade in normal animals and in the same animals after induction of HTN.

Normal: control vs. α_1-adrenergic blockade. In control, HR, CO, NIVC, CBF, CVC, dP/dt_{max} and CP increased from rest to exercise, while MAP and SV remained unchanged. Metaboreflex activation elicited increases in all cardiovascular parameters. Following α_1-adrenergic blockade, resting MAP and SV were lower, HR and CVC were higher and CO, NIVC, CBF, dP/dt_{max} and CP were similar with respect to control. MAP, HR, CO, NIVC, CBF, CVC, dP/dt_{max} and CP increased from rest to exercise, while SV remained unchanged. Metaboreflex activation increased all cardiovascular parameters. MAP and SV were lower, HR, CO, NIVC CBF, CVC and dP/dt_{max} were higher and CP was similar with respect to control during metaboreflex activation.

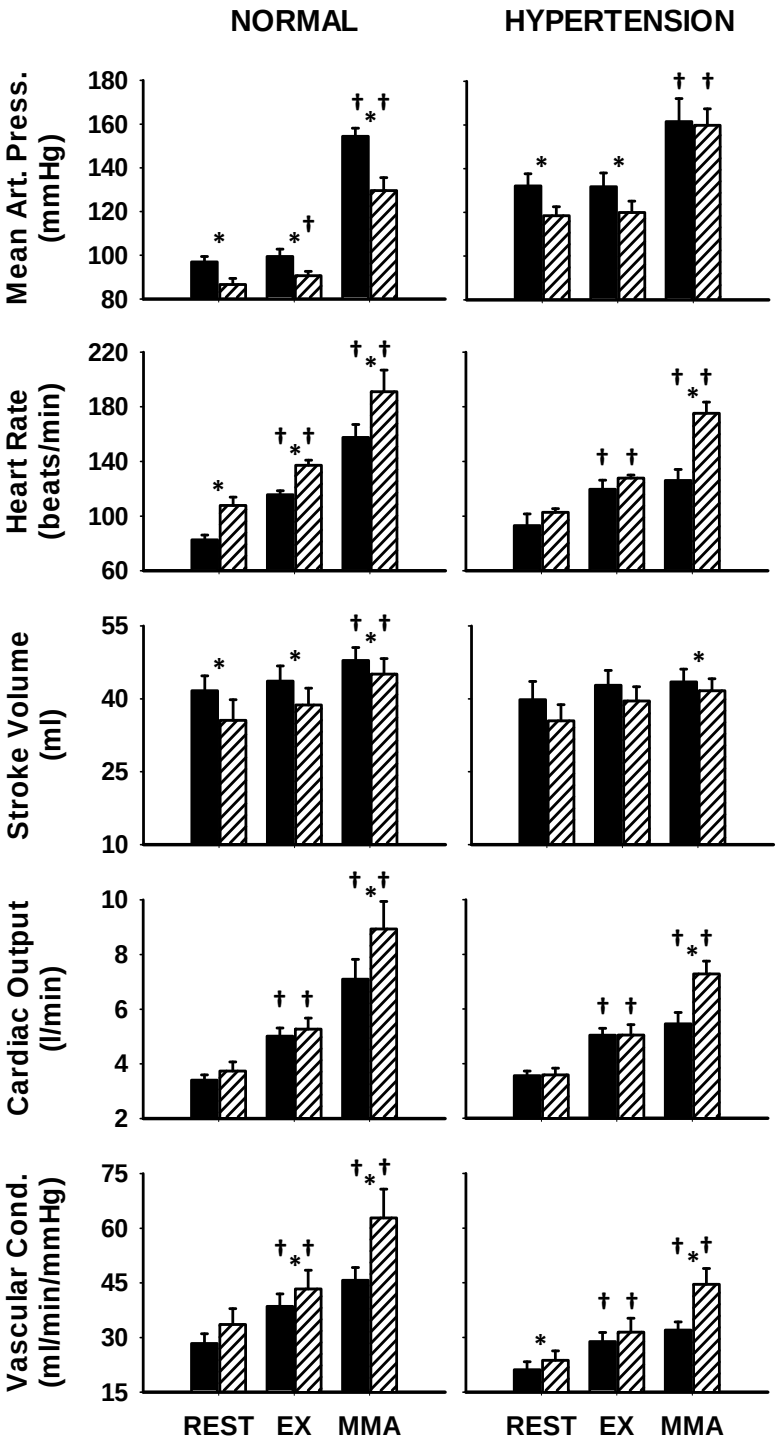

Figure 4.1. Average steady-state mean arterial pressure (MAP), heart rate (HR), stroke volume (SV), cardiac output (CO) and non-ischemic vascular conductance (NIVC) values during rest, mild exercise (EX) and muscle metaboreflex activation (MMA) in control (filled bars) and after α_1-adrenergic blockade (striped bars) in the same animals before (normal) and after induction of hypertension. * - $p<0.05$ between control and α_1-adrenergic blockade; † - $p<0.05$ from previous setting.

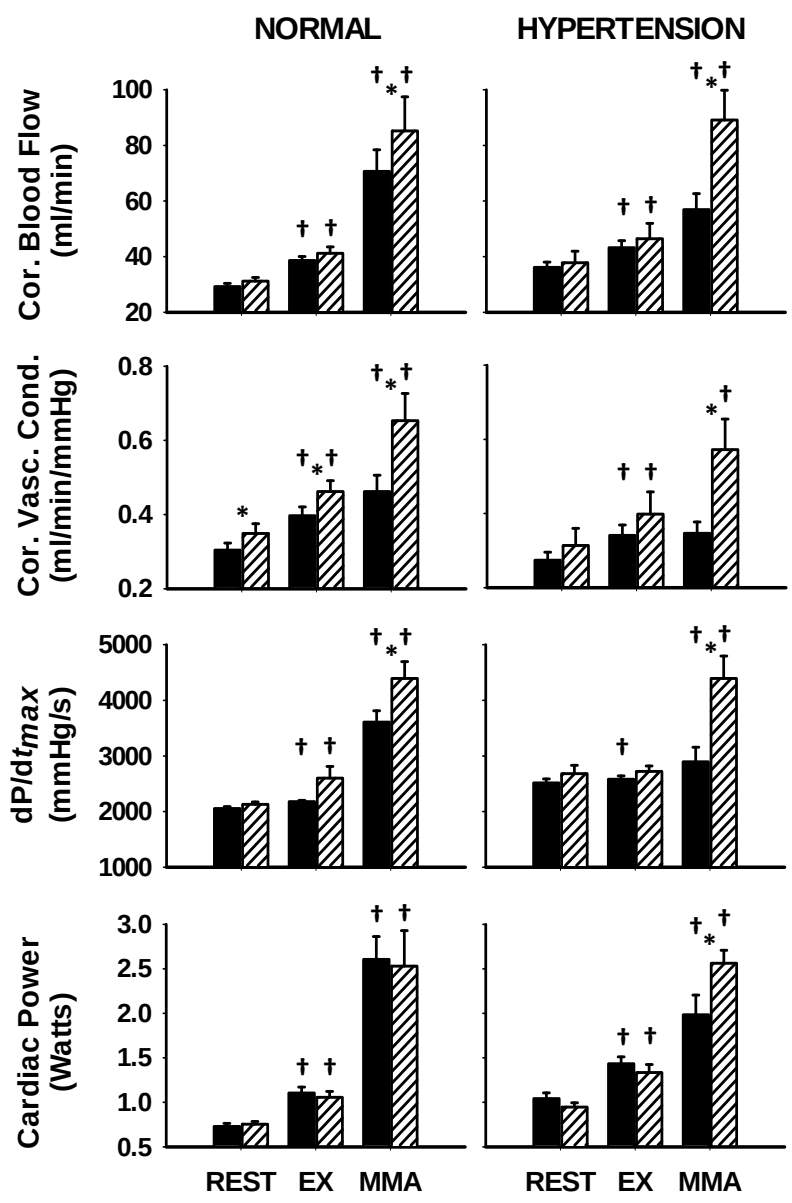

Figure 4.2. Average steady-state coronary blood flow (CBF), coronary vascular conductance (CVC), dP/dt_{max} and cardiac power (CP) values during rest, mild exercise (EX) and muscle metaboreflex activation (MMA) in control (filled bars) and after α_1-adrenergic blockade (striped bars) in the same animals before (normal) and after induction of hypertension. * - $p<0.05$ between control and α_1-adrenergic blockade; † - $p<0.05$ from previous setting.

Hypertension: control vs. α_1-adrenergic blockade. In HTN, HR, CO, NIVC, CBF, CVC, dP/dt_{max} and CP increased from rest to exercise, while MAP and SV remained unchanged. Metaboreflex activation elicited increases in MAP, HR, CO, NIVC, CBF, dP/dt_{max} and CP, but did not affect SV and CVC. Following α_1-adrenergic blockade, resting MAP was lower, NIVC was higher and HR, SV, CO, CBF, CVC, dP/dt_{max} and CP were similar with respect to control. HR, CO, NIVC, CBF, CVC and CP increased from rest to exercise, while MAP, SV and dP/dt_{max} remained unchanged. Metaboreflex

activation increased MAP, HR, CO, NIVC, CBF, CVC, dP/dt_{max} and CP, but did not affect SV. HR, CO, NIVC, CBF, CVC, dP/dt_{max} and CP were higher, SV lower and MAP similar with respect to control during metaboreflex activation.

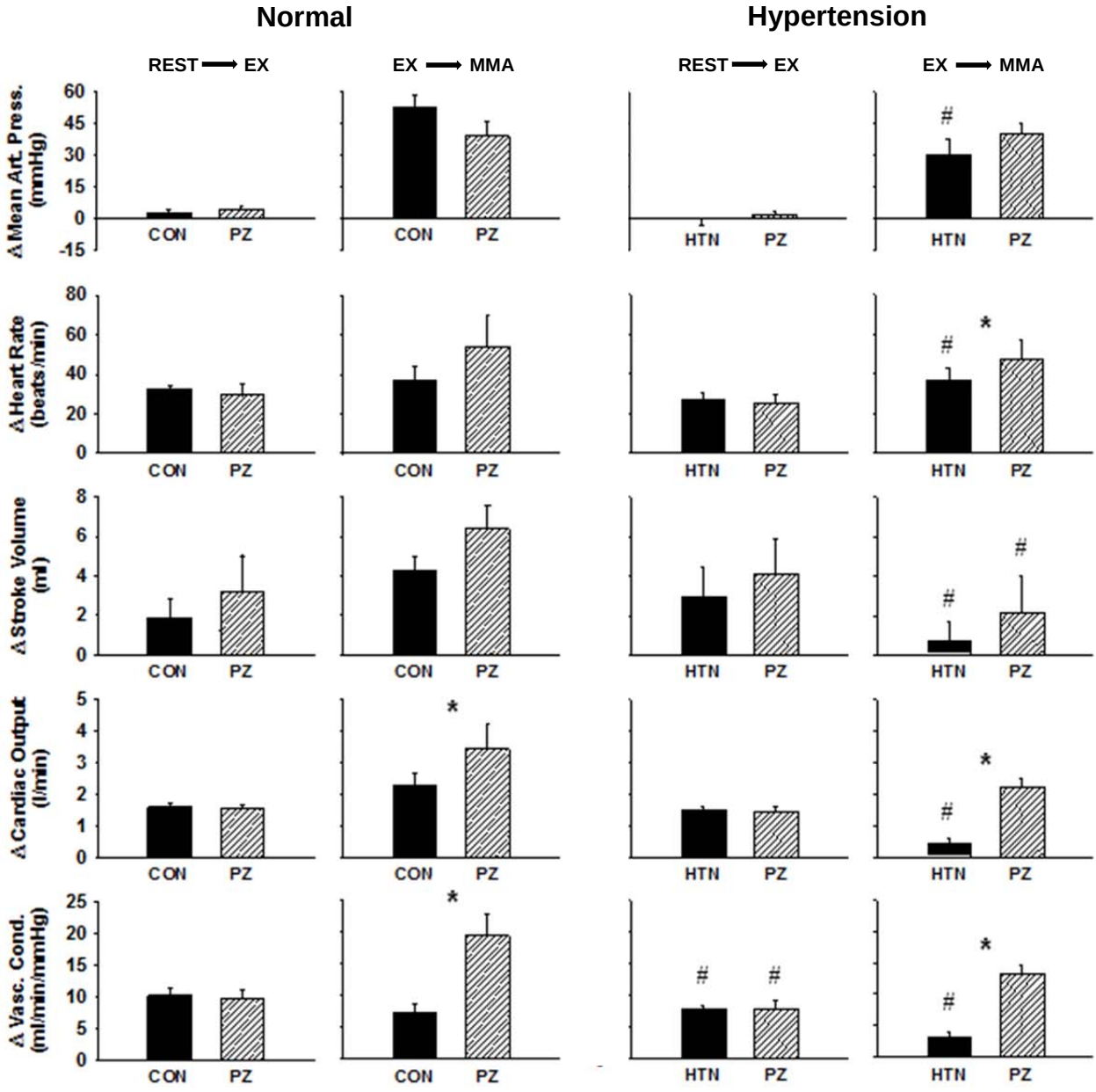

Figure 4.3. Average steady-state changes in MAP, HR, SV, CO and NIVC from rest to mild exercise (EX) and mild exercise to muscle metaboreflex activation (MMA) in control (filled bars) and after α_1-adrenergic blockade (striped bars – prazosin (PZ)) in the same animals before (normal) and after induction of hypertension. * - $p<0.05$ between control and prazosin; # - $p<0.05$ between normal and hypertension.

Average steady-state changes in MAP, HR, SV, CO and NIVC (Figure 4.3) and CBF, CVC, dP/dt_{max} and CP (Figure 4.4) are shown from rest to mild exercise and mild exercise to muscle metaboreflex activation in control and after α_1-adrenergic blockade in normal animals and in the same animals after induction of HTN.

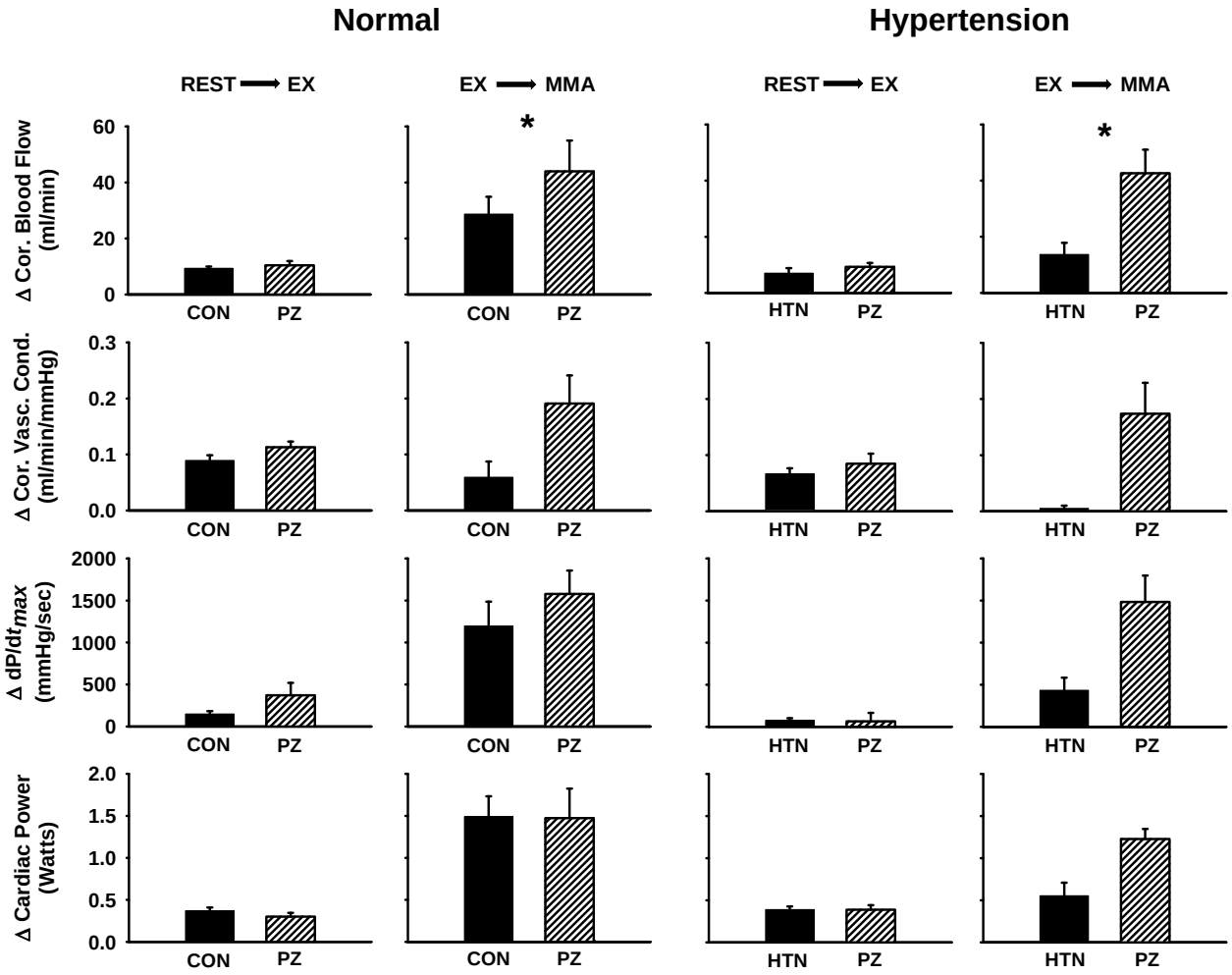

Figure 4.4. Average steady-state changes in CBF, CVC, dP/dt_{max} and CP from rest to mild exercise (EX) and mild exercise to muscle metaboreflex activation (MMA) in control (filled bars) and after α_1-adrenergic blockade (striped bars – prazosin (PZ)) in the same animals before (normal) and after induction of hypertension. * - $p<0.05$ between control and prazosin; # - $p<0.05$ between normal and hypertension.

<u>*Normal vs. hypertension*</u>. Increases in NIVC from rest to exercise in normal animals was significantly lower in the same animals following induction of HTN.

Metaboreflex-induced increases in MAP, HR, SV, CO, NIVC, CBF, dP/dt_{max} and CP were significantly attenuated following induction of HTN, while CVC was unaffected. Following α_1-adrenergic blockade, increases in MAP, HR, CO, NIVC, CBF, CVC, dP/dt_{max} and CP from mild exercise to metaboreflex activation were not any different from normal levels following α_1-adrenergic blockade.

Figure 4.5 (left panel) shows stimulus-response relationships for MAP, CO, HR, CBF, CVC and dP/dt_{max} during free-flow exercise, threshold and max, from right to left, respectively. Data were collected following graded reductions in HLBF during mild exercise in control and after α_1-adrenergic blockade in normal animals and in the same animals after induction of HTN. The slopes of the metaboreflex-response lines are indicative of the gain of the metaboreflex for each individual cardiovascular parameter. Figure 4.5 (right panel) shows the changes in the slopes of the metaboreflex-induced response lines for MAP, CO, HR, CBF, CVC and dP/dt_{max} in control and after

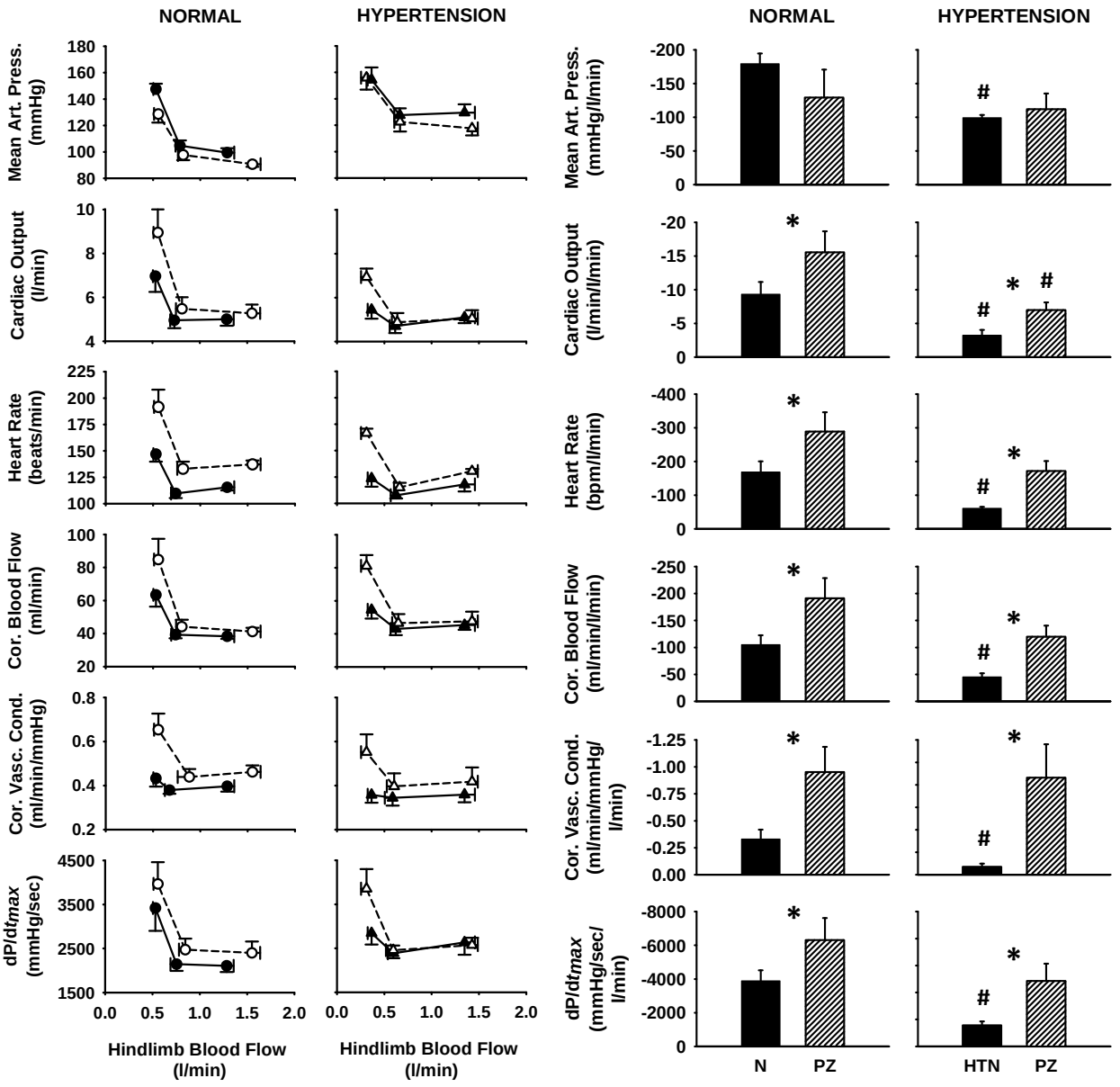

Figure 4.5. (Left panel) Stimulus-response relationships for MAP, CO, HR, CBF, CVC and dP/dt_{max} as a function of graded reductions in hindlimb blood flow (HLBF). Data plotted are free-flow exercise, threshold and max (from right to left, respectively) in control (solid lines/filled symbols) and after α_1-adrenergic blockade (dashed lines/open symbols) in the same animals before (normal) and after induction of hypertension. (Right panel) Changes in the slopes of the metaboreflex-induced response lines for MAP, CO, HR, CBF, CVC and dP/dt_{max} in control (filled bars) and after α_1-adrenergic blockade (striped bars – prazosin (PZ)) in the same animals before (normal) and after induction of hypertension. * - $p<0.05$ between control and prazosin; # - $p<0.05$ between normal and hypertension.

α_1-adrenergic blockade in normal animals and in the same animals after induction of HTN. Following α_1-adrenergic blockade, the slopes of the metaboreflex-induced response lines for CO, HR, CBF, CVC and dP/dt_{max} were significantly higher while MAP was unchanged. These slopes were significantly lower for all cardiovascular parameters following induction of HTN, while α_1-adrenergic blockade fully restored the slopes (except CO; p=0.042) to normal levels following α_1-adrenergic blockade.

The relationship between CVC and CP in one animal is shown in Figure 4.6. A straight line was ascribed from rest to free-flow exercise. Linear regression analysis was used fit a straight line through free-flow exercise and all subsequent reductions in HLBF. The relationship from rest to max was exceedingly nonlinear. Therefore, resting data were excluded and a straight line was ascribed to the remaining data points (i.e., free-flow exercise and all HLBF reductions) via linear regression analysis in order to appropriately compare the slopes of the relationship before and after induction of HTN and with and without α_1-adrenergic blockade (Figure 4.7).

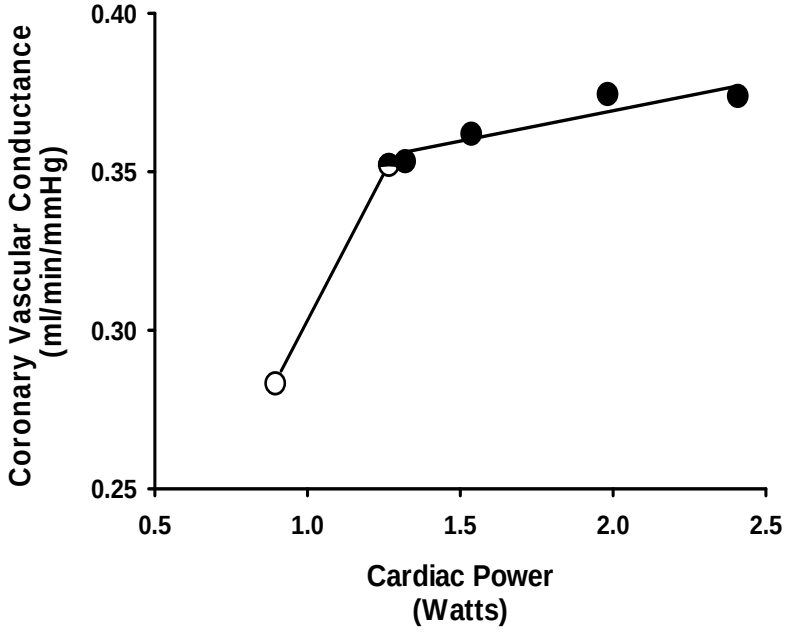

Figure 4.6. The relationship between CVC and CP in one animal during rest (open circle), free-flow exercise (half-filled circle) and all subsequent reductions in HLBF (filled circles). A straight line was ascribed from rest to free-flow exercise. Linear regression analysis was used fit a straight line through free-flow exercise and all subsequent reductions in HLBF.

Figure 4.7 shows the relationship between CVC and CP in control and after α_1-adrenergic blockade in normal animals and in the same animals after induction of HTN during rest, free-flow exercise and max (left to right, respectively). After induction of HTN, the slope of the relationship (from free-flow exercise to max) was significantly lower and the increase in CP was markedly attenuated. Following α_1-adrenergic blockade, the slope of the relationship was significantly shifted upward and not any different than during α_1-adrenergic blockade prior to induction of HTN. Moreover, the increase in CP was not any different following α_1-adrenergic blockade before and after induction of HTN.

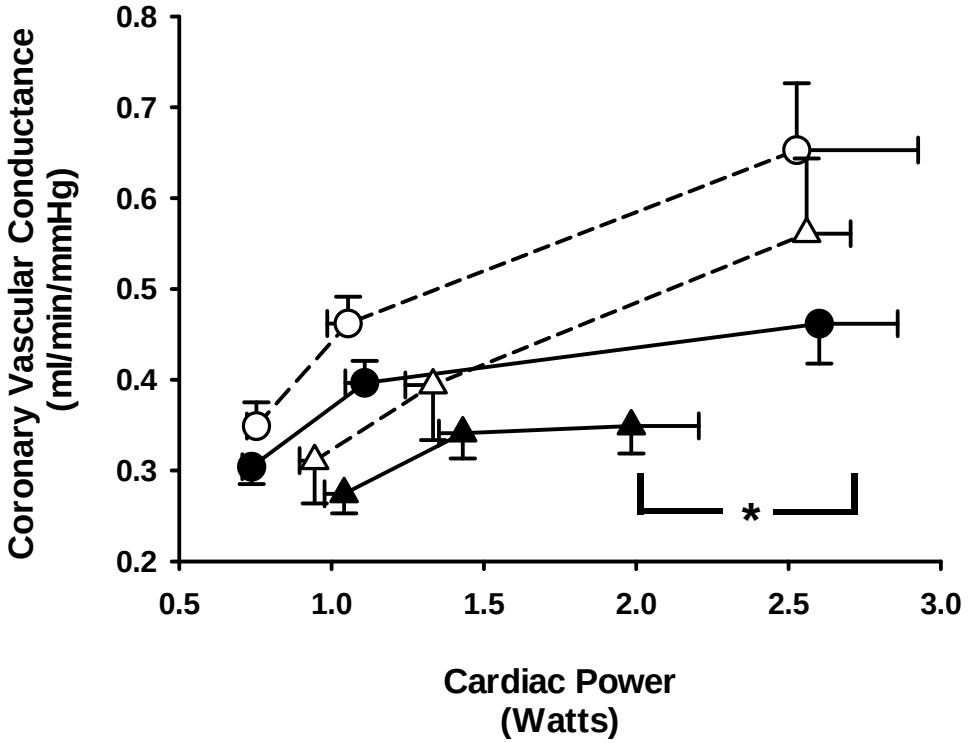

Figure 4.7. The relationships between CVC and CP during rest, free-flow exercise and max (left to right, respectively) in control (solid lines/filled symbols) and after α_1-adrenergic blockade (dashed lines/open symbols) in the same animals before (circles) and after induction of hypertension (triangles). * - $p<0.05$ max CP before and after induction of hypertension.

Figure 4.8 shows the ratio of the changes in CVC and CP from free-flow exercise to max (i.e., ΔCVC:ΔCP) in control and following α_1-adrenergic blockade before and after induction of HTN. Following α_1-adrenergic blockade, ΔCVC:ΔCP was significantly greater than in control. Following induction of HTN, ΔCVC:ΔCP was significantly reduced. Following α_1-adrenergic blockade, ΔCVC:ΔCP was not any different than during α_1-adrenergic blockade prior to induction of HTN.

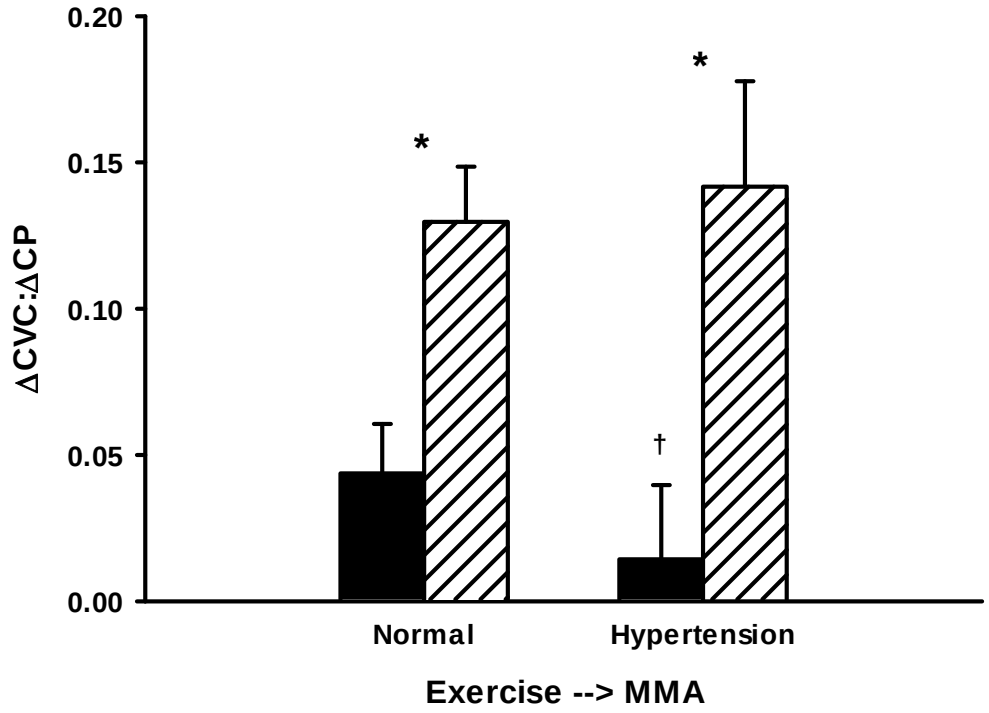

Figure 4.8. Ratio of changes in CVC and CP from free-flow exercise to max (i.e., ΔCVC:ΔCP) in control (filled bars) and following α_1-adrenergic blockade (striped bars) in the same animals before (normal) and after induction of hypertension. * - $p<0.05$ between control and α_1-adrenergic blockade; † - $p<0.05$ between normal and hypertension.

Figure 4.9 shows the relationship between dP/dt_{max} and CBF in control and after α_1-adrenergic blockade in normal animals and in the same animals after induction of HTN. The slope of the relationship between dP/dt_{max} and CBF was exceedingly linear

with all data points considered. After induction of HTN, metaboreflex-induced increases in both dP/dt_{max} and CBF were significantly attenuated. However, metaboreflex-induced increases in both dP/dt_{max} and CBF were not any different following α_1-adrenergic blockade before and after induction of HTN.

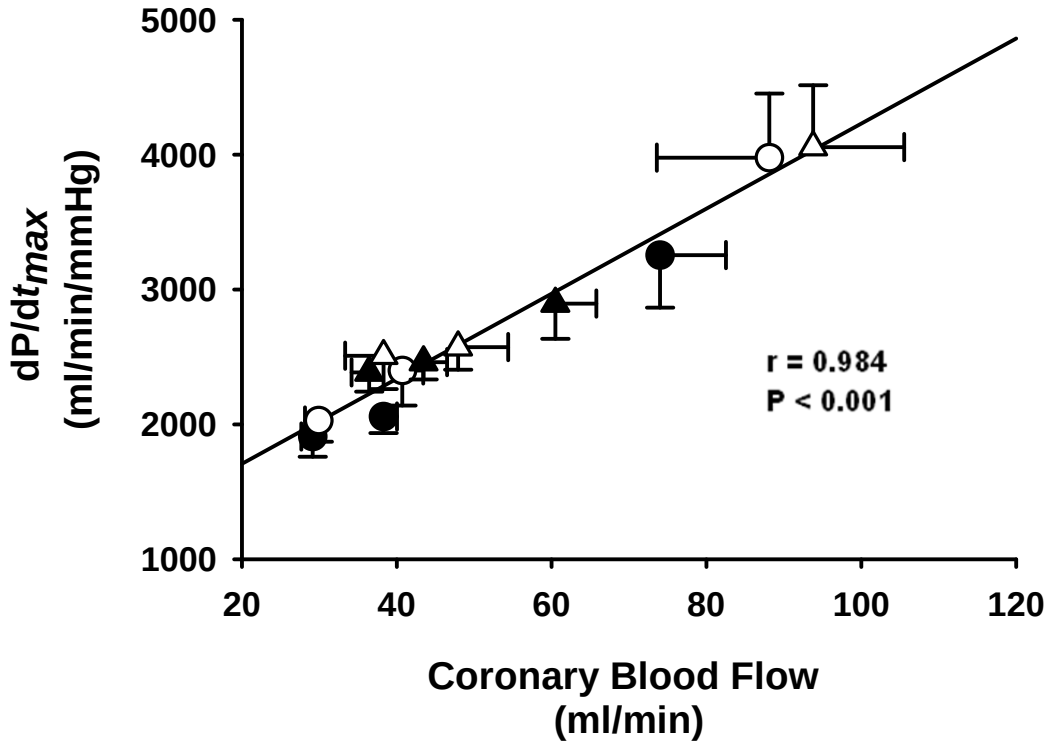

Figure 4.9. Relationship between dP/dt_{max} and CBF in control (filled symbols) and after α_1-adrenergic blockade (open symbols) in the same animals before (normal) (circles) and after induction of hypertension (triangles).

Discussion

Our major new findings are that after induction of HTN, increases in CBF during metaboreflex activation are attenuated and the ability to increase ventricular contractility is reduced. Blockade of α_1-adrenergic receptors restored the increases in CBF and ventricular function to normal levels indicating that the primary mechanism mediating the impaired metaboreflex-induced increases in ventricular function in HTN is coronary vasoconstriction. Inasmuch as metaboreflex-induced increases in CO and MAP are

also attenuated in HTN, the ability of the reflex to restore blood flow to ischemic working muscle is also likely impaired which may contribute to exercise intolerance. Moreover, coronary vasoconstriction during exercise in HTN may produce adverse cardiovascular events (e.g., coronary vasospasms, arrhythmias and myocardial ischemia).

The increase in myocardial oxygen demand during submaximal dynamic exercise is principally met by increases in CBF inasmuch as myocardial oxygen consumption is highly blood flow dependent since oxygen extraction is already near maximal at rest (37; 40; 156). The rise in CBF is largely mediated by coronary metabolic vasodilation (while ~25% of coronary vasodilation is feed-forward, β_2-adrenergic-mediated) as there is little increase in MAP (49). At the same time, the rise in cardiac SNA with exercise limits coronary vasodilation as it activates vascular α_1-adrenergic receptors inducing a coronary constrictor tone (15; 33; 57; 65; 110; 154). Gwirtz et al. demonstrated an α_1-adrenergic-mediated coronary constrictor tone during dynamic exercise in normal animals (57) and in animals with renovascular HTN (55). Moreover, a coronary constrictor tone during exercise has also been shown to attenuate increases in CBF (56; 57; 65; 84; 110), myocardial oxygen delivery (65), ventricular function (27; 56; 57; 84) and CO (27; 84). Paradoxically, it has been demonstrated that this constrictor tone plays a beneficial role in redistributing left-ventricular CBF from the epicardium to the more metabolically-challenged subendocardium (41; 66). Clearly, whether or not the presence of a coronary constrictor tone during exercise in normal individuals is advantageous or merely a cardiac-limiting consequence of elevated SNA during exercise remains debatable.

Following muscle metaboreflex activation, substantial increases in cardiac SNA, CO and afterload intensify cardiac work (4; 114; 116; 119; 132; 158; 159; 163). In this

setting, myocardial oxygen demand is met by increases in CBF which are solely driven by increases in MAP as no coronary vasodilation is observed (10; 27; 119). In fact, Coutsos et al. (27) reported an α_1-adrenergic-mediated functional coronary vasoconstriction with metaboreflex activation during exercise as metaboreflex-mediated increases in CVC and CBF markedly improved following α_1-adrenergic blockade. Metaboreflex activation in heart failure, coupled with attenuated coronary vasodilation due to a decrease in cardiac work, leads to frank coronary vasoconstriction (28). Therefore, during muscle metaboreflex activation, there is a "push-pull" dynamic for the control over coronary tone. It is likely that with the increase in cardiac work, coronary vasodilation is opposed by α_1-adrenergic-mediated coronary vasoconstriction.

Is muscle metaboreflex function attenuated in HTN? While few studies have investigated the effects of HTN on metaboreflex control of cardiovascular function, most report that its function is accentuated (35; 52; 89; 106; 107; 139; 148) rather than attenuated (126). We analyzed the gain (or strength) of metaboreflex-mediated cardiovascular responses in HTN by determining the slope of the metaboreflex-induced response line for each parameter we measured. After induction of HTN, the slopes were significantly lower for all cardiovascular parameters (Figure 4.5) indicating that metaboreflex function is attenuated in HTN. However, following α_1-adrenergic blockade the slopes of all metaboreflex-induced response lines (except CO; $p=0.042$) were fully restored to the levels observed in control experiments following α_1-adrenergic blockade. These data suggest that chronotropic and inotropic function are attenuated during metaboreflex activation in HTN due to a restriction in CBF stemming from exaggerated coronary constrictor tone, not an attenuation in metaboreflex function *per se* nor any inherent reduction in intrinsic myocardial function (e.g., heart failure).

While changes in CBF can directly influence ventricular function, changes in ventricular function can indirectly influence CBF via changes in coronary metabolic vasodilation. As an increase in perfusion pressure can increase CBF independent of any change in coronary vasomotor tone (10), the extent of coronary vasodilation can only accurately be determined by calculating changes in conductance (or resistance)(113). Feigl and colleagues (49; 50) have shown the utility of quantifying coronary vasodilation as a function of myocardial oxygen consumption. As in our previous studies (27; 28), we used cardiac power (CP) which is well correlated to myocardial oxygen consumption (81). In normal animals, CVC increased from rest to exercise and a small further increase occurred with metaboreflex activation similar to the response observed previously (with exception of the small increase in CVC which was not statistically significant in our previous study). After induction of HTN, the slope of the relationship (from free-flow exercise to max) was significantly lower and the increase in CP was markedly attenuated indicating attenuated myocardial oxygen delivery as a result of a restrained coronary vasodilation. Following α_1-adrenergic blockade, the slope of the relationship was significantly shifted upward and not any different than during α_1-adrenergic blockade prior to induction of HTN. Moreover, the increase in CP was not any different following α_1-adrenergic blockade before and after induction of HTN. The findings demonstrate that attenuation of metaboreflex-induced increases in CP are largely, if not solely, dependent on heightened α_1-adrenergic-mediated coronary vasoconstriction in HTN.

The ΔCVC:ΔCP relationship was significantly lower after induction of HTN. As the rise in CP with metaboreflex activation is smaller in HTN, this would tend to increase ΔCVC:ΔCP. Therefore, it is clear that the significant decrease in ΔCVC:ΔCP following

induction of HTN is due to a decrease in the rise in CVC with metaboreflex activation. The ΔCVC:ΔCP relationship (from free-flow exercise to max) in HTN was significantly greater following α_1-adrenergic blockade and was not any different than during α_1-adrenergic blockade prior to induction of HTN. This suggests that the rise in CVC with metaboreflex activation is markedly restrained in HTN and that this exaggerated coronary vasoconstriction can be ameliorated following α_1-adrenergic blockade.

An increase in CBF can improve ventricular function via an increase in myocardial oxygen delivery (112). To analyze the relationship between ventricular function and coronary perfusion, we plotted dP/dt_{max} versus CBF (Figure 4.8). This relationship was exceptionally linear. After induction of HTN, metaboreflex-induced increases in both dP/dt_{max} and CBF were significantly attenuated, without any change in the slope of the relationship. Following α_1-adrenergic blockade, metaboreflex-induced increases in dP/dt_{max} and CBF were restored to the same levels following α_1-adrenergic blockade prior to induction of HTN. These data support hypothesis that exaggerated coronary vasoconstriction in HTN with metaboreflex activation restrains coronary perfusion thereby limiting myocardial oxygen delivery and increases in ventricular function. While the highly linear relationship between dP/dt_{max} and CBF suggests that ventricular function is intimately dependent on changes in CBF, the fact that the slope of this relationship did not change after induction of HTN suggests that most, if not all, of the ventricular impairment observed in this study was solely due to exaggerated coronary vasoconstriction, not myocardial dysfunction *per se*. Coutsos et al. (28) reported that exaggerated metaboreflex-mediated coronary vasoconstriction attenuates the rise in CBF and impairs increases in ventricular function in a heart failure model. Furthermore, they demonstrated that this impairment in ventricular function was partially

ameliorated following α_1-adrenergic blockade. They concluded that the impaired ventricular function in heart failure was due to both intrinsic ventricular dysfunction as well as exaggerated coronary vasoconstriction.

The pathology of HTN is wide-ranging. Structural and/or functional changes have been well documented in the heart and the vasculature (122). Moreover, alterations in arterial baroreflex (22; 53; 73; 109; 149; 164; 164) and muscle metaboreflex function (35; 52; 89; 106; 107; 126; 136; 139; 148) as well as brainstem processing of this reflex information has been demonstrated (53). The attenuation of chronotropic and inotropic function after induction of HTN observed in the present study is clearly not due to overt pathology of the heart and the vasculature as α_1-adrenergic blockade fully restored chronotropic and inotropic function to the same levels observed after α_1-adrenergic blockade prior to induction of HTN. The arterial baroreflex buffers muscle metaboreflex-mediated pressor responses by attenuating its capacity to increase peripheral resistance by constricting the peripheral vasculature (82; 142). There are reports of reduced baroreflex function in HTN (73; 109; 149; 164), however, to the best of our knowledge, there are no studies demonstrating accentuated baroreflex function. Therefore, it is unlikely that the attenuated cardiovascular responses reported in the present study are a result of exaggerated arterial baroreflex buffering of the muscle metaboreflex. Moreover, Smith et al. (148) demonstrated that impaired baroreflex function in HTN has little, if any, effect on skeletal muscle reflex function. Dysfunctional metaboreflex afferent signal processing could at least partially explain the attenuated reflex-mediated increases in cardiovascular function, however data suggest that metaboreceptors are actually sensitized in HTN (106). While we did not study β-adrenergic function in the present study, studies have demonstrated

impaired cardiac responses potentially stemming from reduced β-adrenergic receptor function (18; 165) and density (103).

Potential limitations. By chance and availability, all animals in the present study were female. Data were not collected from animals during estrus. Our group (88) has shown that in dogs muscle metaboreflex function is not influenced by gender.

Systemic α_1-adrenergic blockade can alter loading conditions as it reduces total peripheral resistance. Gwirtz and colleagues (36; 56; 84) administered prazosin via the intracoronary route. However, results from such studies on changes in ventricular function following α_1-adrenergic blockade are limited to the particular segment of the left ventricle infused with drug. In the present study, we were interested in the effects of α_1-adrenergic blockade on global left-ventricular function, and therefore we infused prazosin systemically. We employed a selective α_1-adrenergic blocker, as α_2-adrenergic receptor blockade has negligible effects on coronary vasomotor tone during exercise (33; 154). Following α_1-adrenergic blockade in normal animals, MAP was lower at rest, during exercise and during exercise with metaboreflex activation with respect to control. After induction of HTN in the same animals, MAP was lower at rest and during exercise with respect to control. Interestingly, during exercise with metaboreflex activation, MAP following α_1-adrenergic blockade was not any different than control (likely due to the blunted rise NIVC in HTN). Our hypothesis was that increases in ventricular function would be impaired due to exaggerated coronary vasoconstriction during metaboreflex activation in HTN. Therefore, the major potential concern with systemic prazosin administration would be its effects on our index of contractility (dP/dt_{max}) as this measurement is sensitive to loading conditions. However, while changes in preload can significantly affect dP/dt_{max} (124), changes in afterload

have little, if any, direct effect on this parameter (1; 70; 96; 124).

In summary, we found that coronary vasoconstriction in HTN with metaboreflex activation restrains coronary perfusion and thereby limits myocardial oxygen delivery and increases in ventricular function during submaximal dynamic exercise. Accentuated coronary vasoconstriction in HTN could partially explain exercise intolerance and potentially precipitate adverse cardiovascular events during exercise such as coronary vasospasms, arrhythmias, myocardial ischemia or sudden cardiac death (64).

APPENDIX 1

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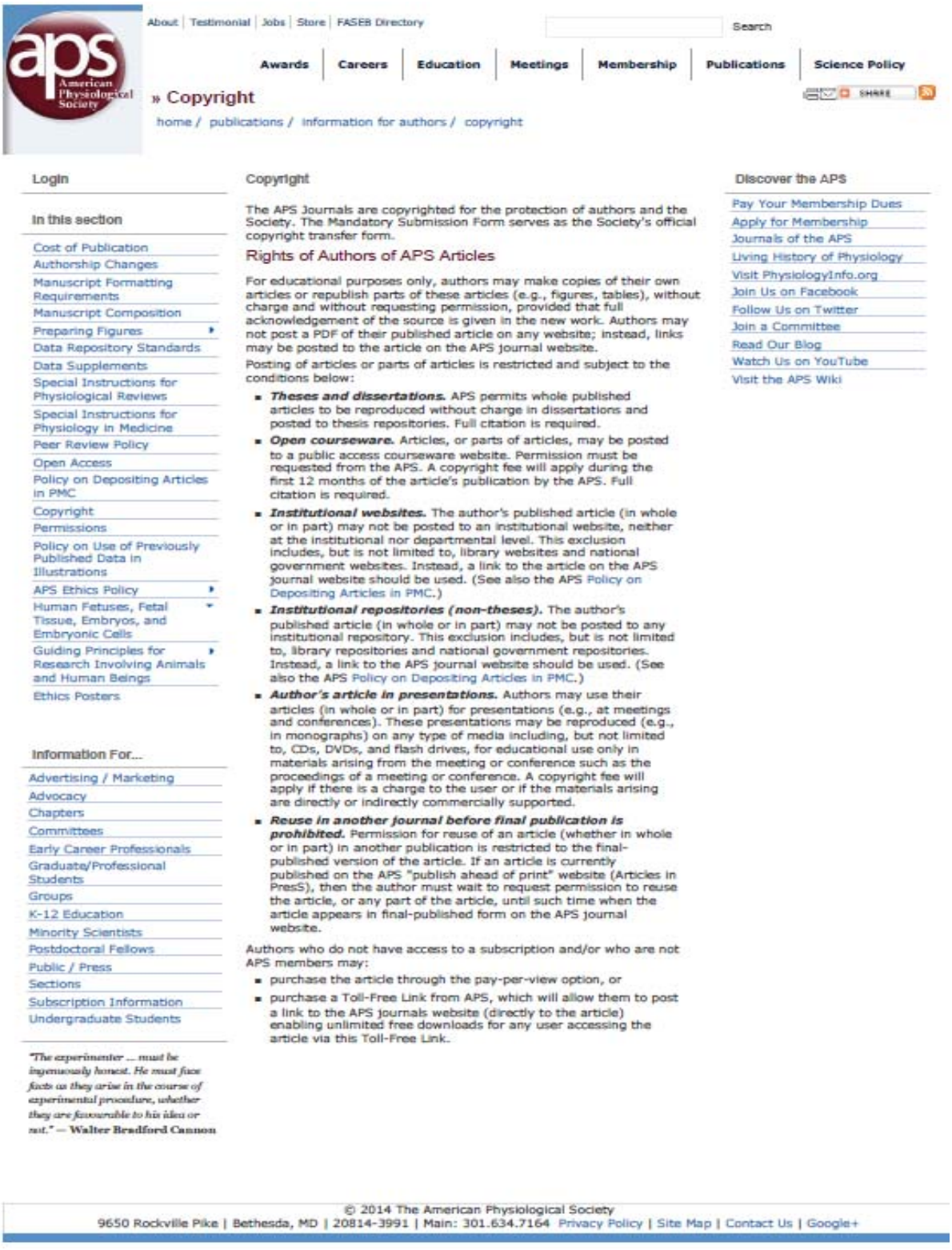

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APPENDIX 2

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 # A 3310-01

PROTOCOL # A 09-01-11

Protocol Effective Period: October 31, 2011 – September 30, 2014

TO: Dr. Donal S. O'Leary
Department of Physiology
4126 Scott Hall

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

SUBJECT: Approval of Protocol # A 09-01-11
"Integrative Cardiovascular Control During Exercise in Hypertension"

DATE: October 31, 2011

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 **October 31, 2011** through **September 30, 2014**. 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	Intact mongrel, either gender (20-25kg)	65	D
*To be purchased			
DOGS	Intact mongrel, adult females btw. 21-25 kg	3	D
**To be transferred from Dr. O'Leary's WSU protocol #A 10-16-08			

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).

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ABSTRACT

MUSCLE METABOREFLEX CONTROL OF CARDIOVASCULAR FUNCTION IN HYPERTENSION

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Skeletal muscle ischemia during or immediately following exercise leads to the accumulation of metabolites (e.g., lactate, proton and diprotonated phosphate) which activate chemoreceptive afferents within the muscle leading to a reflex increase in sympathetic outflow generating substantial increases in mean arterial pressure (MAP), cardiac output (CO) and heart rate (HR) - termed the muscle metaboreflex. When the reflex is activated during submaximal dynamic exercise, the pressor response occurs via increased CO with no net peripheral vasoconstriction. When metaboreflex activation is sustained during the recovery from exercise (i.e., post-exercise muscle ischemia – (PEMI)), whereas MAP remains elevated for as long as the ischemia is maintained, HR precipitously declines towards resting levels drawing into question the role of CO in supporting the pressor response. When the reflex increase in CO is attenuated (e.g., in heart failure), heightened peripheral vasoconstriction supports the pressor response. In hypertension (HTN), metaboreflex-mediated increases in ventricular function and CO are markedly attenuated during mild exercise. As sympathetic nerve activity (SNA) is exaggerated in both heart failure and HTN, it is plausible that the mechanisms mediating the attenuation of CO in HTN with muscle metaboreflex activation are similar

to those previously described in heart failure. Employing a chronically-instrumented canine model, data were collected from control animals and the same animal after induction of HTN. We addressed three specific questions: 1) what are the mechanism(s) mediating the muscle-metaboreflex-induced pressor response observed during PEMI in normal subjects, 2) what are the mechanism(s) mediating the muscle-metaboreflex-induced pressor response observed during PEMI in hypertensive subjects and 3) does metaboreflex activation during submaximal exercise in hypertensive animals functionally restrain coronary vasodilation further limiting increases in ventricular performance. We found that: 1) the sustained increase in MAP during PEMI is driven by a sustained increase in CO not peripheral vasoconstriction, 2) the attenuated pressor response in HTN is not sustained during PEMI as HR and CO fall to normal recovery levels and 3) in HTN, exaggerated metaborelfex-induced increases in SNA functionally vasoconstrict the coronary vasculature impairing increases in CBF which limits oxygen delivery and ventricular performance.

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Education

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Publications

1. **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 Reg Integ Comp Physiol 304-8: R657-63,* 2013.
2. 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. **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, 2014.
2. **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, 2013.
3. **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. FASEB, 2012.
4. 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 (MMA). FASEB, 2014.
5. 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 β_2-mediated vasodilation. FASEB, 2014.