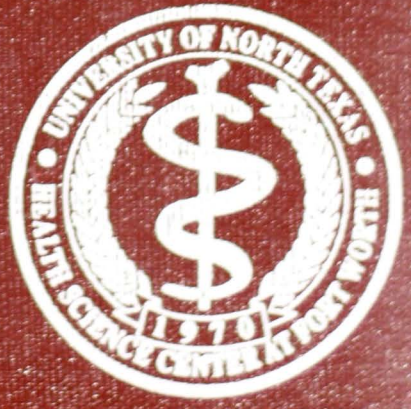
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The carotid baroreflex (CBR) exerts control of arterial blood pressure primarily as a result of changes in total vascular conductance. In humans, understanding CBR control of the vasculature supplying a given vascular bed, such as the leg, remains unclear. Furthermore, it appears that metabolic attenuation of sympathetic vasoconstriction may modulate the CBR of the vasculature supplying contracting skeletal muscle during exercise. However, the balance between baroreflex-mediated vasoconstriction and the mechanisms responsible for the metabolic attenuation has not been fully elucidated. Therefore, the purpose of the investigations within this dissertation was to: i) explain CBR control of leg vascular conductance (LVC) and the relationship between changes in LVC and muscle sympathetic nerve activity at rest and during one-legged knee extension exercise, ii) examine the CBR control of the vasculature supplying an exercising leg and a non-exercising leg during exercise, and iii) demonstrate the role of the ATP-sensitive potassium channel in contributing to the metabolic attenuation of CBR-mediated vasoconstriction in the vasculature supplying contracting skeletal muscle. In the first investigation, we demonstrated: i) the stimulus response relationships for CBR control of LVC and MSNA at rest and during two intensities of one-legged knee extension exercise; ii) that CBR control of LVC was preserved during exercise; iii) that the attenuation of CBR-mediated vasoconstriction was no different between 7W and 25W exercise in the vasculature supplying an exercising leg; and iv) that the contribution of changes in LVC

to CBR changes in mean arterial pressure was no different from rest to exercise in both the exercising leg and the non-exercising leg. In the second investigation, we examined the role of the ATP-sensitive potassium channel in modulating sympathetically-mediated vasoconstriction at rest and during exercise in the vasculature supplying an exercising leg and a non-exercising leg. The attenuated vasoconstictor response to the carotid baroreceptor stimulated hypotension observed in the vasculature supplying an exercising leg was partially restored two to four hours after the oral ingestion of glyburide (5mg). This finding indicates that ATP-sensitive potassium channel activation plays a primary role in the effects of functional sympatholysis during leg exercise in humans. We further demonstrated that CBR control of MAP was not altered by oral glyburide administration in healthy subjects.

CAROTID BAROREFLEX CONTROL OF LEG VASCULATURE

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CAROTID BAROREFLEX CONTROL OF LEG VASCULATURE

DISSERTATION

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By

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Does Reflex Sympathoexcitation Evoke Corresponding Changes in Blood Flow and Tissue Oxygenation in Human Forearm

Presented to Department of Kineisiology, UTA, Arlington, TX – *Graduate Student Seminar, 2003*

Carotid Baroreflex Control of Leg Vasculature: a proposal of studies

Presented to Department of Kineisiology, UTA, Arlington, TX – *Exercise Science Seminar, 2003*

Recent Insights into Carotid Baroreflex Function in Humans

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LIST OF ABBREVIATIONS

ABP	arterial blood pressure
CBR	carotid baroreflex
EL	exercising leg
FBV	femoral blood velocity
HR	heart rate
LVC	leg vascular conductance
LBF	leg blood flow
K_{ATP}	ATP-sensitive potassium
MAP	mean arterial pressure
MSNA	muscle sympathetic nerve activity
NEL	non-exercising leg
NO	nitric oxide
NP	neck pressure
NS	neck suction
NTS	nucleus tractus solitarius
Q	cardiac output
SE	standard error
SV	stroke volume
SVC	systemic vascular conductance
TPR	total peripheral resistance

VO_2	oxygen uptake
PO2	pressure of oxygen
PCO2	pressure of carbon dioxide
H^+	hydrogen ion
yr	year
%	percent

CHAPTER I

INTRODUCTION

The arterial baroreflex is a classic negative feedback reflex system that consists of stretch-sensitive receptors located in the carotid sinus and the aortic arch that send afferent information to the mid-brain (i.e. nucleus tractus solitarius) in regards to the degree of stretch on these receptors, resulting from changes in transmural pressures. This information is then integrated with information from other inputs (i.e., exercise pressor, central command) and in turn, results in efferent changes in both parasympathetic and sympathetic nerve activity in an effort to maintain arterial blood pressure through changes in cardiac output and systemic vascular resistance.

Using the well-established chamber technique, which utilizes 5-sec pulses of neck pressure and neck suction to selectively disengage and engage the carotid baroreflex (CBR), Fadel et al. [8] demonstrated that CBR control of muscle sympathetic nerve activity (MSNA) was unchanged from rest to exercise. Furthermore, Ogoh et al. [29, 30] identified that the vasomotor arm of the CBR (i.e. systemic vascular conductance) is the primary mediator of changes in arterial blood pressure both at rest and during dynamic exercise. Keller et al. [18] demonstrated that 5-sec of neck pressure (NP) and neck suction (NS) reduced and increased, respectively, leg blood flow and leg vascular conductance at rest and during one-legged knee extension exercise. In addition, the CBR control of the leg vasculature appeared to be altered during exercise compared to rest. However, complete CBR control of the leg vasculature had not been assessed, both at

rest, or during exercise. Furthermore, the mechanisms responsible for the alteration in CBR control during exercise remain unclear. Therefore, the purpose of this dissertation was to: i) examine CBR control of the leg vascular conductance at rest and during dynamic, one-legged knee extension exercise over a range of carotid sinus pressures in both an exercising and non-exercising leg; ii) examine the CBR control of MSNA during dynamic, one-legged knee extension exercise over a wide range of carotid sinus pressures; iii) examine the role of ATP-sensitive potassium channel inhibition on CBR control of leg vascular conductance at rest and during dynamic, one-legged knee extension exercise.

REVIEW OF RELATED LITERATURE

The Autonomic Nervous System and the Hemodynamic Response to Exercise

A number of investigations have demonstrated that many of the cardiovascular adjustments to exercise, such as increases in blood pressure, heart rate and regional vascular resistance, are mediated by decreased parasympathetic and increased sympathetic neural activity [12, 23, 47]. These autonomic adjustments to exercise are governed by two distinct mechanisms of neural control: one originating within the central nervous system and the other originating from the exercise pressor reflex [23]. Thus, exercise-induced increases in sympathetic nerve activity are mediated in part by the increased central neural drive, or central command, that accompanies voluntary motor effort [14, 15, 20, 31, 38, 42, 58] and the other mediated by a reflex mechanism that

arises from stimulation of both mechanically- and metabolically-sensitive afferent nerve endings in the contracting skeletal muscles, generally described as the exercise pressor reflex [1, 24, 25]. These excitatory signals from central command and the exercise pressor reflex are thought to be modulated by the arterial baroreflex, thus increasing the complexity of the neural control mechanisms engaged during exercise [32, 50, 54].

In exercising muscle, increased metabolic demand is matched closely by increased blood flow and oxygen delivery. Some investigations have indicated that muscle blood flow can increase up to 100-fold over resting values during intense exercise [40, 49]. This large vasodilator capacity presents a significant challenge to blood pressure control during dynamic exercise involving large muscle mass, when the blood flow needs of the exercising muscles could potentially approach or exceed maximal cardiac output [47, 49].

In the resting skeletal muscle, reflex sympathetic activation produces vasoconstriction [16]. However, the functional consequence of sympathetic activation in the exercising skeletal muscle has become a subject of considerable debate. Previous studies have *suggested* that in the exercising muscle, sympathetic vasoconstriction is well preserved [5], while others have found attenuation of sympathetic activation by the local metabolites produced during exercise [16]. Remensnyder et al. [43] first introduced the idea that sympathetic vasoconstriction in active skeletal muscles may be attenuated by local control factors, causing the vascular smooth muscle to be less sensitive to catecholamines released from the sympathetic nerve terminals. This "*functional sympatholysis*" model of vascular control implies that the local metabolic by-products

produced by contracting skeletal muscle may somehow interfere with the signal transduction pathways mediating alpha-adrenergic vasoconstriction. Thus it appears a balance exists between sympathetic vasoconstriction and local metabolic vasodilation as the exercising muscle attempts to optimize flow within the active muscle without sacrificing control of systemic arterial blood pressure.

Arterial Baroreflex Regulation of Sympathetic Nerve Activity and Blood Flow During Exercise

External neck pressure and neck suction (NP/NS) are commonly used to evaluate carotid baroreflex (CBR) control of heart rate (HR) and mean arterial pressure (MAP) at rest and during exercise [35]. Typically, the changes in MAP caused by alterations in carotid sinus transmural pressure are used to assess carotid-vasomotor responses (i.e. reflex changes in systemic vascular resistance). This approach is based upon the findings that: i) acute changes in carotid sinus transmural pressure have minimal effects on stroke volume [29]; ii) the peak HR response NP/NS occurs within the first 2-3s and is back to baseline at the time the peak blood pressure response occurs [29, 42]; and iii) the application NP/NS causes reflex-mediated changes in muscle sympathetic nerve activity (MSNA) that are not significantly different between rest and exercise [8]. Thus, the CBR-induced changes in arterial blood pressure are mainly a function of changes in vascular smooth muscle tone (vascular conductance) generated by reflex alterations in sympathetic nerve activity, whereas CBR-mediated changes in HR in response to the 5-sec NP/NS are primarily a result of alterations in the vagal control of the heart and

provide minimal contribution to baroreflex control of blood pressure during orthostasis or exercise [29, 30].

In 1990, Rowell and O'Leary [48] proposed a hypothetical model of arterial baroreflex control of sympathetic nerve activity being 'reset' during exercise. Subsequently, this model was tested and based upon findings from both Gallagher et al. and Querry et al. in 2001, [13, 14, 38], modified. In experiments from our laboratory, we modeled the CBR stimulus response curves for heart rate (HR) and blood pressure and were able to demonstrate that resetting had occurred. Resetting of the CBR was said to have occurred, because the responding range (the maximum to minimum change in the dependent variable, i.e. HR, or MAP), was relocated vertically upward to a higher pressure or HR and the operating range (the maximum to minimum change in the independent variable, i.e. carotid sinus pressure), was relocated horizontally rightward to the prevailing pressure of the exercise. This occurred without a change in the slope of the curve at the centering point (maximal gain, G_{MAX}). This allows the baroreflex to respond to a wide range of changes in blood pressure during exercise as effectively as at rest.

Strange et al. [54] demonstrated that during leg cycling between 40-70% VO_{2max}, R-wave gated pulses of NS (–50 mmHg) significantly increased leg vascular conductance. (LVC). However, at a higher workload (-88% VO_{2max}) there was no significant increase in LVC to the same NS stimulus, suggesting that NS had failed to withdraw the sympathetic control of the vasculature. Strange et al. [54] concluded that the high sympathetic activity during near maximal exercise may not be under the control of the arterial baroreflex. However, Sheriff et al. [52] had previously demonstrated the

important restraining role of the arterial baroreflex in the regulation of sympathetic nerve activity during exercise, using an ischemic hind-limb dog model and sino-aortic denervation. In the baroreceptor denervated dog model [52], the arterial blood pressure response to hind-limb ischemia during exercise was markedly augmented at higher workloads, clearly demonstrating the role of tonic inhibitory arterial baroreflex regulation of sympathetic nerve activity. Furthermore, in 2001, Collins et al. [5], using bilateral carotid occlusion, were able to demonstrate the contribution of cardiac output and peripheral vasoconstriction to the carotid sinus hypotension-induced pressor response at rest and during treadmill exercise in the dog. Collins et al [5] demonstrated that hindlimb vasoconstriction, expressed as absolute conductance, contributed progressively more to total vascular conductance from rest to exercise in an intensity-dependent manner. They concluded that the relative importance of CBR-induced vasoconstriction in the vasculature supplying the active skeletal muscle increased with increasing exercise intensity. Because the author did not present changes in hindlimb vascular conductance in response to bilateral carotid occlusion as a percent change from baseline at rest, or during exercise, they found no indication of a *functional sympatholysis*. However, if one reviews the graphical data presented by Collins et al. [5] it appears that functional sympatholysis was present.

Fadel et al., [8] recently demonstrated that the CBR control of MSNA is preserved during steady-state, dynamic arm cycle ergometry (50%VO_{2MAX}). The authors of this investigation confirmed that the CBR-induced changes in MSNA were no different for a range of NP and NS (+45 to –80Torr) at rest, or during arm cycle

ergometry when expressed as a percentage change from the respective resting and exercising baselines. However, because the authors demonstrated an increase in the steady-state MSNA during exercie (~50%), the absolute change in MSNA to any given NP/NS would have been greater. More recently, an investigation by Keller et al. [18] demonstrated, for the first time, CBR-mediated changes in leg blood flow and leg vascular conductance. Using NP (+40Torr) and NS (-60Torr) in conjunction with Doppler ultrasound technology, the authors demonstrated that the CBR alters both leg blood flow and vascular conductance at rest and during dynamic, one-legged knee extension exercise. From this investigation, the data suggested that the percent change in leg vascular conductance was attenuated during exercise in the vasculature supplying the exercising leg when compared to rest. More importantly, and taking into consideration that the absolute MSNA response to NP/NS was not measured in this investigation. In addition, the vasoconstrictor response in the exercising leg was also attenuated when compared to the non-exercising leg during the same exercise. However, the investigation employed only one intensity of NP and NS each, and therefore, did not generate complete CBR function curves at rest or during exercise. Furthermore, the MSNA was not measured and, therefore, no direct measure of the sympathetic activity during the exercise, or in response to NP/NS was obtained. However, the work of Keller et al. [18] confirmed the findings of Tschakovsky et al. [56], who demonstrated a blunted sympathetic neural vasoconstriction during rhythmic handgrip exercise and identified a progressive blunting of endogenous norepinephrine-mediated vasoconstriction (tyramine infusion) during increasing workloads of rhythmic handgrip exercise compared to rest.

Clearly, a number of questions remain regarding the balance between an impaired vasoconstrictor response in active skeletal muscle, yet which may possess sufficient vasoconstrictor reserve in the vasculature supplying both exercising and non-exercising muscle during exercise to maintain arterial blood pressure [5, 30]. Recent work by Pawelczyk and Levine in 2002 [33] demonstrated, in humans, a differential vasoconstrictor responses between the arm and the calf to phenylephrine, a selective alpha-1 agonist. Recently, Wray et al. [63] demonstrated a clear, progressive attenuation of alpha-adrenergic vasoconstriction in the vasculature of the leg during dynamic one-legged exercise using selective drug infusions. The authors demonstrated similar degrees of the exercise-induced blunting of both alpha-1 and alpha-2-mediated vasoconstriction with alpha-2-mediated vasoconstriction being attenuated at relatively lower workloads than alpha-1. Rosenmeier et al., demonstrated similar findings in the forearm during handgrip exercise [45]. In humans, the legs represent a significantly more relevant vascular bed for the regulation of arterial blood pressure; however, the degree of sympatholysis and the functional relevance of the blunting of the *CBR-mediated vasoconstriction* remains to be answered. Furthermore, the mechanisms by which local metabolic factors modulate adrenergic vasoconstriction has not been fully elucidated.

Functional Sympatholysis and ATP-Sensitive Potassium Channels

The ATP-sensitive potassium (K_{ATP}) channel has been identified in vascular smooth muscle and is widely distributed throughout the cardiovascular system [26]. The K_{ATP} channel has been suggested to be a major factor in mediating functional

sympatholysis because: *i)* opening of potassium channels in vascular smooth muscle generally results in hyperpolarization and vasorelaxation [36]; and *ii)* K_{ATP} channel activity is sensitive to changes in the concentrations of intracellular metabolites (e.g. ATP, nucleotide diphosphates), as well as to changes in extracellular factors such as O_2, H^+, adenosine, prostacyclin and nitric oxide [26, 37].

Thomas et al. [55] investigated the possibility that activation of K_{ATP} channels by some metabolic product of skeletal muscle contraction was a key mechanism by which sympathetic vasoconstriction was attenuated in exercising skeletal muscle. They reported that pharmacologic activation of K_{ATP} channels by diazoxide in resting hindlimb of anaesthetized rats attenuated sympathetic vasoconstriction in a dose-dependent manner [55]. In contrast, sympathetic vasoconstriction was well preserved during peak reactive hyperemia following a 60s occlusion of the iliac artery or during infusion of the β-adrenoceptor agonist isoproterenol, used as vasodilator controls. Together these data suggest that the diazoxide-induced attenuation of sympathetic vasoconstriction was a specific effect of K_{ATP} channel activation rather than a non-specific effect of vasodilation. Furthermore, they also demonstrated that the contraction-induced attenuation of sympathetic vasoconstriction (i.e. sympatholysis) normally observed during muscle contraction was partially prevented by the K_{ATP} channel blocker glibenclimide [55]. In addition, skeletal muscle hypoxia may play an important role in K_{ATP} channel activation during contraction [22]. Thus, contraction-induced activation of K_{ATP} channels appears to be a central mechanism underlying functional sympatholysis in the rat. However, sympathetic vasoconstriction was unaffected by glibenclimide in

resting muscle, suggesting that blocking K_{ATP} channels has a greater inhibitory effect on sympathetic vasoconstriction when channels were more likely open than when channels were more likely closed, as in resting muscle.

However, in 1995, Kosmas et al. [19] used on oral dose of glyburide, a K_{ATP} channel blocker commonly used as an antidiabetic agent, to demonstrate the role of the K_{ATP} channel on the regulation of peripheral blood flow in humans at rest. In this investigation, it was found that K_{ATP} channel blockade with oral glyburide decreased basal calf blood flow, as well as peak post-occlusive (reactive hyperemia) flow for 2 and 3 hours, respectively, post administration. The authors suggested that the decreased calf blood flow and hyperemic response was directly affected by K_{ATP} channel blockade in the vasculature of the calf, implying a more active role for the K_{ATP} in the regulation of peripheral blood flow at rest.

It is unlikely that the intracellular concentration of ATP plays a primary role in K_{ATP} channel activation in contracting muscle as ATP concentrations are well maintained, even during ischemic contraction [22]. In contrast, contracting skeletal muscle produces a number of metabolites that have been postulated to act at least in part by opening K_{ATP} channels. These include hydrogen ion, lactate, adenosine, prostacyclin and nitric oxide [26, 37]. Buckwalter et al., in 2004, demonstrated that the inhibition of NO production in L-NAME treated dogs, eliminated the attenuation of alpha-1 mediated vasoconstriction during treadmill exercise [4]. However, alpha-2 mediated vasoconstriction remained attenuated during even moderate treadmill exercise after L-NAME administration. Recent findings have indicated that nitric oxide may not

contribute significantly to the blunting of alpha-adrenergic vasoconstriction in the peripheral vasculature in humans [6, 46]. In this investigation, sodium nitroprusside, an exogenous nitric oxide donor, was infused at rest in an effort to increase forearm blood flow to a degree similar to that observed during handgrip exercise. It was concluded that nitric oxide did not significantly attenuate selective alpha-1 and alpha-2 mediated vasoconstriction, nor did nitric oxide attenuate endogenous norepinephrine-mediated vascoconstriction (tyramine).

However, the experimental evidence of the role of K_{ATP} channels in the modulation of sympathetic vasoconstriction in humans is deficient. An investigation by Banitt et al. [2] in 1996 demonstrated that K_{ATP} channels do contribute to reactive hyperemia in humans in response to forearm cuff release. Other investigations, however, have shown conflicting results in the human forearm [9-11]. Whether K_{ATP} channels play a role in the baroreflex regulation of sympathetic control of skeletal muscle blood flow during exercise in humans remains a question. In animal studies, the role of the ATP-sensitive potassium (K_{ATP}) channels have been implicated in the reduced sympathetic control of the coronary [44, 57] and peripheral vasculature [16].

SPECIFIC AIMS

A number of investigations have demonstrated a primary role for the arterial baroreflex control of blood pressure as providing the necessary regulation of sympathetic neural outflow to active and inactive tissue beds during dynamic exercise [5, 8, 51]. In the past ten years, many investigations from our laboratory have identified carotid

baroreflex (CBR) regulation of blood pressure at rest and during exercise and the physiologic mechanisms involved in the resetting of the CBR that occurs during exercise [13, 14, 27-30, 34, 35, 38, 42, 61, 62]. We have recently demonstrated [8] that CBR control of muscle sympathetic nerve activity (MSNA) was the same at rest as during exercise at 50% maximal aerobic capacity (VO_{2max}). However, the functional significance of CBR control of MSNA as it relates to changes in skeletal muscle blood flow and vascular tone at rest and during exercise within the active and inactive tissue has not been fully elucidated.

There is a growing body of evidence suggesting that the regulatory role of the sympathetic neural outflow is modulated by metabolic by-products released within the active tissue which involve mechanisms of *functional sympatholysis* [3, 16]. Numerous investigations have identified the ATP-sensitive potassium (K_{ATP}) channel as being a major channel by which vascular smooth muscle tone is altered [16, 57]. A suggested mechanism of functional sympatholysis is that the contraction-induced metabolites within the active muscle activate the K_{ATP} channels and thereby partially inhibit the sympathetically mediated vasoconstriction. Thus, we are seeking to establish the contribution of baroreflex (neural) and local (metabolic and hormonal) control mechanisms in muscle perfusion and oxygen demand in active skeletal muscle during exercise.

The goal of this dissertation is to demonstrate the role of the CBR in the control of the vasculature supplying skeletal muscle both at rest and during exercise. Furthermore, we suggest that CBR control of the vasculature supplying exercising muscle is altered by

local metabolic by-products and their interaction with the K_{ATP} channels of the vascular smooth muscle within the active tissue. To test this fundamental hypothesis the following specific aims will be accomplished:

I. To test the hypothesis that the CBR function curve for control of the peripheral vasculature will reset during dynamic exercise; furthermore, the resetting of the CBR function curves will be altered with respect to the range of response in a vascular bed supplying exercising muscle compared to a vascular bed supplying non-exercising muscle during exercise

II: To test the hypothesis that a contraction-induced activation of ATP-sensitive potassium channels (K_{ATP}) will modulate CBR control of the vasculature within the active muscle during dynamic exercise via an alteration in the balance between sympathetic vasoconstriction and local metabolic vasodilation

The experiments designed to explore specific aims I and II are specifically explained in chapters 2and 3. However, we propose below a general description of the experimental design, experimental protocols and methods used to address the specific aims.

EXPERIMENTAL DESIGN

Resetting of Carotid Baroreflex Control of Leg Vascular Conductance in Active and Inactive Vascular Beds During Exercise

Five second pulses of NP and NS were used to demonstrate CBR control of LVC and MSNA both at rest and during dynamic, one-legged knee extension exercise. Doppler ultrasound technology was utilized to measure leg blood flow at the common femoral artery and therefore, used to determine changes in LVC in response to NP/NS in the exercising and non-exercising leg during steady-state exercise. We have previously shown (unpublished findings) that 5-sec pulses of NP/NS do not change the diameter at the common femoral artery and therefore, changes in blood flow as determined at this site are the result of changes in *downtream* vascular resistance. By measuring and comparing changes in LVC during exercise in the non-exercising leg to those from the exercising leg, we can consider possible alterations in CBR control of the vasculature of the exercising leg that are specific to the exercise (i.e. circulating hormones, catecholamines, etc.) that are not accounted for when making comparisons to rest.

Muscle sympathetic nerve activity will be obtained at rest and during exercise from the peroneal nerve of the non-exercising leg using standard microneurographic techniques. While one-legged exercise provides movement artifact limitations to maintaining MSNA recordings, special care was taken to secure subjects legs and torso, in an effort to minimize movement of the instrumented leg.

The Acute Effect of Oral Glyburide Administration On Carotid Baroreflex Control of Leg Vascular Conductance

To investigate the importance of ATP-sensitive potassium channels in modulating vascular responsiveness (LVC) to CBR-induced changes in MSNA, we utilized the NP/NS technique before and after subjects were administered oral doses (5mg) of the second-generation sulfonylurea, glyburide. It has been previously demonstrated that oral ingestion of similar doses of glyburide reduced resting calf blood flow, as well as diminished the hyperemic blood flow responses to cuff occlusion in humans [19]. In this investigations, oral administration of glyburide in healthy subjects resulted in decreased resting calf blood flow, reduced the peak hyperemic blood flow response to leg cuff occlusion and increased the duration of the hyperemia. Furthermore, the role of the K_{ATP} channel has been implicated as an important mediator of functional sympatholysis during exercise in the rat model [55]. However, the role of K_{ATP} channel inhibition in modulating arterial baroreflex control of leg vasculature had not been previously demonstrated.

METHODS

A brief description of the assessment of carotid baroreflex function, leg blood flow and muscle sympathetic nerve activity, as determined in these investigations, is provided in this section of the chapter.

Five-second pulses of NP/NS was applied to the subject's neck using a malleable lead collar that encases the anterior 2/3 of the subjects neck. The pressures in the collar

were generated using two vacuum motors controlled by variable autotransformers. Two-way solenoid valves were controlled by custom software developed for use with a laboratory computer. Each 5-sec pulse of NP/NS was engaged 50ms after the R-wave. The order of delivery of the pressure was as follows: +40, +20, -20, -40, -60, -80, -80, -60, -40Torr, etc. This order of delivery was used to ensure an equal number of trials throughout a condition (i.e. rest, exercise).

Doppler ultrasound technology has been used in a number of investigations to determine leg blood flow at rest and during exercise [7, 18, 21, 39, 63]. In order to determine leg blood flow using Doppler ultrasound technology, a probe is commonly placed on the skin over the common femoral artery, 2 to 3 cm proximal to the bifurcation of the femoral into the profundus and superficial femoral artery. This location is chosen for multiple reasons. First, during one-legged knee extension exercise, static and dynamic, contraction and relaxation of the leg muscles do not alter the diameter of the femoral artery at this location. Furthermore, using a site 2 to 3 cm proximal of the bifurcation of the profundus and superficial femoral artery limits the amount of noise and artifact resulting from turbulence originating from the bifurcation.

Commonly, duplex pulsed and echo Doppler ultrasound probes are used to continuously measure vessel diameter and mean leg blood flow velocity (LBV) simultaneously. Mean leg blood flow (LBF) is calculated using the formula: $LBF=LBV * \pi * radius^2$. Therefore, continuous measures of LBV and vessel diameter allows for the determination of LBF on a beat-to-beat basis for a given unit of time (i.e. cardiac cycle). To estimate vessel diameter, and therefore determine the radius of the vessel,

peak systolic and end diastolic diameters can be used with the following formula: D=(systole/3)+2*(diastole/3).

Using Doppler ultrasound technology and thermodilution during different one-legged knee extension exercise intensities, Radegran [39] demonstrated a high relationship between the two measures of flow (r=0.974) Radegran further stated that Doppler ultrasound can be used to accurately measure blood flow during steady-state dynamic knee extension exercise with high temporal resolution [39]. Doppler ultrasound technology measures of LBF has further been utilized in a number of investigations in which beat-to-beat determination of LBF was necessary [17, 21, 41, 53, 59, 60].

Muscle sympathetic nerve activity was measured in the peroneal nerve (located on the upper and outer aspect of the leg near the fibular head) by standard microneurographic techniques. First, the course of the nerve was determined by stimulating through the skin with a pencil shaped electrode. When the nerve was stimulated, involuntary twitching of the calf or foot and/or tingling sensations in these areas will occur. The twitching or sensations disappeared when the stimulation is stopped. Once the nerve was localized, two tiny, sterile, wire electrodes were inserted through the skin. This was done without local anesthesia sine the electrodes are so small they did not produce appreciable pain when inserted (tip diameter of approximately 5-10 um). One electrode was connected to the stimulator and weak electrical shocks will be given through this electrode. The position of the electrode was adjusted to elicit muscle twitches without tingling sensations. Characteristics of muscle SNA include pulse-synchronous bursts of activity occurring 1.2-1.4 seconds after a QR complex,

reproducible activation during phase II and III of the Valsalva maneuver, and no response to a pinch, skin stroking, or startle which elicits skin sympathetic activation. SNA was normalized within subjects to allow comparisons.

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CHAPTER II

CAROTID BAROREFLEX ALTERATIONS OF LEG VASCULATURE DURING ONE-LEGGED EXERCISE IN HUMANS

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ABSTRACT

The present investigation examined carotid baroreflex (CBR) control of leg vascular conductance (LVC) via changes in muscle sympathetic nerve activity (MSNA). CBR function was examined in five men and three women (25 ± 1yr, mean ± SE) using the variable pressure neck collar technique at rest and during dynamic, one-legged knee extension exercise at 7W and 25W. The CBR exhibited control of LVC at rest and during exercise in an exercising leg (EL) and a non-exercising leg (NEL). Yet, CBR-mediated decreases in LVC (percentage change) in response to carotid baroreceptor stimulated hypotension was attenuated to a similar degree from rest (~40%) in the EL at both 7W (~15%) and 25W (~12%), $P < 0.05$. However, the absolute change in LVC in response to NP was similar between rest and during exercise in the EL and NEL ($P > 0.05$). The CBR control of LVC in the NEL was no different from rest to exercise at 7W and 25W ($P > 0.05$). Furthermore, CBR control of MSNA (percentage change) was preserved from rest to 7W, despite a trend for decreased steady-state MSNA from rest (29bursts/min) to exercise (24bursts/min), $P = 0.059$. The gain of the %MSNA-%LVC relationship was reduced in the EL compared to rest ($P < 0.05$), while the gain of the %MSNA-LVC (absolute units) relationship was similar between rest and 7W exercise in the EL and NEL ($P > 0.05$). Therefore, we concluded that CBR control of LVC was preserved from rest to exercise at 7W and 25W despite a clear attenuation of the vascular response to MSNA in the EL.

INDEX TERMS
leg vascular conductance, carotid baroreceptors, neck collar technique

INTRODUCTION

With exercise, there is resetting of the arterial baroreflex control of mean arterial pressure (MAP) [20], heart rate (HR) [15, 20] and muscle sympathetic nerve activity (MSNA) [7]. Using the well-established variable pressure neck collar technique, Fadel et al. [7] eloquentyly demonstrated that the percentage change in MSNA was similar over a wide range of carotid sinus pressures at rest and during 50% of peak oxygen uptake (VO_{2peak}) arm cycling concomitant with classic rightward and upward resetting of carotid baroreflex (CBR) control of MAP.

Subsequently, Ogoh et al. [16] demonstrated that CBR-mediated changes in MAP were primarily the result of changes in vascular conductance, as opposed to changes in cardiac output, in resting humans in seated and supine positions. Ogoh et al. [17] went on to demonstrate that the CBR becomes increasingly more reliant on changes in vascular conductance in contributing to changes in MAP during steady-state leg cycling at workload intensities as low as 90 bpm compared to rest. In these investigations, Ogoh et al. [16, 17] measured changes in cardiac output, as well as changes in MAP and determined the relative contribution of CBR-induced changes in cardiac output and total vascular conductance to the changes in MAP. They demonstrated that at the time of the peak MAP response to neck pressure (NP) and neck suction (NS), the change in vascular conductance contributed 100% to the blood pressure change. However, changes in MSNA and their effects on leg vasculature during leg cycling were not reported.

With exercise, there is an increase in blood flow to the vasculature of the exercising skeletal muscle. This increase results in an increased fraction of cardiac

output directed to the skeletal muscle compared to rest. Because the maximal flow capacity of the exercising human quadriceps has been found to exceed 2.5 L/min/kg [1], it becomes imperative that a balance exist between regulation of blood flow to the exercising muscles and the regulation of arterial blood pressure. While it is clear that baroreflex control of blood pressure is preserved during exercise, the CBR control of the vasculature supplying exercising skeletal muscle, and non-exercising skeletal muscle during exercise is incompletely understood. In 2003, Keller et al. [12] used the variable pressure neck collar technique to demonstrate that the CBR exhibits control of leg vascular conductance at rest and during one-legged knee extension exercise. In the investigation of Keller et al. [12], the data indicated clear decreases and increases in leg vascular conductanca (LVC) in response to NP (hypotensive stimulus) and NS (hypertensive stimulus); however, the functional response of the CBR control of the vasculature across a range of carotid sinus pressure was not examined. Furthermore, the investigation provided no indices of MSNA from rest to exercise, or in response to the NP/NS and, therefore, did not demonstrate the relationship between changes in MSNA and the effect on LVC. Further evidence of direct CBR control of the leg vasculature during exercise has recently been demonstrated by Wray et al. [36] using intermittant 5sec pulses of +40Torr NP over five minutes to entrain the cascade of physiological responses including MSNA, LVC and tissue oxygenation. However, the functional range of response of LVC and MSNA to carotid baroreceptor stimulation remains to be defined.

O'Leary et al. [18] and Collins et al. [5] demonstrated the importance of baroreflex control of hindlimb vasculature to changes in total vascular conductance

during treadmill exercise in the dog. From these investigations, the authors concluded that changes in hindlimb vascular conductance contributed progressively more to changes in total vascular conductance in response to bilateral carotid artery occlusion (BCO). Collins et al. [5] further indicated that the vasculature supplying *inactive* tissue (renal) contributed relatively less to BCO-induced changes in total vascular conductance during steady-state exercise with increased workloads. However, the role of active and inactive skeletal muscle in baroreflex control of blood pressure in humans is not completely understood. While the baroreflex control of *inactive* vascular beds, such as the renal bed, may be altered during exercise, it is unclear how baroreflex control of *inactive* skeletal muscle is altered over a range of carotid sinus pressures at rest, or during light workload exercise of a relatively small muscle mass.

Therefore, the aim of the current investigation was to examine CBR control of leg vasculature at rest and during two low intensity workload (7W and 25W) trials of one-legged knee extension exercise over a range of carotid sinus pressures. We further sought to examine the relationship between changes in MSNA and LVC at rest and during exercise in an exercising and non-exercising leg over a wide range of carotid sinus pressures. We hypothesized that CBR control of LVC of the exercising leg and non-exercising leg would be reset during exercise in order to regulate the prevailing blood pressure required to match perfusion of the exercising muscle to it's oxygen demand.

METHODS

Subjects.

Five men and three women (age, 25 ± 1yr; height, 156 ± 9cm; weight, 68 ± 1kg; mean ± SE) voluntarily participated in the present investigation. Each subject was familiarized with the testing protocols and informed of the potential risks of participating in the current study. Subjects were provided a written explanation of the investigation and its potential risks. The subjects were requested to provide a signature to the document, thereby signing informed consent approved by the University of North Texas Health Science Center's Institutional Review Board. All subjects were healthy, nonsmokers, free of known cardiovascular and respiratory disease, and were not using prescription or over-the-counter medications. Subjects were advised not to participate in any strenuous physical activity, as well as alcohol consumption, twenty-four hours before any of the scheduled experiments. Subjects were also asked to refrain from the consumption of caffeinated beverages twelve hours before any of the scheduled experiments. Each subject visited the laboratory on two, or three separate occasions. Statistical procedures were used to estimate a sample size required to obtain sufficient statistical power based on data obtained from four subjects. This analysis predicted a subject number of five required to obtain statistical significant differences. A subject number of eight (N = 8) were recruited in the current investigation to ensure the necessary power of N = 5 was reached because of the difficulties of obtaining MSNA during exercise with and without femoral artery catheterization.

Experimental Protocol.

Each subject visited the laboratory on two separate days.

Experimental day one: Carotid baroreflex control of LVC was determined in each subject using the variable pressure neck collar technique at rest and during all exercise trials on experimental day one. After a period of resting NP/NS trials (~1hr), subjects performed 4 trials of one-legged knee extension exercise in a randomized order. Subjects performed 2 bouts of 7W workload exercise and 2 bouts of 25W workload exercise at a kicking rate of 30 kicks per minute (kpm) using a modified cycle ergometer (Ergomedic 874 E, Monark) described by Saltin et al. [26]. While kicking, the subjects were provide an audible cue using a metronome and verbally encouraged when necessary to maintain a consistency of each knee extension. The rate of kicking was set to 30 kpm to allow for adequate time in which the exercising leg was relaxed in order to optimize the integrity of the Doppler ultrasound measures during exercise. The effect of contraction rate on leg blood flow has previously been examined [10, 19]. Osada and Radegran [19] demonstrated that leg blood flow was linearly matched to workload, regardless of contraction frequency. On this experimental day, a resting data collection period of ~1hr was completed before the exercise trials. All exercise trials lasted approximately 25min. The time of the exercise trials was limited to ~25 minutes in order to eliminate the confounding effects of fatigue, or cardiovascular drift on CBR function. In an effort to minimize changes in skin blood flow, laboratory temperature on experimental days was maintained between 24 to 25°C. Each exercise trial was separated by a recovery period of ~30min to ensure return of cardiovascular variables to baseline. Two exercise trials at

each workload (7W and 25W) were performed for the collection of data from an exercising leg (EL) and a non-exercising leg (NEL) during separate trials.

Experimental day two: Carotid baroreflex control of MSNA was determined in each subject using the variable pressure neck collar technique at rest and during all exercise trials on experimental day two. The resting and exercise protocols on experimental day two were the same as experimental day one. However, only two exercise trials were performed, one at 7W and one at 25W for the collection of data of steady-state MSNA and CBR control of MSNA from each exercise intensity. Exercise trials were performed in random order. Microneurographic recordings of MSNA were successfully obtained in eight subjects at rest, in seven subjects during 7W exercise and three subjects during 25W exercise.

Measurements and Procedures.

All testing was performed with subjects in a semi-recumbent ~60 degree back supported seated position, resulting in an ~120 degree leg-to-torso angle to optimize one-legged exercise performance, as well as Doppler ultrasound measurements. Cardiovascular variables were monitored beat-to-beat and recorded on a personal computer (PC) equipped with customized software (Necsuc3) that collects and records data on each R-wave, as well as a second PC equipped with an on-line data acquisition program (DI-720, Dataq Instruments, Akron, OH). Heart rate (HR) was monitored with a standard lead II electrocardiogram (ECG). The ECG signal was output to a monitor (model 78342A, Hewlett-Packard, Andover, MA) interfaced with the PC. Arterial blood pressure (ABP) in seven subjects was measured using a Teflon catheter (18 gauge, 1.35-mm) connected

to a pressure transducer (Maxxim Medical, Athens, TX) placed in the femoral artery of the exercising leg. In one subject, arterial blood pressure was obtained using finger-cuff photoplethysmography (Finapres, Ohmeda 2300) on the middle finger of the right hand and calibrated to match the diastolic blood pressure achieved from brachial auscultation. Subjects were fitted with a malleable lead neck collar for the application of NP and NS. Carotid baroreflex function was assessed at rest and during one-legged knee extension exercise after steady-state hemodynamic conditions had been achieved (~5 minutes) as previously described by Potts et al. [20].

Leg Blood Flow.

Leg blood flow was determined using pulsed Doppler ultrasound velocimetry using the product of the femoral artery mean blood velocity and diameter. Femoral blood velocity (FBV) was obtained using a Doppler unit (model MD6 D.E. Hokanson, Inc., Bellevue, WA, USA) with a bidirectional probe operating at a frequency of 5 MHz and calculated using the formula $V = f_d/(64.9\cos\text{Ø})$, were f_a is the audio frequency, Ø is the angle of insonation and V is the blood velocity in cm/sec. The Doppler probe was placed to the skin over the common femoral artery distal to the inguinal ligament. The angle of the transducer crystal relative to the skin was ~60 degrees. Femoral artery diameter was measured using a 2.5 MHz probe (model RT 6800, GE) at a site matching that at which velocity was measured. Average femoral artery diameter was determined at rest and during one-legged knee extension exercise in the EL and the NEL. All ultrasound data of femoral arterial diameters were recorded onto VHS tape and further analyzed using custom software. The femoral artery radius was determined for each subject at each

condition using the formula: radius = diamter/2. All resting FBV and resting femoral artery diameter data were measured from one leg of each of the subjects (i.e., right, or left) before any exercise trials were performed. Femoral artery diameter was not changed to 5-sec pulses of either NP and the following formula was used to calculate leg blood flow (LBF): LBF = π * radius^2 * FBV.

Muscle sympathetic nerve recordings

Postganglionic MSNA was recorded using standard microneurographic techniques [33]. A microelectrode was inserted into the peroneal nerve near the fibular head of the non-exercising leg (left leg in all subjects). The nerve signal was processed by a preamplifier and an amplifier (nerve traffic analyzer model 662C-3, Department of Bioengineering, University of Iowa, Iowa City, IA) with a total gain of 90,000. Amplified signals were band-pass filtered (700-2,000 Hz), rectified and discriminated. Raw nerve signals were integrated by a resistance-capacitance circuit with a time constant of 0.1sec. Muscle sympathetic nerve recordings were recognized by their pulse-synchronous burst pattern and increased burst frequency with end-expiratory breath holds without any responses to arousal or skin stroking. These characteristics were used to discriminate between muscle and skin sympathetic nerve fibers while positioning the microelectrode.

Carotid Baroreflex Responsiveness.

Carotid baroreflex control of MAP and LBF were assessed at rest by applying single 5-sec pulses of NP and NS ranging from (+40 to –80Torr) as described by Potts et al. [20]. Under resting conditions, NP/NS was applied during a 10- to 15-second breath hold at end expiration, in order to minimize the respiratory modulation of HR and mean arterial

pressure (MAP). During exercise, NP/NS was applied without the presence of a breath hold. A minimum of 45 seconds was allowed to pass between each NP/NS trial to allow physiological variables to return to pre-stimulus values. Peak responses for MAP were determined as the greatest change over a four-second period of time that occurred from the application of NP/NS and changes from each trial were averaged to provide a mean response for each subject. Changes in FBV were determined during the four-second at which the peak MAP response occurred. An average FBV over the four-second interval was used to assess peak FBV changes for each trial compared to an average FBV for the four seconds immediately preceding the NP/NS stimulus. A four-second interval was chosen in an effort to minimize the effect of kicking frequency, and therefore the contraction relative to the relaxation phase (30kpm, 2 second kicking cycle). These changes were then averaged to provide a mean response for each NP and NS for each subject. Leg Vascular Conductance (LVC) was calculated using the following formula was used: LVC = LBF ÷ MAP.

On experimental day two, the MSNA response to NP/NS was determined during the 5sec neck chamber stimulus and calculated as the burst frequency and burst amplitude, and expressed in arbitrary units. At rest, the MSNA responses for each NP/NS were averaged to provide mean responses for each subject, which was expressed as a percentage change from the mean MSNA obtained during only a breath hold (control). During one-legged knee extension exercise, the average MSNA for each NP/NS was compared with the time interval immediately preceding the neck chamber stimulus (~20 to 30sec). Estimated changes in carotid sinus pressure were calculated as the pre-stimulus MAP minus the

chamber pressure. This estimated carotid sinus pressure (ESCP) was used in the representation of CBR control of MAP, LVC and MSNA.

Data Analyses

Stimulus-response curves for CBR control of MAP were fit for individual subject to a four-parameter logistic function described by Kent et al. [13], using the following equation: $MAP = A_1\{1 + \exp[A_2(ECSP\text{-}A_3]\}\text{-}1 + A_4$

A_1 is the MAP response range (maximum-minimum), A_2 is the gain coefficient, A_3 is the centering point (ECSP required to elicit equal pressor and depressor responses) and A_4 is the minimum MAP response. The individual data were fit to this model by nonlinear least-squares regression which minimized the sum-of-squares error to predict a curve of "best fit" for each data set. The gain of the CBR-MAP stimulus-response curve was derived from the first derivative of the logistic function of Kent et al. [13], and the maximal gain (Gmax) was calculated as the gain at the centering point (A_3). The threshold (i.e. point where no further increase in MAP occurred, despite reductions in ECSP), as well as the saturation (i.e. point where no further decrease in MAP occurred, despite increases in ECSP) were also determined. All parameters were averaged and presented as group means.

For CBR control of MSNA the logistic function of Kent et al. was not fit for each individual due to the large variability obtained in individual MSNA responses [7]. Therefore, estimates of CBR-MSNA reflex sensitivity at rest and during one-legged knee extension exercise, linear regression analysis was utilized. Similarly, we chose not to utilize the logistic model of Kent et al. [13] to generate function curves for CBR control

of LVC due to the limitations observed with MSNA. The relationship between percentage changes in MSNA and LVC (percentage change and absolute) were examined using linear regression analysis. The analysis was performed between the responses to a range of neck chamber pressures of +40, +20, 0, -20 and –40Torr. The responses to –60 and –80Torr were not utilized for the regression analysis, so as to minimize reductions in calculated gain within a given condition.

Statistical Analyses.

Comparisons of physiological variables, CBR-MAP stimulus-response parameters and CBR-LVC reflex sensitivity between rest and exercise were made using paired *t*-tests. A two-way analysis of variance was used to determine significant differences in CBR control of %LVC between rest and exercise (7W and 25W) in the exercising and non-exercising leg. For comparisons of steady-state MSNA between rest and exercise at 7W, a paired *t*-test was used for the seven subjects that maintained nerve recordings during 7W exercise. Comparisons of steady-state MSNA at 25W exercise were not made due a to low subject number. Statistical significance was set at $P < 0.05$. Values are means ± SE.

RESULTS

Cardiovascular and MSNA responses to one-legged knee extension exercise

Resting HR, MAP, LBF and LVC at rest are presented in Table 1. During one-legged knee extension exercise HR increased from a resting value of 72 ± 4 bpm to 81 ± 5 bpm at 7W and 87 ± 5 bpm at 25W ($P < 0.05$). In the exercising leg, LBF and LVC progressively increased above rest during 7W and 25W exercise ($P < 0.05$). The MAP

during 7W exercise was no different than rest (86 ± 4 vs. 87 ± 3) while MAP during 25W exercise was significantly increased compared to rest and 7W exercise (93 ± 3, $P < 0.05$). Muscle sympathetic nerve activity demonstrated a tendency to decrease during 7W exercise compared to rest (29 ± 2 vs. 24 ± 3 bursts/min), but was not statistically significant ($P = 0.059$), see figure 1. During the 25W exercise, three subjects maintained sympathetic nerve recordings (27 ± 6 bursts/min vs. 28 ± 4 at rest in same subjects); however, because of the reduced number of subjects, statistical comparisons were not made.

Carotid baroreflex control of LVC

CBR-mediated changes in LVC are presented in Figure 3 (absolute) and Figure 4 (percentage change). At rest, the application of NP (+40Torr) resulted in a decrease in LVC (~40%). During one-legged knee extension exercise, the application of NP (+40 and +20Torr) decreased LVC in both the exercising leg (~15% and ~12%) and non-exercising leg (~36% and ~39%). While this decrease in %LVC in the exercising leg was attenuated compared to rest ($P < 0.05$), there was no significant difference between changes in %LVC to NP (+40 and +20Torr) between exercise at 7W and 25W in the exercising leg ($P > 0.05$). The increases in %LVC in response to NS were no different across–20 to –80Torr at rest and during exercise, $P > 0.05$. The absolute change in LVC in response to NP at rest and during exercise in the exercising and non-exercising leg demonstrated no significant differences across conditions ($P > 0.05$). The estimated CBR-%LVC sensitivity over an examined range of neck chamber pressures (+40 to –40Torr) at rest (0.77 ± 0.1 %/mmHg) and was significantly reduced from rest during

exercise in the EL at 7W and 25W (0.54 ± 0.1 %/mmHg and 0.30 ± 0.1 %/mmHg), $P <$ 0.05.

Carotid baroreflex control of MSNA

Figures 5 displays the stimulus-response relationship for CBR control of MSNA at rest and during 7W and 25W exercise. The percentage change in MSNA in response to NP/NS from +40 to –80Torr was not different from rest to exercise at 7W. The estimated CBR-%MSNA sensitivity over an examined range of neck chamber pressures (+40 to –40Torr) at rest and during exercise were no different (-1.72 ± 0.6 vs. -2.88 ± 0.6 %/mmHg, respectively), $P > 0.05$.

Reflex-mediated changes in MSNA and the transduction to changes in LVC

The gain of for the relationship between percentage changes in MSNA and LVC (percentage changes and absolute) at rest and during exercise (7W) were determined using the response to a range of chamber pressures from +40, +20, 0, -20 and –40Torr are summarized in Figure 6. The gain of the relationship between changes in %MSNA and %LVC in the EL was significantly reduced compared to rest (see Figure 6A, $P < 0.05$). However, the gain of the relationship between changes in %MSNA and absolute changes in LVC in the EL was similar to rest (Figure 6B, $P >0.05$). The gain for changes in %MSNA and both percentage and absolute changes in LVC in the NEL were no different than rest ($P > 0.05$).

Carotid baroreflex control of MAP

Figure 2 displays the function curves for CBR control of MAP at rest and during 7W and 25W exercise. Table 2 illustrates CBR stimulus-response curve parameters for the

logistic modeling. All parameters for the CBR-MAP function curves were similar between rest and exercise. However, the operating point (OP) for CBR control of MAP during 25W exercise was shifted rightward and upward to the prevailing MAP of the exercise.

DISCUSSION

The major new findings from this investigation demonstrate: i) that CBR control of LVC is present at rest and during exercise over a wide range of carotid sinus pressures; ii) the static relationship between CBR-mediated changes in MSNA and the consequent change in LVC at rest and during one-legged knee extension exericse; iii) that the attenuated vascular responsiveness to hypontensive stimuli was not different between 7W and 25W in the exercising leg; and iv) that the CBR-mediated decreases in absolute LVC were no different in an exercising leg and a non-exercising leg. Therefore, our initial hypothesis that CBR control of LVC would be reset during exercise in order to regulate the prevailing blood pressure required to match perfusion of the exercising muscle to it's oxygen demand was supported.

Carotid baroreflex control of LVC at rest and during exercise:

In the current investigation, we have demonstrated CBR control of leg vasculature at rest and during one-legged knee extension exercise at 7W and 25W in an exercising and non-exercising leg. In response to hypotensive stimuli (+40 and +20Torr), CBR-induced decreases in %LVC were similarly attenuated during both 7W and 25W in an exercising leg compared to rest. These findings extend the work of Keller et al. [12], who recently demonstrated that NP at +40Torr decreased %LVC at rest and during one-legged knee

extension. Many animal [3, 25, 30] and human investigations [24, 32, 37] have demonstrated the consequence of muscle contraction on modulating pharmacologically-induced *sympathetic* vasoconstriction (i.e. functional sympatholysis). Wray et al. [37] recently demonstrated this attenuated vasoconstrictor response to selective α1- and α2-adrenoreceptor stimulation during one-legged knee extension exercise. These investigations indicated that both receptor subtypes in the leg vasculature are sensitive to functional sympatholysis with the α2-adrenoreceptor mediated vasoconstriction being abolished at much lower workloads (7W) than α1-adrenoreceptors (37W). This important finding may directly influence baroreflex control of the leg vasculature during exercise, both at the onset and during light workload steady-state exercise, in that it has been found that the sympathetic arm of the arterial baroreflex innervates the vascular tree at a position that exhibits a greater density of α2-adrenreceptors near the small nutrient arterioles which appears to be exquisitely sensitive to metabolic inhibition [14, 34]. In the present investigation, we found no further difference in CBR-induced decreases in %LVC in the exercising leg between 7W and 25W exercise. These data interpreted in conjunction with the findings of Wray et al. [37], that α2-adrenoreceptor mediated vasoconstriction was near maximally blunted at 7W, while α1-adrenoreceptor vasoconstriction was preserved at least to workloads equal to, or greater than 25W suggests that the influence of functional sympatholysis on arterial baroreflex control of LVC has reached near maximal effects at relatively light workloads. This may explain the lack of an increase in the absolute change in LVC in the exercising leg from rest to

the 7W exercise and the trend for an increase in absolute LVC at 25W exercise in the current investigation.

In 1991, O'leary et al. [18] demonstrated that with increasing exercise intensity, the hindlimb vasculature of an exercising dog to bilateral carotid artery occlusion became significantly greater during exercise in a workload intensity dependent manner. In 2001, Collins et al. [5] reiterated the importance of the vasculature supplying exercising skeletal muscle in its contributing to baroreflex-mediated changes in total vascular conductance. Collins et al. [5] clearly demonstrated an increased reliance on changes in exercising hindlimb vascular conductance compared to the relatively *inactive* renal vascular bed. In the current investigation, the CBR-hypertensively induced changes in absolute LVC of the exercising leg were no different during both 7W and 25W exercise compared to rest. While there was a trend for an increased absolute LVC response to NP at 25W in the exercising leg, the changes did not reach statistical significance. However, one might speculate that if the effect of functional sympatholysis on baroreflex control of leg vasculature was nearly complete at 25W (no difference between 7W and 25W), as absolute conductance in an exercising leg increases, such as with higher workloads, the consequence would be a greater relative contribution to changes in total vascular conductance.

In response to neck suction (NS), CBR-mediates increases in both absolute LVC and %LVC over a range of neck chamber pressures at rest and during exercise in an exercising and non-exercising leg. However, in the present investigation there was no significant difference between %LVC at rest and during exercise at 7W and 25W in both

the exercising leg and non-exercising leg. These findings indicate that vascular responsiveness to CBR-induced withdrawal of MSNA is unchanged during light exercise. This proposed discrepancy between NP and NS is likely due to the *active* and *passive* nature of the end-organ response. With NP, the result is an increase in MSNA directed to the vasculature causing some degree of active vasoconstriction. Certainly, during exercise, this vasoconstrictor response is counter-balanced by the influence of functional sympatholysis [2, 3, 6, 9, 11, 23, 25, 37]. However, the vascular response to the withdrawal of MSNA appears not to be altered during light workload exercise. Strange et al. [29], using repetitive pulses of NS, demonstrated that as exercise intensity increased, CBR-mediated increases in LVC were diminished. However, Strange et al. [28] used leg cycling as the exercise model and achieved much greater absolute workloads involving a much greater muscle mass. It is likely that during high intensity exercise involving a large muscle mass, competitive vasoconstrictor influences which are not present during exercise at light workloads, modulate the vascular response to baroreflex-mediated withdrawal of MSNA.

Carotid baroreflex control of MSNA at rest and during exercise:

In this investigation, CBR control of MSNA was unchanged during low workload 7W exercise compared to rest. This finding is similar to the work of Fadel et al. [7], that demonstrated a clear preservation of CBR control of MSNA during arm cycling at 50% VO_{2peak}. However, unlike the workload achieved by Fadel et al. [7], the current investigation utilized an exercise model that incorporated a light workload and a much

smaller exercising muscle mass that resulted in a slight, but not significant, reduction in steady-state MSNA ($P = 0.059$). This is markedly different than the approximate 50% increase in steady-state MSNA seen with arm cycling at 50% VO_{2peak} [7]. However, in the present investigation, the percentage changes in MSNA to a given neck chamber pressure at rest and during exercise were no different between rest and exercise confirming the preservation of CBR control of MSNA during exercise initially identified by Fadel et al. [7].

Previous investigations have demonstrated a reduced MSNA during steady-state one-legged knee extension exercise [21, 22]. It has been demonstrated in humans using handgrip exercise [35] that MSNA was not increased from resting values until workloads greater than 30% of maximal voluntary contraction were acheived. Furthermore, it has been suggested that the muscle-metaboreflex is the primary mechanism that stimulates MSNA during isometric exercises which utilize a small muscle mass, but the effect of the muscle-metaboreflex was not as affective during dynamic exercise [27]. Ray et al. [22] demonstrated a decrease in steady-state MSNA during 4min trials of upright one-legged knee extension exercise at 20, 30 and 40W workloads and suggested that the fall in MSNA was likely in response to a loading of the cardiopulmonary baroreceptors via an increase in venous return. This inhibition has been previously demonstrated by Fu et al. [8] in response to lower body positive pressure-induced increases in central blood volume. The data of the present investigation suggests increased cardiopulmonary baroreceptor load reduced MSNA during exercise. However, the decrease in steady-state MSNA during the 7W exercise did not alter CBR control of MSNA. While only

collected in three subjects, steady-state MSNA at 25W appeared to return to resting values. These findings confirm the presence of CBR control of MSNA over a wide range of carotid sinus pressure during exercise, even at workloads that result in decreased steady-state sympathetic neural outflow.

Tranduction of changes in MSNA to changes in LVC

Recently, Ogoh et al. [16] demonstrated that in resting humans, CBR-induced changes in MAP over a wide range of carotid sinus pressures are primarily the result of changes in vascular conductance. The authors further demonstrated that the reliance on changes in vascular conductance contributed entirely to the CBR-induced changes in MAP during leg cycling, at workloads eliciting heart rates as low as 90 bpm [17]. Keller et al. [12] demonstrated that NP and NS decreased and increased, respectively, LVC at rest and during one-legged knee extension. However, Keller et al. [12] did not present CBR control of MSNA, and used only one CBR hypotensive NP of +40Torr and one hypertensive NS of –60Torr stimulus. In the present investigation, it is clear that CBR control of MSNA resulted in clear changes in LVC at rest and during one-legged knee extension exercise. Recently, Wray et al. [36] used sinusoidal NP to dynamically entrain CBR control of MAP, MSNA, LVC and tissue oxygenation at rest and during one-legged knee extension exercise. Using spectral analysis, Wray et al. [36] demonstrated a reduction in the power of entrainment of CBR control of LVC at the frequency of the sinusoidal NP stimulus, while CBR control of MSNA (power) was unchanged at the frequency of the sinusoidal stimulus. In this investigation, we have demonstrated for the first time the static relationship between CBR-mediated changes in MSNA and the

consequent change in LVC at rest and during light workload one-legged knee extension exercise. Figure 6 indicates estimated relationships between changes in MSNA and LVC at rest and in an exercising and non-exercising leg at 7W. Importantly, the gain between percentage change in MSNA and absolute change in LVC was no different in either the exercising leg or the non-exercising, despite the increase in steady-state LVC of the exercising leg. Therefore, an exquisite balance must exist between changes in the conductance of a given vascular bed and the influence of contraction-induced attenuation of sympathetically-mediated vasoconstriction. In the present investigation, the effect of functional sympatholysis is evident, even at very low exercise workloads (7W and 25W). While many mechanisms have been proposed regarding the specific modulators involved in functional sympatholysis, the exact mechanisms remain unclear. The role of nitric oxide (NO) in mediating functional sympatholysis has been examined in both animal [4, 31] and human [6] investigations. Recent work by Dinenno et al. [6] addressed the role of nitric oxide (NO) in the modulation of sympathetcally-mediated vasoconstriction and demonstrated that NO is not obligatory for the presence of functional sympatholysis. However, it has clearly been demonstrated that flow-mediated vasodilation is not the mechanism by which sympathetic vasoconstriction is attenuated [32].

Potential Limitations:

In this investigation, measures of MSNA were collected from the non-exercising leg. While it has been demonstrated that MSNA is global throughout the body during exercise [27], it remains possible that the activity between vascular beds is different. Another limitation in this investigation was our inability to model CBR-MSNA and CBR-LVC

relationships using logistic function described by Kent et al. [13]. However, while it is apparent that this logistic function can be utilized to describe CBR control of MAP, HR and RRI, the use of this logistic function to describe CBR control of MSNA and LVC appears unsuitable in small subject populations because of large variability of individual responses to NP/NS for the specific variable. Fadel et al. [7] compared CBR-mediated changes in MSNA between rest and exercise as the percentage change in MSNA from the respective baseline sympathetic outflow. We too have chosen to report changes in CBR control of MSNA in a similar fashion. Also, we identified potential changes in CBR control of LVC in a similar fashion to MSNA in that both percentage changes and absolute changes in LVC in response to NP/NS were compared during rest and exercise at each chamber pressure. In terms of physiological significance of the contribution of the leg vasculature responses to arterial baroreflex control of blood pressure, we suggest that this was an appropriate choice. Another methodological limitation involves the lack of our ability using Doppler ultrasound measures of leg blood flow to distinguish the supply of other tissue in the leg (i.e. skin, bone, inactive skeletal muscle, etc.). However, with respect to CBR-mediated changes in LVC, it is likely that any modulation during exercise would originate in the active skeletal muscle.

In summary, we have demonstrated that CBR control of LVC over a wide range of carotid sinus pressures is present at rest and during low workload steady-state exercise and that the CBR-mediated decreases in absolute LVC were no different in an exercising leg and a non-exercising leg during low workload exercise. For the first time in humans, we have identified that changes in MSNA in response to arterial baroreceptor stimulation

result in similar changes in LVC at rest and during low workload exercise. A delicate balance between baroreflex-induced changes in MSNA and blood pressure regulation must exist, in the face of an immediate impact (7W) of functional sympatholysis on physiological regulation of leg vasculature. Furthermore, it appears that the vascular responsiveness to CBR-mediated withdrawal of sympathetic nerve activity is unchanged from rest to exercise at low workloads.

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FIGURE LEGENDS

Figure 1. Steady-state muscle sympathetic nerve activity (MSNA) in bursts per minute at rest (N = 8) and during one-legged knee extension exercise at 7W (N = 7) and 25W (N = 3).

Figure 2. Carotid baroreflex-MAP response curves determined with the variable pressure neck collar technique at rest and during one-legged knee extension exercise at 7W and 25W. Lines represent fitted logistic functions generated from the CBR-MAP curve parameters for the group. ECSP, estimated carotid sinus pressure; OP, operating point.

Figure 3. Carotid baroreflex-mediated changes in absolute leg vascular conductance (ml/min/mmHg) determined with the variable pressure neck collar technique at rest and during one-legged knee extension exercise at 7W and 25W in an exercising leg (EL), ***A***; carotid baroreflex-mediated changes in absolute leg vascular conductance at rest and during one-legged knee extension exercise at 7W and 25W in a non-exercising leg (NEL), ***B***. *Significantly different from rest, $P < 0.05$.

Figure 4. Carotid baroreflex-mediated percentage changes in leg vascular conductance (% change) determined with the variable pressure neck collar technique at rest and during one-legged knee extension exercise at 7W and 25W in an exercising leg (EL), ***A***; carotid baroreflex-mediated percentage changes in leg vascular conductance (% change) at rest

and during one-legged knee extension exercise at 7W and 25W in a non-exercising leg (NEL), ***B***. *Significantly different from rest, $P < 0.05$.

Figure 5. Carotid baroreflex-mediated changes muscle sympathetic nerve activity (MSNA) determined with the variable pressure neck collar technique at rest and during one-legged knee extension exercise at 7W and 25W in a *non-exercising leg*. Data are expressed as a percentage change from baseline.

Figure 6. Group average gain of baroreflex-mediated changes in muscle sympathetic nerve activity (expressed as a percentage change, %MSNA) and percent changes in leg vascular conductance (%LVC) at rest and during one-legged knee extension exercise, ***A***; group average gain of baroreflex-mediated changes in muscle sympathetic nerve activity (expressed as a percentage change, %MSNA) and absolute changes in LVC at rest and during one-legged knee extension exercise, ***B***. Gains represent group average slopes for linear regressions of percentage changes in MSNA and changes in LVC across neck chamber pressure +40, +20, 0, -20 and –40Torr, *Significantly different from rest, $P < 0.05$.

Table 1. *Physiological responses to one-legged knee extension exercise*

	Rest	Exercise 7W	Exercise 25W
HR, bpm	72 ± 4	81 ± 5*	87 ± 5*†
MAP, mmHg	86 ± 4	87 ± 3	93 ± 3*†
LBF, mL/min			
Exercising leg	261 ± 55	717 ± 75*	1185 ± 129*†
Nonexercising leg	261 ± 55	254 ± 34	344 ± 33*†
LVC, mL/min/mmHg			
Exercising leg	3.1 ± 0.7	8.4 ± 1.0*	12.8 ± 1.5*†
Nonexercising leg	3.1 ± 0.7	3.0 ± 0.4	3.7 ± 0.4

Values are means ± SE. HR, heart rate; MAP, mean arterial pressure; LBF, leg blood flow; LVC, leg vascular conductance. *Significantly different from rest ($P < 0.05$); †Significantly different from 7W ($P < 0.05$).

Table 2. *Average Carotid Baroreflex-MAP function curve parameters*

	Rest	Exercise 7W	Exercise 25W
Threshold, mmHg	65 ± 6	61 ± 4	59 ± 7
Saturation, mmHg	125 ± 5	122 ± 7	144 ± 7†
Operating point, mmHg	86 ± 4	87 ± 3	93 ± 3*†
Centering point, mmHg	95 ± 4	92 ± 4	101 ± 4†
G_{max}, mmHg/mmHg	-0.39 ± .05	-0.39 ± .04	-.034 ± .03
Response range, mmHg	22 ± 3	23 ± 2	27 ± 2*
Minimum MAP response, mmHg	73 ± 4	74 ± 3	77 ± 3

Values are means ± SE. *Significantly different from rest ($P < 0.05$); †Significantly different from 7W.

Figure 1

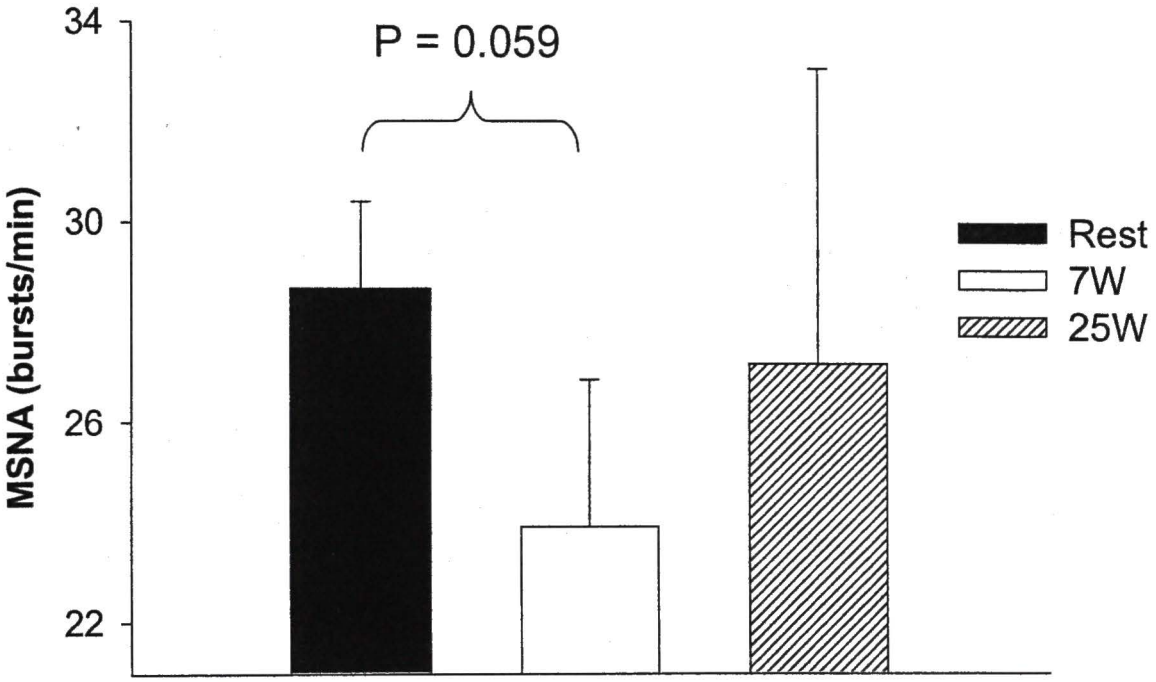


Figure 2

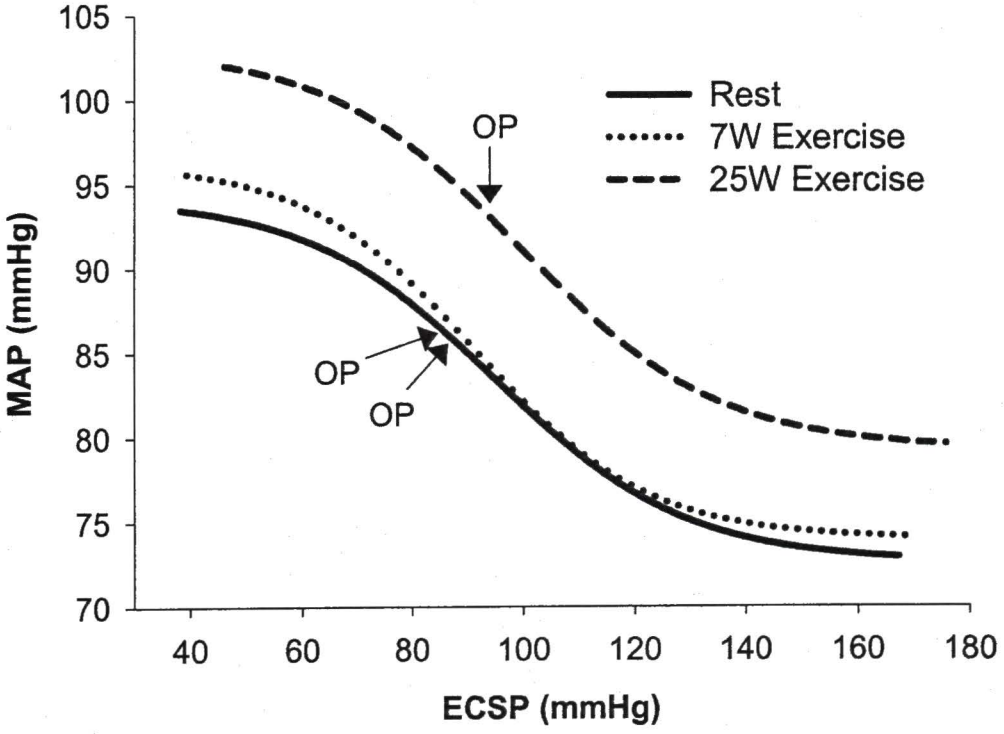

105
100
95
90
85
80
75
70
MAP (mmHg)
40
60
80
100
120
140
160
180
ECSP (mmHg)
Rest
7W Exercise
25W Exercise
OP
OP
OP

Figure 3

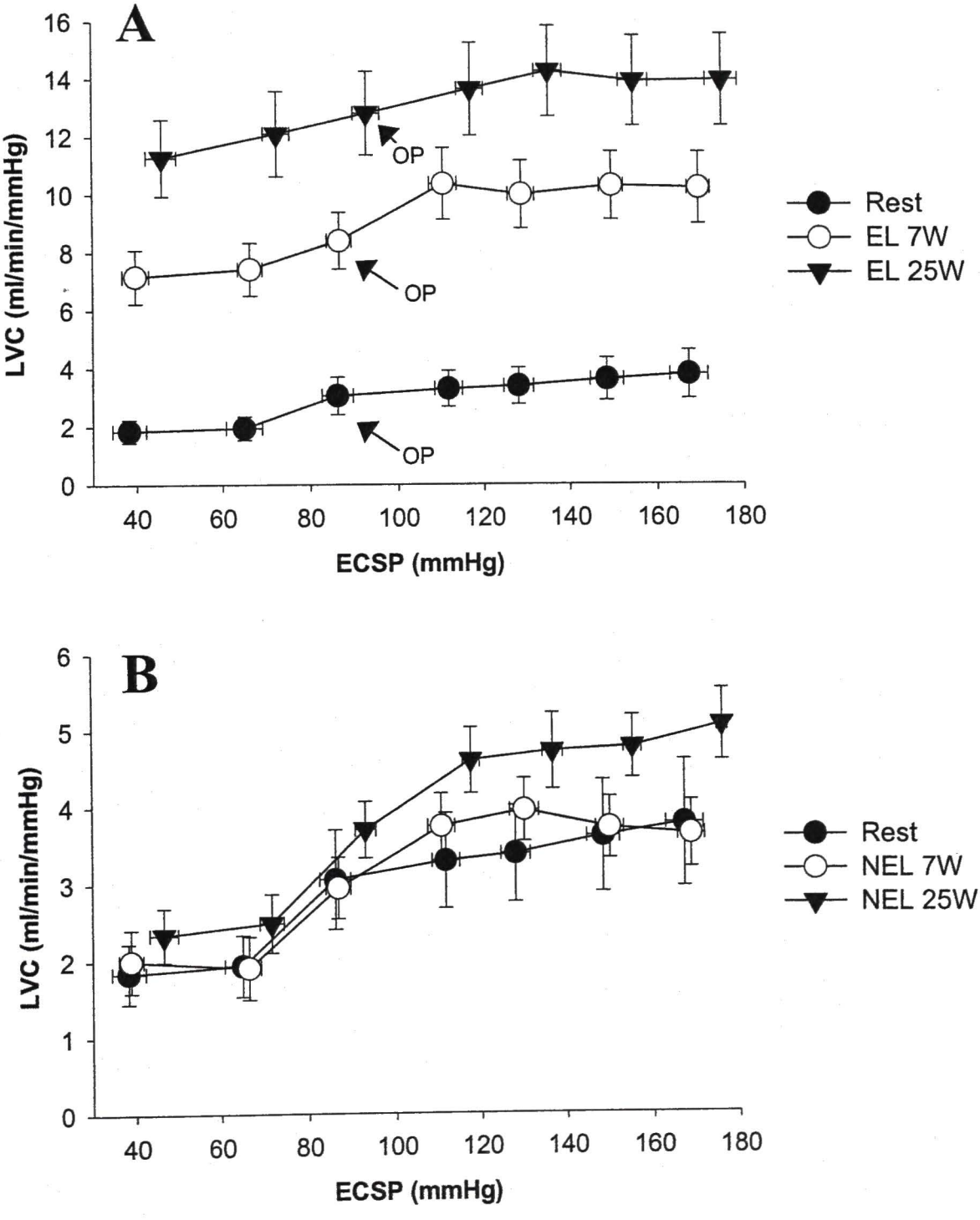
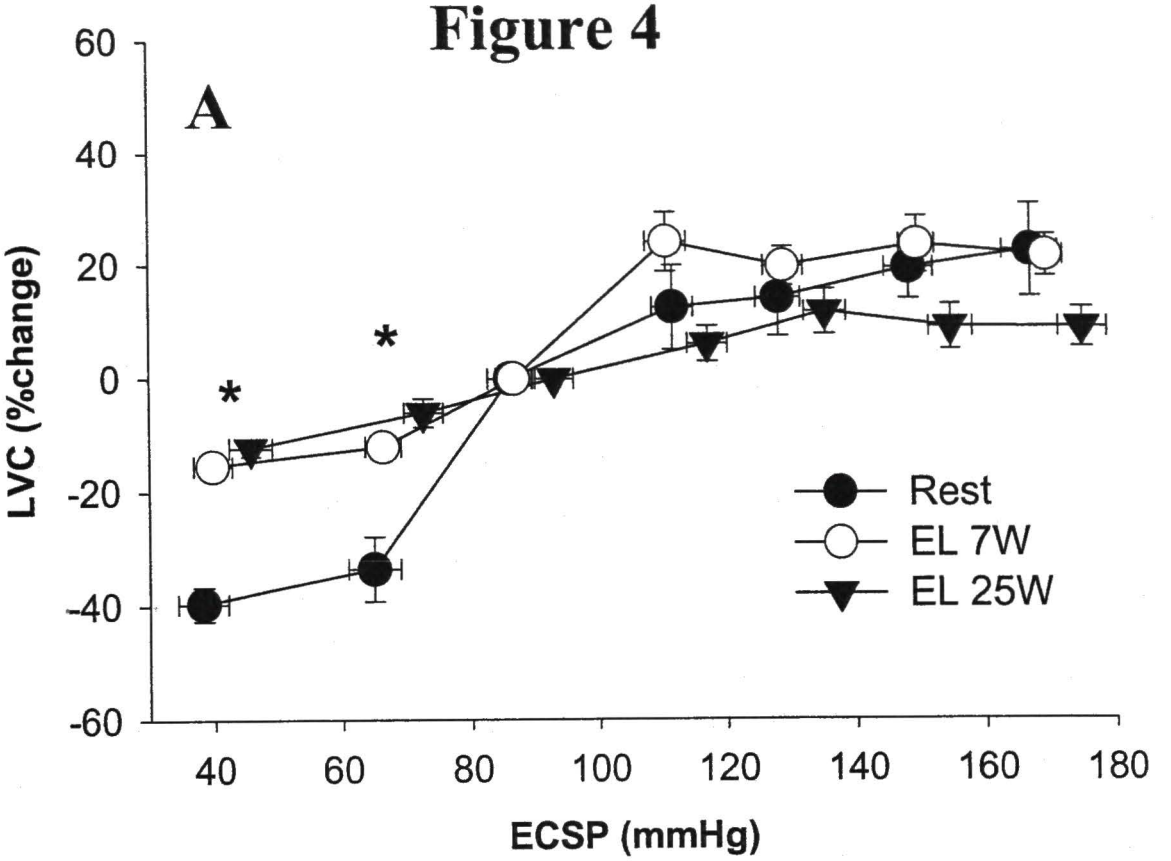

A
LVC (ml/min/mmHg)
ECSP (mmHg)
OP
OP
OP
Rest
EL 7W
EL 25W
B
LVC (ml/min/mmHg)
ECSP (mmHg)
Rest
NEL 7W
NEL 25W

Figure 4


A
60
40
20
0
-20
-40
-60
LVC (%change)
*
*
Rest
EL 7W
EL 25W
40
60
80
100
120
140
160
180
ECSP (mmHg)

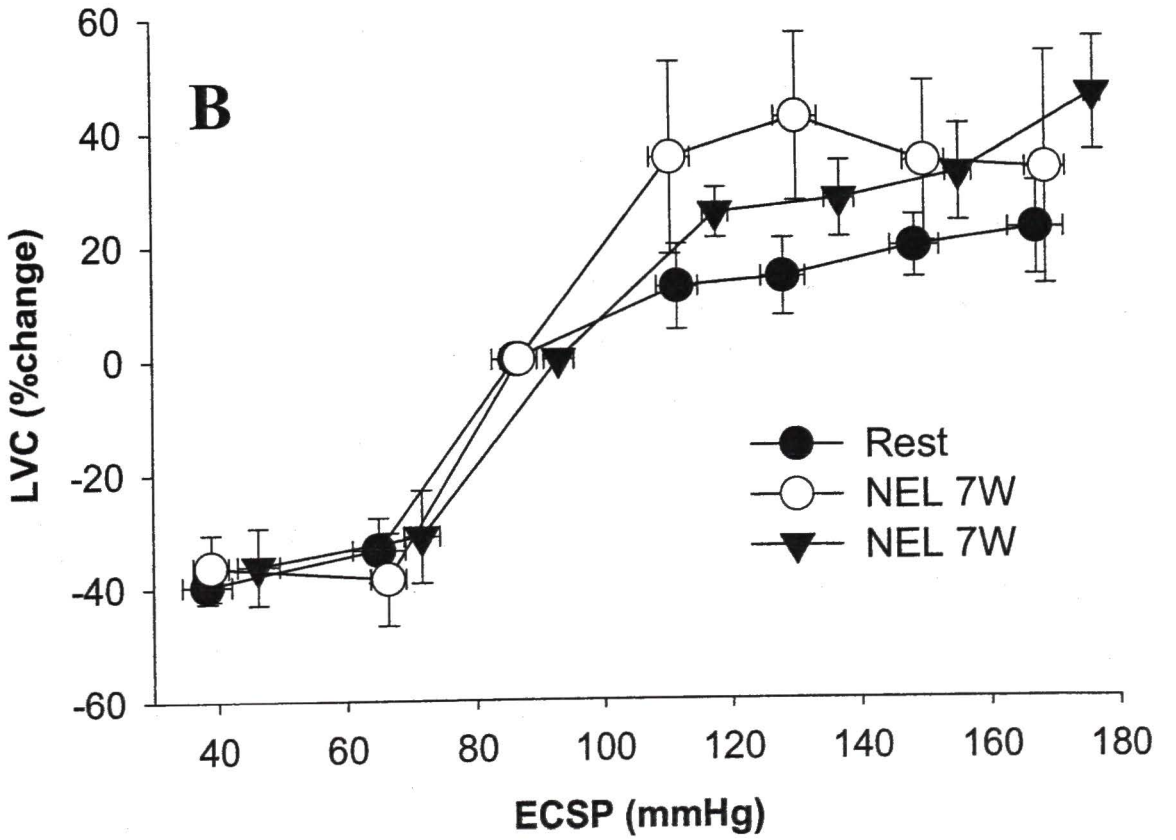

B
60
40
20
0
-20
-40
-60
LVC (%change)
Rest
NEL 7W
NEL 7W
40
60
80
100
120
140
160
180
ECSP (mmHg)

Figure 5

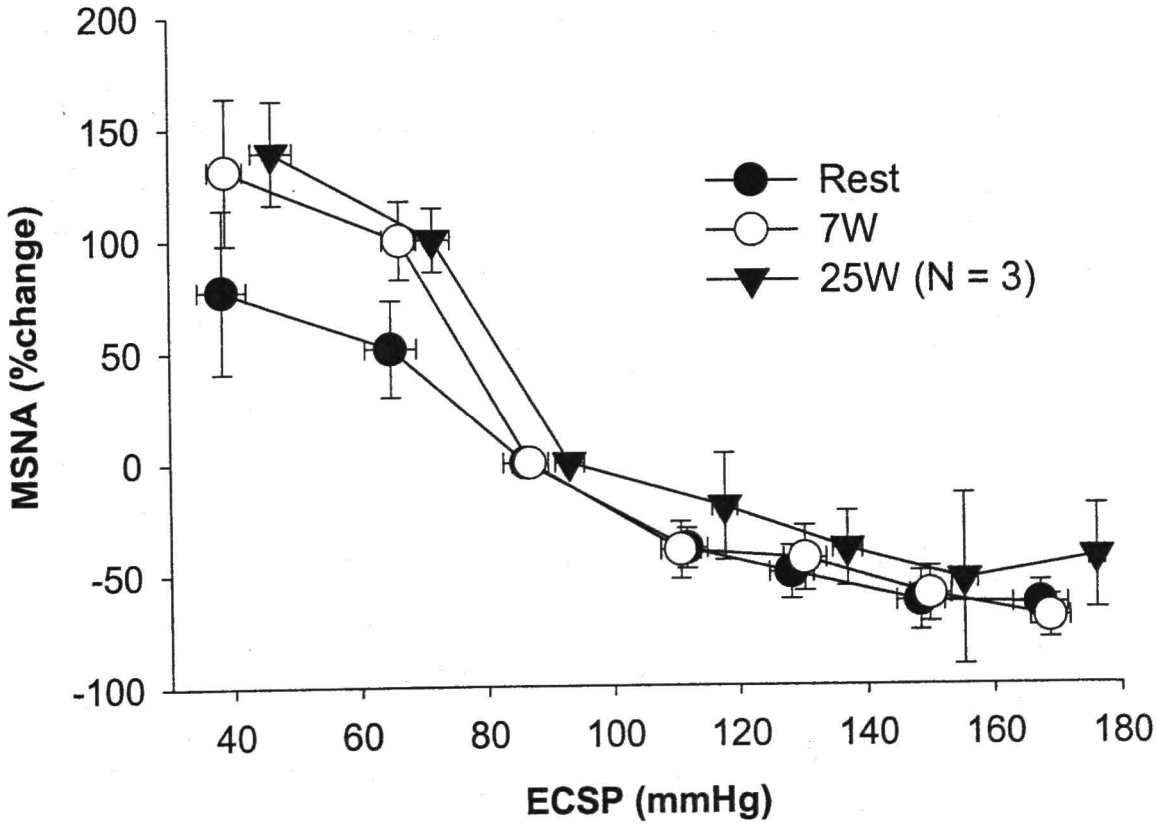
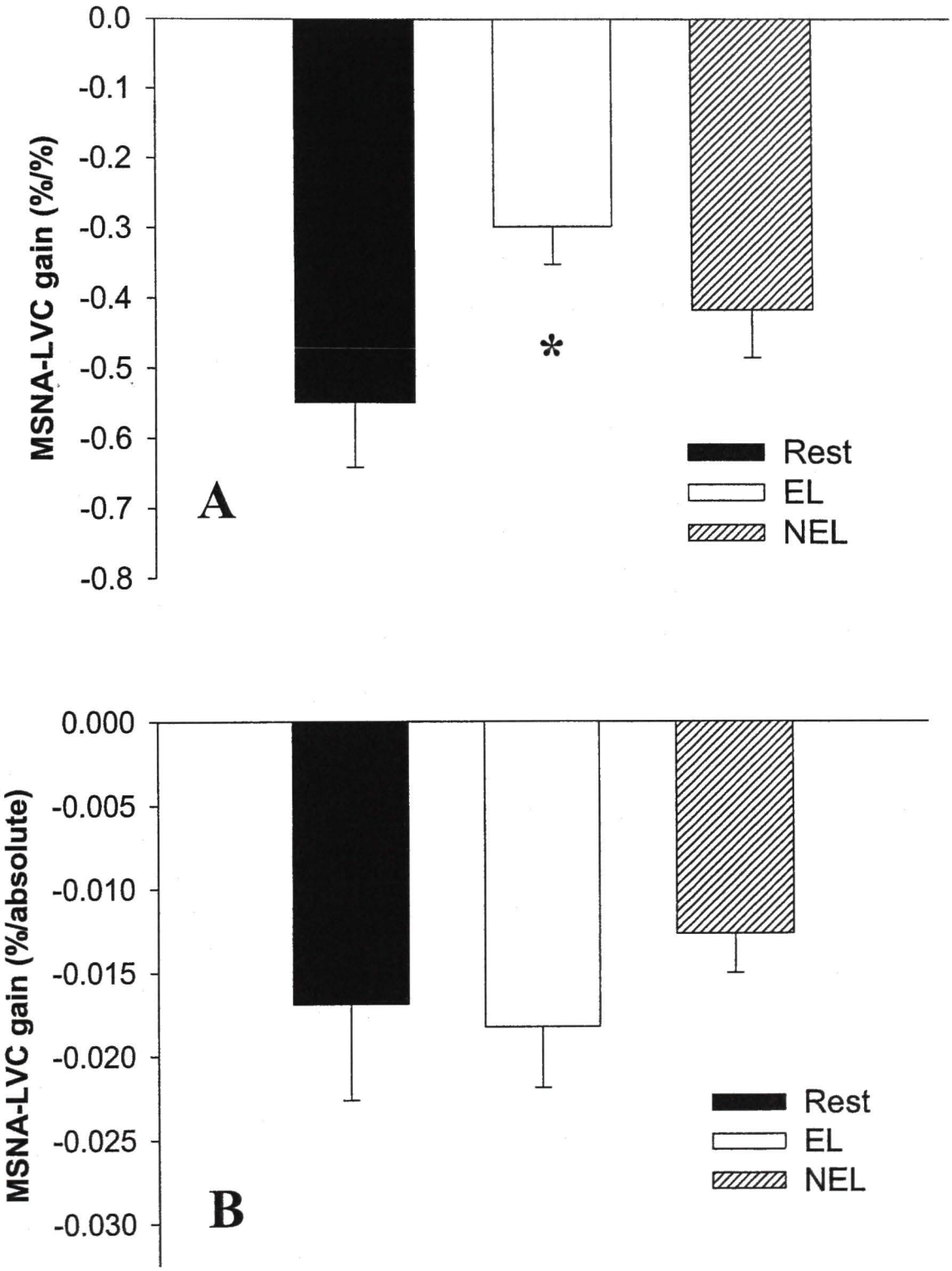

200
150
100
50
0
-50
-100
MSNA (%change)
40
60
80
100
120
140
160
180
ECSP (mmHg)
Rest
7W
25W (N = 3)

Figure 6


0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
MSNA-LVC gain (%/%)
A
*
Rest
EL
NEL
0.000
-0.005
-0.010
-0.015
-0.020
-0.025
-0.030
MSNA-LVC gain (%/absolute)
B
Rest
EL
NEL

CHAPTER III

INHIBITION OF ATP-SENSITIVE POTASSIUM CHANNEL ACTIVITY AUGMENTS BAROREFLEX-MEDIATED VASOCONSTRICTION IN THE VASCULATURE SUPPLYING EXERCISING SKELETAL MUSCLE

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Journal of Physiology

ABSTRACT

In the present investigation we sought to examine the role of ATP-sensitive potassium (K_{ATP}) channel activity in modulating carotid baroreflex (CBR) induced vasoconstriction in the vasculature of the leg. CBR control of mean arterial pressure (MAP) and leg vascular conductance (LVC) was determined in seven subjects (25 ± 1yr, mean ± SE) using the variable pressure neck collar technique at rest and during one-legged knee extension exercise. The oral ingestion of glyburide (5mg) did not change mean arterial pressure (MAP) at rest (86 vs. 89), $P > 0.05$), or during exercise (87 vs. 92), $P = 0.053$. CBR-MAP function curves were similar at rest before and after glyburide ingestion. The CBR-mediated decreases in LVC observed at rest (~39%) was attenuated during exercise in the exercising leg (EL) (~15%), $P < 0.05$. However, K_{ATP} channel inhibition partially restored CBR-mediated vasoconstriction in the exercising leg (~40% restoration) compared to control exercise. These findings indicate that K_{ATP} channel activity is important in modulating sympathetic vasoconstriction in humans and may prove to be the fundamental mechanism by which functional sympatholysis operates in humans during exercise.

INDEX TERMS

carotid baroreflex, leg vascular conductance, functional sympatholysis, blood pressure

INTRODUCTION

Contracting skeletal muscle produces vasodilatory metabolites which attenuate sympathetically-mediated vasoconstriction. This phenomenon was first demonstrated by Remensnyder et al. [29] in 1962 and was defined as functional sympatholysis. Since that time, a number of investigations have demonstrated functional sympatholysis in rats [34, 35], dogs [3, 32] and humans [7, 12, 42]. Functional sympatholysis appears to provide a mechanism by which adequate perfusion of the active muscle can occur and, yet, systemic arterial blood pressure can be supported [15]. However, the mechanisms by which the contraction-induced modulation of sympathetic vasoconstriction becomes manifest remains obscure.

Recent findings [31, 37] have demonstrated a clear effect of functional sympatholysis in the human forearm during handgrip exercise, resulting specifically from muscle contraction, and not elevated flow 'per se', as pharmacologic vasodilation did not result in an attenuated vascular responsiveness. In the forearm, it has been demonstrated that α1- and α2-adrenergic vasoconstriction is attenuated to a similar degree during relatively low intensity exercise [31]. However, a clear difference in the effect of the metabolic attenuation on α-adreneceptor subtype responsiveness has recently been found in both animal preparations [4, 35], and the human one-legged exercise model [42]. These investigations have demonstrated that the α-2 adrenoreceptor, located more densely in the proximity of the nutrient arterioles [1, 21], is sensitive to sympatholysis at relatively low intensities of exercise, whereas attenuation of the α-1 adrenergic receptor requires greater intensities of exercise [4, 42].

Two prominent mechanisms for causing functional sympatholysis during exercise have been proposed. One involves the endogenous vasodilator nitric oxide (NO), while the other involves the ATP-sensitive potassium (K_{ATP}) channel [13]. A body of evidence exists from animal experiments which strongly supports the role of NO as having a role in the phenomenon of functional sympatholysis [5, 36]. In contrast, human experiments indicate that NO is not obligatory as the primary mechanism underlying functional sympatholysis [8]. However, it has been shown that K_{ATP} channel activity is sensitive to a range of vasodilator metabolites, including NO [22], which may, therefore, be one of the factors contributing to the activation of K_{ATP} channel in humans during muscle contraction.

Activation of potassium channels, which are widely distributed in vascular smooth muscle [22] generally results in hyperpolarization and relaxation of vascular smooth muscle [27]. Furthermore, it has been demonstrated that K_{ATP} channel activity is sensitive to changes in the concentrations of both intra- and extracellular factors such as ATP, nucleotide diphosphates, molecular oxygen (O_2), hydrogen ions (H^+), adenosine, prostacyclin and NO [22, 28]. Thomas et al. [34] investigated the possibility that activation of K_{ATP} channels by some metabolic product of skeletal muscle contraction was a key mechanism by which sympathetic vasoconstriction, especially that mediated by activation of α-2 adrenoceptors, was attenuated in contracting skeletal muscle. They reported in anaesthetized rats, that pharmacologic activation of K_{ATP} channels via diazoxide administration attenuated sympathetic vasoconstriction in resting hindlimb in a dose-dependent manner [34]. Furthermore, they demonstrated that functional

sympatholysis was sensitive to modulation by changes in the activity of the K_{ATP} channel and that contraction-induced sympatholysis was moderately prevented by the K_{ATP} channel blocker, glibenclamide [34]. Thus, contraction-induced activation of K_{ATP} channels appears to be a central mechanism underlying functional sympatholysis in the rat. Furthermore, K_{ATP} channel activity appears to be important in modulating coronary blood flow [30, 38]. Investigations using glibenclamide demonstrated that K_{ATP} channel inhibition reduced resting coronary blood flow [30] and reduced myocardial oxygen delivery during exercise in a diabetic dog model [38]. Collectively, these animal studies demonstrate that K_{ATP} channel activity within vascular smooth muscle modulates steady-state hemodynamics and alters vascular responsiveness to sympathetic stimulation.

Investigations examining the role of K_{ATP} channel inhibition in reactive hyperemia in humans have generated inconsistent findings [2, 10, 19] with results indicating reduced [19], or unchanged baseline limb blood flow [2, 10], as well as either no change [10], or decreases [2, 19] in hyperemic responses to cuff occlusion, or post-exercise hyperemia. Taken together, these studies demonstrate that K_{ATP} channel activity on vascular smooth muscle may modulate steady-state hemodynamics [19, 30], as well as alter vascular responsiveness to sympathetic stimulation in humans [34].

Recently, we have identified the effectiveness of functional sympatholysis during disengagement of the carotid baroreflex (CBR) using neck pressure (NP) and the resulting vasoconstrictor response of active skeletal muscle [17]. However, whether the K_{ATP} channel plays a role in the functional sympatholysis observed in the baroreflex-

mediated sympathetic vasoconstriction during exercise in humans [17] remains to be explored.

The concept of an attenuated sympathetically-mediated vasoconstriction graded to contraction intensity, as opposed to a complete elimination of vasoconstriction, is important to whole body dynamic exercise [15]. Marhsall et al. [20] reported that a patient that was treated for malignant hypertension by total sympathectomy was unable to maintain arterial pressure at resting values while performing supine or head-down tilt dynamic leg cycling. Furthermore, in both animals [6, 24] and in humans [23] the importance of baroreflex-mediated control of total vascular conductance in maintaining arterial blood pressure during exercise has been confirmed. Consequently, despite the effect of functional sympatholysis in exercising skeletal muscle, a reliance on sympathetically-mediated vasoconstriction is necessary for adequate blood pressure maintenance, while the presence of functional sympatholysis within the contracting muscle insures adequate tissue perfusion during exercise.

Using the well-established neck chamber technique, Fadel et al. [9] demonstrated that CBR control of muscle sympathetic nerve activity (MSNA) was preserved during arm cyclying at 50% peak oxygen uptake (VO_{2peak}). Wray et al. [41] recently demonstrated the importance of the CBR in the dynamic regulation of MSNA, femoral blood flow and tissue oxygenation during one-legged exercise. Similarly, we have demonstrated the presence of functional sympatholysis during the modeling of CBR regulation of leg vasculature during one-legged knee extension exercise [16]. Therefore, based upon the animal work of Thomas et al. [34], and the human work of Kosmas et al.

[19], we hypothesize that K_{ATP} channel inhibition will restore CBR-mediated vasoconstriction in a vascular bed supplying exercising skeletal muscle during one-legged knee extension exercise. We addressed this hypothesis in humans performing one-legged knee extension exercise, using quantifiable neck pressure stimulation of the carotid baroreceptors with and without ingestion of oral glyburide with the expectation of partially restoring sympathetically-mediated vasoconstriction in the exercising leg.

METHODS

Subjects.

Five men and two women (age, 25 ± 1yr; height, 153 ± 9cm; weight, 67 ± 1kg; mean ± SE) voluntarily participated in the present investigation. Each subject was familiarized with the testing protocols and informed of potential risks of participating in the current study. Subjects were provided a written explanation of the investigation and its potential risks. The subjects were requested to provide a signature to the document, thereby signing informed consent approved by the University of North Texas Health Science Center's Institutional Review Board. All subjects were healthy, non-smokers, free of known cardiovascular and respiratory disease, and were not using prescription or over-the-counter medications. Subjects were advised not to participate in strenuous physical activity, as well as alcohol consumption, twenty-four hours before any of the scheduled experiments. Subjects were also asked to refrain from the consumption of caffeinated beverages twelve hours before any of the scheduled experiments.

Experimental Protocol.

Each subject visited the laboratory on two separate days (random order).

Experimental day one: Carotid baroreflex control of MAP and LVC was determined in each subject using the variable pressure neck collar technique [26] at rest and during exercise trials on experimental day one. At rest, CBR control of MAP was determined using NP/NS ranging from +40 to –80Torr as described by Potts et al. [26]. Also at rest, CBR control of LVC was determined in response to NP (+40Torr) only. After a period of resting data collection (~1hr), subjects performed 2 trials of one-legged knee extension exercise. During exercise, CBR control of MAP and LVC were determined using only NP (+40Torr). Subjects performed 2 bouts of 7W workload exercise at a kicking rate of 30 kicks per minute (kpm) using a modified cycle ergometer (Ergomedic 874 E, Monark) described by Saltin et al. [33]. While kicking, the subjects were provided an audible cue using a metronome and verbally encouraged when necessary to maintain a consistency of each knee extension. The rate of kicking was set to 30 kpm to allow for adequate time in which the exercising leg was relaxed in order to optimize the integrity of the Doppler ultrasound measures in response to NP during exercise. The effect of contraction rate on leg blood flow has previously been examined [14, 25]. Osada and Radegran [25] demonstrated that leg blood flow was linearly matched to workload, regardless of contraction frequency. On this experimental day, a resting data collection period of ~1hr was completed before the exercise trials. Each exercise trial lasted approximately 25min. The time of the exercise trials was limited to ~25 minutes in order to eliminate the confounding effects of fatigue, or cardiovascular drift on CBR function. In an effort to

minimize changes in skin blood flow, laboratory temperature on experimental days was maintained between 24 to 25°C. Each exercise trial was separated by a recovery period of ~30min to ensure return of cardiovascular variables to baseline. Two exercise trials at the 7W workload were performed for the collection of data from an exercising leg (EL) and a non-exercising leg (NEL) during separate trials.

Experimental day two: After instrumentation, subjects ingested a 5mg dose of glyburide. On this experimental day, CBR control of MAP was determined at rest after glyburide ingestion in an effort to demonstrate any potential direct effects of glyburide ingestion on CBR function. At rest, CBR control of LVC was determined in response to NP (+40Torr) only. The experimental protocol on experimental day two was similar to experimental day one. During exercise, CBR control of MAP and LVC were determined using only NP (+40Torr). All data collection (resting and exercise trials) was performed during hours 3-4 post glyburide ingestion.

Glyburide administration.

On experimental day two, after instrumentation, subjects ingested a 5mg dose of glyburide. All resting and one-legged knee extension exercise trials were performed during hours 3-4 post administration of the drug. This time frame has been shown to significantly alter calf blood flow and hyperemic responses to leg cuff occlusion [19]. Blood glucose for each subject was determined with finger stick blood sampling (Ascensia Elite XL, Bayer) every thirty-minutes for hours 1 and 2 post-admin. During hours 3 and 4, blood glucose was determined every twenty-minutes. Glyburide administration did not change blood gases variables at rest, or during 7W exercise in

three subjects (see Table 2). Meals were provided to each subject at the time of glyburide ingestion, and a carbohydrate supplement drink was given to subjects as needed to prevent hypoglycemia in response to the glyburide.

Techniques of Measurements

All testing was performed with subjects in a semi-recumbent ~60 degree back supported seated position, resulting in an ~120 degree leg-to-torso angle to optimize one-legged exercise performance, as well as Doppler ultrasound measurements. Cardiovascular variables were monitored beat-to-beat and recorded on a personal computer (PC) equipped with customized software (Necsuc3) that collects and records data on each R-wave, as well as a second PC equipped with an on-line data acquisition program (DI-720, Dataq Instruments, Akron, OH). Heart rate (HR) was monitored with a standard lead II electrocardiogram (ECG). The ECG signal was output to a monitor (model 78342A, Hewlett-Packard, Andover, MA) interfaced with the PC. Arterial blood pressure (ABP) in six subjects, as well as arterial blood sample collection (PaO_2, $PaCO_2$, pH, Hct and O_2Sat) in three subjects, was measured using a Teflon catheter (18 gauge, 1.35-mm) connected to a pressure transducer (Maxxim Medical, Athens, TX) placed in the femoral artery of the exercising leg. A second Teflon catheter was inserted into the femoral vein of the same three subjects used for arterial blood sampling for whole leg venous blood samples (PO_2, PCO_2, pH, Hct and O_2Sat). In one subject, arterial blood pressure was obtained using finger-cuff photoplethysmography (Finapres, Ohmeda 2300) on the middle finger of the right hand and calibrated to match the diastolic blood pressure achieved from brachial auscultation. Subjects were fitted with a malleable lead neck

collar for the application of NP/NS. Carotid baroreflex function was assessed at rest and during one-legged knee extension exercise after steady-state hemodynamic conditions had been achieved (~5 minutes) as previously described by Potts et al. [26].

Leg Blood Flow.

Leg blood flow (LBF) was determined using pulsed Doppler ultrasound velocimetry using the product of the femoral artery mean blood velocity and diameter. Femoral blood velocity (FBV) was obtained using a Doppler unit (model MD6, D.E. Hokanson, Inc., Bellevue, WA, USA) with a bidirectional probe operating at a frequency of 5 MHz and calculated using the formula $V = f_a/(64.9\cos\text{Ø})$, were f_a is the audio frequency, Ø is the angle of insonation and V is the blood velocity in cm/sec. The Doppler probe was placed to the skin over the common femoral artery distal to the inguinal ligament. The angle of the transducer crystal relative to the skin was ~60 degrees. Femoral artery diameter was measured using a 2.5 MHz probe (model RT 6800, GE) at a site matching that at which velocity was measured. Average femoral artery diameter was determined at rest and during one-legged knee extension exercise in the EL and the NEL. All ultrasound data of femoral arterial diameters were recorded onto VHS tape and further analyzed using custom software. The femoral artery radius was determined for each subject at each condition using the formula: radius = diameter/2. All resting FBV and resting femoral artery diameter data were measured from one leg of each of the subjects (i.e., right, or left) before any exercise trials were performed. Femoral artery diameter was not changed in response to 5-sec pulses of NP and the following formula was used to calculate LBF: $\text{LBF} = \pi * \text{radius}^2 * \text{FBV}$.

Carotid Baroreflex Responsiveness.

Carotid baroreflex control of MAP was assessed only at rest by applying single 5-sec pulses of NP/NS ranging from (+40 to –80Torr) as described by Potts et al. [26]. Under resting conditions, NP/NS was applied during a 10- to 15-second breath hold at end expiration, in order to minimize the respiratory modulation of HR and mean arterial pressure (MAP). A minimum of 45 seconds was allowed to pass between each NP/NS trial to allow physiological variables to return to pre-stimulus values. Peak responses for MAP were determined as the greatest change over a four-second period of time that occurred from the application of NP/NS and compared to an average MAP for the four seconds immediately preceding the NP/NS stimulus. The responses for each trial were averaged to provide a mean response for each subject. Changes in FBV were determined during the four-second at which the peak MAP response occurred. An average FBV over the four-second interval was used to assess peak FBV changes for each trial compared to an average FBV for the four seconds immediately preceding the NP/NS stimulus.

During exercise, only trials of NP of +40 Torr were applied, and was done so without the presence of a breath hold. A four-second interval was chosen in an effort to minimize the effect of kicking frequency, and therefore the contraction relative to the relaxation phase (30kpm, 2 second kicking cycle). These changes were then averaged to provide a mean response for each NP (+40 Torr) for each subject. LVC was calculated using the following formula: LVC = LBF ÷ MAP.

Data Analyses

Stimulus-response curves for CBR control of MAP were fit for individual subject to a four-parameter logistic function described by Kent et al. [18], using the following equation: $MAP = A_1\{1 + \exp[A_2(ECSP\text{-}A_3]\}\text{-}1 + A_4$

A_1 is the MAP response range (maximum-minimum), A_2 is the gain coefficient, A_3 is the centering point (ECSP required to elicit equal pressor and depressor responses) and A_4 is the minimum MAP response. The individual data were fit to this model by nonlinear least-squares regression which minimized the sum-of-squares error to predict a curve of "best fit" for each data set. The gain of the CBR-MAP stimulus-response curve was derived from the first derivative of the logistic function of Kent et al. [18], and the maximal gain (Gmax) was calculated as the gain at the centering point (A_3). The threshold (i.e. point where no further increase in MAP occurred, despite reductions in ECSP), as well as the saturation (i.e. point where no further decrease in MAP occurred, despite increases in ECSP) were also determined. All parameters were averaged and presented as group means. We chose not to utilize the logistic model of Kent et al. [18] to generate function curves for CBR control of LVC due to the limitations observed with muscle sympathetic nerve activity [9].

Statistical Analyses.

Comparisons of physiological variables, CBR-MAP stimulus-response parameters and CBR-LVC reflex sensitivity between rest and exercise were made using paired *t*-tests. Based on our power calculation from pilot data, we *a priori* expected a significant effect of glyburide administration on baroreflex-induced percentage changes in LVC of the

exercising leg with an N = or > 4. This *a priori* expectation was tested using paired t-tests with significance set at $P < 0.05$ following a two-way analysis of variance used to determine significant differences in CBR-%LVC values between rest, non-exercising leg and exercising leg with and without glyburide. Statistical significance was set at $P < 0.05$. Values are means ± SE.

RESULTS

Cardiovascular responses to glyburide at rest and during one-legged knee extension exercise

Glyburide administration did not alter resting HR, MAP, LBF and LVC (Table 1). Furthermore, oral glyburide administration did not alter HR, LBF and LVC during one-legged knee extension exercise at 7W (Table 1). The MAP at rest was not different after glyburide administration, while the MAP during control exercise was similar to ($P > 0.05$). The MAP during exercise after glyburide ingestion appear to be increased; however, statistical significance was not reached ($P = 0.054$).

Carotid baroreflex control of MAP after glyburide administration

The calculated parameters of the CBR-MAP reflex function curve parameters are presented in Table 3. At rest, CBR stimulus-response curves for MAP were unaffected by glyburide administration (Figure 1), except for a significant decrease in the centering point pressure (90 ± 6mmHg) compared a control value of 95 ± 5mmHg, $P < 0.05$). Furthermore, the MAP response to NP (+40mmHg) during exercise was no different with glyburide (8 ± 1mmHg) compared to control exercise (8 ± 1mmHg), $P > 0.05$.

Carotid baroreflex control of LVC after glyburide administration

The CBR-mediated changes in LVC expressed as a percentage change from baseline are presented in Figure 2. At rest, the application of +40 Torr NP resulted in a decrease in LVC (39 ± 3%). During one-legged knee extension exercise, the application of the +40 Torr NP decreased LVC in both the exercising leg (15 ± 2%) and non-exercising leg (40 ± 6%). The decrease in %LVC in the exercising leg (15%) was significantly less than at rest (39%, $P < 0.05$). Furthermore, the percentage decrease in LVC in the exercising leg after glyburide (25 ± 4%) was significantly greater than control exercise (15 ± 2%, $P < 0.05$) and represented a 40% reinstatement of vasoconstriction.

The absolute change in LVC in response to NP at rest (1.0 ± 0.2) and during exercise in the exercising (1.23 ± 0.2) and non-exercising leg (1.0 ± 0.1) were not significantly different ($P > 0.05$), see Figure 3. The absolute change in LVC after glyburide at rest (1.36 ± 0.34) was no different than control (1.02 ± 0.23, $P > 0.05$). The absolute change in LVC after glyburide during exercise in the exercising leg (2.09 ± 0.6) was not statistically different from control exercise in the exercising leg (1.23 ± 0.2, $P = 0.13$).

DISCUSSION

The primary finding of this investigation is that K_{ATP} channel inhibition with oral glyburide partially restored (40%) baroreflex-mediated vasoconstriction in the vasculature supplying exercising skeletal muscle. This finding supports the hypothesis that K_{ATP} channel activity is important in modulating sympathetic vasoconstriction in humans and may prove to be the fundamental mechanism by which functional sympatholysis operates in humans during exercise.

Thomas et al. [34], using lumbar sympathetic stimulation, demonstrated the importance of K_{ATP} channel activity in causing functional sympatholysis in rats. To our knowledge, the data of the present investigation is the first to demonstrate a role of K_{ATP} channel activity in modulating functional sympatholysis in humans. Similar to Thomas et al. [34] using lumbar sympathetic stimulation, the findings of the current investigation using CBR-mediated increases in MSNA, demonstrated an attenuation of sympathetic vasoconstriction in the vasculature supplying exercising skeletal muscle. Data from the present investigation identified a clear attenuation of CBR-mediated decreases in LVC (% change) at very low exercise workloads (7W). Recently, it has been demonstrated in both dogs [4] and humans [42], that functional sympatholysis occurs at low workloads and that α2-adrenergic receptors were susceptible to sympatholysis at lower workloads than α1-adrenergic receptors. This may be especially important when considering baroreflex control of vascular tone in that, presumably, there may be a shift in the relative contribution of α-adrenergic receptor subtypes to changes in vasomotor tone between rest and exercise at increasing exercise intensities. While there is a clear diminution of vascular responsiveness, as indicated by a percentage change in LVC in the exercising leg compared to rest, CBR control of the leg vasculature remains functional [16, 41]. This attenuation in CBR control of the vasculature supplying exercising skeletal muscle occurs despite obvious preservation of arterial blood pressure control during exercise. Ogoh et al. [23] demonstrated the importance of CBR-mediated changes in total vascular conductance as the sole contributor to changes in arterial blood pressure during cycling at an exercise intensity equivalent to HR of 90bpm. Furthermore, Collins et al. [6]

demonstrated an increased contribution of changes in hindlimb vascular conductance to baroreflex-mediated (bilateral carotid occlusion) changes in total vascular conductance. Collectively, these findings demonstrate a delicate balance between the effects of functional sympatholysis and baroreflex control of the vasculature in regulating arterial blood pressure.

The effect of K_{ATP} channel inhibition on reactive hyperemic responses in the forearm has produced variable findings. Banitt et al. [2] demonstrated a reduction in the total hyperemic volume in the forearm with cuff occlusion release after local infusion of tolbutamide (K_{ATP} channel inhibitor) into the brachial artery. In contrast, Farouque and Meredith [10] demonstrated that K_{ATP} channel inhibition with glibenclamide lyophilisate did not alter hyperemia during recovery from wrist flexion/extension exercise. However, comparison of findings between the arm and the leg, as well as vascular responses to hyperemia or sympathetic activation must be made with caution. In the current investigation, an oral dose of glyburide (5mg) enhanced baroreflex-mediated vasoconstriction in the EL as indicated by a 40% increase in the leg vasoconstrictor response to 5sec pulses of NP (hypotensive stimuli) compared to control exercise. There are several possible explanations for the remaining 60% effect of sympatholysis. First, and possibly the major factor, is that the oral dose used in the current investigation (5mg) did not completely inhibit all vascular smooth muscle K_{ATP} channel activity. Therefore, the effect of K_{ATP} activation on functional sympatholysis was only partially prevented, while the remaining K_{ATP} channel activation prevented complete expression of baroreflex vasoconstriction observed at rest. However, as the population studied in this

investigation were young, healthy adults, increases in the oral dosage may have further complicated experimental side effects by exaggerating the insulin response to the drug and resulting in clinically significant hypoglycemia. A second possible explanation for the remaining 60% attenuation is that the pathways involved in functional sympatholysis are not limited to the K_{ATP} channel. While the data from this investigation, similar to the work by Thomas et al. [34] indicate an important role of K_{ATP} activation in contributing to functional sympatholysis, the vasoconstictor response was not completely restored in either investigation. It is therefore possible that other mechanisms, along with K_{ATP} channel activation, result in attenuated α-adrenergic vasoconstriction during muscle contraction. A third, less likely explanation, is that increased flow in and of itself is partially responsible for the attenuated vasoconstrictor response during muscle contraction. Although Tschakovsky et al. [37] convincingly demonstrated that dilation with both adenosine and sodium nitroprusside at rest resulted in no sympatholysis, it is possible, that the mechanical and physical consequence of skeletal muscle contraction in conjunction with increased flow results in altered vascular responsiveness.

Collins et al. [6] clearly demonstrated that changes in hindlimb vascular conductance with bilateral carotid artery occlusion contribute progressively more to the changes in total vascular conductance during treadmill exercise compared to rest. The data of the investigation [6] indicated that from rest to exercise, despite potential alterations in vascular responsiveness due to functional sympatholysis, the vasculature supplying exercising skeletal muscle becomes a more important site for regulation of arterial blood pressure compared to rest. Collins et al. [6] further demonstrated that

concomitant changes in renal vascular conductance became relatively less important, contributing progressively less to changes in total vascular conductance.

In the current investigation, baroreflex control of the NEL was also determined and indicate CBR control of the vasculature supplying NEL was unchanged by both the exercise and glyburide administration. It has been suggested that K_{ATP} channel inhibition at rest does not effectively augment sympathetically-mediated vasoconstriction, due to the low probability of K_{ATP} channel activation in vascular smooth muscle supplying resting skeletal muscle [34]. Although K_{ATP} channel activity is likely enhanced during muscle contraction, it remains possible that K_{ATP} channel activity in the vasculature supplying skeletal muscle at rest and in the NEL varies across species and within a given population. K_{ATP} channel inhibition has been previously shown to not change resting forearm blood flow [2, 10]. Kosmas et al. [19], using a slightly greater oral dosage of glyburide (7.5mg) than used in the current investigation, demonstrated that resting calf blood flow was reduced 1 to 4 hours post-administration. Unlike the findings in the forearm [2, 10], the findings of Kosmas et al. [19] indicate a potential role of K_{ATP} channel activity in modulating resting vascular tone. However, our data does not support the findings of Kosmas et al. [19] at rest, in that resting leg blood flow was not changed after glyburide ingestion compared to control conditions. Two possible explanations for this discrepancy are as follows. First, it is possible that the dosage used in the current investigation, although enough to result in enhanced baroreflex-mediated vasoconstriction during exercise in a contracting skeletal muscle, was not great enough to reduce steady-state blood flow via changes in K_{ATP} channel activity at rest. A second

explanation pertains to the methods used for measuring flow between the two investigations. In the present investigation, we used Doppler ultrasound to measure LBF to the whole leg. Kosmas et al. [19] used strain-gauge plethysmography at the calf for measures of calf blood flow. While both methods are commonly utilized for indices of blood flow at rest, Doppler ultrasound estimates flow to all leg skeletal muscle, especially the upper leg which constitutes the majority of leg muscle mass, whereas strain gauge plethysmography at the calf is specific to the muscle and tissue in the lower leg. It is probable that the different findings at rest between these two investigations is partially the result of using this different measurement technologies and dosages of oral glyburide.

Somewhat unique to the one-legged knee extension exercise model is the ability to determine steady-state hemodynamics, as well as baroreflex control of the vasculature supplying the NEL. This determination potentially allows for more unique interpretation of the data in the current investigation. Keller et al. [17] had previously suggested that with functional sympatholysis, the vascular responses in the NEL may serve as a more appropriate control compared to rest in that, the comparison of the EL to the NEL accounts for potential non-specific effects of the exercise on functional sympatholysis that are not related to muscle contraction (i.e., hormonal, temperature related, etc.). In this study, there was no significant increase in LVC in the NEL during 7W exercise. Green et al. [11] demonstrated that brachial artery blood flow of an inactive forearm was reduced during low workload leg cycling and reported that an increase in the retrograde flow in the brachial artery during exercise with little to no change in anterograde flow. These findings suggested that the decrease in total flow was the result of either wave

reflection, or the result of a 'stealing' phenomenon during exercise [11]. However, based upon the flow recordings observed in the present investigation, we suggest that the lack of an increase in flow in the NEL during exercise is the result of increased retrograde flow during diastole. Nonetheless, the CBR control of LVC was not altered during exercise compared to rest (see figures 2 and 3).

Potential Limitations

The primary limitation of the current investigation pertains to the use of oral glyburide as the K_{ATP} channel inhibitor. As with many orally ingested drugs, the actual delivery of the drug to the site of interest (vascular smooth muscle) is subject to a number of influences (i.e., digestion, blood volume, etc). Furthermore, we did not challenge the effect of K_{ATP} channel inhibition of the vascular smooth muscle in this investigation with a K_{ATP} channel agonist, such as diazoxide, and therefore, have no direct indication of the degree of channel inhibition. However, the 5mg dosage used in this investigation resulted in clear reproducible decreases in blood glucose within subjects. While the relationship between K_{ATP} channel inhibition at the pancreas and peripheral smooth muscle have not been examined, ensuing hypoglycemia, as well as modulated CBR-mediated vasoconstriction in the EL indicate that some significant inhibition had occurred at this dosage. One methodological limitation involves the lack of our ability, using Doppler ultrasound measures of leg blood flow, to distinguish the supply of other tissue in the leg (i.e. skin, bone, inactive skeletal muscle, etc.). It is unclear how changes in vascular responsiveness of the skin during exercise contributes, if at all to the measured effects of functional sympatholysis. It has been previously demonstrated that the skin

vasoconstrictor response to norepinephrine is reduced with whole body heating [40]. However, the role that skin blood flow and whole body or limb temperature play in the effect of functional sympatholysis in this experimental protocol remain unknown. We suggest that the modulation of CBR-mediated changes in LVC during exercise originates in the active skeletal muscle due to a lack of evidence for CBR control of skin sympathetic nerve activity [39].

In summary the findings of the current investigation demonstrate that K_{ATP} channel activity is important in modulating baroreflex-mediated vasoconstriction in the vasculature supplying exercising skeletal muscle in humans. The data suggest that K_{ATP} channel activation is an underlying mechanisms by which functional sympatholysis occurs in humans during exercise and partial blockade of the K_{ATP} channel results in a moderate restoration of the sympathetically-mediated vasoconstriction in the exercising leg. Furthermore, K_{ATP} channel inhibition with glyburide did not alter CBR control of MAP at rest. These findings are important in the understanding of skeletal muscle blood flow control during exercise in healthy individuals, and potentially provide a clinically relevant baseline response by which the treatment of Type II diabetic patients can be evaluated.

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FIGURE LEGENDS

Figure 1. Carotid baroreflex-MAP response curves determined with the variable pressure neck collar technique at rest before (Control) and after glyburide administration (Glyburide). Lines represent fitted logistic functions generated from the CBR-MAP curve parameters for the group. ECSP, estimated carotid sinus pressure; OP, operating point.

Figure 2. Carotid baroreflex-mediated changes in leg vascular conductance expressed as a percentage change from baseline (% change) at rest and during one-legged knee extension exercise in the exercising leg (EL) and the non-exercising leg (NEL) before (Control) and after glyburide administration (Glyburide). *Significantly different from Rest, $P < 0.05$.). †Significantly different from control, $P < 0.05$.

Figure 3. Carotid baroreflex-mediated changes in absolute leg vascular conductance at rest and during one-legged knee extension exercise in the exercising leg (EL) and the non-exercising leg (NEL) before (Control) and after glyburide administration (Glyburide).

Table 1. *Physiological responses to one-legged knee extension exercise with and without glyburide*

	Rest	Rest w/ Glyburide	Control Exercise	Exercise with Glyburide
HR, bpm	74 ± 4	78 ± 4	84 ± 5*	86 ± 4*
MAP, mmHg	86 ± 4	89 ± 4	87 ± 3	92 ± 5†
LBF, mL/min				
Exercising leg			731 ± 101*	720 ± 131*
Nonexercising leg	224 ± 47	244 ± 30	237 ± 34	298 ± 29
LVC, mL/min/mmHg				
Exercising leg			8.5 ± 1.3*	8.0 ± 1.7*
Nonexercising leg	2.6 ± 0.6	2.8 ± 0.4	2.8 ± 0.4	3.3 ± 0.4

Values are means ± SE. HR, heart rate; MAP, mean arterial pressure; LBF, leg blood flow; LVC, leg vascular conductance. *Significantly different from rest ($P < 0.05$); †denotes P value of 0.053, from control condition.

Table 2. *Blood gas and metabolic variables at rest and during 7W exercise with and without glyburide*

	Rest	7W	Recovery	Rest w/ Glyburide	7W w/ Glyburide
PO_2 (mmHg)					
Arterial	97 ± 4	97 ± 2	98 ± 3	101 ± 1	104 ± 3
Venous	33 ± 2	29 ± 2	38 ± 3	37 ± 2	30 ± 1
PCO_2 (mmHg)					
arterial	38 ± 1	39 ± 2	39 ± 2	38 ± 1	39 ± 1
venous	47 ± 2	50 ± 1	45 ± 3	44 ± 3	51 ± 3
O_2 Sat (%)					
arterial	96.7 ± 0.5	96.6 ± 0.3	97.0 ± 0.2	96.9 ± 0.3	96.9 ± 0.3
venous	63.0 ± 3.6	52.4 ± 3.4	72.5 ± 3.9	70.3 ± 3.2	53.8 ± 3.6
pH					
arterial	7.44 ± 0.01	7.42 ± 0.01	7.43 0.004	7.43 ± 0.01	7.42 ± 0.01
venous	7.39 ± 0.01	7.37 ± 0.01	7.39 ± 0.01	7.39 ± 0.01	7.36 ± 0.02
Hct (%)					
arterial	44.7 ± 2.6	45.3 ± 3.0	45.2 ± 2.3	45.5 ± 2.5	45.7 ± 2.9
venous	45.5 ± 2.6	44.2 ± 1.9	44.7 ± 2.3	44.0 ± 2.0	45.8 ± 2.5
Glucose (mmol/L)					
Arterial	5.6 ± 0.7	4.9 ± 0.1	4.6 ± 0.2	5.3 ± 0.1	5.0 ± 0.1
Venous	5.3 ± 0.6	4.4 ± 0.6	4.4 ± 0.4	4.5 ± 0.6	4.4 ± 0.3
Lactate (mmol/L)					
arterial	1.3 ± 0.5	1.1 ± 0.4	0.9 ± 0.3	1.5 ± 0.4	1.1 ± 0.4
venous	1.3 ± 0.4	1.1 ± 0.3	1.0 ± 0.3	1.5 ± 0.5	1.1 ± 0.3

Values are means ± SE.

Table 3. *Average Carotid Baroreflex-MAP function curve parameters*

	Rest	Rest w/ Glyburide
Threshold, mmHg	66.5 ± 8.1	65.9 ± 8.4
Saturation, mmHg	123.0 ± 7.0	114.5 ± 8.5
Operating point, mmHg	87.5 ± 3.6	89.0 ± 4.3
Centering point, mmHg	94.7 ± 5.1	90.2 ± 5.7*
G_{max}, mmHg/mmHg	-0.44 ± 0.1	-0.45 ± 0.04
Response range, mmHg	23.1 ± 3.5	22.9 ± 4.0
Minimum MAP response, mmHg	73.3 ± 5.0	76.01 ± 6.8

Values are means ± SE. *Significantly different from rest ($P < 0.05$).

Figure 1

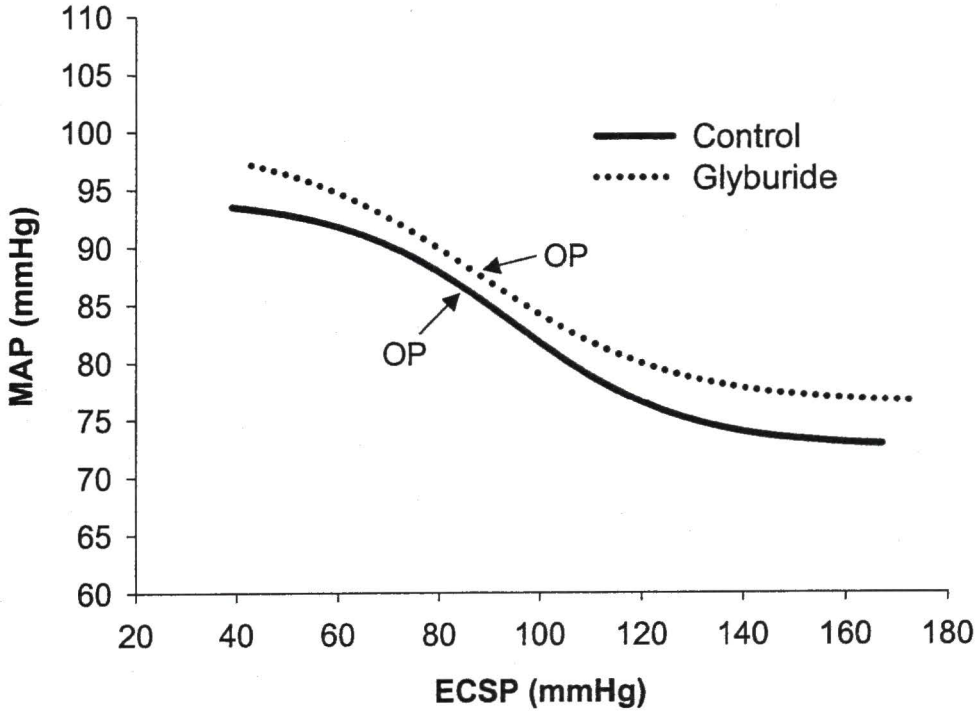

110
105
100
95
90
85
80
75
70
65
60
MAP (mmHg)
20
40
60
80
100
120
140
160
180
ECSP (mmHg)
Control
Glyburide
OP
OP

Figure 2

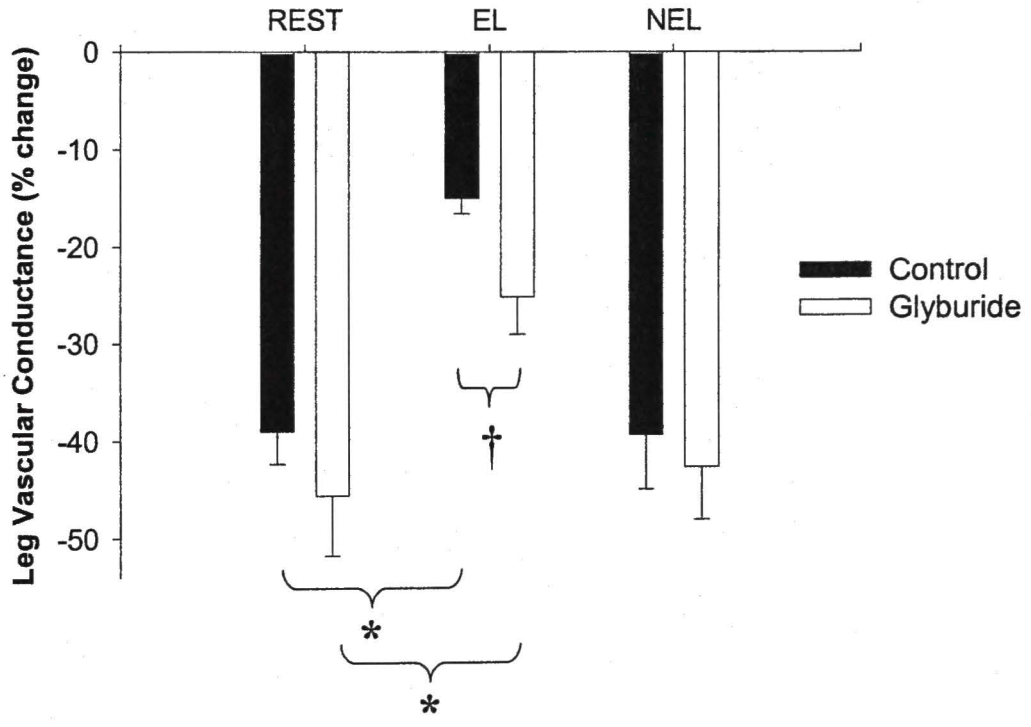
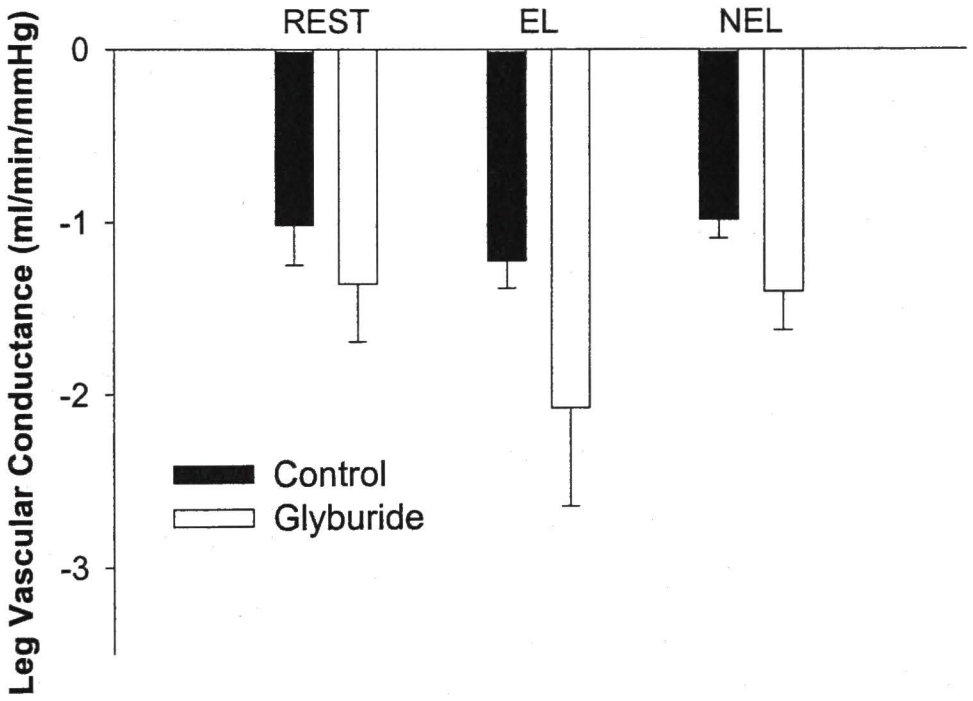

REST
EL
NEL
0
-10
-20
-30
-40
-50
Leg Vascular Conductance (% change)
Control
Glyburide
*
*
†

Figure 3


REST
EL
NEL
0
-1
-2
-3
Leg Vascular Conductance (ml/min/mmHg)
Control
Glyburide

CHAPTER IV

CONCLUSIONS

The findings from the two investigations described in this dissertation demonstrate the presence of CBR control of the leg vasculature at rest and during one-legged knee extension exercise in the vasculature supplying both an exercising leg (EL) and a non-exercising leg (NEL). The findings also indicate that ATP-sensitive potassium (K_{ATP}) channel activity is an important mediator of functional sympatholysis in an exercising leg.

In the first investigation, we demonstrated that engagement and disengagement of the CBR modulates leg vascular conductance (LVC) over a wide range of carotid sinus pressures. We further demonstrated that CBR control of LVC was present during one-legged knee extension exercise in an EL and NEL. While CBR control of LVC in the NEL was preserved during exercise, the effect of functional sympatholysis reduced CBR-induced vasoconstriction in the EL at workload intensities of 7W and 25W compared to rest. Additionally, CBR control of MSNA was preserved from rest to exercise at 7W. This observation was apparent despite a tendency for steady-state MSNA to decrease during exercise at 7W. Interestingly, the CBR-mediated changes in absolute LVC were similar from rest to exercise in the EL and NEL at 7W and 25W. These findings indicate that at low workloads involving a relatively small skeletal muscle mass (one leg), the

contribution of an EL and a NEL to CBR-mediated changes in arterial blood pressure are similar compared to rest. Therefore, a balance between baroreflex-induced changes in MSNA and arterial blood pressure regulation must exist, in the face of functional sympatholysis in leg vasculature supplying exercising skeletal muscle. Furthermore, it appears that the vascular responsiveness to CBR-mediated withdrawal of sympathetic nerve activity was unchanged from rest to exercise at low workloads.

The second investigation examined the role of K_{ATP} channel activity in modulating the effect of functional sympatholysis on CBR-mediated vasoconstriction. While the ingestion of the K_{ATP} channel inhibitor, glyburide, resulted in no change in CBR control of mean arterial pressure at rest, the inhibition of K_{ATP} channel activity during exercise partially restored the CBR-mediated vasoconstriction of the vasculature supplying the EL. Therefore, for the first time in humans, we have demonstrated the role of K_{ATP} channel activity as an underlying mechanism by which functional sympatholysis occurs in humans during low workload leg exercise. Although the vasoconstrictor response to sympathetic activation was enhanced in the EL after glyburide, the CBR-mediated increases in MAP were similar between rest and exercise. However, it is likely that during higher intensity exercise involving a greater muscle mass, activation of the K_{ATP} channel is a necessary component of oxygen delivery to active skeletal muscle while enabling CBR control of MSNA to maintain its role in arterial blood pressure regulation.

CHAPTER V

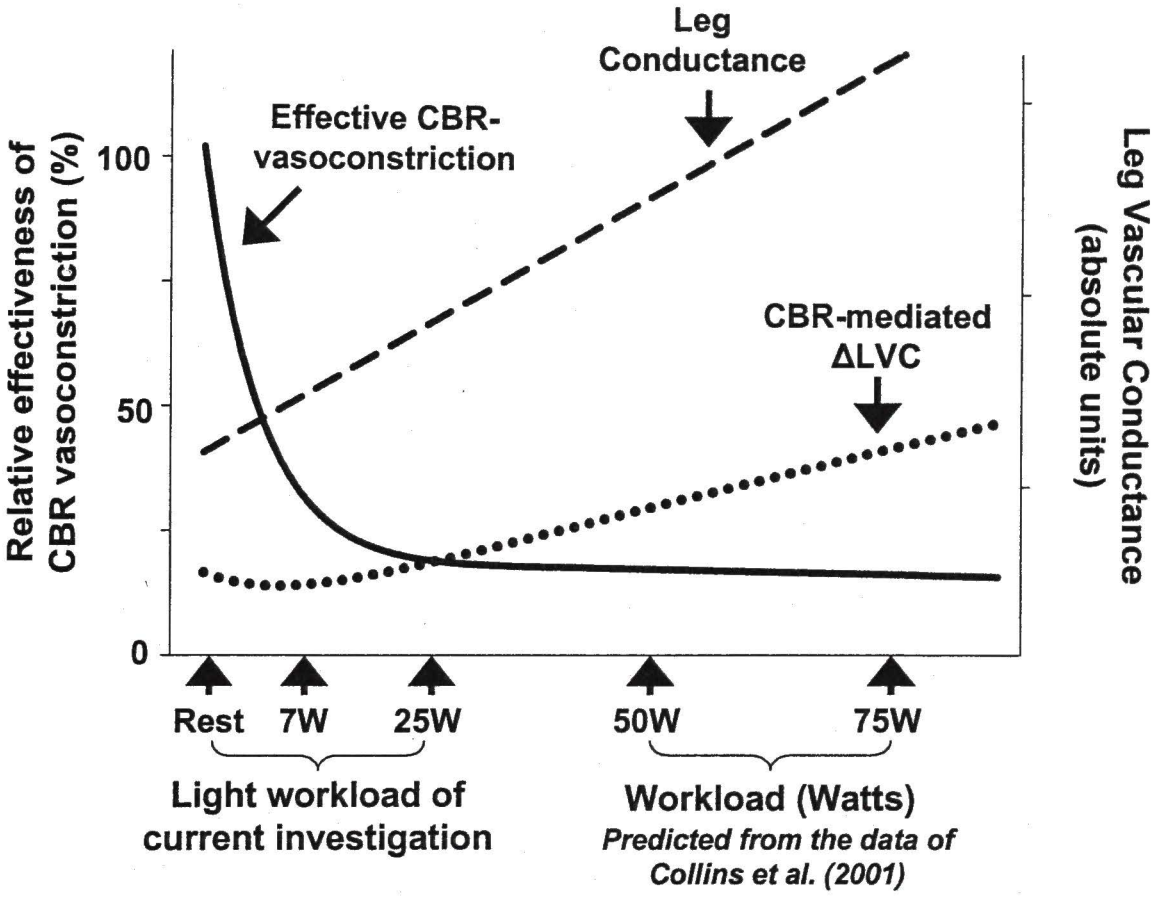
SUGGESTIONS FOR FUTURE RESEARCH

Many questions remain unanswered regarding CBR control of the peripheral vasculature, especially during dynamic exercise. Listed below are suggestions for future research intended to address specific questions that would expand the work of this dissertation, as well as the knowledge pertaining to adrenergic and non-aderenergic control of the peripheral vasculature at rest and during exercise.

I. To expand our understanding of carotid baroreflex control of leg vasculature, an experimental model that incorporates higher workload intensity, as well as a greater muscle mass (e.g. resistance against quadriceps and hamstring contraction). Also, by using an experimental design that addressed CBR-mediated changes in total vascular conductance, as well as simultaneous measures of CBR-mediated changes in leg, arm or renal vascular conductance, it would be possible to determine the actual contribution of a given vascular bed to changes in arterial blood pressure. Unfortunately, the one-legged knee extension exercise model has fundamental limitations when used in conjunction with the variable pressure neck collar technique (i.e., limitation on kicking frequency). However, it is possible that combinations of different applications of neck pressure and neck

suction with different one-legged exercise workloads and kicking frequencies may be utilized to examine these unanswered questions. Figure 1 demonstrates predicted CBR-mediated changes in absolute LVC in an exercising leg with increasing exercise intensity.

Figure 1



Note that with a proposed plateau of the influence of sympatholysis on baroreflex-mediated control of leg vascular conductance in an exercising leg, the absolute changes beyond a *threshold leg conductance* become progressively larger with increasing steady-state leg vascular conductance.

II. Investigations using the variable pressure neck collar may also be designed to examine the role of non-adrenergic modulators of peripheral vasculature at rest and during exercise. An experimental design to address this concern could utilize neck suction, a carotid baroreceptor hypertensive stimulus, to examine the role of angiotensin II-mediated vasoconstriction in the face of sympathetic withdrawal at rest and during exercise. It is possible that with either angiotensin converting enzyme inhibitors, or selective angiotensin receptor blockade, the importance of non-adrenergic vasoconstriction in modulating baroreflex control of vascular tone could be examined. While drug-induced hypotension is a potential problem in this experimental design, a balance between drug dosages and effective inhibition may be possible with healthy populations and particular exercise protocols.

III. To further examine the role of the ATP-sensitive potassium channel as a primary mechanism involved in functional sympatholysis, an experiment could be designed in which selective blockade of a given vascular bed (i.e., leg) would be examined. The findings from this dissertation regarding the role of ATP-sensitive potassium channel in mediating functional sympatholysis were based on an experiment that used an orally ingested drug (glyburide). This resulted in a global, rather than isolated, inhibition of the channel activity, and therefore, limited our ability to completely isolate the ATP-sensitive potassium channel in the exercising leg. At the time of the investigation, it was impractical, if not impossible, to obtain an infusible form of a ATP-sensitive potassium channel

inhibitor. However, if one were to utilize an infusible form of the drug, a more complete blockade of channel activity may be obtainable without spillover and its systemic effects (i.e., enhanced insulin release) would enable a more specific analysis of the effects of K_{ATP} channel blockade.