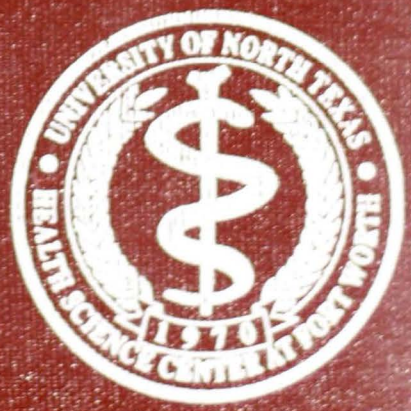
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Arterial baroreflex control of sympathetic nerve activity is dependent on afferent nerve activity emanating from both the aortic and carotid baroreceptors. While several investigations have reported that the aortic baroreceptor reflex dominates in the baroreflex control of heart rate in humans, the role of the carotid and the aortic baroreceptors in the control of sympathetic nerve activity remains unclear. In addition, the effect of exercise and long term endurance training on baroreflex-sympathetic nerve activity responses requires further definition. Therefore, the purpose of the investigations described within this dissertation was to: i) describe carotid baroreflex (CBR) control of muscle sympathetic nerve activity (MSNA) at rest and during exercise, ii) examine the relative contribution of the carotid and aortic baroreflexes to the overall arterial baroreflex control of MSNA during acute hypotension, and iii) determine the effect of fitness on arterial baroreflex control of MSNA. In the first investigation, we constructed stimulus-response relationships for CBR control of MSNA at rest and during dynamic arm cycling and demonstrated that carotid baroreflex control of MSNA was reset to function at the higher arterial pressures induced by exercise without a change in reflex sensitivity. Thus, we concluded that the carotid baroreflex control of MSNA was preserved during dynamic exercise. In the second investigation, acute hypotension was induced non-pharmacologically by releasing a unilateral arterial thigh cuff (300 Torr)

following nine minutes of resting ischemia under two conditions: control (aortic and carotid baroreflex deactivation) and suction (aortic baroreflex deactivation alone). The application of neck suction to negate the CBR during cuff release caused a significant attenuation of the MSNA response and a greater decrease in mean arterial pressure; thereby signifying the importance of the CBR in the control of MSNA and maintenance of arterial blood pressure. However, when the drop in carotid sinus pressure was counteracted with neck suction a significant MSNA response was noted, indicating the dominance of the aortic baroreflex in the baroreflex control of MSNA. Furthermore, a comparison between high-fit (HF) and average-fit (AF) subjects indicated that despite an augmented baroreflex control of MSNA, HF subjects exhibited a greater decrease in mean arterial pressure compared to AF subjects. Thus, it appeared that although the arterial baroreflex appropriately increased the MSNA response to hypotension, the regulation of blood pressure remained attenuated in the HF subjects. We contend that an impaired control of vasomotion hinders blood pressure regulation in high-fit subjects.

ARTERIAL BAROREFLEX CONTROL OF MUSCLE
SYMPATHETIC NERVE ACTIVITY

Paul Joseph Fadel, Jr., M.S.

APPROVED:

Major Professor

Committee Member

Committee Member

Committee Member

Committee Member

University Member

Chair, Department of Integrative Physiology

Dean, Graduate School of Biomedical Sciences

ARTERIAL BAROREFLEX CONTROL OF MUSCLE SYMPATHETIC NERVE ACTIVITY

DISSERTATION

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For the Degree of

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By

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The Effects of Chronic Endurance Exercise Training on the Myocardium With and Without Beta-Adrenergic Receptor Blockade. Southern Region Experimental Biology and Medicine Meeting. New Orleans, Louisiana, March 2000.

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List of Abbreviations

ABR	aortic baroreflex
ABP	arterial blood pressure
ANOVA	analysis of variance
ANCOVA	analysis of covariance
ANS	autonomic nervous system
AF	average-fit
CBR	carotid baroreflex
CVP	central venous pressure
DBP	diastolic blood pressure
Δ	change
ECG	electrocardiogram
ECSP	estimated carotid sinus pressure
ET	endurance trained
HR	heart rate
HF	high-fit
LBNP	lower body negative pressure
VO_{2max}	maximal oxygen uptake
MAP	mean arterial pressure
MSNA	muscle sympathetic nerve activity
NP	neck pressure
NS	neck suction

NTS	nucleus tractus solitarius
%	percent
PVR	peripheral vascular resistance
RSNA	renal sympathetic nerve activity
rVLM	rostral ventral lateral medulla
s	seconds
SBP	systolic blood pressure
SNA	sympathetic nerve activity
SE	standard error
SV	stroke volume

CHAPTER I

INTRODUCTION

The arterial blood pressure (ABP) is an outcome measure of cardiac output (i.e., heart rate x stroke volume) and peripheral vascular resistance (PVR). Arterial baroreceptors, located in the carotid sinus and aortic arch, have been reported to alter both cardiac function and vasomotion in response to sudden changes in arterial blood pressure (17,27). Thus, when ABP is elevated or lowered, these receptors sense changes in mechanical deformation caused by the alteration in ABP and respond through the sympathetic and parasympathetic branches of the autonomic nervous system to return ABP to its original regulated value (27). More specifically, Rea & Eckberg (26) noted that at rest the application of 5-s periods of neck pressure and neck suction to alter carotid sinus transmural pressure produced carotid baroreflex (CBR) mediated changes in muscle sympathetic nerve activity (MSNA) that were described by an inverse sigmoid relationship. However, the carotid baroreflex-MSNA stimulus-response curve has yet to be characterized during exercise. Additionally, due to the complexity of the system and limitations in techniques that can be employed in humans, the relative contribution of the CBR and aortic baroreflex (ABR) to the overall arterial baroreflex control of MSNA remains unclear. Moreover, while several investigations have indicated alterations in arterial baroreflex control of heart rate (HR) in endurance trained individuals (32,33,38);

limited information is available pertaining to the effects of endurance training on CBR and ABR control of MSNA. Therefore, the intent of this dissertation is to: i) quantify CBR control of MSNA at rest and during dynamic exercise; ii) delineate the relative contribution of the CBR and ABR to overall arterial baroreflex mediated changes in MSNA; and iii) determine if endurance exercise training alters arterial baroreflex control of MSNA.

REVIEW OF RELATED LITERATURE

Autonomic Pathways

The arterial baroreceptors are unencapsulated free nerve endings located at the medial-adventitial border of blood vessels in the aortic arch and the carotid sinus (12). These mechanoreceptors function as the sensors in the negative feedback control system which responds to moment to moment changes in arterial blood pressure by reflexively altering sympathetic and parasympathetic nerve activity. The carotid and aortic baroreceptor afferent neurons travel to the nucleus tractus solitarius (NTS) in the medulla via the glossopharyngeal (cranial nerve IX) and the vagus (cranial nerve X) nerves, respectively (2,14). Efferent parasympathetic outflow has been reported to travel through the nucleus ambiguous to postganglionic neurons next to or in the walls of the heart chambers (13). In contrast, the sympathetic efferent nerve projections from the NTS stimulate the caudal ventral lateral medulla, which forms a direct inhibitory projection to sympathoexcitatory neurons in the rostral ventral lateral medulla (rVLM) (21). The

preganglionic sympathetic fibers project from the rVLM to the intermediolateral cell columns of the spinal cord and synapse with postganglionic neurons in the sympathetic ganglion (21). Therefore, an increase in ABP will increase afferent baroreceptor nerve activity and reflexively cause an increase in parasympathetic nerve activity and a decrease in sympathetic nerve activity to produce a bradycardia and vasodilatory response in order to return blood pressure back to its original regulated value (i.e., set point). Conversely, a reduction in ABP will decrease afferent nerve activity, which reflexively causes a tachycardia via parasympathetic withdrawal and a sympathetic mediated vasoconstrictor response to maintain arterial blood pressure around its set point value.

Carotid Baroreflex Function

In 1957, Ernsting and Parry (7) developed a neck chamber that, although cumbersome, permitted the application of neck suction (NS) to selectively stimulate the carotid baroreflex (CBR) in humans. Subsequently, Eckberg et al. (5) designed a simplified neck chamber model devised initially for only NS but modified to also sustain positive pressures. This allowed investigators to selectively examine CBR responses to hypo- and hyper-tensive stimuli by applying variable amounts of neck pressure (NP) and NS, respectively. However, an important methodological concern of this new technique was that sustained NP or NS of greater than 5–10 s compromises the selectivity of the CBR responses because the aortic baroreceptor will be activated and, subsequently, will counteract the CBR mediated changes in ABP (25). Therefore, brief 5-10 s periods of

NP/NS are necessary to isolate CBR responses. A major advantage of applying these brief periods of NP/NS was that it made it possible to non-invasively examine CBR control of arterial blood pressure in humans, a distinct difference from techniques involving injections of vasoactive drugs (27) which directly alter ABP in addition to activating the aortic baroreflex (ABR). In addition, the 5-s stimulus to the carotid sinus region does not result in alterations in central venous pressure (CVP) and thereby eliminates any interaction emanating from cardiopulmonary baroreceptor activation (20,35).

Numerous studies have utilized the application of 5-s periods of NP/NS to evaluate carotid baroreflex (CBR) mediated changes in heart rate (HR) and arterial blood pressure (ABP) both at rest and during exercise (18,19,22,23). Peak changes in HR occurred consistently before the peak changes in ABP and have primarily been attributed to CBR mediated changes in parasympathetic nerve activity (25). More specifically, Potts et al. (22) noted that the time to the peak changes in HR were not different between rest and exercise and consistently occurred 2-3 s after the application of NP/NS. Therefore, the peak change in HR repeatedly occurred during the 5-s NP/NS stimuli, while the peak change in ABP invariably occurred after the NP/NS had been turned off. Due to the findings that stroke volume remained constant during NP and NS (15), this phasic lag between the peak HR and ABP responses indicated that some variable other than cardiac output must be producing the ABP response. Thus, the changes in ABP induced by altering carotid sinus transmural pressure with NP/NS have been primarily attributed to reflex mediated changes in sympathetic nerve activity and subsequently

peripheral vascular resistance. However, limited information is available pertaining to carotid baroreflex control of sympathetic nerve activity.

CBR Control of MSNA at Rest

Wallin and Eckberg (43) reported that alterations in carotid sinus transmural pressure with NP and NS caused profound and transitory changes in MSNA. In addition, it has been reported that the sympathetic response to alterations in carotid sinus pressure are asymmetrical with increases in MSNA associated with NP being much greater than the reductions in activity associated with NS (26). Thus, it appears that CBR control of MSNA was more important in protecting against hypotension rather than hypertension. Collectively, these data suggested that the CBR was capable of altering MSNA and was better suited to respond to decreases in ABP. However, the investigations that evaluated the MSNA responses to NP/NS were performed at rest in the supine position (26,43). Therefore, interpretation of the results only applied to the resting supine position and was not applicable to other resting positions such as seated upright or to upright exercise. Additionally, in the resting supine position baseline MSNA is low presumably due to loading of the cardiopulmonary baroreceptors (42) and therefore, the ability of the CBR to respond to hypertensive stimuli (NS) could falsely appear to be attenuated. In other words, a low baseline MSNA in the supine position will limit the ability to see the carotid baroreflex's influence on MSNA during hypertensive stimuli, whereas CBR responses to NP (hypotensive stimuli) will be greater as there is a larger range for MSNA to be

increased. Thus, the posture of the subjects may play an important role in examining the CBR mediated changes in MSNA.

CBR Control of MSNA during Exercise

Previous investigations have demonstrated that the CBR-HR and CBR-MAP stimulus-response curves were reset to the prevailing systemic pressure during exercise with no attenuation in baroreflex sensitivity (18,23). Even though the overall sensitivity for the baroreflex control of HR was unaltered during exercise from rest to maximal intensity exercise, Potts et al. (22) noted that the reflex tachycardia to NP decreased during exercise, while the reflex bradycardia to NS was augmented. These alterations in CBR control of heart rate were highly correlated with the functional level of cardiac vagal tone. Thus, under resting conditions a high degree of cardiac vagal tone provides an enhanced ability to increase HR compared to exercise when vagal control is diminished and conversely, the high cardiac vagal tone at rest limits the capacity to decrease HR in comparison to the low amount of vagal tone that exists during exercise. Therefore, while the carotid baroreflex control of blood pressure remains operable during exercise, the mechanisms by which the CBR responds to changes in ABP may be different from resting conditions. However, the effect of exercise on CBR control of efferent sympathetic nerve activity remains unclear.

Aortic Baroreflex Function

Previously several studies have indicated that the aortic baroreceptor reflex predominates in the baroreflex control of heart rate (HR) in humans (8,16,36,38). However, human investigations of arterial baroreflex (CBR and ABR) control of MSNA have been limited almost exclusively to the influence exerted by the carotid baroreflex because there are few techniques that allow aortic baroreflex mediated changes in MSNA to be evaluated (6). In an attempt to evaluate the relative importance of the CBR vs. the ABR on the control of MSNA, Sanders and coworkers (28,29) used steady-state infusions of phenylephrine and nitroprusside alone (aortic and carotid baroreceptor activation and deactivation) and in combination with neck pressure and neck suction, respectively (aortic baroreceptor activation and deactivation). From these investigations, it was concluded that the ABR dominates in the arterial baroreflex control of MSNA. However, there remains some question as to the specific interpretation of these data. First, Eckberg and Sleight (6) noted that the period of time used to evaluate baroreflex function (three minutes) may have caused baroreflex adaptation, particularly for the CBR which has been found to adapt to sustained stimulation (4). Second, the arterial baroreceptors are known to respond to moment to moment changes in ABP and therefore, averaging the data for three minutes may have obscured any differences between the CBR and ABR response to the initial change in ABP. Third, the steady-state infusions of vasoactive drugs caused sustained alterations in arterial blood pressure despite the counteractive reflex responses. These continuous and constant increases and decreases in ABP may not represent the true physiological stimulus to the ABR and CBR during dynamic changes in ABP (i.e.,

standing) because by maintaining a fixed pressure at the baroreceptors the interaction between these two baroreceptor populations was probably altered. In addition, MSNA responses to NP/NS appear to be more dependent on the actual change in CBR afferent activity rather than the absolute level of the ABP (43). Therefore, sustained alteration in ABP may have confounded these results.

Carotid Baroreflex Function and Chronic Endurance Exercise Training

Chronic endurance exercise training results in numerous adaptations that affect the heart, the peripheral vasculature, the skeletal muscle, and the autonomic nervous system (1). These adaptations greatly improve an individual's ability to perform physical work, however, they may also result in the endurance trained (ET) individual being more susceptible to orthostatic intolerance (3,10). One explanation for the increased prevalence of orthostatic intolerance in ET individuals may be an attenuation of the arterial baroreflex control of HR and vascular resistance (24). Recently our laboratory has demonstrated that CBR control of HR was unaffected by endurance training and that an attenuation in arterial baroreflex control of HR in ET individuals was exclusively due to the aortic baroreflex (33,38). While these investigations clearly delineated a functional difference in HR control between the CBR and ABR, limited information is available pertaining to CBR and ABR mediated changes in vascular resistance. However, Stevens et al. (39) noted that after eight months of endurance training subjects exhibited a decreased tolerance to LBNP that was primarily attributed to a reduced vasoconstrictor response, suggesting that a training-induced alteration in the control of vasomotion occurred. In

addition, Shi et al. (34) demonstrated that the reflex control of forearm vascular resistance by cardiopulmonary baroreceptors was diminished in ET individuals compared to their sedentary counterparts. Collectively, these data indicate that baroreflex control of vascular resistance may be altered in ET individuals. However, it was unclear if the altered control of vascular resistance was due to an alteration in autonomic nervous system function (i.e, SNA) or end organ responsiveness (i.e, vascular smooth muscle).

Currently, only two studies have examined the effects of endurance training on arterial baroreflex control of sympathetic nerve activity in humans and the results are equivocal. In addition, no attempt was made to isolate the carotid baroreflex control of MSNA from aortic baroreflex control. Grassi et al. (9) noted a potentiation of arterial baroreflex control of MSNA whereas Sheldahl et al. (31) reported no changes in the baroreflex-MSNA response to hypo- and hypertensive stimuli. Both studies were longitudinal in design (10 and 12 weeks, respectively) and even after undergoing the training regimen the subjects maximal oxygen uptake (VO_{2max}) values were no greater than 41 $ml \cdot kg^{-1} \cdot min^{-1}$. Therefore, it remains unclear to what extent long-term high intensity endurance exercise training, resulting in a VO_{2max} of greater than 60 $ml \cdot kg^{-1} \cdot min^{-1}$, affects baroreflex control of MSNA and whether or not the CBR or ABR control of MSNA is altered with endurance training.

Summary

Arterial baroreceptors, located in the carotid sinus and aortic arch, respond to moment to moment changes in ABP by altering sympathetic and parasympathetic nerve

activity to return blood pressure back to its set point value. However, the role of the carotid and the aortic baroreceptors in the control of muscle sympathetic nerve activity (MSNA) remains unclear. While the MSNA responses to NP/NS have been reported to be asymmetrical at rest, the posture of the subjects may have confounded these results. In addition, the effect of exercise on CBR control of efferent sympathetic nerve activity remains unclear. Therefore, by examining the CBR-mediated changes in MSNA at rest with the subjects in the upright seated position and during upright arm cycling, we propose to determine carotid baroreflex control of MSNA more completely. Moreover, by implementing a non-pharmacological stimulus to produce a dynamic and transient decrease in ABP, we propose to partition the importance of each of the baroreceptor populations (carotid and aortic) in the reflex control of MSNA. Furthermore, we will examine the effects of long-term endurance exercise training on arterial baroreflex control of MSNA to further our understanding of the altered baroreflex control of vasomotion previously reported in high-fit individuals.

SPECIFIC AIMS

Given the lack of information regarding carotid baroreflex control of MSNA during exercise and the limited techniques available to study aortic baroreflex control of MSNA in humans, two primary objectives were developed for this dissertation. These are: i) to determine if CBR control of muscle sympathetic nerve activity (MSNA) is altered during dynamic exercise; and ii) to develop a technique that provides a dynamic

and transient decrease in ABP in order to examine arterial baroreflex control of MSNA and to partition the importance of each of the baroreceptor populations (carotid and aortic) in the reflex control of MSNA. Another goal of this dissertation was to determine if long-term endurance exercise training alters arterial baroreflex function. In order to investigate these specific aims, the following hypotheses were proposed:

I. To test the hypothesis that the carotid baroreflex-muscle sympathetic nerve activity stimulus-response curve is reset and unaltered during dynamic exercise.

II. To test the hypothesis that a dynamic and transient decrease in arterial blood pressure would elucidate the discrete importance of the carotid baroreflex in the muscle sympathetic nerve activity response to acute hypotension.

III. To test the hypothesis that chronic endurance exercise training attenuates the arterial baroreflex control of MSNA by attenuating the aortic baroreceptors.

EXPERIMENTAL DESIGN

Two individual experiments were designed to investigate specific aims I, II, and III. These experiments are discussed in detail in chapters 2 and 3, however, a brief description of the experimental rationale and methodology is provided below.

Carotid Baroreflex Control of MSNA during Dynamic Exercise

In order to determine if carotid baroreflex control of MSNA is altered during dynamic exercise we used brief 5-s periods of NP and NS to perturb the CBR at rest and during arm cycling and measured the ensuing changes in MSNA. Arm exercise was chosen as the mode of exercise because measurements of MSNA to contracting muscle are not possible and the peroneal nerve near the fibula head is the preferred site for MSNA measurements. Therefore, taking these limitations into consideration we developed an apparatus that enables subjects to perform dynamic arm exercise with minimal leg movement, particularly the right leg in which the peroneal nerve is instrumented with wire electrodes for the MSNA recordings. Previously, it has been reported that the release of SNA is global (30) and therefore, we reasoned that the inactive muscle would provide an effective site to measure MSNA changes and quantify CBR-MSNA responses during exercise.

Carotid and Aortic Baroreflex Control of MSNA

In order to determine the importance of the carotid and aortic baroreceptors in the reflex control of MSNA during acute hypotension, we designed a non-pharmacological method to induce a dynamic and transient decrease in ABP. This protocol consisted of nine minutes of unilateral arterial thigh cuff occlusion (300 Torr) and release under two conditions: control and suction. Release of the thigh cuff produced a sudden drop in ABP that was reproducible within 1.7 ± 1.2 mmHg between trials during pilot studies (N=9). We reasoned that during the initial control trial both the aortic (ABR) and carotid (CBR) baroreflexes were deactivated by the acute decrease in ABP and therefore, the response would accurately characterize the arterial baroreflex control of MSNA. The protocol was then repeated with the application of neck suction to the anterior two-thirds of the neck during the initial 14 seconds after cuff release to counteract the changes in carotid sinus transmural pressure induced by the release of the arterial thigh cuff. The amount of NS utilized was derived from the nadir of the MAP response during the control trial. This procedure enabled us to negate the cuff release induced alterations in MAP at the carotid sinus and therefore, functionally isolate the aortic baroreflex (ABR deactivation alone). Therefore, these procedures allowed us to examine the arterial, aortic, and carotid baroreflex control of MSNA during acute hypotension.

Comparison of Baroreflex Control of MSNA in Average Fit and High Fit Individuals

The unilateral arterial cuff occlusion-release protocol described above was utilized to discern fitness related differences in baroreflex control of MSNA during acute

hypotension. Several investigations have reported alterations in baroreflex control of vascular resistance in endurance trained individuals (34,37,39), however, it is unclear if control of vascular resistance is altered at the level of the autonomic nervous system (i.e, SNA) or at the level of the end organ (i.e, vascular smooth muscle). Therefore, our intent was to examine arterial baroreflex control of MSNA to determine if autonomic control of blood pressure was altered in high fit individuals compared to their average fit counterparts.

METHODS

The methodology used in each investigation is described in detail within the following chapters, however, it is appropriate to discuss the major aspects of the microneurographic techniques used to obtain the muscle sympathetic nerve activity recordings and the neck pressure/neck suction technique used to examine carotid baroreflex function. In the late 1960's Vallbo and colleagues (40,41) developed a microneurographic technique for the direct measurements of efferent postganglionic sympathetic nerve traffic to muscle beds in humans. Recordings can be made from any peripheral nerve, but typically the peroneal nerve near the fibula head is used, as it is a relatively superficial nerve site and subjects experience only minimal discomfort during the procedure. First the course of the nerve is determined by stimulating through the skin with a pencil shaped electrode. When the nerve is stimulated, involuntary twitching or tingling sensations occur in the calf or foot. Once the nerve is localized, two tiny, sterile,

wire electrodes (tip diameter of approximately 5-10 μm) are inserted through the skin. One electrode is a reference electrode and the other is the recording electrode that will be inserted into the peroneal nerve to obtain the muscle sympathetic nerve activity (MSNA) recordings. Criteria used to document that the recording is MSNA include: (i) pulse-synchronous bursts occurring 1.2-1.4 s after a QRS complex, (ii) tapping or stretching of the muscle evokes mechanoreceptor afferent discharge, (iii) nerve activity increases in response to end-expiratory breathholds and Valsalva maneuvers, and (iv) no responses to arousal or skin stroking as this elicits skin sympathetic nerve activity. The raw nerve signals are amplified, bandpass filtered (700 - 2000 Hz), rectified, and integrated by a resistance-capacitance circuit with a time constant of 0.1 s. Muscle sympathetic nerve activity is expressed as burst frequency and as total activity, which is calculated as the product of burst frequency and burst amplitude and expressed in arbitrary units.

Carotid baroreflex function was assessed through the use of 5-s periods of NP/NS delivered to the region of the carotid sinuses encased by a malleable lead collar around the anterior 2/3 of the neck. Graded levels of pressure and suction pulses were generated by a variable pressure source and delivered to the neck collar through large bore two-way solenoid valves (Asco, Florham Park, NJ). The NP/NS system was controlled by a custom software package developed for use with a laboratory minicomputer. Pulses were delivered to the neck precisely 50 ms after the R-wave of the ECG, with each pulse duration timed to a 5-s period. The 5-s periods of NP/NS were delivered in a pseudo-random fashion at eight discrete levels of pressure ranging between +45 and –80 Torr. The generated level of pressure within the neck collar was manually controlled and

measured by a pressure transducer. Multiple trials were performed at each of the selected pressure levels and responses were averaged to provide a mean response for each subject. The estimated carotid sinus pressure for a given pulse was calculated as the mean arterial pressure minus the chamber pressure. Carotid baroreflex stimulus-response curves for MAP were individually fit for each subject to a four parameter logistic function described by Kent et al. (11). This function incorporates the following equation:

$$MAP = A_1(1 + e^{[A_2(ECSP-A_3)]})^{-1} + A_4$$

where MAP is the dependent variable, ECSP is the estimated carotid sinus pressure, A_1 is the range of response of the dependent variable (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 response of the dependent variable. This develops a sigmoid reflex model for the carotid sinus baroreflex including an operating range, response range, threshold, saturation, and gain value for the reflex. Unfortunately, the CBR-MSNA responses were unable to be modeled by the Kent logistic model. Therefore, in order to derive an estimate of CBR-MSNA reflex sensitivity at rest and during exercise a simple linear regression analysis was used.

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CHAPTER II

CAROTID BAROREFLEX REGULATION OF SYMPATHETIC NERVE ACTIVITY DURING DYNAMIC EXERCISE IN HUMANS

P.J. Fadel, S. Ogoh, D. Watenpaugh, W. Wasmund, A. Olivencia-Yurvati, M.L. Smith, and P.B Raven

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ABSTRACT

The present investigation was designed to determine if carotid baroreflex control of muscle sympathetic nerve activity (MSNA) was altered during dynamic exercise. In five men and three women, (mean±SE) age of 23.8±0.7 yrs, carotid baroreflex (CBR) function was evaluated at rest and during 20 minutes of arm cycling at 50% peak arm oxygen uptake using 5-s periods of neck pressure (NP) and neck suction (NS). From rest to steady-state arm cycling, mean arterial pressure (MAP) was significantly increased from 90.0±2.7 to 118.7±3.6 mmHg and MSNA burst frequency was elevated by 51±14% ($p<0.01$). However, despite the marked increases in MAP and MSNA during exercise, CBR-Δ%MSNA responses elicited by the application of varying levels of NP/NS ranging from +45 to –80 Torr were not significantly different from rest. Furthermore, estimated baroreflex sensitivity for the control of MSNA at rest was the same as during exercise ($p=0.74$) across the range of neck chamber pressures. Thus, we concluded that carotid baroreflex control of sympathetic nerve activity was preserved during moderate intensity dynamic exercise.

INDEX TERMS

mean arterial pressure, carotid baroreceptors, neck pressure, neck suction

INTRODUCTION

In 1966, Bevegard and Shepherd (1) reported that carotid baroreflex (CBR) regulation of arterial blood pressure (ABP) was unaltered during exercise in humans. The application of neck suction (NS) caused CBR mediated decreases in heart rate (HR) and ABP that were similar both at rest and during supine leg cycling. In an attempt to more completely define CBR function during dynamic exercise, Potts et al. (20) defined the open-loop stimulus-response relationship for the carotid baroreflex during 25 and 50 % VO_{2peak} leg cycling. These investigators demonstrated that the CBR was reset to the prevailing level of systemic pressure and was able to respond to transient changes in ABP as effectively as at rest. This resetting of the CBR without a change in sensitivity has been confirmed for moderate to high intensity dynamic exercise (13,15) as well as static exercise (2). Therefore, it appears that carotid baroreflex function is preserved during exercise.

While carotid baroreflex control of blood pressure remains operable during exercise, the mechanisms by which the CBR responds to changes in ABP may be different from resting conditions. Several investigations have reported that efferent baroreflex control of heart rate was altered during leg cycling (5,19,23) and it has been suggested that during dynamic exercise the magnitude of CBR mediated changes in HR was dependent upon the level of cardiac vagal tone such that the reflex tachycardia to neck pressure (NP) decreased during exercise, while the reflex bradycardia to NS was augmented (19). However, the effect of exercise on CBR control of efferent sympathetic

nerve activity remains unclear as studies examining CBR mediated changes in sympathetic nerve activity during exercise have been limited.

Eckberg and Wallin (4) noted that isometric handgrip exercise at 30% of maximum voluntary contraction resulted in an attenuated muscle sympathetic nerve activity (MSNA) response to + 30 Torr NP and an augmented MSNA response to – 30 Torr NS. These investigators concluded that exercise caused small but significant alterations of CBR mediated neural responses that appear to limit increases in MSNA. In contrast, Papelier et al. (16) reported that sustained activation of the muscle chemoreflex with post-exercise ischemia caused diminished CBR-mean arterial pressure (MAP) responses to hypertension and enhanced responses to hypotension without alterations in the carotid-HR response. Although MSNA was not recorded it was suggested that the augmentation in sympathetic nerve activity caused by muscle chemoreflex activation overpowered the CBR response to hypertension and added to the CBR mediated vasoconstriction induced by hypotension. Although equivocal these investigations suggest possible alterations in CBR control of MSNA during exercise.

Previously it has been demonstrated that at rest 5-s periods of NP/NS produced CBR mediated changes in MSNA that were described by a typical sigmoid baroreflex relationship (22). However, the stimulus-response curve for the CBR control of MSNA has yet to be characterized during dynamic exercise. Therefore, the purpose of the present investigation was to determine if dynamic exercise altered the CBR control of MSNA in humans. Our intent was to construct stimulus-response relationships for carotid baroreceptor control of MSNA using brief 5-s perturbations of carotid sinus

pressure generated by the application of NP and NS (from +45 to –80 Torr) at rest and during 20 minutes of dynamic arm cycling at 50% VO_{2peak}. Given the previous findings in human investigations that indicate a resetting of the CBR-HR and MAP stimulus-response curve to the prevailing arterial blood pressure without a change in reflex sensitivity, we hypothesized that the CBR-MSNA response would also be reset and unaltered during dynamic exercise.

METHODS

Subjects. Five men and three women with a mean (± SE) age of 23.8 ± 0.7 yrs, height of 174.7 ± 3.8 cm, and weight of 71.3 ± 5.3 kg, voluntarily participated in the present investigation. Each subject was advised of the testing protocols and potential risks of participation in the study and provided written informed consent, which was approved by the University of North Texas Health Science Center Institutional Review Board for the use of Human Subjects. All subjects were non-smokers, were free of any known cardiovascular or respiratory disease and were not using prescription or over the counter medication. Strenuous physical activity and alcohol consumption was prohibited 24 hours prior to any scheduled testing session and subjects were asked to abstain from caffeinated beverages 12 hours before any testing. A total of 20 subjects initially entered this study. However, in five subjects placement of the femoral arterial catheter was unsuccessful, in three subjects nerve recordings were unobtainable and in four subjects we were unable to maintain nerve recordings during exercise. Only the eight subjects for whom we had nerve recordings at rest and during exercise were used for the analyses.

Exercise Testing. Subjects were administered a seated incremental exercise test on an arm cycle ergometer (Intellifit, Inc., Houston, Texas) mounted to a table for the determination of peak arm oxygen uptake ($VO_{2armpeak}$). The arm cycle work rate was set at 20 watts for men and 10 watts for women and was increased 20 and 10 watts for men and women, respectively each minute as the subject maintained an arm cycling rate of 60 rpm. The exercise test was terminated when the subject could no longer maintain a work rate at 60 rpm despite strong verbal encouragement.

Subjects respired through a mouthpiece attached to a low-resistance turbine volume transducer (Sensor Medics VMM E-2A, Anaheim, CA) for measurement of breath volumes while respiratory gases were continuously sampled from the mouthpiece for determinations of fractional concentrations of O_2, CO_2, and N_2 with mass spectrometry (Perkin-Elmer MGA1100B, St. Louis, MO). The analog signals of the mass spectrometer underwent analog-to-digital conversion and computer analysis (Dell OptiPlex GXi) for on-line, breath-by-breath determination of respiratory gases. For each subject the work rate at which 50% $VO_{2armpeak}$ occurred was determined and used as the work rate for 20 minutes of dynamic arm exercise.

Experimental Measurements. All testing was performed with the subjects in an upright seated position. Cardiovascular variables were monitored beat-to-beat and recorded by a personal computer (PC) equipped with customized software as well as a second PC equipped with an online data acquisition program (Dataq Instruments DI-720, Akron, OH). Heart rate was monitored with a standard lead II electrocardiogram (ECG). The ECG signal was output to a pressure monitor (Hewlett-Packard 78342A, Andover,

MA) interfaced with the personal computers. Arterial blood pressure was measured directly from the femoral artery of the left leg using an 18 gauge (1.35 mm) 12 cm Teflon catheter connected to a pressure transducer (Maxxim Medical, Athens, TX) interfaced with the aforementioned pressure monitoring system. Prior to placement of the femoral catheter Lidocaine (1%) was injected subcutaneously to minimize subject discomfort. The catheter was kept patent by a continuous drip of heparinized saline (2 ml/hr) and before obtaining blood pressure measurements; the transducer was zeroed to the mid-axillary line of the subject.

Sympathetic Nerve Recordings. Post-ganglionic muscle sympathetic nerve activity was recorded with standard microneurographic techniques, as described previously (27). A tungsten microelectrode was inserted into the peroneal nerve near the fibular head of the right leg. The nerve signal was processed by a preamplifier and an amplifier (Nerve Traffic Analyzer, Model 662C-3, University of Iowa Bioengineering, Iowa City, IA) with a total gain of 70,000. Amplified signals were bandpass filtered (700 - 2000 Hz), rectified, and discriminated. Raw nerve signals were integrated by a resistance-capacitance circuit with a time constant of 0.1 s. Muscle sympathetic nerve recordings were readily recognized by their pulse-synchronous burst pattern and an increase in burst frequency with end-expiratory breathholds and Valsalva maneuvers without any response to arousal or skin stroking. These characteristics were used to discriminate between muscle and skin sympathetic nerve fibers.

Procedures. On the experimental day, after being instrumented with ECG leads and a femoral arterial catheter subjects were seated in a chair positioned in front of the

arm cycle ergometer and proper seat and ergometer adjustments were made. At this point, in order to verify the work rate (50% $VO_{2armpeak}$) and to identify optimal leg positioning to minimize movement of the right leg which was to be instrumented for muscle sympathetic nerve activity (MSNA) measurements, breath by breath measurements of oxygen uptake were collected during six minutes of exercise at the desired work rate. Work rate and leg positioning adjustments for each subject were made accordingly. After this initial validation exercise bout, the peroneal nerve of the right leg was instrumented for continuous recordings of MSNA. Following obtainment of a muscle nerve site, the subjects were fitted with a malleable lead neck collar for the application of neck pressure (NP) and neck suction (NS). Carotid baroreflex (CBR) responsiveness was then assessed at rest and followed by measurements obtained during steady-state arm exercise at 50% VO_{2peak} (after ~ 6 min of exercise).

Carotid Baroreflex Responsiveness. Carotid baroreflex control of MSNA and mean arterial pressure (MAP) was assessed by applying random ordered single 5-s pulses of NP and NS to the anterior 2/3 of the subject's neck, a technique previously described in detail (20). Briefly, NP and NS ranging from +45 to –80 Torr were applied to simulate a reflex stimulus response to +45, +30, +15, 0, -10, -20, -40, -60, -80 Torr. Under resting conditions each level of pressure and suction was delivered to the carotid sinus for a period of five seconds during a 10 to 15 second breath hold at end expiration. The breath hold was performed to minimize the respiratory related modulation of HR and MAP; however, slight but consistent increases in MSNA were noted during the breath hold in some subjects. Therefore, multiple control trials (ambient pressure in collar) were

performed and the average of these trials was used as the baseline pre-stimulus MSNA values for each subject. During exercise, the breath hold was eliminated as Eckberg et al. (3) has reported that at a breathing frequency greater than 24 breaths/min no differences existed between the responses to neck collar stimuli during inspiration and expiration. In addition, our laboratory has previously identified the repeatability of applying NP and NS without a breath hold during high intensity exercise (14). Four to five perturbations were performed at each level of NP/NS at rest whereas during exercise only two to three perturbations were performed. The reduced time for carotid sinus stimulation during exercise (~ 12 to 14 min) was designed to allow subjects to be at steady-state before CBR testing began and also, to minimize any confounding effects of cardiovascular drift on CBR function. Peak MAP responses to each stimulus were determined and averaged to provide a mean response for each subject. The MSNA responses during each 5-s period of NP and NS were identified according to their appearance and timing in relation to preceding R-waves and calculated as total activity, which was computed as the product of burst frequency and burst amplitude and expressed in arbitrary units. At rest the MSNA responses for each level of NP and NS were averaged to provide a mean response for each subject, which was then expressed as a percent change from the mean MSNA value obtained during the breath hold alone (control trials). During steady-state exercise the average MSNA value for each level of NP and NS was compared to a mean baseline value determined from a segment taken at the beginning and end of the CBR stimulation period and also, presented as a percent change in total activity. Estimated changes in

carotid sinus pressure (ECSP) were calculated as MAP minus neck chamber pressure and used to build CBR stimulus-response curves for MSNA and MAP.

Data Analyses. Carotid baroreflex stimulus-response curves for MAP were fit for each subject to a four-parameter logistic function described by Kent et al. (8), which uses the following equation:

$$MAP = A_1 (1+e^{[A_2(ECSP-A_3)]})^{-1} + A_4$$

where A_1 is the MAP response range (maximum – minimum), A_2 is the gain coefficient, A_3 is the centering point or carotid sinus pressure (CSP) required to elicit equal pressor and depressor responses, and A_4 is the minimum MAP response. Individual data were fit to this model by non-linear 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 Kent logistic function and the maximal gain (G_{max}) was calculated as the gain value at the centering point (A_3). In addition, a CBR threshold, point where no further increase in MAP occurred despite reductions in CSP, and saturation, point where no further decrease in MAP occurred despite increases in CSP, were identified. All parameters were averaged and presented as group means. Unfortunately, the CBR-MSNA responses were unable to be modeled by the Kent logistic model. Therefore, in order to derive an estimate of CBR-MSNA reflex sensitivity at rest and during exercise a simple linear regression analysis was used.

Statistical Analyses. Comparisons of selected physiological variables and CBR-MAP stimulus-response parameters between rest and exercise were made utilizing paired

t tests. An analysis of covariance (ANCOVA) was employed to determine significant differences in CBR- Δ % MSNA values between rest and exercise. In addition, a *t* test was used to determine significant differences in CBR-MSNA reflex sensitivity between rest and exercise and to identify significant differences between the $VO_{2armpeak}$ values of the men and women. Statistical significance was set at $p<0.05$. Results are presented as means ± SE.

RESULTS

Arm VO_2 Peak. The average peak arm oxygen uptake ($VO_{2armpeak}$) for the group was 29.1 ± 2.5 $ml \cdot kg^{-1} \cdot min^{-1}$ with the men demonstrating significantly higher values (33.7 ± 1.5 $ml \cdot kg^{-1} \cdot min^{-1}$) compared to the women (21.3 ± 1.8 $ml \cdot kg^{-1} \cdot min^{-1}$). As such, the steady-state exercise work rate at 50% $VO_{2armpeak}$ was 16.8 ± 1.1 $ml \cdot kg^{-1} \cdot min^{-1}$ (equivalent to 44 ± 1.9 Watts) for the men and 11.4 ± 0.8 $ml \cdot kg^{-1} \cdot min^{-1}$ (equivalent to 20 ± 0.0 Watts) for the women.

Selected physiological variables. The MSNA and cardiopulmonary responses to steady-state dynamic arm exercise at 50% VO_{2peak} are presented in Table 1. Muscle SNA expressed as either total activity (TA) or burst frequency (BF) exhibited approximately a 50% increase during the arm exercise ($p<0.05$). This increase in MSNA was consistent throughout the steady-state exercise bout as measurements recorded at 5 to 6 minutes (1380 ± 306 TA (N=5) and 41.7 ± 4.0 BF) were not significantly different from measurements obtained at 18 to 19 minutes (1359 ± 302 TA (N=5) and 43.7 ± 4.1 BF). Mean arterial pressure (MAP) and HR were also significantly elevated from rest to steady-state exercise, see Table 1.

Carotid Baroreflex Control of MAP. The stimulus-response relationship for the carotid baroreflex control of MAP at rest and during steady-state dynamic arm cycling is presented in Figure 1. The logistic parameters describing the carotid baroreflex-MAP relationship are shown in Table 2. Carotid sinus threshold and saturation pressures were significantly increased from rest to steady-state exercise. In addition, during exercise the operating point (OP) and centering point (CP) were significantly increased from rest and the OP moved away from the CP towards the threshold region of the reflex (CP-OP relationship 0.01 ± 2.6 at rest and 4.2 ± 1.9 during exercise, $p>0.05$). The maximal gain of the CBR-MAP stimulus-response relationship was similar at rest and during the 50% VO_{2peak} arm cycling ($p=0.40$).

Carotid Baroreflex Control of MSNA. The stimulus-response relationship for the carotid baroreflex control of MSNA at rest and during arm cycling is presented in Figure 2. The CBR- Δ % MSNA responses elicited by the application of varying levels of neck pressure and neck suction from +45 to –80 Torr were not significantly different between rest and exercise. In addition, estimated maximal gain across the range of neck chamber pressures was similar at rest and during exercise (-1.24 ± 0.21 and –1.24 ± 0.18 Δ % MSNA/mmHg, $p=0.74$). Sample recordings depicting the maintained baroreflex sensitivity during exercise are presented in Figures 3 and 4.

DISCUSSION

The major accomplishment of this investigation was that for the first time, carotid baroreflex mediated changes in muscle sympathetic nerve activity (MSNA) have been quantified during steady-state dynamic exercise in humans. More importantly, our

findings indicate that carotid baroreflex control of sympathetic nerve activity was reset to function at the higher arterial pressures induced by dynamic arm exercise without a change in reflex sensitivity. In addition, similar to dynamic leg exercise, the CBR-MAP stimulus-response curve responding range was displaced upwards and the operating range was shifted to the right during the steady-state arm cycling (i.e., classical resetting).

Effect of exercise on CBR-MSNA responses. The finding that the CBR- Δ % MSNA responses were not significantly different between rest and steady-state exercise indicated that the carotid baroreflex control of MSNA was preserved during dynamic exercise. In contrast, Eckberg and Wallin (4) reported that although subtle baroreflex control of MSNA was altered during brief isometric handgrip exercise, with a heightened ability to inhibit MSNA and a diminished capacity to increase MSNA. The reason for differences in results of Eckberg and Wallin (4) and those of the present investigation is unclear. However, the different types of exercise employed or posture of the subjects during experiments may have been responsible. The differences in physiological responses between static and dynamic exercise have been well-documented (12), however, carotid baroreflex responsiveness as determined from CBR-MAP and HR responses have been reported to be unaltered during both static and dynamic exercise in humans (2,13,20). These reports suggest that carotid baroreflex control was not dependent on the type of exercise being performed. Therefore, the more likely explanation of the differences between investigations may be due to the subjects being in a supine position in the Eckberg and Wallin (4) study compared to the seated position in the present investigation. The seated position results in a higher resting baseline muscle

sympathetic nerve activity compared to the supine position, presumably due to cardiopulmonary unloading (26). Thus, it was plausible that the greater and smaller MSNA responses to 30 Torr NP and NS, respectively at rest compared to handgrip exercise in the Eckberg and Wallin investigation (4) were a consequence of the low baseline MSNA in the resting supine position. Whereas in the present investigation, the seated position resulted in a higher resting baseline activity that provided more comparable baseline MSNA values at rest to those obtained during steady-state exercise.

The influence of subject posture on CBR control of MSNA may be further realized by a comparison of the resting CBR-MSNA responses in the present investigation with carotid-MSNA responses obtained at rest in a group of subjects in the supine position. Rea and Eckberg (22) reported that sympathetic nerve activity responses to changes in carotid sinus pressure from +40 to –65 Torr were asymmetric with increases in MSNA during NP being much greater than reductions in MSNA with NS. In contrast, as depicted in Figure 2, we found more symmetric changes in MSNA with the application of NP and NS. We suggest that these differences were a result of the higher baseline values of MSNA induced by the seated position, which permitted more graded responses to the application of varying levels of NS pressures. Whether this was a result of a central interaction between the cardiopulmonary baroreceptors and the carotid baroreflex or a consequence of higher baseline nerve activity remains unknown.

Even though Eckberg and Wallin (4) reported differences in CBR-MSNA responses during isometric exercise, the differences were minor and when evaluated completely the carotid baroreflex control of MSNA was maintained. These findings

combined with several human (2,13,15,20) and animal studies (11) that have reported continued baroreflex control of arterial pressure at varying intensities of exercise, suggest that the control of blood pressure via the sympathetic nervous system was unaltered during exercise. By utilizing a range of pressure inputs from +45 to –80 Torr, the present investigation has identified the stimulus-response relationship for the carotid baroreflex control of MSNA during arm cycling. Although we were unable to model the MSNA responses using the Kent logistic model, examination of figure 2 indicated that the CBR-MSNA stimulus-response curve was relocated to higher carotid sinus pressures (i.e., to the right) during exercise. However, classical resetting describes an upward relocation on the response axis as well as a rightward relocation on the operating axis of the CBR stimulus-response curve. Even though an upward relocation was not evident in figure 2, if absolute values for MSNA total activity were used the upward relocation would be apparent as MSNA was significantly elevated during steady-state exercise (Table 1). However, the inter-subject variability of total activity among subjects combined with the use of different nerve sites during exercise compared to rest in three subjects warranted the use of Δ % MSNA changes for group comparisons.

Analysis of the CBR- Δ % MSNA stimulus-response curves constructed for each subject indicated that the relocation of the response curve to operate at the higher pressures induced by exercise occurred without a change in reflex sensitivity. These findings are in agreement with previous investigations that have reported a resetting of the CBR-MAP stimulus-response curve without changes in the maximal gain of the

reflex (13,20). Thus, it appeared that despite marked increases in MSNA and MAP during exercise, the carotid baroreflex control of MSNA was preserved.

Effect of exercise on CBR-MAP responses. In addition to the MSNA responses, we also measured CBR mediated changes in MAP. During steady-state arm exercise the CBR-MAP operating point, centering point, and minimal response were all significantly increased from rest (Table 2.). Furthermore, the CSP-MAP threshold and saturation pressures were elevated by exercise without any change in maximal reflex gain. Collectively, these alterations in the CBR-MAP stimulus-response curve represent the classic rightward and upward resetting of the CBR without changes in reflex sensitivity (Figure 1.). While this resetting of the CSP-MAP curve has been identified during dynamic leg exercise from low to maximal intensity (13,20) this is the first study to confirm resetting of the CBR during dynamic arm exercise. This is of considerable interest as arm exercise evokes different metabolic, thermal, circulatory, and perceptive responses compared to leg exercise, which leads to greater cardiovascular strain (17,18). Thus, at a similar percentage of VO_{2peak} blood pressure, ratings of perceived exertion (RPE) and heart rate were higher during arm compared to leg exercise (17). However, despite these differences the carotid baroreflex appropriately resets to regulate blood pressure at the prevailing MAP.

Interestingly, a comparison of the CSP-MAP stimulus-response curve from the present investigation with that reported by Norton et al. (13) for a group of subjects performing leg cycling at 50% VO_{2max} in the seated position indicates that the CBR-MAP response curve was reset rightward and upward during both forms of exercise performed

at the same relative workload. The major difference was that the stimulus response curve was clearly relocated further upward and rightward during arm exercise. This indicates that the CBR resetting was independent of exercise mode and intensity and occurred in direct relationship to the prevailing exercise pressure.

It has been suggested that central command, a feedforward mechanism that acts through central processes to activate in parallel the cardiovascular and somatomotor responses to exercise, and the exercise pressor reflex, a negative feedback reflex that originates in the active skeletal muscle and responds to chemical and mechanical stimuli, are involved in CBR resetting during exercise (7,24). Nevertheless it remains unclear as to the extent to which central command and the exercise pressor reflex affect resetting of the CBR. Both appear to be more active during arm compared to leg exercise, as indicated by the greater metabolic strain (exercise pressor reflex) and perceived effort (central command) of small muscle mass exercise (17,18). However, recent findings that indicate that lactate and hydrogen ions do not contribute to the stimulation of the exercise pressor reflex challenge the role of a greater exercise pressor activation during arm exercise (10). The primary metabolic differences between leg and arm exercise were delayed oxygen kinetics and greater recruitment of fast-twitch glycolytic muscle fibers, which lead to elevated concentrations of plasma lactic acid at any given work rate during arm exercise (17). Therefore, it is possible that the metabolic strain did not potentiate the exercise pressor reflex. However, the consistently higher heart rates and ratings of perceived exertion at any given steady-state VO_2 during arm exercise compared to leg exercise indicated an elevated central command (17,18). As our laboratory has recently

demonstrated that central command actively reset the CBR during dynamic exercise, we suggest that an elevated central command was the primary reason for the augmented resetting of CBR-MAP stimulus-response curve during arm exercise compared to leg exercise.

Gallagher et al. (6) reported a significantly greater upward and rightward shift in the CBR-MAP stimulus-response curve when Norcuron was used to induce partial neuromuscular blockade and thereby increase central command during dynamic leg cycling at 20% VO_{2peak}. This augmentation in CBR resetting reported by Gallagher et al. (6) was very similar to the greater resetting noted in the comparison of the CBR-MAP stimulus-response curves during 50% VO_{2peak} arm cycling in the present investigation with the curves reported by Norton et al. (13) during 50% VO_{2max} leg cycling. Therefore, a greater activation of central command during arm exercise may actively reset the CBR to the greater pressures induced by the small muscle mass exercise. However, this by no means excludes involvement of the exercise pressor reflex in the resetting of the CBR during arm exercise.

Potential Limitations. In the present investigation, we utilized sympathetic measurements from an inactive skeletal muscle bed to describe overall carotid baroreflex control of sympathetic outflow. This does not preclude the possibility that CBR-mediated changes in sympathetic nerve activity differ among vascular beds, however, it has been suggested that the release of sympathetic nerve activity was uniform throughout the body (25). Therefore, we suggest that the MSNA measurements of the present investigation provided an effective approximation of CBR-control of sympathetic neural

outflow both at rest and during arm exercise. Another potential limitation was our inability to model the CBR-MSNA responses by using the logistic function described by Kent et al. (8) to predict a curve of best fit and determine the typical parameters and derived variables to describe carotid baroreflex control of MSNA. However, with the use of arbitrary units to quantify MSNA, it is unclear how much information would be obtained from the parameters and variables derived from the curve of best fit. We were able to estimate the responsiveness of the CBR control of MSNA by fitting each subject's data to a simple linear regression model. Although this model does not take into account the typical sigmoid baroreflex relationship, it enabled us to compare changes in reflex responsiveness between rest and exercise.

A final potential limitation was the controversy over the precise pressure transmission to the carotid sinus with NP and NS. Previous findings using a fluid-filled catheter have indicated incomplete transmission to the carotid sinus, with 86% of positive pressure being transmitted and only 64% of negative pressure transmitted (9). However, more recently, Querry et al. (21) measured pressure transmission with a pressure-tipped (Millar) catheter at rest and during exercise and reported greater than 90% transmission for both NP and NS. Taking these new findings into consideration, we considered neck pressures and suctions to be faithfully transmitted to the carotid sinus at rest and during steady-state exercise.

In summary, we demonstrated that across a range of pressures from +45 to –80 Torr, carotid baroreflex mediated changes in sympathetic nerve activity were not significantly different between rest and exercise. Moreover, estimated baroreflex

sensitivity for the control of MSNA at rest was similar to that obtained during steady-state dynamic arm exercise. Thus, CBR control of sympathetic outflow was reset to function at the higher arterial pressures induced by dynamic exercise. In addition, the CBR-MAP stimulus-response curve was shifted upwards and to the right during steady-state arm cycling, which appears to be augmented in comparison to steady-state leg exercise performed at the same relative workload. We speculate that this augmentation results from a greater activation of central command during exercise performed with a small muscle mass. We conclude that carotid baroreflex control of sympathetic nerve activity and mean arterial pressure was maintained during moderate intensity dynamic exercise.

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After August 2000 the address for Paul J. Fadel, Ph.D. will be University of Texas Southwestern Medical Center at Dallas, Division of Cardiology, 5323 Harry Hines Blvd. Dallas, TX 75390.

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FIGURE LEGENDS

Figure 1. Reflex responses in mean arterial pressure (MAP) elicited by perturbations to the carotid sinus baroreceptors at rest (•) and during arm cycling at 50% VO_{2peak} (○). Symbols denote group means ± (SE), and lines represent fitted logistic functions developed from the mean baroreflex curve parameters for all subjects. OP, Operating point; ECSP, estimated carotid sinus pressure (MAP – chamber pressure).

Figure 2. Reflex responses in muscle sympathetic nerve activity (MSNA) elicited by perturbations to the carotid sinus baroreceptors at rest and during arm cycling at 50% VO_{2peak}. MSNA is presented as a percent change from baseline. A) Group means ± (SE) changes in MSNA across the full range of neck chamber pressures at rest and during arm cycling at 50% VO_{2peak}. B) The typical carotid baroreflex stimulus-response curve presented as percent changes in MSNA plotted against estimated carotid sinus pressure (ECSP) calculated as MAP minus chamber pressure. Symbols denote group means ± (SE), (•) rest and (○) 50% VO_{2peak} arm cycling. No differences in carotid baroreflex-MSNA responses were found between rest and exercise.

Figure 3. Muscle sympathetic nerve activity (MSNA) and arterial blood pressure (ABP) responses of one subject to the application of –80 Torr neck suction at rest (A) and during 50% VO_{2peak} arm cycling (B). Data segment represents 30-second period of nerve activity followed by 15-second segment with the application of neck suction for 5s. ChP, neck chamber pressure.

Figure 4. Muscle sympathetic nerve activity (MSNA) and arterial blood pressure (ABP) responses of one subject to the application of +30 Torr neck pressure at rest (A) and during 50% VO_{2peak} arm cycling (B). Data segment represents 30-second period of nerve activity followed by 15-second segment with the application of neck pressure for 5s. ChP, neck chamber pressure.

Table 1. Physiological responses to 50% VO_{2peak} arm cycling.

	Rest	Arm Cycling	Change (Δ)
MSNA, Total Activity (units·min^{-1}) ‡	921 ± 283	1,369 ± 302	+ 448 ± 130 *
MSNA, Burst Frequency (bursts·min^{-1})	30.1 ± 3.6	42.6 ± 3.8	+ 12.5 ± 2.8 *
MAP (mmHg)	90.0 ± 2.7	118.7 ± 3.6	+ 28.7 ± 2.7 *
HR (beats·min^{-1})	74.4 ± 2.4	122.4 ± 4.6	+ 48.0 ± 4.4 *
Oxygen Uptake (ml·min^{-1})	351 ± 36	1,063 ± 120	+ 711 ± 100 *

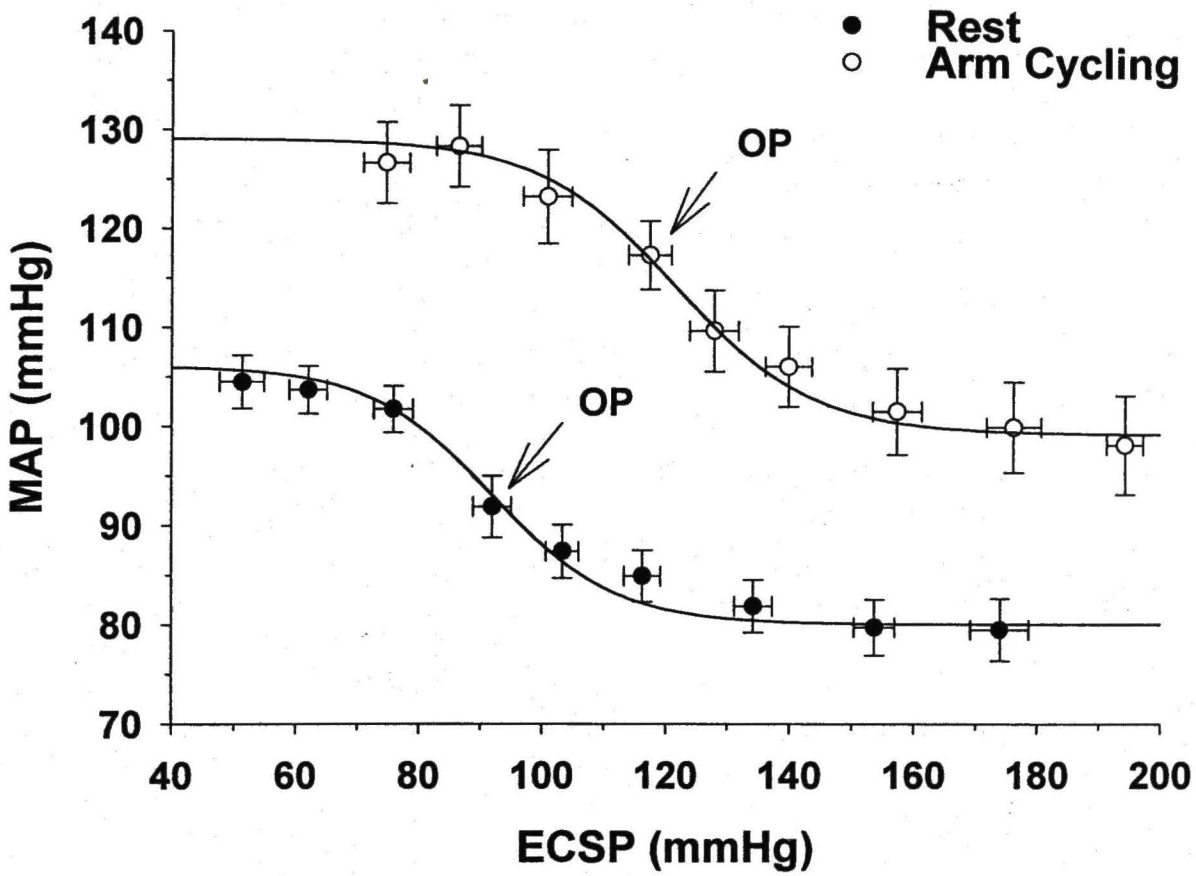
Values are mean ± SE. MSNA, muscle sympathetic nerve activity; MAP, mean arterial pressure; HR, heart rate. ‡ N=5, different nerve site was used for three subjects during exercise. * Denotes significant difference from rest ($p<0.05$).

Table 2. Carotid-MAP baroreflex function curve parameters.

	Rest	50% VO_{2peak} Arm Cycling
Threshold (mmHg)	70.3 ± 5.6	97.5 ± 5.4 *
Saturation (mmHg)	113.5 ± 6.1	145.7 ± 1.5 *
Operating Point (mmHg)	91.9 ± 3.1	117.4 ± 3.5 *
Centering Point (mmHg)	91.9 ± 5.1	121.6 ± 3.3 *
Max Gain (mmHg/mmHg)	-0.62 ± 0.05	-0.65 ± 0.07
Response Range (mmHg)	26.0 ± 3.0	29.8 ± 3.1
Min. MAP Response (mmHg)	80.1 ± 2.7	99.3 ± 4.8 *

Values are mean ± SE. MAP, mean arterial pressure. Response range equals maximum minus minimum MAP response. * Denotes significant difference from rest ($p<0.05$).

Figure 1.


Rest
Arm Cycling
OP
OP
MAP (mmHg)
ECSP (mmHg)
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130
120
110
100
90
80
70
40
60
80
100
120
140
160
180
200

Figure 2.

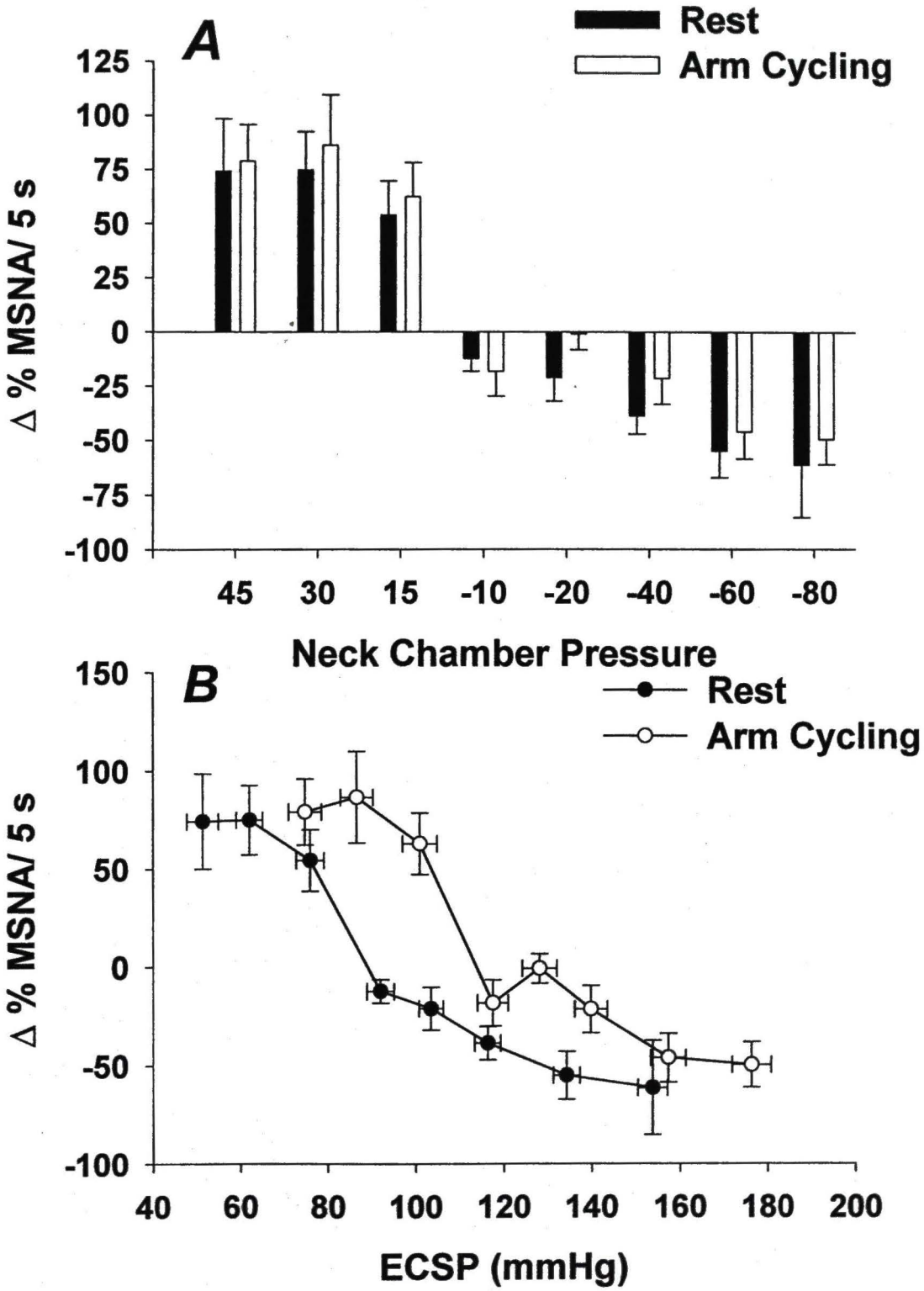

A
Rest
Arm Cycling
125
100
75
50
25
0
-25
-50
-75
-100
Δ % MSNA/ 5 s
45
30
15
-10
-20
-40
-60
-80
Neck Chamber Pressure
B
Rest
Arm Cycling
150
100
50
0
-50
-100
Δ % MSNA/ 5 s
40
60
80
100
120
140
160
180
200
ECSP (mmHg)

Figure 3.

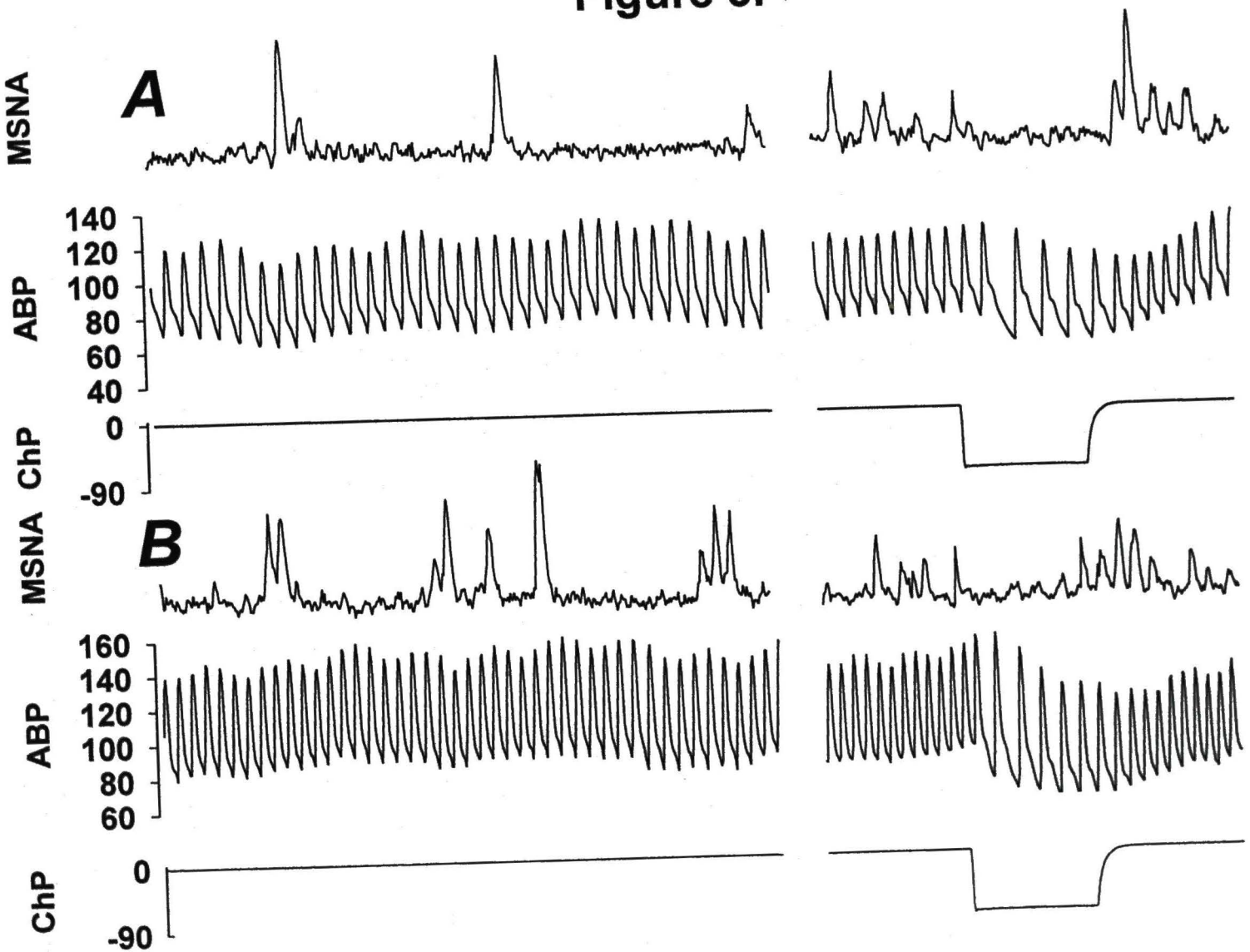
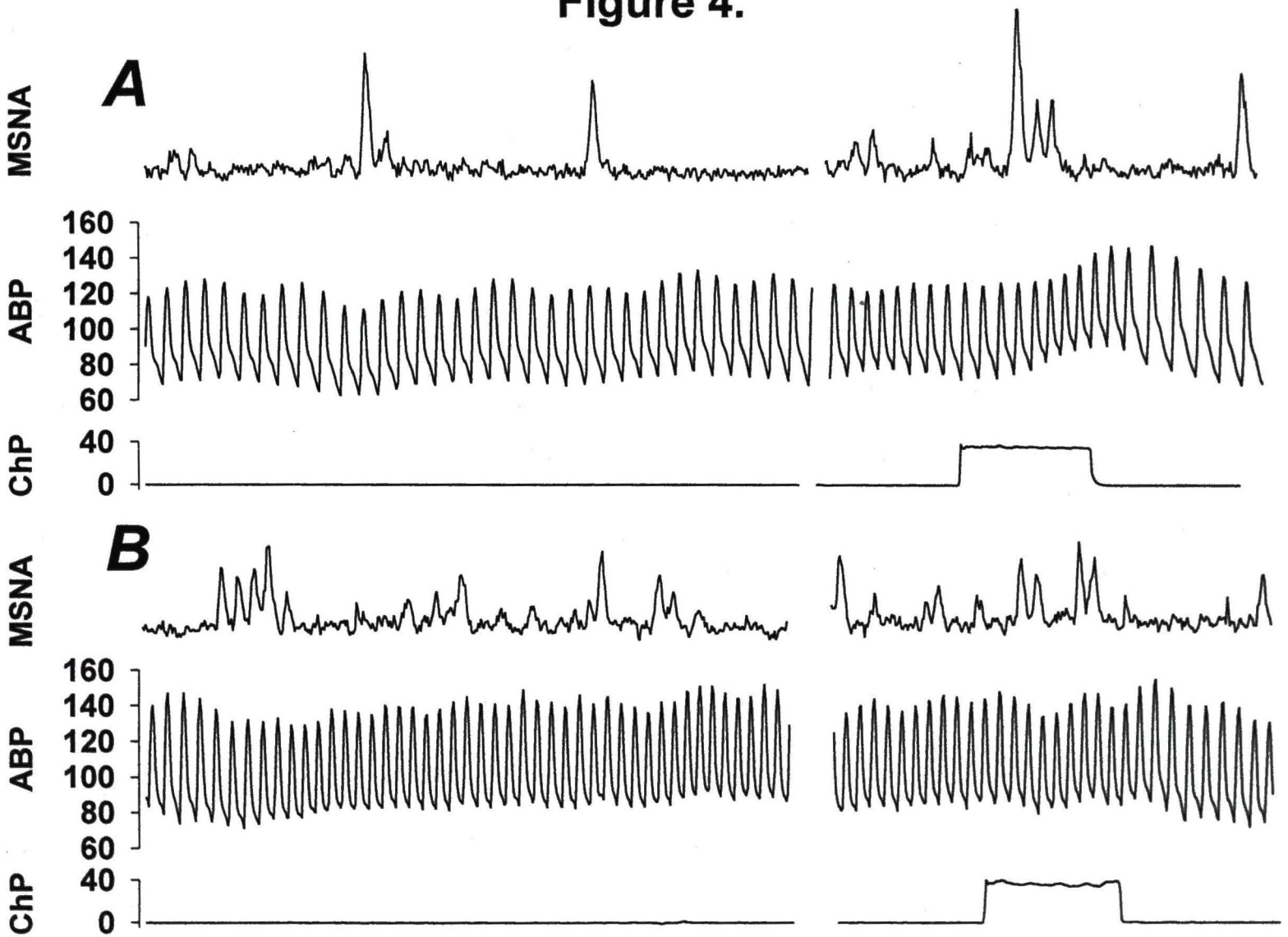

A
MSNA
ABP
140
120
100
80
60
40
ChP
0
-90
B
MSNA
ABP
160
140
120
100
80
60
ChP
0
-90

Figure 4.


A
MSNA
ABP
160
140
120
100
80
60
ChP
40
0
B
MSNA
ABP
160
140
120
100
80
60
ChP
40
0

CHAPTER III

ARTERIAL BAROREFLEX CONTROL OF SYMPATHETIC NERVE ACTIVTY DURING ACUTE HYPOTENSION: EFFECT OF FITNESS

P.J. Fadel, M. Stromstad, J. Hansen, M. Sander, K. Horn, S. Ogoh,

M.L. Smith, N.H. Secher and P.B. Raven

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ABSTRACT

We examined arterial baroreflex control of muscle sympathetic nerve activity (MSNA) during abrupt decreases in mean arterial pressure (MAP) and evaluated if endurance training alters baroreflex function. Acute hypotension was induced non-pharmacologically in fourteen healthy subjects, seven high-fit (HF) and seven average-fit (AF), by releasing a unilateral arterial thigh cuff following 9-minutes of resting ischemia under two conditions: control (aortic (ABR) and carotid (CBR) baroreflex deactivation) and suction (ABR deactivation alone). The application of neck suction to negate the CBR during cuff release significantly attenuated the MSNA response (increased 134.3±31.9 units/14-s) compared to control (increased 195.2±43.4 units/14-s) and caused a greater decrease in MAP (19.4±1.7 vs. 15.2±1.7 mmHg, $p<0.05$). Furthermore, during both trials HF subjects exhibited a greater decrease in MAP compared to AF subjects despite an augmented baroreflex control of MSNA. These data indicate that while the CBR contributes importantly to the MSNA response during acute hypotension, the ABR is more dominant. Additionally, we suggest that an impaired control of vasomotion hinders blood pressure regulation in HF subjects.

INDEX TERMS

carotid baroreceptors, aortic baroreceptors, endurance training, neck suction

INTRODUCTION

Previously several studies have indicated that the aortic baroreceptor reflex predominates in the baroreflex control of heart rate (HR) in humans (8,18,29,31). However, the role of the carotid vs. the aortic baroreceptors in the control of muscle sympathetic nerve activity (MSNA) requires further definition. Human investigations of arterial baroreflex control of MSNA have been limited almost exclusively to the influence exerted by the carotid baroreflex (CBR) because there are few techniques that allow aortic baroreflex (ABR) mediated changes in MSNA to be evaluated (7). Using the variable pressure neck chamber Wallin and Eckberg (36) reported that alterations in carotid sinus transmural pressure with neck pressure and neck suction caused profound and transitory changes in MSNA. Furthermore, since CBR activation with neck suction completely suppressed MSNA, these investigators suggested that afferent baroreceptor neurons (ABR and CBR) influence the same efferent postganglionic sympathetic motorneuron pool. However, the relative importance of the CBR compared to the ABR in the reflex control of MSNA is unclear.

Sanders et al. (24,25) used steady-state infusions of phenylephrine and nitroprusside alone (aortic and carotid baroreceptor activation and deactivation) and in combination with neck pressure and neck suction, respectively (aortic baroreceptor activation and deactivation) to evaluate the relative importance of the CBR vs. the ABR on the control of MSNA. From these investigations it was concluded that the ABR dominates in the arterial baroreflex control of MSNA. However, there remains some question as to the specific interpretation of these data. In particular, questions have arisen

about the use of three-minute periods to evaluate baroreflex function, and the use of steady-state infusions of vasoactive drugs that produced sustained alterations in arterial blood pressure despite counteractive reflex responses (7). Therefore, one purpose of the present investigation was to use a non-pharmacological method to induce acute hypotension to examine arterial baroreflex control of MSNA during a more transient and dynamic change in ABP.

Another purpose of the present investigation was to examine whether chronic endurance exercise training alters arterial baroreflex function. Chronic endurance exercise training has been reported to place the endurance trained (ET) individual at risk for orthostatic intolerance (3,12). One explanation for the increased prevalence of orthostatic intolerance in ET individuals appears to be an attenuation of the arterial baroreflex control of HR and vascular resistance (21). Several investigations have demonstrated that CBR control of HR was unaffected by endurance training, however in these same investigations it was reported that an attenuation of the aortic baroreflex resulted in a diminished arterial baroreflex control of HR in ET individuals (27,31). While these investigations clearly delineated an endurance training induced functional difference in HR control between the CBR and ABR, limited information pertaining to differences in CBR and ABR control of MSNA due to training has been reported. Currently, only two studies have examined the effect of endurance training on baroreflex control of MSNA in humans and the results are equivocal and appear confounded by the subjects' relatively low maximal oxygen uptakes even after the training periods (10,26).

In order to address some of these issues pertaining to arterial baroreflex control and the effects of endurance training on baroreflex function, we used a non-pharmacological method to induce acute hypotension with and without neck suction to deactivate the aortic baroreceptors alone (suction) and in combination with the carotid baroreceptors (control). One goal was to examine arterial baroreflex control of MSNA during a dynamic and transient decrease in ABP and to partition the importance of each of the baroreceptor populations (carotid and aortic) in the reflex control of MSNA. We hypothesized that the dynamic nature of the ABP change would elucidate the discrete importance of the CBR and ABR control of MSNA in response to acute hypotension. A second goal was to determine if long-term endurance exercise training alters arterial baroreflex function. We hypothesized that chronic endurance exercise training would attenuate arterial baroreflex control of MSNA and this attenuation would be a result of an attenuated ABR control of MSNA.

METHODS

Subjects. Fourteen healthy men participated in the present study. The group mean (± SE) age, height, and weight were 25.5 ± 1.0 yr, 183.8 ± 1.7 cm, and 77.2 ± 1.9 kg, respectively. Each subject was advised of the testing protocols and provided written informed consent for this investigation, which was approved by the Ethics Committee of Copenhagen. All subjects were free of any known cardiovascular disease and were not taking any medications. Subjects were administered an incremental exercise test on a cycle ergometer (Monark 818e) for the determination of maximal oxygen uptake (VO_{2max}). The cycle work rate was set at 100 watts and was increased 50 watts every two

minutes until the subject could no longer maintain a work rate at 60 rpm despite strong verbal encouragement. To assess fitness differences subjects were divided into two groups. Subjects involved in competitive sports competition were considered to be high-fit (HF = 7, group VO_{2max} = 67.8 ± 2.3 ml·kg^{-1}·min^{-1}), while those not performing exercise training regularly were considered average-fit (AF = 7, group VO_{2max} = 49.4 ± 2.1 ml·kg^{-1}·min^{-1}). The actual experimental protocol was scheduled on a separate day from the exercise test. Strenuous physical activity and alcohol consumption was prohibited 24 hours prior to the experiment and subjects were asked to abstain from caffeinated beverages 12 hours before testing. Each subject was familiarized with the equipment and procedures prior to the start of each experimental protocol.

Experimental Procedures

Measurements. All testing was performed with the subjects in the supine position. The subjects were instrumented with standard electrocardiogram (ECG) electrodes and HR measurements were processed by an ECG data computer (Dialogue 2000; IBC-Dancia, Denmark) interfaced with a personal computer (PC). Arterial blood pressure was measured from the brachial artery of the non-dominant arm using a 19 gauge (1mm) Teflon catheter connected to a pressure transducer (PX-260, Baxter, The Netherlands) and pressure monitoring system (Dialogue 2000; IBC-Dancia, Denmark) interfaced with the PC. Systolic (SBP), diastolic (DBP), and mean (MAP) pressures were calculated for each cardiac cycle using custom made software. The catheter was kept patent by a continuous drip of heparinized saline (3 ml/hr) and the transducer was zeroed to the mid-axillary line of the subject. Central venous pressure (CVP) was measured by a sterile

disposable pressure transducer (PX-260, Baxter, The Netherlands) interfaced with the aforementioned monitoring system via a single-lumen catheter. The central catheter was placed in the median antecubital vein of the left arm and advanced to an intrathoracic position. The reference point was zeroed at the midaxillary line and patency was maintained by a continuous drip of heparinized saline.

Sympathetic Nerve Recordings. Post-ganglionic muscle sympathetic nerve activity (MSNA) was recorded with standard microneurographic techniques, as described previously (36). A tungsten microelectrode was inserted into the peroneal nerve near the fibular head of the non-cuffed leg. The nerve signal was processed by a preamplifier and an amplifier (Nerve Traffic Analyzer, Model 662C-3, University of Iowa Bioengineering, Iowa City, IA) with a total gain of 90,000. Amplified signals were bandpass filtered (700 - 2000 Hz), rectified, and integrated by a resistance-capacitance circuit with a time constant of 0.1 s. Muscle sympathetic nerve recordings display a pulse-synchronous burst pattern and an increase in burst frequency with end-expiratory breathholds and Valsalva maneuvers. However, there is no response to arousal or skin stroking. These characteristics were used to discriminate between muscle and skin sympathetic nerve fibers. Sympathetic nerve activity was expressed as burst frequency and as total activity, which was calculated as the product of burst frequency and burst amplitude and expressed in arbitrary units.

Experimental Protocol. Following instrumentation, the subjects rested quietly (~ 10 min) before any testing commenced. The arterial baroreflex, carotid sinus (CBR) and aortic arch (ABR), control of MSNA during acute hypotension was then assessed non

-pharmacologically by releasing a unilateral arterial thigh cuff (300 Torr) following nine minutes of resting leg ischemia. Release of the thigh cuff produced a sudden drop in MAP that was reproducible within 1.7 ± 1.2 mmHg between trials during pilot studies (N=9). A unilateral thigh cuff was used to limit the drop in CVP, as unloading of the cardiopulmonary baroreceptors alters MSNA (35). The protocol started with a five minute baseline period followed by inflation of the thigh cuff for nine minutes, after which the cuff was deflated and measurements were continued for an additional four minutes. Cuff deflation was initiated at a normal end-expiration as observed from the subjects' diaphragm movement. This was to ensure that all subjects were at the same point in the breathing cycle to minimize the effects of respiration on the comparison of responses between cuff release trials. The initial trial served as a control from which the nadir of the MAP response was calculated and used to determine the level of neck suction (NS) needed during the suction trial. We assumed that during this phase of testing both the CBR and ABR were deactivated by the acute drop in MAP and therefore, the response would characterize the arterial baroreflex control of MSNA and HR.

After approximately 25 minutes to allow for all cardiovascular variables to return to basal levels, the protocol was repeated with the application of NS to the anterior two-thirds of the neck through a malleable lead collar (6). The NS was applied to the carotid sinus close to the nadir of the pressure change as estimated from the initial cuff release trial. Suction was continued for 14-s after cuff release to counteract the changes in carotid sinus transmural pressure induced by the release of the arterial thigh cuff. This procedure enabled us to negate the cuff release induced alterations in MAP at the carotid

sinus and therefore, functionally isolate the aortic baroreflex (ABR deactivation alone). The amount of NS utilized was derived from the nadir of the MAP response during the control trial, using a pressure transmission value of 68% (NS = control Δ MAP/0.68). Incomplete transmission of neck pressure and suction to the carotid sinus region has been well documented (7,16). Therefore, we felt it important to correct the NS if we were to successfully negate the fall in pressure at the carotid sinus and functionally isolate the aortic baroreflex control of MSNA. The amount of NS used in this investigation ranged from –15 to –35 mmHg. To offset an effect of order on the MSNA responses, in three subjects the control condition was repeated after the suction and compared to the initial control trial. No significant differences were found in the changes in MSNA due to cuff release ($p>0.05$). Muscle SNA comparisons were made between equivalent time periods for baseline, cuff inflation (pre-cuff release), and cuff release (i.e. 14-s). Comparisons of MSNA to the value obtained during the pre-cuff release periods during the control and suction periods minimized the potential confounding effect of a shift of the nerve recording site between conditions and also, accounted for changes in MSNA from baseline to nine minutes of cuff inflation (Table 1).

Data analyses. Control and suction cuff release trials were matched according to the time NS was initiated and terminated and the period of the cardiac cycle at that time. Due to the pulse synchronicity of MSNA bursts (36) we felt it important to start and end the data analyses periods at the same period of the cardiac cycle. Muscle SNA was calculated as burst frequency and total activity for the 14-s of cuff release with and without NS and compared to the average 14-s MSNA value between eight and nine

minutes of the cuff inflation period. The MSNA responses are presented as changes from this pre-cuff release period. Nadir and peak responses for MAP and HR, respectively, were calculated as five-second averages for the time period at 6 to 10 s cuff release and compared to the 60 second mean MAP and HR at 8-9 min of cuff inflation. Central venous pressure was calculated in the same manner as MAP and HR and all three variables are reported as changes from the pre-cuff release period. The values are all presented as differences from pre-cuff release in order to account for any differences in trials and changes provoked by the nine minutes of cuff inflation. Baroreflex responsiveness was assessed as a change in HR or MSNA per change in diastolic blood pressure (DBP) and comparisons were made between HF and AF subjects at 0-5, 6-10, and 11-14 s after cuff release. The Δ HR/Δ DBP response was used for comparisons between fitness groups for two reasons: (i) there were no significant differences between Δ HR/Δ MAP and Δ HR/Δ DBP and, (ii) the changes in MSNA were related to the DBP. During baseline a mean 60-s MAP, HR, and, CVP and 14-s MSNA value were calculated and compared to the value obtained at 8-9 min of cuff inflation.

Statistical Analyses. **Group.** Statistical comparison of cardiovascular variables (HR, MAP, and CVP) between the control and suction trials were made utilizing a repeated measures two-way analysis of variance (ANOVA) with a 2 x 2 design (condition x time). Comparisons of the change in MSNA between cuff-release with NS (suction) and without NS (control) and the cardiovascular and MSNA variables between baseline and 8 to 9 min cuff inflation were made by paired *t* tests. **Fitness Effect.** The effect of fitness on the changes in MSNA, HR, MAP, and CVP were made utilizing a

repeated measures two-way ANOVA with a 2 x 2 design (fitness group x condition). Further analysis of fitness differences over the time segments of 0-5, 6-10, and 11-14 s after cuff release were made by executing a three-way ANOVA (fitness, condition, time segment). Following all ANOVA analyses, a Student Neumen-Keuls (SNK) test was employed *post hoc* when main effects were significant. Statistical significance was set at $p<0.05$. Results are presented as means ± SE.

RESULTS

Cuff Inflation. The MSNA and cardiovascular responses to nine minutes of complete vascular occlusion in one leg during control and suction trials are presented in Table 1. No significant differences were found between the trials at baseline or at nine minutes of cuff inflation. However, during the resting leg ischemia mean arterial pressure (MAP) increased 3.5 ± 0.8 and 4.5 ± 0.8 mmHg ($p<0.05$) from baseline to nine minutes of cuff inflation during control and suction trials, respectively. This increase in MAP during control and suction corresponded to increases in MSNA of 26.8 ± 8.8 and 24.4 ± 7.4 units ($p<0.05$), respectively. Heart rate (HR) was not significantly altered during suction and increased 3.0 ± 1.0 beats·min^{-1} in the control trial ($p<0.05$). Central venous pressure (CVP) decreased approximately 1.0 mmHg during both conditions ($p<0.05$).

Cuff Release. During the initial deflation of the thigh cuff MAP rapidly decreased and reached a nadir of –17.8 ± 1.5 mmHg within three seconds during the control trial (ABR and CBR deactivation), see Figure 1. The application of neck suction to offset changes in carotid sinus pressure and isolate ABR mediated responses caused a greater

decrease in MAP (-20.6 ± 1.7 mmHg $p<0.05$) which occurred at five seconds. This greater decrease in MAP persisted throughout the 14 seconds with the greatest difference between trials occurring over the six to ten second time period (decreased 15.2 ± 1.7 and 19.4 ± 1.7 mmHg during control and suction, respectively; $p<0.05$). Estimations of carotid sinus pressure (ECSP) calculated as MAP minus chamber pressure (Figure 1) indicate that we were successful in maintaining carotid sinus pressure fairly constant during the suction trials, thereby functionally isolating the aortic baroreceptor reflex mediated responses to the acute hypotension induced by cuff release.

The larger fall in MAP during ABR deactivation alone (suction) corresponded to an attenuated reflex mediated increase in MSNA from 195.2 ± 43.4 units/14s during control (ABR and CBR deactivation) to 134.3 ± 31.9 units/14s during the suction trial ($p<0.05$), see Figure 2. This decrease in MSNA was also evident by the significant reduction in burst frequency noted during the suction trial. Although not significant, the reflex mediated increases in HR induced by cuff release also tended to be less when the ABR was deactivated alone with the greatest differences occurring over the six to ten second time period (increased 11.1 ± 1.1 and 9.6 ± 1.1 beats·min^{-1} during control and suction, respectively), see Figure 3. Central venous pressure was unaltered during both cuff release trials (Figure 3).

Fitness differences: During both cuff release trials (control and suction) high-fit (HF) subjects exhibited a significantly larger drop in MAP compared to their average-fit (AF) counterparts (Figure 4). Under control conditions when the ABR and CBR were deactivated MAP decreased 18.6 ± 1.7 and 10.9 ± 1.8 mmHg in the HF and AF subjects,

respectively ($p<0.05$). During suction the application of NS to negate the CBR caused a greater decrease in MAP in both fitness groups with a decrease of 21.6 ± 2.7 mmHg in the HF group and 16.0 ± 1.4 mmHg in the AF group ($p<0.05$). The larger decrease in MAP in HF subjects during both control and suction conditions was accompanied by a greater increase in MSNA compared to the AF subjects ($p<0.05$), see Figure 5. On the other hand, despite the larger fall in MAP, reflex mediated increases in HR were not significantly different between the HF and AF subjects and even tended to be lower in the HF group during control conditions (Figure 4).

When both the aortic and carotid baroreceptors were deactivated together (control) both HF and AF subjects exhibited large increases in MSNA that were significantly attenuated when neck suction was used to discretely disengage the CBR (suction), see Figure 5. In contrast, the application of NS during cuff release had no effect on the HR response in the HF subjects (Figure 4). However, the HR response was significantly diminished in the AF group with the greatest difference occurring between five and seven seconds (increased 11.7 ± 1.8 and 8.4 ± 0.9 during control and suction, respectively).

The overall responsiveness of the arterial baroreflex assessed during control conditions indicated distinct differences in the baroreflex control of HR and MSNA in the HF and AF subjects ($p<0.05$), see Figure 6. During the acute decrease in MAP induced by the cuff release, arterial baroreflex (ABR and CBR) control of HR was significantly attenuated in the HF subjects, while the baroreflex control of MSNA was significantly augmented. This was consistent throughout the 14 second period with the greatest differences occurring between six and ten seconds following cuff deflation ($p<0.05$).

When NS was applied to isolate the aortic baroreflex mediated response no significant differences in the Δ HR/Δ DBP response were noted between the HF and AF subjects. However, the Δ MSNA/Δ DBP response remained elevated in the HF subjects with the greatest differences occurring between eleven and fourteen seconds after cuff deflation ($p<0.05$).

DISCUSSION

The major findings of the present investigation were that i) unilateral arterial cuff deflation provided a reproducible non-pharmacological method for evaluating arterial baroreflex function during acute hypotension that is independent of significant stimuli to the cardiopulmonary baroreceptors; ii) the carotid baroreflex contributed importantly to the MSNA response during acute hypotension; iii) the aortic baroreflex, when deactivated alone, produced a significant sympathetic response to dynamic decreases in arterial blood pressure (ABP); and iv) chronic endurance exercise training augments arterial baroreflex control of MSNA and attenuates baroreflex control of HR.

Carotid and Aortic Baroreflex Responses The application of NS to negate the decrease in pressure at the carotid sinus caused a greater decrease in MAP in all subjects. Thus, the elimination of the CBR contribution to the arterial baroreflex mediated response during acute hypotension allowed MAP to decline further; thereby signifying the importance of the CBR in the maintenance of ABP. This larger decrease in MAP was accompanied by an attenuated MSNA response compared to control conditions when both the CBR and ABR were deactivated. Considering that the reflex increases in HR were not significantly different between control (CBR and ABR deactivation) and suction

(ABR deactivation alone), it is likely that the decreased MSNA response produced by eliminating the decrease in pressure at the carotid sinus accounted for the greater fall in MAP. This indicates the importance of the CBR in the reflex control of MSNA and subsequent correction of sudden decreases in MAP. These findings confirm previous reports that have indicated the importance of the CBR in the control of MSNA (9,22,36).

In contrast, Sanders et al. (25) indicated that the aortic baroreflex was the more important arterial baroreflex controller of MSNA responses during six minutes of nitroprusside induced hypotension. The first three minutes represented the aortic and carotid baroreceptor muscle sympathetic nerve response after which NS was applied for the remaining three minutes to offset any changes in carotid sinus pressure and therefore, isolate the ABR. Sanders et al. (25) concluded that the aortic baroreceptors dominated the arterial baroreflex control of MSNA and that the CBR contributed minimally. We suggest that differences between the findings of the present investigation and those reported by Sanders et al. (25) were most likely due to differences in experimental methodologies. In the 3-minute steady-state nitroprusside infusions the vascular smooth muscle was continuously relaxed and resulted in a maintained decrease in MAP and it was possible that adaptation of the CBR occurred (9). In contrast, the present investigation used a unilateral arterial thigh cuff occlusion and release method that allowed for more dynamic and transient decreases in ABP. Moreover, we limited the time of NS to fourteen seconds to avoid prolonged stimulation of the CBR during which adaptations between carotid sinus transmural pressure and afferent nerve firing may occur.

The rationale for developing a protocol that provided for a more dynamic and transient decrease in ABP than those induced by the use of steady-state nitroprusside infusions was based upon the following: (i) a sustained drop in ABP (three minutes) and subsequent application of NS for three minutes during nitroprusside infusions most likely caused both aortic and carotid baroreflex adaptations to occur (7,36); (ii) a continuous and constant drop in ABP despite counteractive reflex responses is unphysiological and may have altered baroreflex function as well as the interaction between the ABR and CBR; (iii) a central adaptation may have occurred as investigations using anaesthetized dogs have shown that during sustained electrical carotid sinus nerve stimulation central adaptation of sympathetic responses developed (23); and (iv) the sustained drops in ABP may dampen any effects of the CBR on the control of MSNA as it is the changes in afferent carotid baroreceptor activity that are important in determining the sympathetic outflow rather than the absolute arterial pressure (9,36). Any or all of these factors may have contributed to the results and interpretation of the findings of previous work utilizing steady-state drug infusions (25,27). We expected that by employing a method of using a more dynamic and transient decrease in ABP we would eliminate the concerns raised by the unique technique of aortic isolation used previously (25,27). In addition, the unilateral arterial thigh cuff release did not alter CVP. Therefore, unlike nitroprusside infusions, there was no need to counteract decreases in CVP with volume infusion (25) or lower body positive pressure (27). Moreover, it was of particular importance to maintain CVP constant when examining arterial baroreflex control of MSNA because unloading of the cardiopulmonary baroreceptors causes reflex mediated increases in MSNA (35). In

addition, Pawelczyk and Raven (20) have reported that reductions in CVP augmented carotid baroreflex sensitivity.

Even though we were able to define the importance of the CBR in the arterial baroreflex control of MSNA, our overall findings are in agreement with the conclusions of previous investigations (25). As indicated by the absolute changes in the total activity of MSNA, as well as burst frequency, it appeared that in the present investigation the aortic baroreflex was responsible for approximately 69% of the increased MSNA after cuff release. Thus, both a dynamic decrease in MAP and a steady-state reduction in MAP induced by nitroprusside infusion (25) produced significant increases in MSNA that appear to be primarily due to aortic baroreflex deactivation. These findings are in agreement with several investigations that have examined the aortic and carotid cardiac responses during hypo- and hypertensive stimuli and concluded that the ABR accounted for approximately two-thirds of the arterial baroreflex control of HR (8,18,29,31). Thus, it appeared that while the CBR contributed importantly, in normal healthy adults the arterial baroreflex control of MSNA and HR was dominated by the ABR.

Fitness differences High-fit (HF) subjects demonstrated a greater fall in MAP compared to their average-fit (AF) counterparts. This occurred when both the CBR and ABR were deactivated together (control) as well as when the ABR was deactivated alone (suction). These findings are in agreement with numerous studies that have reported changes in blood pressure regulation and orthostatic tolerance after endurance exercise training (21,30,33,34). Both animal and human studies have reported larger decreases in blood pressure during varying levels of lower body negative pressure after endurance

exercise training (33,34). In agreement, several cross sectional studies have noted a reduced orthostatic tolerance in HF subjects (14,27,30). While these studies clearly link endurance training and orthostatic intolerance, the exact mechanism(s) responsible for the reduction in orthostatic tolerance remain unclear. However, several potential contributing factors have been identified. It has been suggested that the greater decreases in MAP observed after endurance training are associated with training induced alterations in myocardial compliance (15), baroreceptor function (21), α-receptor sensitivity (4), or vascular compliance (1).

The most likely reasons for the greater decrease in MAP observed in the HF subjects in the present investigation were an altered arterial baroreflex control of HR and MSNA combined with an impaired control of vasomotion. When both the ABR and CBR were deactivated together (control) arterial baroreflex control of HR was significantly attenuated in the HF subjects, see figure 6. At the nadir of the MAP response the Δ HR/ Δ DBP was approximately 50% less in the HF subjects (-1.33 ± 0.23 and -0.65 ± 0.09 Δ HR/ Δ DBP for AF and HF subjects, respectively ($p<0.05$)). These findings were in agreement with previous work in our laboratory that has indicated a reduced arterial baroreflex control of HR during vasoactive drug infusions in endurance trained subjects (27,31). However, in those studies it was an attenuated ABR that contributed to the diminished arterial baroreflex HR responses. In the present investigation, the reduction in arterial baroreflex responsiveness was primarily attributed to a reduced CBR responsiveness, as no significant differences were found in the Δ HR/ Δ DBP responses between the AF and HF subjects when the ABR was deactivated alone.

The probable reason for the differences between the previous studies and the present study is likely due to differences in methodologies used to isolate the ABR (27) and assess reflex responsiveness (31). By using a steady-state infusion of nitroprusside to induce hypotension an altered interaction between the ABR and CBR may have occurred (27). In addition, the conclusions of Smith et al. (31) were based on calculated responding ranges for the arterial, ABR and CBR control of HR whereas in the present investigation estimated gain calculations were used for comparisons. Whether the responding range or gain measurement provides a more accurate mathematical calculation for assessing baroreflex function in humans remains questionable. Therefore, it may be that both the CBR and ABR control of HR are susceptible to alterations with chronic endurance exercise training.

Interestingly, in contrast to the attenuated baroreflex control of HR, the HF subjects exhibited an augmented arterial baroreflex control of MSNA. During the control trial when both the ABR and CBR were deactivated together the Δ MSNA/ Δ DBP was significantly greater in the HF subjects compared to the AF subjects. As reported for the Δ HR/ Δ DBP the greatest differences occurred over the six to ten second period after cuff release. However, unlike the HR responses, the Δ MSNA/ Δ DBP remained greater in the HF subjects when NS was applied to isolate the ABR response. Thus, it appears that both CBR and ABR control of MSNA were augmented in the HF subjects.

These findings were unexpected and were contrary to our hypothesis. However, we suggest that changes in central neural processing that occur with endurance training may augment the arterial baroreflex control of MSNA. Recently Chen et al. (2) reported

that exercise trained rats exhibited a reduced central gain of the baroreflex regulation of HR that was attributed to an altered central processing of afferent signals without changes in the reactivity of afferent nerves. In addition, Moffitt et al. (19) has reported an attenuated baroreflex control of renal sympathetic nerve activity (RSNA) following deconditioning induced by hindlimb unloading in rats that was due to changes in central processing of baroreceptor afferent information. Collectively, these animal investigations indicate that alterations in central nervous system processing may play a role in modifying arterial baroreflex function after endurance exercise training which may provide an explanation for the altered baroreflex responses in the HF subjects in the present investigation.

Further evidence suggesting an increased baroreflex control of MSNA with training has been reported by Grassi and colleagues (10). These investigators reported a potentiation of the baroreceptor-sympathetic reflex responses in a group of young subjects after undergoing a 10-week running program. However, in contrast, Sheldahl et al. (26) has reported no changes in baroreflex control of MSNA after training in middle aged men and Dicarlo and Bishop (5) noted an attenuated baroreflex control of RSNA in conscious rabbits following 8 weeks of endurance training. Therefore, the effect of endurance training on baroreflex control of SNA is unclear, especially in humans (10,26). Furthermore, in these previous investigations, no attempt was made to maintain CVP constant and therefore, these findings include cardiopulmonary baroreceptor induced changes in SNA. This is an important distinction as other studies have reported

alterations in the cardiopulmonary baroreceptor reflex in endurance trained subjects (17,28).

Another reason for the augmented CBR and ABR mediated Δ MSNA/ Δ DBP responses of the HF subjects may be related to a change in the control of vasomotion in the HF subjects. Previous investigations have reported an attenuated vasoconstrictor response and an increased capacity for vasodilation in endurance trained individuals (14,30,32,33). Thus, the arterial baroreflex may be increasing the MSNA to compensate for the greater reduction of blood pressure that occurs because of a reduced vasoconstrictor response or a greater pooling of blood in the ischemic leg. One possible explanation for the reduced vasoconstrictor response may be an attenuated α-adrenergic receptor sensitivity. However, results from studies examining alterations in α-adrenergic receptors with training are equivocal indicating a decrease (4), increase (13) or no change (30) in responsiveness with endurance exercise training. Alternatively it may be that changes in local metabolites interfered with or overrode the α-adrenergic receptor's ability to vasoconstrict ("functional" sympatholysis) and resulted in a greater pooling in the ischemic leg. Previous studies have indicated that vascular responsiveness to endothelium-dependent vasodilators was enhanced in endurance trained animals (4). Therefore, it may be that the production of metabolites induced by the nine minutes of cuff inflation caused a greater vasodilatory response in the HF subjects that could not be compensated for by the sympathetically mediated α-adrenergic receptor vasoconstriction. This in turn may have led to the arterial baroreflex increasing MSNA to help override the greater vasodilation in the HF subjects.

Aside from these metabolic differences, structural changes in the vasculature may also be responsible for a greater vasodilatory response in HF subjects. Snell et al. (32) has reported an increased capacity for vasodilation in endurance trained subjects and suggested that the increased vascular conductance reflects a structural adaptation at the arteriolar level that increases the capacity of the vascular system to perfuse exercising muscle. This adaptation would provide endurance-trained individuals with an increased ability to supply exercising muscle with blood flow thereby supporting large amounts of muscle metabolism and subsequently muscle work. However, this increased ability to vasodilate may be disadvantageous under resting conditions and predispose the endurance trained individual to a greater pooling of blood during orthostatic challenges. In agreement, Levine et al. (14) has reported a greater maximal vascular conductance (G_{max}) of the calf in HF subjects and noted that G_{max} was a strong independent predictor of lower body negative pressure tolerance. A large G_{max} would result in greater vascular pooling in the legs during orthostatic stress.

Another important vascular adaptation that has been found to occur after endurance training is an increase in arterial compliance (1). It can be reasoned that an augmented vasodilatory capacity combined with an increased arterial compliance leaves the endurance-trained subject susceptible to decreases in arterial blood pressure (i.e, orthostatic intolerance). Therefore, one could speculate that as a compensatory mechanism the arterial baroreflex adapts by increasing its control of MSNA to assist in the maintenance of blood pressure. However, despite the increased MSNA blood

pressure remains low because the MSNA cannot overcome the changes in vascular remodeling that occur with chronic endurance exercise training.

Potential limitations in the design and interpretation of the present investigation should be considered: (i) estimations of carotid sinus pressure during the suction trial indicated that the technique was very successful in negating the change in pressure at the carotid sinus, however, some deactivation or activation of the CBR cannot be discounted despite our correcting for incomplete transmission, and (ii) recent findings suggest an inhibitory interaction between the carotid and aortic reflex control of HR in humans (31). This indicates a non-linear relationship exists between the CBR and ABR and therefore, our quantitative analysis of CBR and ABR control of MSNA may not be applicable over a full range of hypo- and hypertensive stimuli. However, Guo et al. (11) has indicated that there are major differences in baroreflex control of HR and vascular resistance in rabbits. Therefore, as the entire response ranges for CBR and ABR control of MSNA have not been fully characterized in humans, it remains unknown if an inhibitory interaction exists in the arterial baroreflex control of MSNA. As such, our results reflect an initial attempt to defining the interactive relationship between the CBR and ABR in the baroreflex control of MSNA.

In summary, we demonstrated that the carotid baroreflex contributed significantly to the MSNA response during acute hypotension. However, when the aortic baroreflex was deactivated alone, significant increases in sympathetic nerve activity were noted. These data suggest that while the CBR contributes importantly to arterial baroreflex control of MSNA it appears that the ABR is more dominant. Furthermore, unilateral

arterial cuff deflation provides a reproducible non-pharmacological method for evaluating the reflex control of MSNA by the arterial baroreceptors during acute hypotension. Moreover, it allows for a more dynamic and transient decrease in ABP, which presents more of a true physiological stimulus to the arterial baroreceptors. In addition, CVP is unaltered during this protocol and therefore, limits the confounding affect of deactivation of the cardiopulmonary baroreceptors.

Chronic endurance exercise trained individuals exhibited a greater fall in MAP compared to their average fit counterparts. In response to the greater decrease in MAP arterial baroreflex control of HR was attenuated, while the baroreflex control of MSNA was augmented. However, despite the greater increases in MSNA high fit subjects continued to have a greater fall in MAP. This suggests that although the arterial baroreflex increases the MSNA of the endurance-trained individual the regulation of blood pressure during hypotension remains attenuated. We suggest that the more marked hypotension is a result of endurance exercise training mediated vascular adaptations, namely a greater vasodilatory capacity and a reduced vasoconstrictor response, as well as an attenuated arterial baroreflex control of HR.

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After August 2000 the address for Paul J. Fadel, Ph.D. will be University of Texas Southwestern Medical Center at Dallas, Division of Cardiology, 5323 Harry Hines Blvd. Dallas, TX 75390.

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FIGURE LEGENDS

Figure 1. Changes in mean arterial pressure (MAP) and estimated carotid sinus pressure (ECSP) during cuff release with neck suction (NS) (aortic baroreflex deactivation) and without NS (aortic and carotid baroreflex deactivation). ECSP was calculated as MAP minus chamber pressure and is not plotted for the control condition, as it would be equal to MAP. Symbols denote group means ± (SE) and are presented as a change from the sixty second average recorded between eight and nine minutes of the cuff inflation period. * Denotes significant differences between control and suction ($p<0.05$).

Figure 2. Muscle sympathetic nerve activity (MSNA) responses to cuff release with aortic baroreflex deactivation alone (suction) and in combination with carotid baroreflex deactivation (control). MSNA was assessed as burst frequency and total activity (burst frequency x amplitude) for the initial 14 seconds of cuff release during control and suction trials and presented as a change from the average 14 second MSNA value between eight and nine minutes of the cuff inflation period. * Denotes significant differences between control and suction ($p<0.05$).

Figure 3. Heart rate (HR) and central venous pressure (CVP) changes during cuff release with aortic baroreflex deactivation alone (suction) and in combination with carotid baroreflex deactivation (control). Symbols denote group means ± (SE) and are presented as a change from the sixty second average obtained between eight and nine

minutes of the cuff inflation period. CVP was not significantly different from zero at any time point. * Denotes significant differences between control and suction ($p<0.05$).

Figure 4. Comparison of the mean arterial pressure (MAP) and heart rate (HR) responses to cuff release with neck suction (NS) (aortic baroreflex deactivation) and without NS (aortic and carotid baroreflex deactivation) in the high-fit (HF) and average-fit (AF) subjects. Symbols denote group means ± (SE) and are presented as changes from the sixty second average recorded between eight and nine minutes of cuff inflation. † Indicates significant differences between HF and AF subjects during the control trial; ‡ Indicates significant differences between HF and AF subjects during the suction trial ($p<0.05$).

Figure 5. Comparison of muscle sympathetic nerve activity (MSNA) responses to cuff release with aortic baroreflex deactivation alone (suction) and in combination with carotid baroreflex deactivation (control) in the high-fit (HF) and average-fit (AF) subjects. † Indicates significant differences in MSNA between HF and AF subjects; * Indicates significant differences in the MSNA response between control and suction ($p<0.05$).

Figure 6. Comparison of baroreflex responsiveness between high-fit (HF) and average-fit (AF) subjects. Baroreflex responsiveness was assessed as a change in heart rate (HR) or muscle sympathetic nerve activity (MSNA) per change in diastolic blood pressure

(DBP) and comparisons were made at 0-5, 6-10, and 11-14 seconds of cuff release with neck suction (NS) (suction) and without NS (control). † Indicates significant differences between HF and AF subjects; ‡ Indicates significant differences between control and suction ($p<0.05$).

Table 1. Subject responses to nine minutes of resting leg ischemia (N=14).

		Baseline	9 min Cuff Inflation	Change (Δ)
MAP (mmHg)	Control	89.4 ± 2.5	92.9 ± 2.3	+ 3.5 ± 0.8 *
	Suction	90.1 ± 2.8	94.6 ± 2.7	+ 4.5 ± 0.8 *
MSNA (total act. /14sec) †	Control	102.8 ± 14.5	129.6 ± 20.2	+ 26.8 ± 8.8 *
	Suction	104.4 ± 19.1	128.7 ± 23.5	+ 24.4 ± 7.4 *
MSNA (bursts /14sec) †	Control	4.4 ± 0.3	6.1 ± 0.4	+ 1.8 ± 0.3 *
	Suction	4.4 ± 0.3	6.0 ± 0.5	+ 1.6 ± 0.3 *
HR ($b \cdot min^{-1}$)	Control	56.4 ± 2.1	59.3 ± 2.1	+ 3.0 ± 1.0 *
	Suction	57.4 ± 2.0	58.3 ± 2.1	+ 0.85 ± 1.0
CVP (mmHg) ‡	Control	5.2 ± 1.1	4.2 ± 1.1	- 0.98 ± 0.22 *
	Suction	5.0 ± 1.0	4.2 ± .90	- 0.84 ± 0.24 *

Values are mean ± SE. MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; HR, heart rate; CVP, central venous pressure. † MSNA N=13, resting MSNA recording problem in one subject. ‡ CVP N=13, no CVP data was obtained in one subject. No significant differences were found between the control and suction trials at baseline or nine minutes cuff inflation. * Denotes differences from baseline to nine minutes cuff inflation ($p<0.05$).

Figure 1.

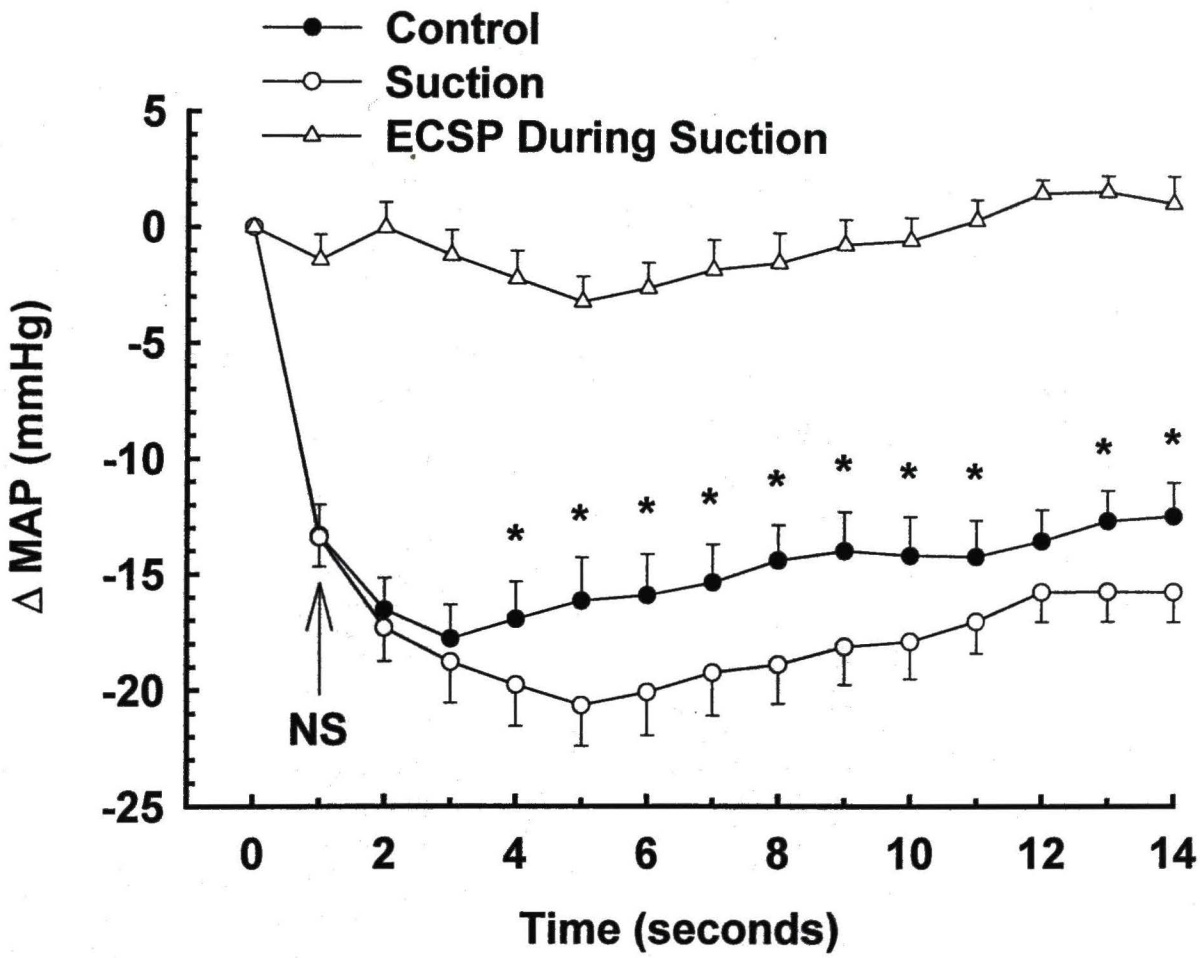

Control
Suction
ECSP During Suction
Δ MAP (mmHg)
5
0
-5
-10
-15
-20
-25
NS
*
0
2
4
6
8
10
12
14
Time (seconds)

Figure 2.

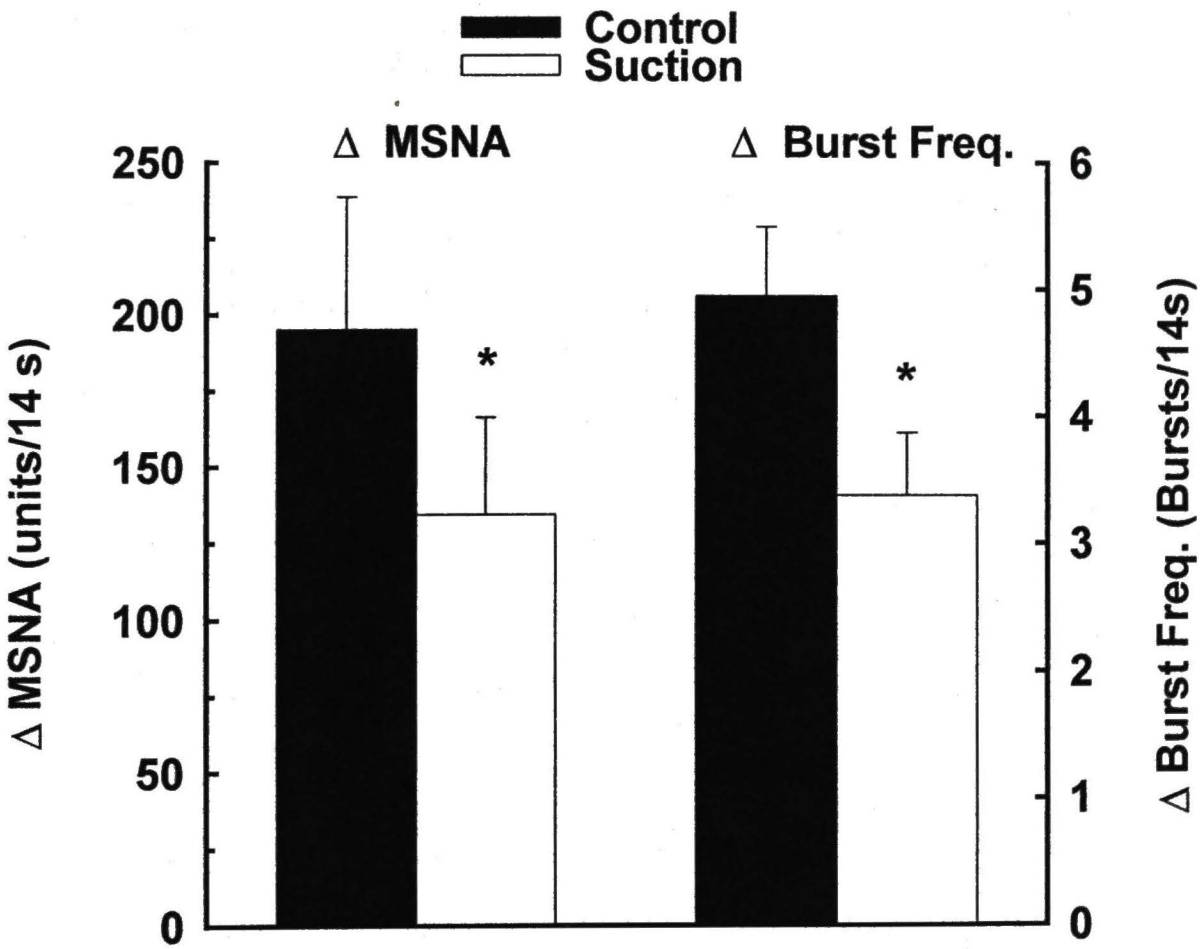

Control
Suction
Δ MSNA
Δ Burst Freq.
Δ MSNA (units/14 s)
Δ Burst Freq. (Bursts/14s)
250
200
150
100
50
0
6
5
4
3
2
1
0
*
*

Figure 3.

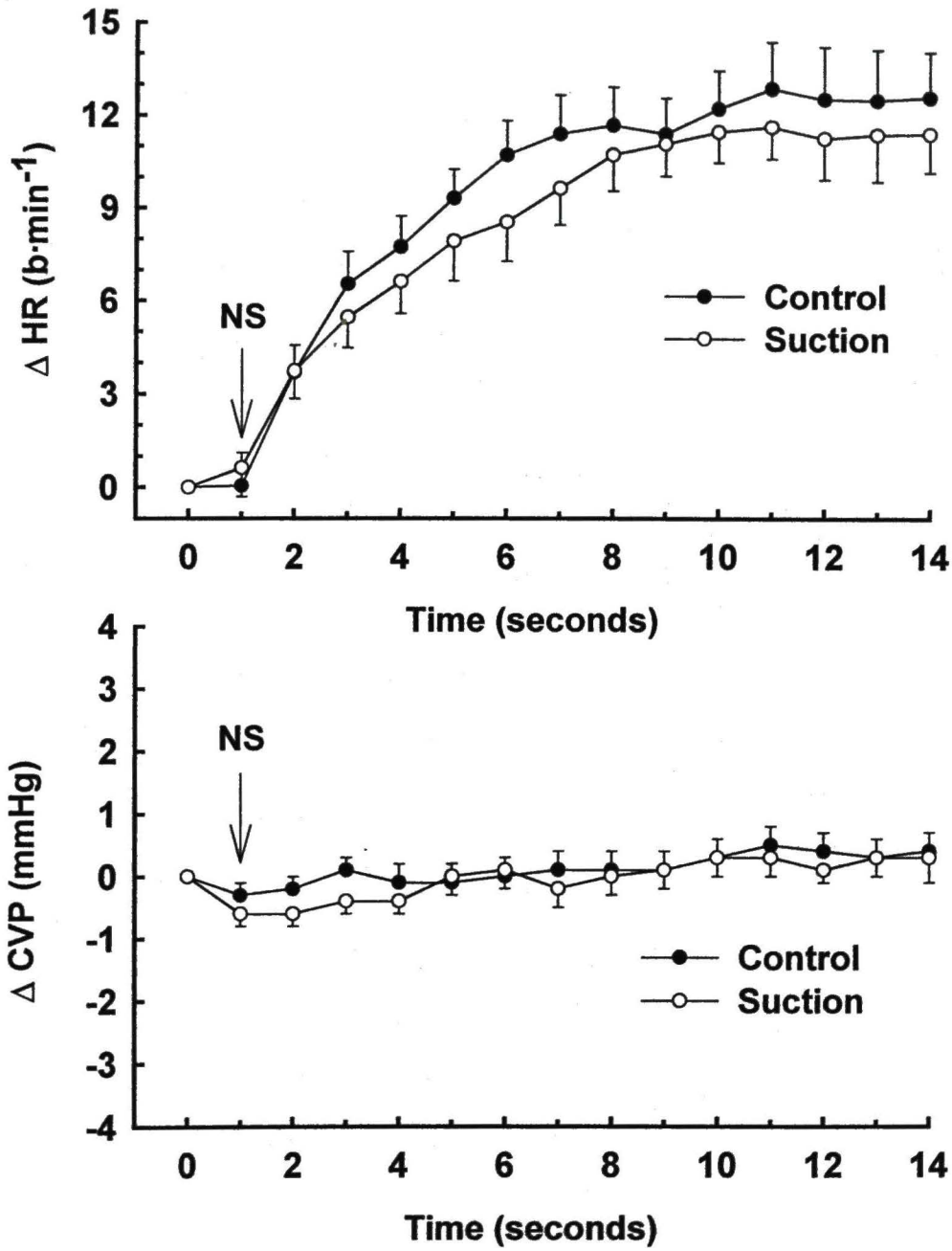

Δ HR (b·min-1)
15
12
9
6
3
0
NS
Control
Suction
0
2
4
6
8
10
12
14
Time (seconds)
Δ CVP (mmHg)
4
3
2
1
0
-1
-2
-3
-4
NS
Control
Suction
0
2
4
6
8
10
12
14
Time (seconds)

Figure 4.

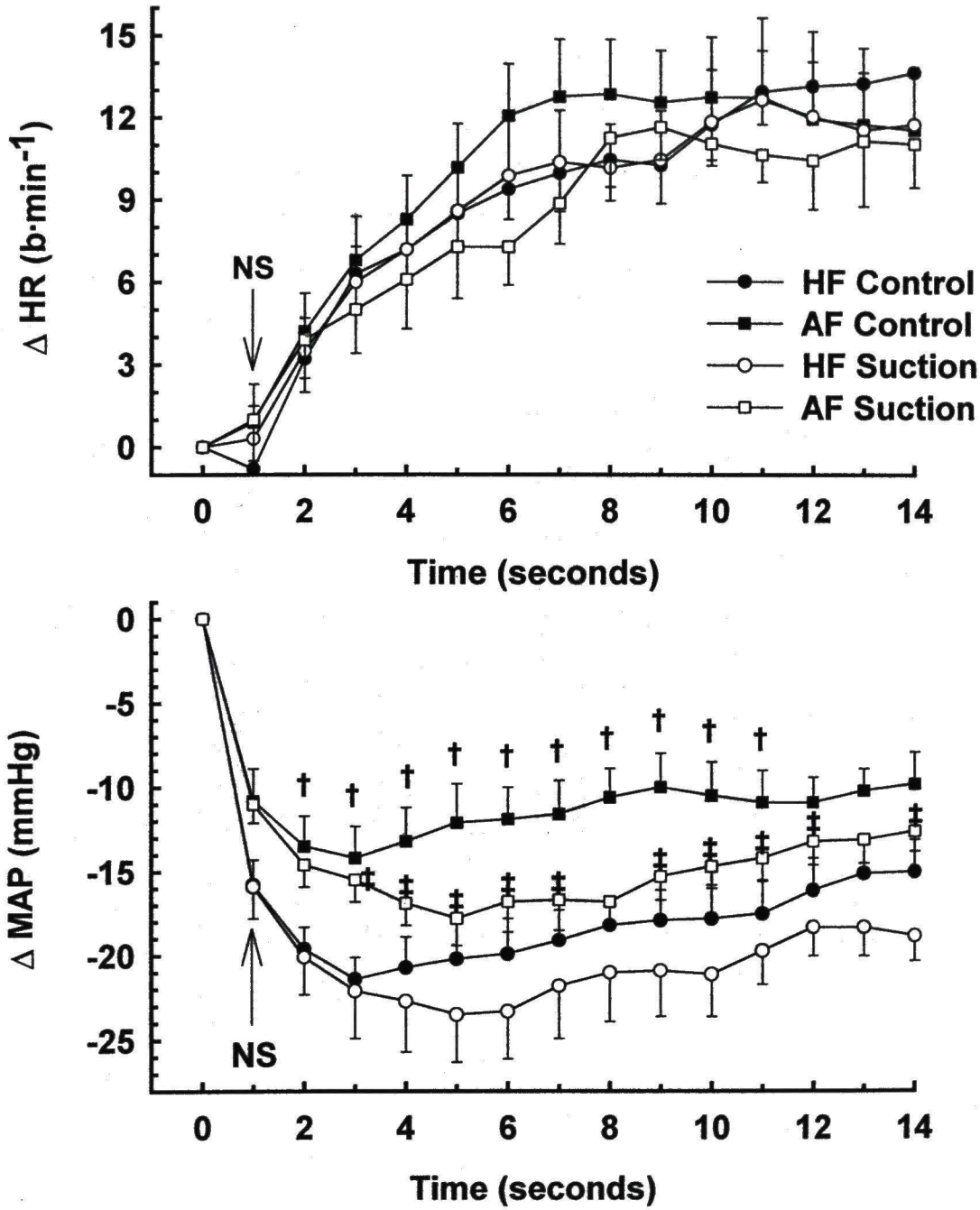

Δ HR (b·min-1)
15
12
9
6
3
0
NS
HF Control
AF Control
HF Suction
AF Suction
0
2
4
6
8
10
12
14
Time (seconds)
Δ MAP (mmHg)
0
-5
-10
-15
-20
-25
NS
†
‡
0
2
4
6
8
10
12
14
Time (seconds)

Figure 5.

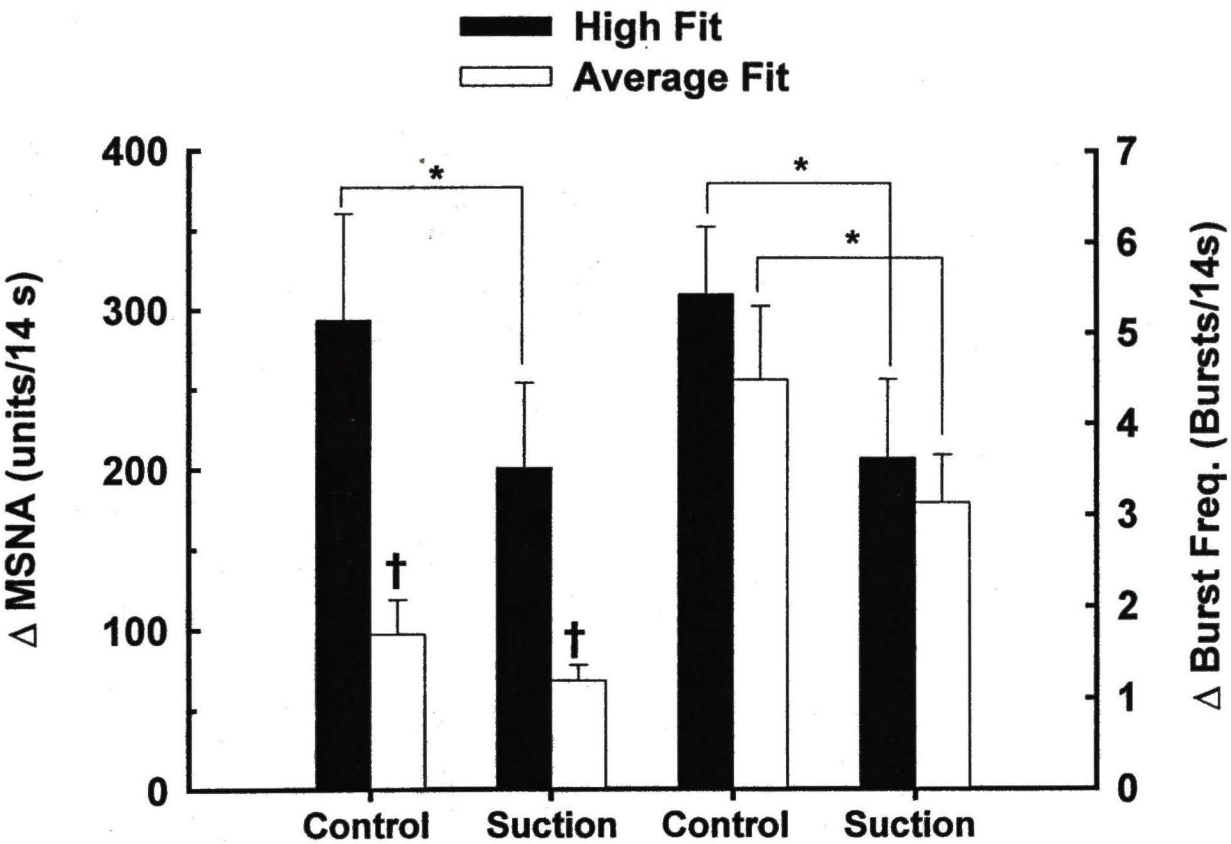
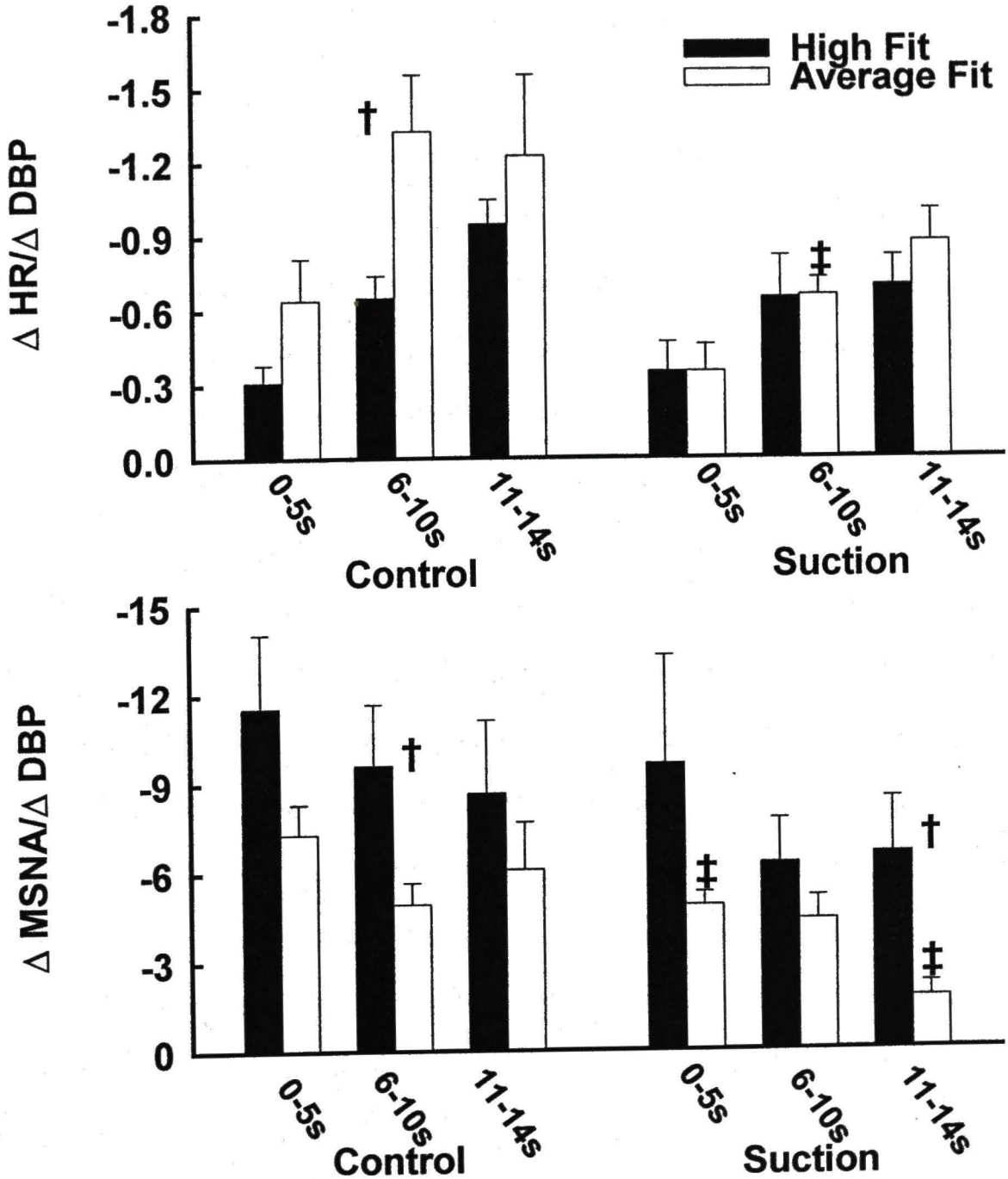

High Fit
Average Fit
Δ MSNA (units/14 s)
Δ Burst Freq. (Bursts/14s)
400
300
200
100
0
7
6
5
4
3
2
1
0
*
*
*
†
†
Control
Suction
Control
Suction

Figure 6.


High Fit
Average Fit
Δ HR/Δ DBP
-1.8
-1.5
-1.2
-0.9
-0.6
-0.3
0.0
†
‡
0-5s
6-10s
11-14s
Control
Suction
Δ MSNA/Δ DBP
-15
-12
-9
-6
-3
0

CHAPTER IV

CONCLUSIONS

The results of the two investigations described within this dissertation demonstrated the importance of the carotid baroreflex in the arterial baroreflex control of MSNA both at rest and during dynamic arm exercise. However, our overall findings indicate that under resting conditions the aortic baroreflex appeared more dominant in mediating the arterial baroreflex-MSNA response to acute hypotension. In addition, we suggest that vascular adaptations incurred by long-term endurance exercise training hinder blood pressure regulation during hypotension in high-fit individuals.

The first investigation demonstrated that carotid baroreflex control of muscle sympathetic nerve activity was reset to function at the higher arterial pressures induced by dynamic arm exercise without a change in reflex sensitivity. Thus, it appeared that despite marked increases in MSNA and MAP during exercise, the carotid baroreflex control of MSNA was preserved. Additionally, the CBR-MAP stimulus-response curve was relocated upwards on the response arm and rightward to higher carotid sinus pressures during steady-state arm cycling. However, in comparison to steady-state leg exercise performed at the same relative workload the resetting of the CBR-MAP stimulus-response curve appeared augmented, presumably a result of a greater activation of central command during exercise performed with a small muscle mass. Collectively, these findings lead to the conclusion that carotid baroreflex control of sympathetic nerve

activity and mean arterial pressure was maintained during moderate intensity dynamic arm exercise.

The second investigation demonstrated that unilateral arterial cuff deflation provides a reproducible non-pharmacological method for evaluating arterial baroreflex function during acute hypotension that was independent of alterations in central venous pressure. The maintenance of central venous pressure was of particular importance when examining arterial baroreflex control of MSNA because an unloading of the cardiopulmonary baroreceptors would cause reflex mediated increases in MSNA. Utilization of this technique elucidated the importance of the carotid baroreflex in the arterial baroreflex control of MSNA during acute hypotension, however, it appeared that the aortic baroreflex dominated the MSNA response to hypotension. Moreover, a comparison of responses between high-fit and average-fit subjects indicated that long-term endurance exercise training augments arterial baroreflex control of MSNA and attenuates baroreflex control of HR. However, despite the greater increases in MSNA high-fit subjects exhibited a greater decrease in mean arterial pressure during cuff release. This suggests that although the arterial baroreflex increases the MSNA response to hypotension of the endurance-trained individual the regulation of blood pressure during hypotension remained attenuated. We contend that the more marked hypotension was a result of a reduced vasoconstrictor response produced by endurance exercise training mediated vascular adaptations.

CHAPTER V

SUGGESTIONS FOR FUTURE RESEARCH

Although the research presented in this dissertation provided several new findings regarding carotid and aortic baroreflex function, many questions remain unanswered. Below is a list of several potential investigations designed to further support the research presented within this dissertation and to expand our understanding of arterial baroreflex control of blood pressure both at rest and during exercise.

I. To further test the hypothesis that the carotid baroreflex control of muscle sympathetic nerve activity is unaltered during dynamic exercise, an experiment could be conducted using the procedures described in Chapter II utilizing different exercise intensities (i.e., 75% VO_{2peak}). In addition, an investigation should be designed to allow carotid baroreflex control of MSNA to be examined during dynamic leg exercise. Differences in physiological responses to exercise with a small muscle mass (arms) versus a large muscle mass (legs) have been well documented and therefore, it would be important to verify the preserved carotid baroreflex control of MSNA during exercise with a larger muscle mass. Unfortunately, the limitation in this experimental design would be the need to use the arm to obtain nerve recordings, as the limb of the impaled nerve must remain relaxed. However, recent investigations have obtained nerve recordings from the median nerve at the cubital fossa. This would provide an ideal site

for the investigation of carotid baroreflex control of MSNA during leg cycling at various exercise intensities.

II. A series of experiments utilizing the methodology of cuff inflation-release to induce acute hypotension, as described within this dissertation, could be performed to determine the contribution of the carotid and aortic baroreflex's to the overall MSNA response during varying levels of hypotension. This would allow a more complete evaluation of each of the baroreceptor populations to the overall arterial baroreflex control of MSNA. The degree of hypotension could be adjusted by varying the amount of muscle mass being exposed to the ischemia. For example, an arm could be used instead of the leg to provide a smaller decrease in blood pressure and then cuffs could be placed around both an arm and leg to induce a proportionally greater decrease in blood pressure. However, a potential limitation in producing a larger drop in blood pressure than those used in this dissertation may be a decrease in central venous pressure and subsequent unloading of the cardiopulmonary baroreceptors. Therefore, the application of the cuff at higher or lower positions on the limbs to adjust the degree of reactive hyperemia upon cuff release would provide a more reasonable method for varying the degree of hypotension to study the baroreflex control of MSNA across a range of pressures.

III. An area that receives continual debate is the question of carotid baroreflex adaptation to sustained neck pressure or neck suction. In order to address this question a study could be performed to examine pulsatile versus non-pulsatile stimuli delivered to the carotid sinus region for varying durations. This investigation would examine the extent to which the pulsatility of the stimuli and the duration of stimulation affect carotid baroreflex mediated changes in heart rate and MSNA in humans. In addition, the prolongation of the stimulus time beyond five seconds would identify any responses emanating from the aortic baroreflex to counteract the carotid baroreflex mediated changes in arterial blood pressure.