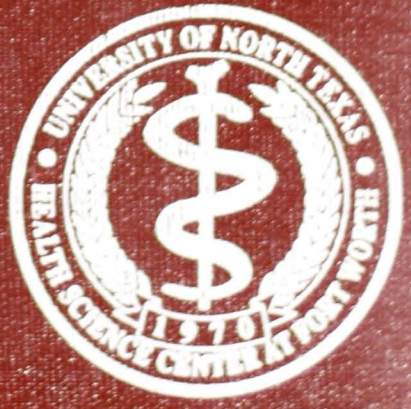
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Raven, Joseph Simon, Baroreflex Mediated Autonomic Modulation by Acute Pain and Orthostatic Stress. Doctor of Philosophy (Integrative Physiology), October 2008, 147 pp.; 2 tables; 23 figures; bibliography; 123 titles.

Nociceptive and baroreceptor afferent neurons are implicated as the components responsible for carotid baroreceptor reflex (CBR) resetting. The purpose of this dissertation was to identify the effect of cold induced pain, and cardiopulmonary baroreceptor (CPBR) unloading accompanied by pain, on CBR resetting.

First, the relationships between cold induced pain to cardiovascular responses, pain perception, and muscle sympathetic nerve activity (MSNA) were investigated. Questions were addressed through use of the cold pressor test (CPT), finger plethysmography, and microneurography. This study demonstrated perceived pain, MSNA, and blood pressure responses to a cold stimulus were reproducible. Furthermore, graded responses observed in mean arterial pressure (MAP) and MSNA directly correlated to the intensity of the pain stimulus.

The next study examined cold induced pain on CBR gain and operational point resetting in healthy normotensive subjects. Using similar experimental methodologies to the previous study, the data demonstrated acute pain shifted the CBR operational point toward the lower limiting value of MSNA. These data also confirmed

an upward-rightward shift and increased gain of the CBR function curve during pain.

Finally, CBR gain and operational point resetting during simultaneous CPBR unloading and cold induced pain in healthy normotensive subjects was addressed. Using the previous experimental paradigm, this investigation revealed CPBR unloading during acute pain did not abolish the shift of the CBR operational point. Thus, the capacity for hypotensive buffering remained enhanced. This study also determined CPBR unloading during acute pain produced higher prevailing blood pressures compared to periods of CPBR unloading alone.

In summary: 1) MSNA and cardiovascular responses were tightly coupled to pain. 2) The CPT was a reliable technique for producing repeated sympathoexcitation within a subject. 3) Acute pain increased CBR gain and induced a shift of the CBR operational point. 4) The CBR operational point shift remained in the presence of CPBR unloading, which precipitated increased MAP during hypotensive stimuli. These findings suggested pain improves blood pressure maintenance during central hypovolemic stress.

BAROREFLEX MEDIATED AUTONOMIC MODULATION BY ACUTE PAIN AND ORTHOSTATIC STRESS

DISSERTATION

Presented to the Graduate Council of the

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Joseph Simon Raven, B.A.

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Original Articles

I. **Raven JS**, Siu JC, Thakre T, Smith M. Pain Perception Determines Sympathoexcitatory Response to Cold, *Exp. Physiol.* (Submission October 2008)

II. **Raven JS,** Nguyen J, Thakre T, Pacchia C, Smith M. Baroreceptor Modulation During Cold Induced Acute Pain, *Am. J. Physiol.* (Submission October 2008)

III. **Raven JS,** Nguyen J, Thakre T, Pacchia C, Smith M. Cold Induced Pain During Cardiopulmonary Baroreceptor Unloading Improves Maintenance of Blood Pressure, *Exp. Physiol.* (Submission October 2008)

IV. Thakre TP, Gonzalez S, Garner M, **Raven JS**, Smith M. Ethnic-Specific Influence of 3'-UTR Polymorphisms of the ATP1A2 Gene on Arterial Blood Pressure, *Exp. Physiol. (*Submission September 2007)

V. Thakre TP, Gonzalez S, Garner M, **Raven JS**, Smith M, Physiologic Phenotype of Polymorphisms of the ATP1A2 Gene in African Americans and Caucasians: Relation to Hypertension, *J. of Hypertension* (Submission September 2007)

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LIST OF ABBREVIATIONS

BP	Blood pressure
CBR	Carotid Baroreceptor
CDP	Carotid Distending Pressure
CP	Cold Pressor
CPBR	Cardiopulmonary Baroreceptor
CPT	Cold Pressor Test
CVP	Central Venous Pressure
ECG	Electrocardiogram
HR	Heart Rate
LBNP	Lower Body Negative Pressure
MAP	Mean Arterial Pressure
MSNA	Muscle Sympathetic Nerve Activity
MSVR	Mean Systemic Vascular Resistance
NE	Norepinephrine
NP	Neck Pressure
NS	Neck Suction
NTS	Nucleus Tractus Solitarii
Q	Cardiac Output
RA	Room Air
RPP	Rating Perceived Pain
RVLM	Rostro-Ventro-Lateral Medulla
SEM	Standard Error of the Mean

CHAPTER I

INTRODUCTION

Pain is a component of daily life but is frequently overlooked as an important survival mechanism. While pain operates in several capacities, its importance as a component of blood pressure regulation remains relatively under-emphasized in cardiovascular physiology. Prior studies have shown that pain modulates the activity of neural components responsible for controlling the carotid baroreceptor (CBR) responses (*Boscan and Paton. , 2001, Boscan, Pickering and Paton. , 2002, Bruehl and Chung. , 2004*). This research project is primarily involved in examining pain as a causative factor for resetting the CBR operational point, leading to a hypertensive state.

The purpose for this dissertation was to examine the effect of acute pain stimuli on blood pressure regulation in normotensive individuals during central hypovolemic stimuli, by assessing the CBR function curve and operating point. By decreasing central venous pressure (CVP) and combining it with pain, I hoped to establish a preliminary model of traumatic injury to determine how blood pressure control was altered. Until now, the effects of cardiopulmonary baroreceptor (CPBR) unloading during simultaneous cold-induced pain on CBR modulation remained unexplored.

Importance of Pain as a Clinical Condition

Pain and the management of pain have received increased attention over the past 70 years. The treatment of pain is a primary component of many clinical visits, and pain itself is diagnostic of many ailments. Acute pain mediates a systemic sympatho-excitation which in turn may lead to 1) neural modulation of areas in the brain such as the Nucleus Tractus Solitarii (NTS), 2) increased heart rate, 3) changes in blood pressure modulation, and 4) a reduction in the signals buffering the cardiovascular system from hypertension (*Conde-Guzon, et al. , 2003, Ghione, et al. , 1988, Victor, et al. , 1987*). Hypertensives, which constitute ~25% of the patient population, have decreased pain sensitivity and an increased risk of mortality following physical trauma (*Terry, et al. , 2007, Thom, et al. , 2006)*. Thus, there remains a clear need to advance the understanding of pain and its effect on cardiovascular autonomic physiology.

Model of Pain Physiology: Cold Pressor Test (CPT)

The CPT is performed by immersing the subject's hand into an ice water bath for 2 minutes. This stimulus elicits large elevations in blood pressure (*Wirch, et al. , 2006*). The primary physiological outcome of a CPT is increased muscle sympathetic nerve activity (*Fagius, Karhuvaara and Sundlof. , 1989, Kregel, Seals and Callister. , 1992, Victor, et al. , 1987*). The effects of the cold pressor test on sympathetic outflow have demonstrated direct relationships between sympathetic nerve discharge and the changes in arterial pressure and plasma norepinephrine (*Victor, et al. , 1987*). Arterial pressure was observed to steadily increase during the entirety of the CPT. However, the autonomic

responses do not simply parallel the arterial pressure responses. At the onset of a CPT parasympathetic withdrawal causes heart rate to increase during the first 30 seconds of the CPT before returning to control values by the second minute (*Victor, et al. , 1987*). In contrast, increases in sympathetic activity are initially delayed, but increase progressively during the second minute until the end of the test. The result is an initial pressure rise mediated primarily by an increase in cardiac output followed by a sympathetic-mediated vasoconstriction. Thus, as a model for increasing sympathetic activity the CPT is an effective stimulus. However, is the autonomic response the direct result of the pain experienced or the temperature of the water?

Kregel *et al.* determined sympathetic excitation during hand immersion in cold water occurs only when skin temperature falls to levels that produce a sensation of intense pain. Studies which determined the relationship between sympathetic activity and pain associated with localized skin cooling in human subjects suggest that increases in MSNA during a CPT is achieved through the activation of high-threshold C-fiber and A-δ nociceptive fibers found within the human hand (*Kregel, Seals and Callister. , 1992, Mengel, et al. , 1993, Simone and Kajander. , 1997*). These findings demonstrate that pain, independent of cold perception, is the primary determinant of sympathoexcitation during a cold pressor stimulus.

Sympathetic Nervous System and Pain

The sympathetic nervous system is activated in response to various stressors, such as exercise, pain, and/or disease. Specifically, pain elicits a strong sympathoexcitatory effect (*Fagius, Karhuvaara and Sundlof. , 1989, Kregel, Seals and Callister. , 1992, Victor, et al. , 1987*). Following sympathetic activation the heart rate increases, and systemic vascular resistance increases as the stimulus persists. Furthermore, through the systemic activation of sympathetic pathways, elevations in catecholamines like nor-epinephrine and epinephrine have been observed when the stimulus is sustained (*Victor, et al. , 1987*). While it is clear that pain induces profound increases in sympathetic activity, it is also important to consider how sympathoexcitation affects pain perception.

Renn *et al.* (2005) demonstrated that the locus coeruleus, located in the pons at the fourth cerebral ventricle, is almost entirely composed of noradrenergic neurons. Anti-nociceptive signals are sent through bilateral projections originating from this area of the brain to the contralateral spinal dorsal horn (*Renn and Dorsey. , 2005*). Further studies have identified α_2 noradrenergic receptors in layers II and IV of the dorsal horn. Through the activation of neurons responsible for descending-inhibitory control of pain, nor-adrenaline is released producing an analgesic effect via the α_2 noradrenergic receptor (*Calvino and Grilo. , 2006*).

Adrenaline is widely used clinically for anesthesia in conjunction with local anesthetics (*Yoshimura, 2006*). Originally used under the premise that adrenaline would reduce the clearance of local anesthetics via vasoconstriction, it has been shown to produce analgesia even when administered intrathecally in the absence of local

anesthetics. Also, clonidine, an α_2 agonist without vasoconstrictive effects, has been shown to prolong the duration of analgesia (*Yoshimura and Furue. , 2006*). Thus, through the activation of α_2-noradrenergic receptors within the spinal column, pain is reduced via sympathoexcitation.

The interplay between the sympathoexcitatory effect of pain and adrenergic analgesia in the nervous system is relevant for the hypotheses addressed by this research. While the relationship between sympathoexcitation and pain is not a simple one, it highlights the integration of two components which directly modulate cardiovascular outcome in the body. Ultimately, pain has a long and well documented history as a method for increasing sympathetic activity and altering cardiovascular variables within human and animal models *(Kregel, Seals and Callister. , 1992, Victor, et al. , 1987*).

Model of Baroreceptor Stimulation (NP/NS)

A method of counterpressure which utilizes a neck chamber for the purpose of non-invasive baroreceptor stimulation was first developed by Ernsting and Parry in 1957 and later modified by Bevegård and Shepherd in 1966 is (*Bevegard and Shepherd. , 1966, Parati and Mancia. , 1992*). The neck chamber technique is a valuable method for studying human carotid baroreflexes. By altering the transmural pressure across the carotid sinus, neck pressure or neck suction respectively increases or decreases the stretch on the carotid arteries and thus load or unload the CBR (*Kober, Dannenberg and Arndt. , 1969, Kober and Arndt. , 1970*). The effect of a positive neck pressure is a net reduction in the transmural pressure in the vascular wall of the carotid sinus, producing a perceived

hypotension by the CBR. Conversely, a negative neck pressure produces a net increase in carotid sinus transmural pressure and produces a perceived hypertension by the CBR. Various forms of this apparatus have been produced. However, the most commonly implemented neck collar design, which consists of a malleable lead collar which isolates the anterior two-thirds of a subject's neck, was developed by Eckberg et al. in 1975 (*Eckberg, et al. , 1975*). Readily reproducible, non-invasive, and with a high degree of pressure transmission (75-85%) the neck chamber technique has been utilized with great success for modeling CBR reflexes for over 40 years.

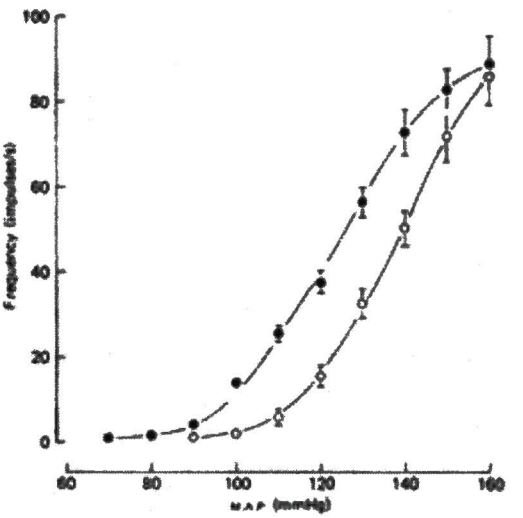
Baroreceptor Assessment and Resetting

The carotid baroreflex operational point is the position on the CBR function curve of sympathetic tone relative to a carotid distending pressure at rest. Normally this point is situated centrally within the reverse-sigmoid relationship between MSNA and carotid distending pressure, at the median and point of highest gain (*Mancia, et al. , 1978, Mancia, et al. , 1985, McDowall and Dampney. , 2006, Mengel, et al. , 1993*).

Baroreceptor resetting consists of altered MSNA relative to a specific amount of arterial wall stress. Resetting frequently results in elevated MAP and can occur acutely and/or chronically (*Chapleau, Hajduczok and Abboud. , 1988*). While hypertension results from changes in the viscoelastic properties of the arterial wall, and/or as the result of paracrine and endocrine function, baroreflex resetting frequently occurs in conjunction with these factors to allow for sustained elevations in MAP (*Chapleau, Hajduczok and Abboud. , 1989).*

Pain, exercise, and hypertension have been shown to mediate a resetting of the baroreflex, and thus alter the ability of an individual to compensate for hypotensive and hypertensive stimuli during these stressors (*Bristow, et al. , 1969, Cui, Wilson and Crandall. , 2002, Grassi, et al. , 2006, Mancia, et al. , 1985, McDowall and Dampney. , 2006*). There are two classifications of baroreflex resetting, peripheral and central.

Under most physiological conditions, peripheral resetting is a shift in the blood pressure-baroreceptor function curve in the direction of the prevailing level of arterial pressure, such that following elevations in pressure, the baroreceptor activity is reduced at equivalent pressures (*Coleridge, et al. , 1984*). Peripheral resetting occurs within two heart beats during the diastolic phase of a cardiac cycle or after brief exposure to elevated pressures, such as those found in exercise (*Gallagher, et al. , 2006*). Acute bouts of peripheral resetting can be prevented or reduced when the elevations in pressure are pulsatile rather than static (*Coleridge, et al. , 1984*).



Coleridge et al. *J. Physiol.* (1984)

During chronic hypertension or when chronic structural changes in the vasculature have occurred, a chronic resetting of the baroreflex is sustained, resulting in the continued elevation of MAP. During central baroreflex resetting there is altered afferent baroreceptor nerve activity relative to efferent sympathetic nerve activity. Central

resetting can involve neural-humoral interactions and/or altered responsiveness of central structures such as the NTS which mediate the baroreflex. Static pressures result in the continuous discharge of the baroreceptors which is followed by a central resetting response. The stereotypical central resetting response is illustrated by relative elevations in MSNA in the face of increased blood pressure. However, when exposed to pulsatile pressures, the phasic baroreceptor discharge minimizes central resetting which results in sustained sympathetic inhibition (*Chapleau, Hajduczok and Abboud. , 1988, Chapleau, Hajduczok and Abboud. , 1989*).

Pain, Hypertension, and Blood Pressure Control

Acute and/or chronic hypertension has been clearly shown to produce hypoalgesia (*Conde-Guzon, et al. , 2003, Guasti, et al. , 2002*) a condition of decreased pain sensitivity and increased pain threshold. Yet, hypoalgesia precedes the clinical manifestation of hypertension in individuals with a family history of hypertension (*France, 1999*). While seemingly contradictory, one implication from these disparate data is that neural components which mediate baroreceptor sensitivity and pain modulation are functionally intertwined within the nervous system. Recent evidence suggests that nociceptive stimulation selectively attenuates the vagal or cardiac parasympathetic, but not the sympathetic limb, of the baroreflex (*Pickering, Boscan and Paton. , 2003*). Thus, in the acute setting of pain, prior to a hypertensive response, there is a nociceptive-mediated resetting of the baroreflex which permits hypertension to occur.

Much of the evidence concerning the interaction between pain and blood pressure regulation suggests that modulation of CBR function during acute pain may be due to the balance struck between the hypertensive effects of pain, and the hypoalgesic effect of hypertension (*Conde-Guzon, et al. , 2003, France, 1999, Guasti, et al. , 2002*).

Clinical Significance of Sympathoexcitation Induced by Pain

The role of pain-mediated sympathoexcitation on clinical outcome and diagnosis is mostly unexplored. Yet, the impact of this relationship remains important. Acute pain has been shown to increase blood pressure by increasing MSNA (*Randich and Maixner. , 1984, Schnitzler and Ploner. , 2000, Woodrow, et al. , 1972*). Thus, pain may exacerbate conditions of elevated arterial pressure and heart rate in the hypertensive population.

The importance of understanding these associations during treatment and diagnosis in the clinical setting are heightened by a recent report published by the American Heart Association which determined that 65,000,000 of the 71,300,000 adult Americans with at least one form of heart disease have been diagnosed with essential hypertension (*Thom, et al. , 2006*). The capability to estimate the effect pain shall have on the regulation of autonomic processes of the body becomes critical to the treatment of individuals suffering from extreme trauma, such as that which may be sustained in automotive accidents and field casualties. The varied and potent responses to pain and changes in blood volume to which hypertensive patients may be exposed result in profound pathophysiological modulation of blood pressure control. Thus, understanding how the mechanisms which contribute to blood pressure modulation are altered between seriously

injured normotensive and hypertensive patients is important. The value of understanding these mechanisms is increasingly substantial in light of the fact that hypertensive patients may exhibit a nearly 50% increased risk of mortality following physical trauma (*Tamosiunas, et al. , 2005, Terry, et al. , 2007*).

Scientific Significance of Sympathoexcitation Induced by Pain

Many studies have been performed to enhance our understanding of how the baroreceptors regulate blood pressure. However, the relationship produced between baroreceptor function and physiological stressors which occur as a component to survival remain mostly unexamined.

Experimental studies, especially those examining the effects of various stimuli on the regulation of blood pressure may utilize techniques such as: arterial or venous catheterization, localized ischemia, thermal effects, and prolonged or intense exercise. Any of these techniques may readily elicit pain stimuli which, if not accounted for, may produce aberrant data and confound analysis. For these reasons, it is important to examine in greater detail, the relationship between pain and its influence on blood pressure regulation.

Summary

The hypothalamus plays a central role in the regulation of cardiovascular variables through its many afferent innervations and efferent activation of preganglionic vagal and sympathetic neurons (*Guyenet, 2006*). For nearly 20 years the site of signal integration

for much of the regulation between CBR and pain has been demonstrated to be the result of polysynaptic integration at the NTS (*Boscan and Paton. , 2001, Boscan, Kasparov and Paton. , 2002, Boscan, Pickering and Paton. , 2002, Guyenet, 2006*). In 2003, studies by Boscan et al. demonstrated that increased nociceptive activity alter the sensitivity of the NTS to the neural impulses originating from the CBR (*Boscan, Pickering and Paton. , 2002*). The interaction between pain and blood pressure regulation described by these studies suggested that modulation of the CBR function curve and possibly its operating point may be due to a balance struck between the hypertensive effects of pain, and the hypoalgesic effect of hypertension (*Conde-Guzon, et al. , 2003, France, 1999, Guasti, et al. , 2002*).

By selectively activating the CBR during episodes of acute pain, and during acute pain accompanying CPBR unloading, we propose to identify the changes in the CBR reflex that may occur during a model of traumatic injury. We predict that pain induces an increase in CBR gain, as well as a resetting of the operational point favoring a hypertensive state to buffer against episodes of hypotension. These findings shall further the understanding of pain and its role in the maintenance of blood pressure during episodes of central hypovolemia, as well as the effect pain has on the modulation of CBR function and several cardiovascular parameters like MAP.

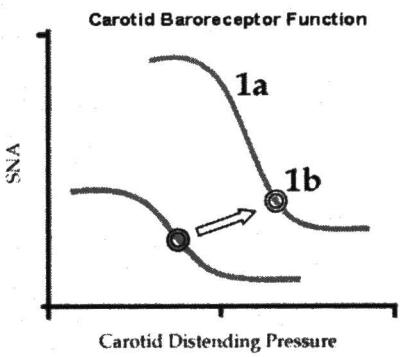
SPECIFIC AIMS

Two primary objectives were developed for this dissertation: a) to examine the effect of cold-induced pain on CBR resetting; b) to determine the response of the CBR reflex to simultaneous cold-induced acute pain and decreased CVP. In order to address these primary objectives a secondary objective to determine the effects of repeated exposure to cold-induced pain on MSNA and MAP was also addressed. To accomplish all of these objectives the following aims were established:

Aim 1: Determine the effect of intermittent cold-pressor induced pain on sympathetic nerve activity, cardiovascular response, and baroreceptor resetting, in normotensives, by testing the following hypotheses:

a) Acute pain stimuli increase CBR gain and cause an upward-rightward shift of the CBR function curve;

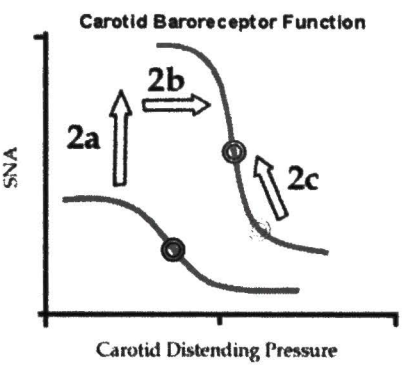
b) Acute pain stimuli reset the operational point of the CBR function curve, relocating it to the lower limiting value of the reverse-sigmoid relationship.



Aim 2: Determine the effects of intermittent cold-pressor induced pain and orthostatic stress on sympathetic nerve activity, cardiovascular response, and CBR modulation, in normotensives, by testing the following hypotheses:

a) CPBR unloading will increase CBR gain and cause an upward shift of the CBR function curve.

b) CPBR unloading, during simultaneous cold-pressor induced pain, will evoke an exaggerated increase in CBR gain, shift the CBR function curve to the right, but will not significantly shift the curve upward.



c) CPBR unloading during an acute pain stimulus will provoke a resetting of the operational point in CBR function curve, relocating it to the median of the reverse-sigmoid relationship.

Aim3: Determine effects of repeated exposure to intermittent cold induced pain on sympathetic nerve activity and cardiovascular response in normotensives, by testing the following hypotheses:

a) Repeated cold pressor stimuli do not change the baseline sympathetic and cardiovascular responses observed during an initial cold pressor stimulus.

b) Increased pain perception evokes graded increases in MSNA.

c) A pain threshold must be achieved or exceeded in order to invoke sympathoexcitation.

EXPERIMENTAL DESIGN

Two individual experiments were designed to investigate the specific aims and are discussed in detail in chapters 2-4. Below are brief descriptions of the experimental designs.

Adaptive MSNA and MAP Responses (Aim 3a)

To test the adaptive responses to a cold pressor test MSNA, arterial blood pressure, heart rate (HR), and respiratory function were continuously recorded while subjects placed their hand in 2°C cold water for 2 minutes. A total of 7 stimuli were applied with either a 10, 20, or 30 minute adaptive recovery phase, which was randomized between each stimulus. The data obtained were used to determine the rate of recovery from pain-induced increases in MSNA and MAP.

Graded MSNA and MAP Responses (Aim 3b and 3c)

To test the graded responses to cold pressor test MSNA, arterial blood pressure, HR, and respiratory function were continuously recorded while subjects placed their hand in varying temperatures of cold water for 2 minutes, with a 20 minute recovery period between each stimulus. The range of temperatures used to test the graded response to CPT included: 2°C, 6°C, 10°C, 14°C, and 18°C. The various water temperatures were presented in random order. The data obtained was used to determine the magnitude of MSNA and MAP response to various intensities of perceived pain.

Pain-Induced Modulation of CBR Gain (Aim 1a) and Operational Point (Aim 1b)

To assess resting CBR gain and operational point, subjects placed their right hand in a thermoneutral (25°C) water bath for 2 min, while MSNA and MAP were recorded. In random order, hypertensive, hypotensive, and sham stimuli, were administered via neck collar during the second minute of immersion. To assess CBR gain and operational point during pain, subjects placed their right hand in a cold pressor (2°C) water bath for 2 min, while MSNA and MAP were recorded. Three hypertensive stimuli, two hypotensive stimuli, and a sham stimulus, were administered via neck collar during the second minute of immersion. Regressions and calculations for CBR gain were compared between the thermoneutral and cold-induced pain conditions. To determine the location of the operational point during cold pressor-induced pain a repeated measures one-way ANOVA compared the differences between each CBR stimulus. Furthermore, regressions to illustrate the location of the CBR operational point were produced.

Modulation of CBR Gain by Pain and CPBR unloading (Aim 2a and 2b)

To assess CBR gain during LBNP, subjects placed their right hand in a thermoneutral (25°C) water bath for 2 min, during which time LBNP was applied. During this stimulus MSNA and MAP were recorded. In randomized order, two hypotensive stimuli and a sham stimulus were administered to the CBR via neck collar during the second minute of the water bath phase. To assess CBR gain during pain and LBNP, subjects placed their right hand in a cold pressor (2°C) water bath for 2 min, while MSNA and MAP were recorded. In randomized order, three hypertensive stimuli, two

hypotensive stimuli, and a sham stimulus, was administered to the CBR via neck collar during the second minute of the water bath phase. Regressions and calculations for CBR gain were compared between the two conditions.

Modulation of CBR Operational Point by Pain and CPBR unloading (Aim 2c)

To assess CBR operational point during LBNP, stimuli were applied as described above. To assess CBR operational point stimuli were applied as described above. To determine the location of the operational point during cold pressor induced pain and CPBR unloading a repeated measures one-way ANOVA compared the differences between each CBR stimulus. Furthermore, regressions to illustrate the location of the CBR operational point were produced.

METHODS

The methodology used in each study is described in the respective chapters, however, it is pertinent to discuss the major aspects of the neck pressure/neck suction (NP/NS) and the microneurography techniques used to examine CBR function.

A malleable lead collar was placed on the anterior 2/3 of the subject's neck. A rapid square wave application of pressure and suction was utilized to precipitate changes in MSNA and MAP. Each NP/NS stimulus consisted of six 10 second cycles of 5 second duration on/off stimuli which were manually controlled. The pressures of NP/NS utilized to map the CBR response were +40. +20, 0, -20, -40, and -60 (mmHg). Respiration was monitored and subjects were instructed to breath normally to reduce the confounding

effects of respiratory sinus arrhythmia (*Saul, et al. , 1989*). CDP was calculated by adding the absolute value of negative neck pressures or subtracting positive neck pressures from pre-stimulus MAP. As the input threshold was not obtained for the CBR stimulus-response curve the Kent logistical function model was not utilized (*Kent, et al. , 1972*). Instead, 1^{st} and 2^{nd} order linear regression were utilized to assess the relative shape of the CBR response at and around the area of input saturation.

Muscle sympathetic nerve activity was measured in the peroneal nerve, located on the upper and outer aspect of the leg near the fibular head using standard microneurographic technique (*Smith, et al. , 1996, Vallbo, et al. , 1979*). First, the course of the nerve was determined by stimulating through the skin with a pencil-shaped electrode. Once located, two tiny, sterile, wire electrodes were inserted through the skin and into the nerve. MSNA burst amplitude was normalized to each individual's baseline condition one minute prior to any stimulus. MSNA was further normalized to heart rate and is expressed as the total activity per 100 heart beats. The normalization of MSNA values allows for accurate comparisons to be made between subjects.

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CHAPTER II

PAIN PERCEPTION DETERMINES SYMPATHOEXCITATORY RESPONSE TO COLD

Raven JS, Siu JC, Thakre T, Smith M.

Department of Integrative Physiology

University of North Texas Health Science Center

3500 Camp Bowie Blvd.

Fort Worth, TX. 76107

(Submitted to Experimental Physiology)

ABSTRACT

The utilization of the Cold Pressor Test (CPT) for diagnostic purposes has a well-documented history over 60 years in length. The CPT is used as a potent cold pain stimulus resulting in increases in sympathetic outflow, which in turn yield elevated mean systemic vascular resistance (MSVR) and heart rate (HR). In order to understand the physiological response to repeated pain stimuli this study attempts to quantify the relationships among acute cold pressor (CP) pain stimuli, cardiovascular responses, and muscle sympathetic nerve activity (MSNA) in healthy individuals. In order to elucidate the relationships among the various components of the CPT response the following hypotheses were tested: 1) Repeated CP stimuli do not change the baseline responses observed during an initial CP stimulus. 2) Increased pain perception evokes graded increases in MSNA. 3) A pain threshold must be achieved or exceeded in order to invoke sympathoexcitation. In this study HR, MSVR, and MSNA returned to baseline values following repeated exposure to the CPT, within a 10 minute time frame. MSVR and MSNA were demonstrated to increase in response to a greater perceived pain. These data support the hypothesis that the physiological response to pain is non-adaptive and highly repeatable.

Keywords: Muscle Sympathetic Nerve Activity, Sympathoexcitation, Mean Systemic Vascular Resistance

INTRODUCTION

Importance of pain as a clinical condition: Pain and the management of pain have received increased attention over the past 60 years. The treatment of pain is a primary component of many clinical visits, and pain itself is diagnostic of many ailments. Chronic pain mediates a systemic sympathoexcitation which in turn may lead to elevated circulating norepinephrine (NE) concentrations, increased heart rate (HR), and increased systemic vasoconstriction. Hypertension has been shown to mediate a resetting of the baroreflex, and thus alter the ability of an individual to regulate blood pressure in response to hypotensive and hypertensive stimuli (*Bristow, et al. , 1969, Grassi, et al. , 2006, Mancia, et al. , 1978*). Thus, there remains a clear need to advance the understanding of pain and its effect on modifying autonomic physiology.

The Sympathetic Nervous System (SNS) and Pain: The SNS responds to stressors like exercise, pain, and disease, by modulating various functions of the cardiovascular, musculoskeletal, pulmonary, adrenergic, gastrointestinal, and renal systems (*Nordin and Fagius. , 1995, Schobel, et al. , 1998*). This study investigates the SNS response to cold pressor pain stimuli and develops a quantitative model of pain-mediated MSNA which is applicable to test the hypotheses related to pain and sympathetic coupled mechanisms. Whether the pain-sympathetic relationship behaves as a linear graded relationship is unknown. Further, whether the response to a single acute pain exposure produces persistent autonomic effects is also unknown and thus, may impact subsequent pain stimuli.

Therefore, the purpose of this study was to determine the relationship of MSNA to graded cold-mediated pain and to determine how quickly autonomic and cardiovascular responses recover after an acute cold stimulus.

METHODS

Subjects: This study was approved by the University of North Texas Health Science Center Institutional Review Board. (Sixteen subjects (7 M and 9 W), normotensive, healthy ages 18-25, volunteered to participate in this investigation). All individuals were studied at the same time of day. All subjects reported pain perception as a function of Borg's 15 point rating of perceived pain (RPP) scale (*Borg, et al. , 1970*) during and prior to each stimulus, every 15 seconds, until no pain was reported. In the adaptive response treatment group, 4 men and 5 women participated. In the graded response treatment group, 3 men and 4 women participated.

Cardiovascular measures: HR was measured by utilizing a standard limb-lead ECG. This method requires three electrodes placed on the ventral surfaces of the left arm, right arm, and left leg. Arterial beat-to-beat BP was measured non-invasively via photoplethysmography at the finger (Finapres Blood Pressure monitor 2300, Ohmeda; Englewood, CO). This method has been demonstrated as a reliable measure of BP (*Imholz, et al. , 1988, Imholz, et al. , 1990, Imholz, et al. , 1998*).

Respiratory measures: Respiratory rate was recorded via a respiratory monitoring band placed around the subject's abdomen. (Grass Instruments; West Warwick, RI). Also, a low-resistance, turbine, volume transducer (model VMM, Alpha

Technologies; Laguna Hills, CA) was attached to a leak-free nasal mask which then recorded movement of air volumes. Respiration was monitored to assure that apneas were not performed, which could have potentially contaminated the MSNA recording of sympathetic nerve traffic (*Cutler, et al. , 2004*).

MSNA Measures: Postganglionic MSNA was directly measured from the peroneal (fibular) nerve at the popliteal fossa or at the fibular head, using standard microneurographic techniques (*Vallbo, et al. , 1979*). Activity for MSNA was obtained as described by Smith et al. (*Smith, et al. , 1996*). Two sterile tungsten microelectrodes (tip diameter 5-10 μm, 35 mm long, Fredrick Haer and Co., Bowdoinham, ME) were inserted; one, inserted subcutaneously, served as a reference and the other was inserted into the peroneal nerve for measurement of MSNA. Due to their small size, microelectrodes were inserted without the use of local anesthesia to avoid any effect the anesthetic may have on local nerve function. Nerve signal were processed by a preamplifier and an amplifier (nerve traffic analyzer model 662C-3, Department of Bioengineering, University of Iowa, Iowa City, IA) with a total gain of 90,000. Amplified signals were band-pass filtered (700-2,000 Hz), rectified, and discriminated. Finally, a resistance-capacitance circuit with a time constant of 0.1 s integrated raw nerve signals. All signals were sampled at a rate of 1,000 Hz. MSNA recordings were confirmed using the following criteria: 1) pulse-synchronous bursts occurring 1.2-1.4 s after the associated QRS complex, 2) reproducible activation during apnea, and 3) no activation following pinch, skin stroking, or startle stimuli (all of which activate skin sympathetic fibers).

Calf Blood Flow Measures: Strain gauge plethysmography was used around the leg contra-lateral to the leg used for microneurography. Changes in leg circumference alter the total stretch measured by the strain gauge, and thus, the electrical resistance of the strain gauge. This resistance was then measured by a plethysmograph (Hokanson EC-4 Plethysmograph; Issaquah, Wa) which outputs the magnitude of stretch as an analog voltage. Calf blood flow was estimated from the relative changes in leg circumference as a function of time. The percent change was then extrapolated as a volume change in ml/(100 ml of tissue/min).

Protocols:

Adaptive Response Study: The first set of experiments tested the adaptive responses to CPT. MSNA (microeurography), beat-to-beat arterial blood pressure (Finapress), heart rate (ECG), calf blood flow (plethysmography), and respiratory function were continuously recorded while subjects were asked to place their hand in 2°C cold water for 2 minutes. A total of 7 stimuli were applied with either a 10, 20, or 30 minute adaptive recovery phase, which was randomized and repeated, between each cold pressor stimulus. Data were collected for 2 min pre-stimulus, 2 min stimulus, and 4 min post stimulus. The total time required to complete the 27 stages of the adaptive response study was approximately 162 minutes.

Graded Response Study: The second set of experiments tested the graded responses to CPT. MSNA (microeurography), beat-to-beat arterial blood pressure (Finapress), heart rate (ECG), calf blood flow (plethysmography), and respiratory function were continuously recorded while subjects were asked to place their hand in

varying temperatures (2°C, 6°C, 10°C, 14°C, and 18°C - in random order) of cold water for 2 minutes each, with 20 min recovery between each stimulus. The 19 stages of the graded response study were completed within approximately 100 minutes.

Analyses: All data are presented as mean ± SEM. Responses after different recovery time periods were obtained by repeated measures analysis of variance (ANOVA). A similar analytical approach was used to compare the responses over time between the different temperatures and perceived pain. Finally, the relation of pain and MSNA was assessed by linear regression.

RESULTS

Arterial Pressure Responses: No significant differences in MAP were observed during the first (p_{10} = 1.0, p_{20} = 1.0, p_{30} = 1.0) and second minute (p_{10} = 0.86, p_{20} = 1.0, p_{30} = 0.93) of the cold pressor test between the three rest paradigms. Furthermore, no significant differences were observed for measures of MAP during the first half (p_{10} = 0.45, p_{20} = 1.0, p_{30} = 0.37) and second half (p_{10} = 1.0, p_{20} = 0.99, p_{30} = 1.0) recovery period for all three rest paradigms. There were no significant differences in MAP between baseline measures and following 4 minutes of recovery for all three rest paradigms (p_{10} = 1.0, p_{20} = 1.0, p_{30} = 1.0) (Figure 1). Finally, the magnitude of MAP increase was dependant on the intensity of the stimulus provided (p = 0.001) (Figure 2).

Heart Rate Responses: No significant differences in HR were observed during the first (p_{10} = 0.98, p_{20} = 1.0, p_{30} = 1.0) and second minute (p_{10} = 1.0, p_{20} = 1.0, p_{30} = 1.0) of the cold pressor test between the three rest paradigms (Figure 3).

Furthermore, no significant differences were observed for measures of HR during the first half ($p_{10} = 1.0$, $p_{20} = 1.0$, $p_{30} = 1.0$) and second half ($p_{10} = 1.0$, $p_{20} = 1.0$, $p_{30} = 1.0$) recovery period for all three rest paradigms (Figure 3). There were no significant differences in MAP between baseline measures and following 4 minutes of recovery for all three rest paradigms ($p_{10} = 1.0$, $p_{20} = 1.0$, $p_{30} = 1.0$) (Figure 3). Finally, while the magnitude of HR increase trended toward a dependence on the intensity of the stimulus provided, the only statistically significant difference between conditions was during the first minute of the 18°C and 2°C CPT. ($p = 0.048$) (Figure 2).

Perceived Pain Responses: No significant differences were observed between the three rest paradigms during examination of perceived pain ratings ($p_{10} = 1.0$, $p_{20} = 1.0$, $p_{30} = 1.0$) (Figure 4). Furthermore, there were no significant differences in perceived pain ratings between baseline measures and following 4 minutes of recovery for all three rest paradigms ($p_{10} = 1.0$, $p_{20} = 1.0$, $p_{30} = 1.0$) (Figure 4). Perceived pain intensity was dependant on water bath temperature ($p = 0.001$) (Figure 5). Finally, MSNA and MAP responses correlated strongly to the intensity of perceived pain experienced by the subjects (Figures 6 and 7).

Sympathetic Neural Responses: MSNA normalized to baseline values demonstrated no statistically significant differences between the three rest paradigms ($p_{10} = 1.0$, $p_{20} = 1.0$, $p_{30} = 1.0$) (Figure 8). Perceived pain intensity was dependant on water bath temperature ($p = 0.001$) (Figure 2). The consistency of the pain perception and MAP responses provide evidence which suggest the functional expression of inter-individual MSNA variability is an expression of end-organ responsiveness. Thus,

individual differences in MSNA do not result in a functional translation to differences in other physiological variables (Figures 1, 3, and 4).

DISCUSSION

These data demonstrate that a cold stimulus can be used to model the pain-mediated sympathetic neural response. Gradations of cold lead to gradations of pain perception and pain-related sympathoexcitation in a linear manner. These data also indicate that when the cold stimuli are repeated, that basal MSNA, HR, and arterial pressure return within 5 min or less, and that subsequent cold stimuli evoke similar physiological responses. Thus, when multiple brief cold stimuli are delivered, there is not an adaptive response.

Pain Physiology Models: Acute cold stimuli are frequently used to provoke intense pain-mediated pressor responses. The standard approach is to submerse the hand in a single temperature water bath of (0-2)°C. Immersion of a hand into an ice water bath (2°C) for 2-3 minutes is a potent stimulus which can lead to increases in arterial pressure (*Barnett, et al. , 1963*). This response is mediated in part by an initial withdrawal of parasympathetic activity and associated rapid increases in heart rate, which is followed by a progressive increase in sympathetic nerve activity and further rise in arterial pressure which is primarily mediated by increases in vascular resistance (*Seals, 1990, Victor, et al. , 1987*). Victor and colleagues also showed that the initial increase in heart rate returns toward control values during the 2nd minute of a cold stimulus (*Seals, 1990, Victor, et al. , 1987*). However, to date, there have been limited studies to assess the effect of

gradations of cold stimuli on the physiologic responses. As this stimulus evokes distinct responses among different populations and is known to test the health of sympathetic efferent nerves, this stimulus holds promise to be another tool for evaluating normal autonomic control mechanisms among selected populations and diseases. The importance of utilizing sub-maximal stimuli must not be overlooked. A graded pain stimulus would be useful in evaluating the responsiveness of the SNS, as well as the nociceptive sensitivity of patients while in a clinical setting. Moreover, most forms of pain do not occur as profoundly intense; yet, the physiologic impact of modest pain is unknown. This merits further investigation and is a focus of this study.

Ordinarily, cold is sensed by type C and A-δ primary sensory/nociceptive neurons through the activation of a network of cold sensitive ion channels. Recent findings have shown that of greatest importance to neural activation at temperatures <17°C are the TRPA1 channels (*Tominaga and Caterina, 2004*). Whether the cold-mediated sympathoexcitation noted above is mediated by cold versus the pain stimulus was addressed by Kregel and colleagues. *Kregel et al.* demonstrated that sympathoexcitation from a CPT only occurs when skin temperature falls to levels that produce a sensation of intense pain (*Kregel, Seals and Callister, 1992*). These results suggest that increased MSNA, resulting from the CPT, is driven by high threshold nociceptive fibers within the hand and wrist. *Fagius et al.* further demonstrated a significant correlation between the rating of perceived pain and increases in SNA during a CPT (*Fagius, Karhuvaara and Sundlof. , 1989*). Together, these studies suggest that the sympathetic activation associated with an acute cold stimulus is primarily mediated by the pain perception rather

than the cold itself. As noted above, pain is recognized as a stimulus for sympathoexcitation and cold-mediated pain evokes significant increases in MSNA. In this study we demonstrated that graded pain perception evokes graded increases in MSNA and MAP. These data further support the concept that pain is the primary component of a cold stimulus contributing to increases in MSNA and MAP, and that self reported pain perception correlated very strongly with sympathetic activity. Supporting the findings of Kregel et al. minimal to moderate increases in MSNA were observed at the low to moderate pain intensities, and it was only at the higher levels of pain (above a rating of 10) that significant sympathoexcitation occurred. Above these levels of pain perception, second and third order linear regressions did not substantially increase the r^2value, which suggests that this is a linear cause-effect relationship as pain is increased.

Reproducibility and Adaption of MSNA Responses to Pain: This study demonstrates that the perceived pain returns very quickly to a non-pain state after termination of the cold stimulus. The return to baseline occurred within 3 min regardless of the intensity of the cold stimulus. Nevertheless, the pain perception persisted beyond the withdrawal of the cold stimulus. Arterial pressure, HR, and MSNA consistently returned to pre-stimulus basal values within 4 min regardless of the pain intensity suggesting that there was rapid recovery independent of the intensity of cold-pain stimuli. The return to baseline for all physiological variables was longer following the more intense cold and pain stimuli, suggesting that the persistence of this sustained effect was related to the stimulus intensity. In subsequent cold stimuli, the pain perception and physiologic responses were comparable regardless of whether the recovery period was

10, 20 or 30 min. The return to baseline occured after repeated exposure of up to 6 consecutive stimuli. Varying the recovery times (10, 20, 30 minutes) did not change the sympathetic and cardiovascular responses to a subsequent cold pressor stimulus. Collectively, these data suggests that there was not a prolonged adaptation to the cold and pain stimuli.

In summary, these data demonstrate that 1) graded increases in pain perception provoke graded increases in SNA above a threshold level of pain perception, and 2) repeated cold-induced pain does not alter the baseline characteristics of the MSNA mediated cold pressor response when the recovery period is > 10 min.

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FIGUERE LEGENDS

FIGURE 1 – Mean arterial pressure (MAP) ± SEM during a 2°C cold pressor test (CPT) as a function of time. Filled Circles = MAP during the CPT before a 10 minute rest period. Hollow Circles = MAP during the CPT after a 10 minute rest period. Filled Triangles = MAP during the CPT before a 20 minute rest period. Hollow Triangles = MAP during the CPT after a 20 minute rest period. Filled Squares = MAP during the CPT before a 30 minute rest period. Hollow Squares = MAP during the CPT after a 30 minute rest period.

FIGURE 2 – Panel A: Heart rate (HR) ± SEM during several cold pressor tests (CPT)s as a function of time. Filled Circles = HR during a 2°C CPT. Hollow Circles = HR during a 10°C CPT. Filled Triangles = MAPs during a 18°C CPT. **Panel B:** Mean arterial pressure (MAP) ± SEM during several cold pressor tests (CPT)s as a function of time. Filled Circles = MAPs during a 2°C CPT. Hollow Circles = MAPs during a 10°C CPT. Filled Triangles = MAPs during a 18°C CPT. **Panel C:** Muscle sympathetic nerve activity (MSNA) ± SEM percentage of baseline, during several cold pressor tests (CPT)s as a function of time. Filled Circles = MSNA during a 2°C CPT. Hollow Circles = MSNA during a 10°C CPT. Filled Triangles = MSNA during a 18°C CPT.

FIGURE 3 – Heart rate (HR) ± SEM during a 2°C cold pressor test (CPT) as a function of time. Filled Circles = HR during the CPT before a 10 minute rest period. Hollow Circles = HR during the CPT after a 10 minute rest period. Filled Triangles = HR during the CPT before a 20 minute rest period. Hollow Triangles = HR during the CPT after a 20 minute rest period. Filled Squares = HR during the CPT before a 30 minute rest period. Hollow Squares = HR during the CPT after a 30 minute rest period.

FIGURE 4 – Mean perceived pain rating (PPR) ± SEM during a 2°C cold pressor test (CPT) as a function of time. Filled Circles = PPR during the CPT before a 10 minute rest period. Hollow Circles = PPR during the CPT after a 10 minute rest period. Filled Triangles = PPR during the CPT before a 20 minute rest period. Hollow Triangles = PPR during the CPT after a 20 minute rest period. Filled Squares = PPR during the CPT before a 30 minute rest period. Hollow Squares = PPR during the CPT after a 30 minute rest period.

FIGURE 5 - Perceived pain rating (PPR) as a Function of Time across several ice water bath temperatures. Data expressed as means ± SEM. Filled Circles = PPR during a 2°C cold pressor test (CPT). Hollow Circles = PPR during a 10°C CPT. Filled Triangles = PPR during a 18°C CPT.

FIGURE 6 - First Order Linear Regression of % change in muscle sympathetic nerve activity (MSNA) as a function of perceived pain. Data expressed as means ± SEM.

FIGURE 7 - First Order Linear Regression of % change in mean arterial pressure (MAP) as a function of Perceived Pain. Data expressed as Means ± SEM.

FIGURE 8 - Muscle sympathetic nerve activity (MSNA) ± SEM expressed as a percentage of baseline during a 2°C cold pressor test (CPT) as a function of time. Filled Circles = MSNA during the CPT before a 10 minute rest period. Hollow Circles = MSNA during the CPT after a 10 minute rest period. Filled Triangles = MSNA during the CPT before a 20 minute rest period. Hollow Triangles = MSNA during the CPT after a 20 minute rest period. Filled Squares = MSNA during the CPT before a 30 minute rest period. Hollow Squares = MSNA during the CPT after a 30 minute rest period.

FIGURE 1

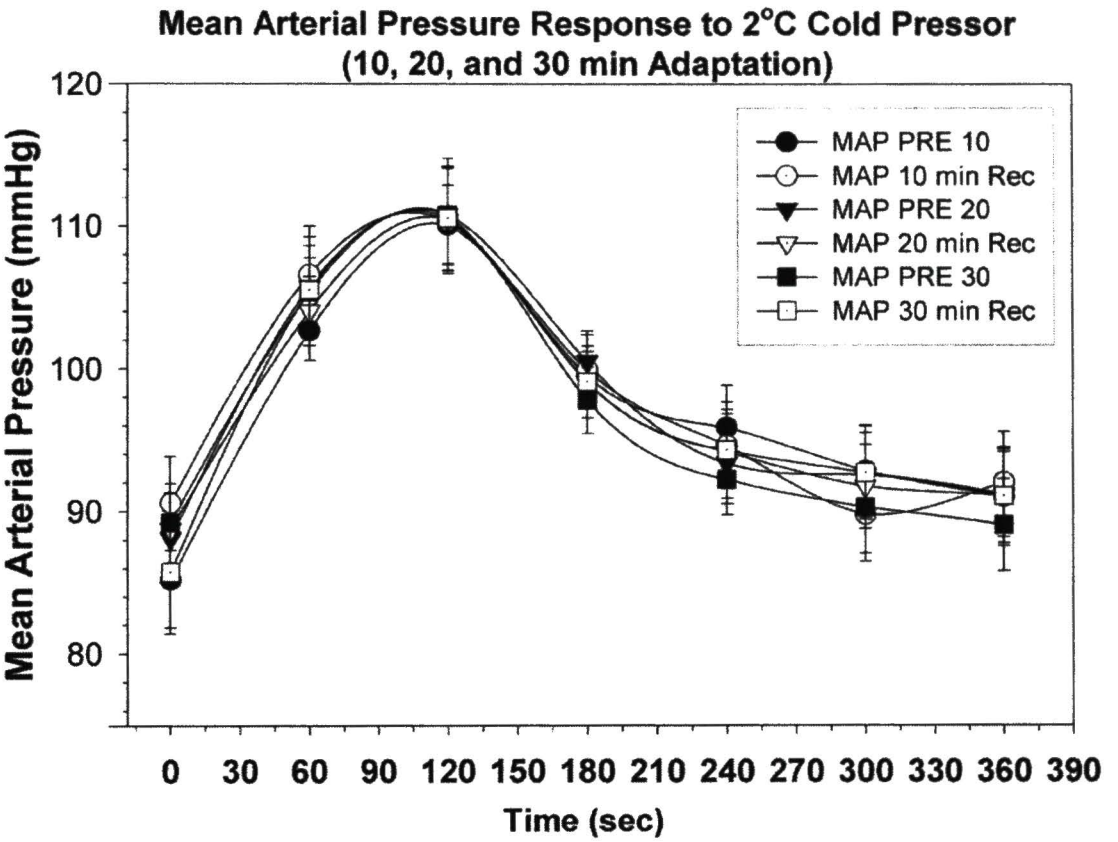

Mean Arterial Pressure Response to 2°C Cold Pressor
(10, 20, and 30 min Adaptation)
Mean Arterial Pressure (mmHg)
120
110
100
90
80
0
30
60
90
120
150
180
210
240
270
300
330
360
390
Time (sec)
MAP PRE 10
MAP 10 min Rec
MAP PRE 20
MAP 20 min Rec
MAP PRE 30
MAP 30 min Rec

FIGURE 2

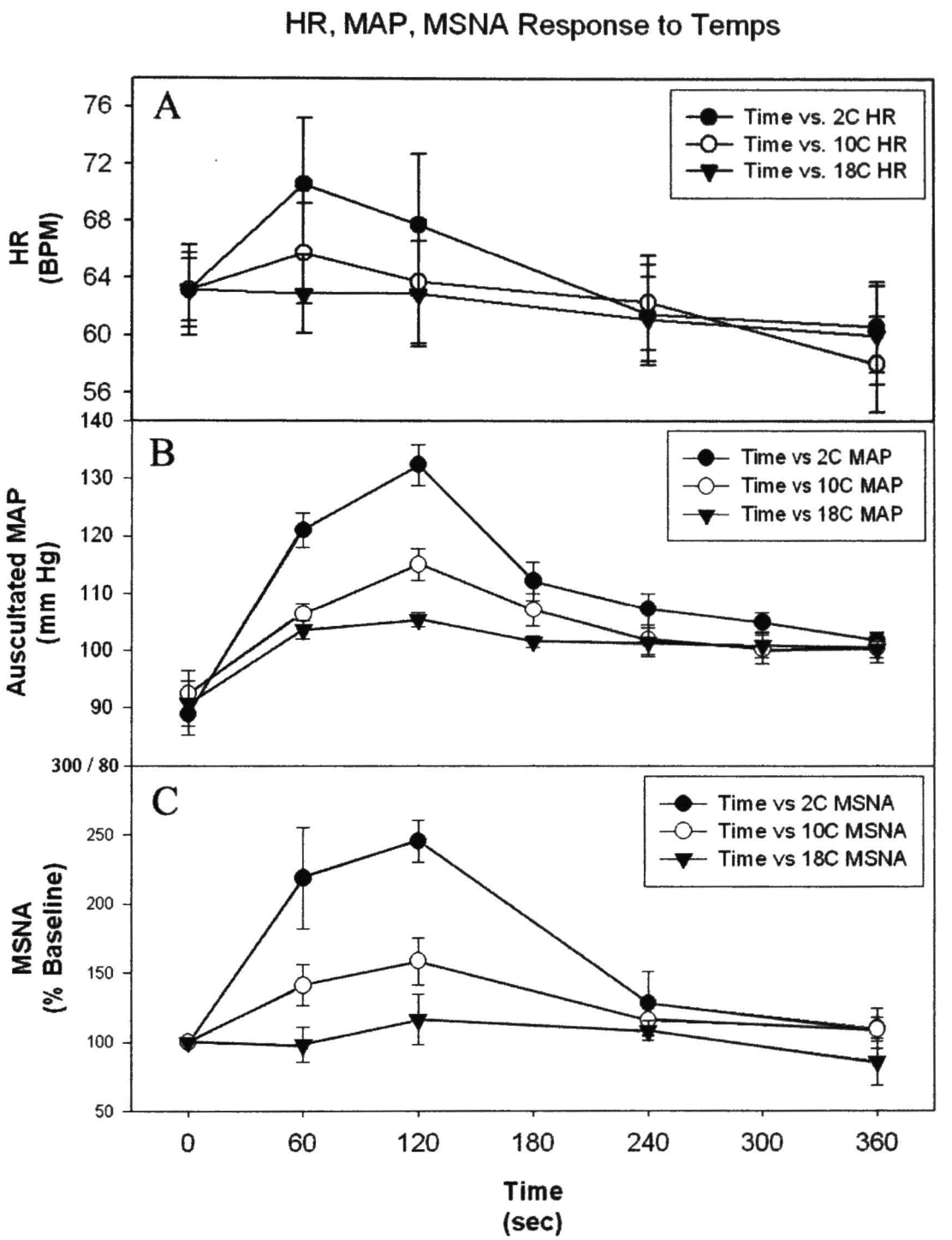

HR, MAP, MSNA Response to Temps
A
B
C
HR (BPM)
Auscultated MAP (mm Hg)
MSNA (% Baseline)
Time vs. 2C HR
Time vs. 10C HR
Time vs. 18C HR
Time vs 2C MAP
Time vs 10C MAP
Time vs 18C MAP
Time vs 2C MSNA
Time vs 10C MSNA
Time vs 18C MSNA
76
72
68
64
60
56
140
130
120
110
100
90
300 / 80
250
200
150
100
50
0
60
120
180
240
300
360
Time (sec)

FIGURE 3

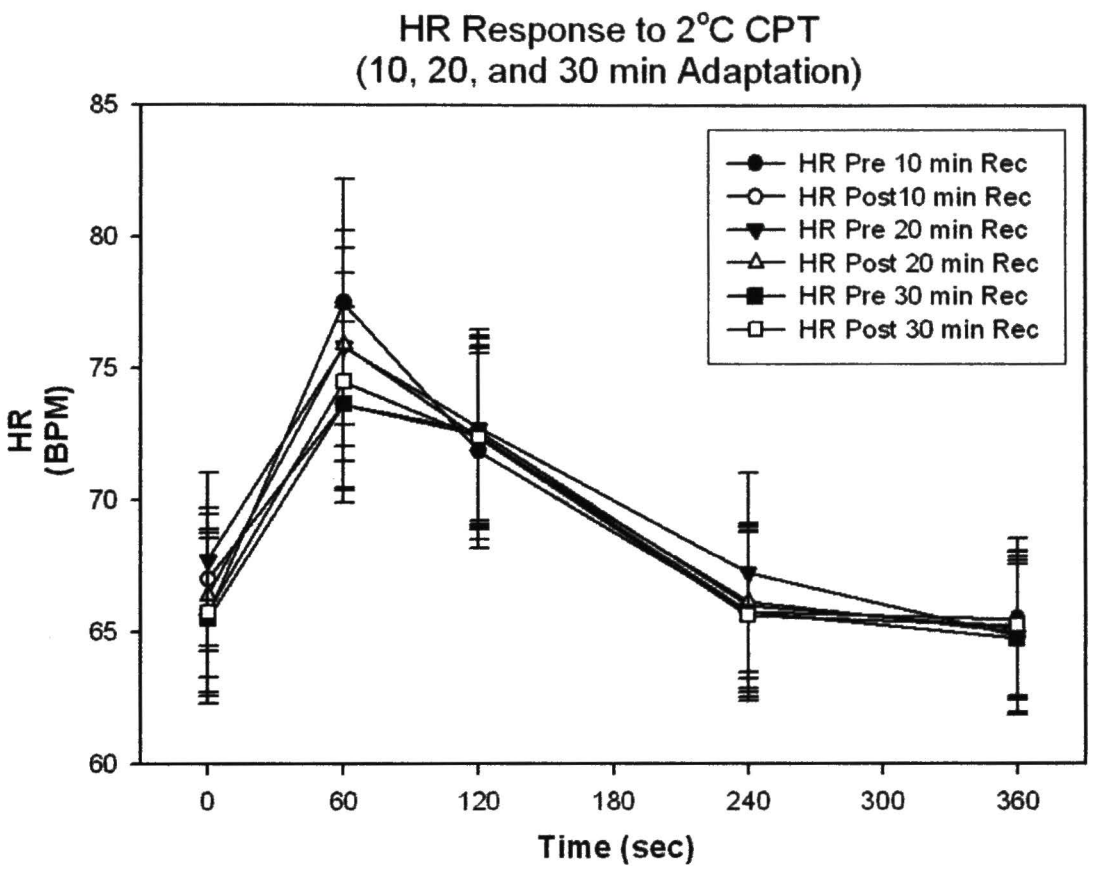

HR Response to 2°C CPT
(10, 20, and 30 min Adaptation)
HR (BPM)
85
80
75
70
65
60
0
60
120
180
240
300
360
Time (sec)
HR Pre 10 min Rec
HR Post10 min Rec
HR Pre 20 min Rec
HR Post 20 min Rec
HR Pre 30 min Rec
HR Post 30 min Rec

FIGURE 4

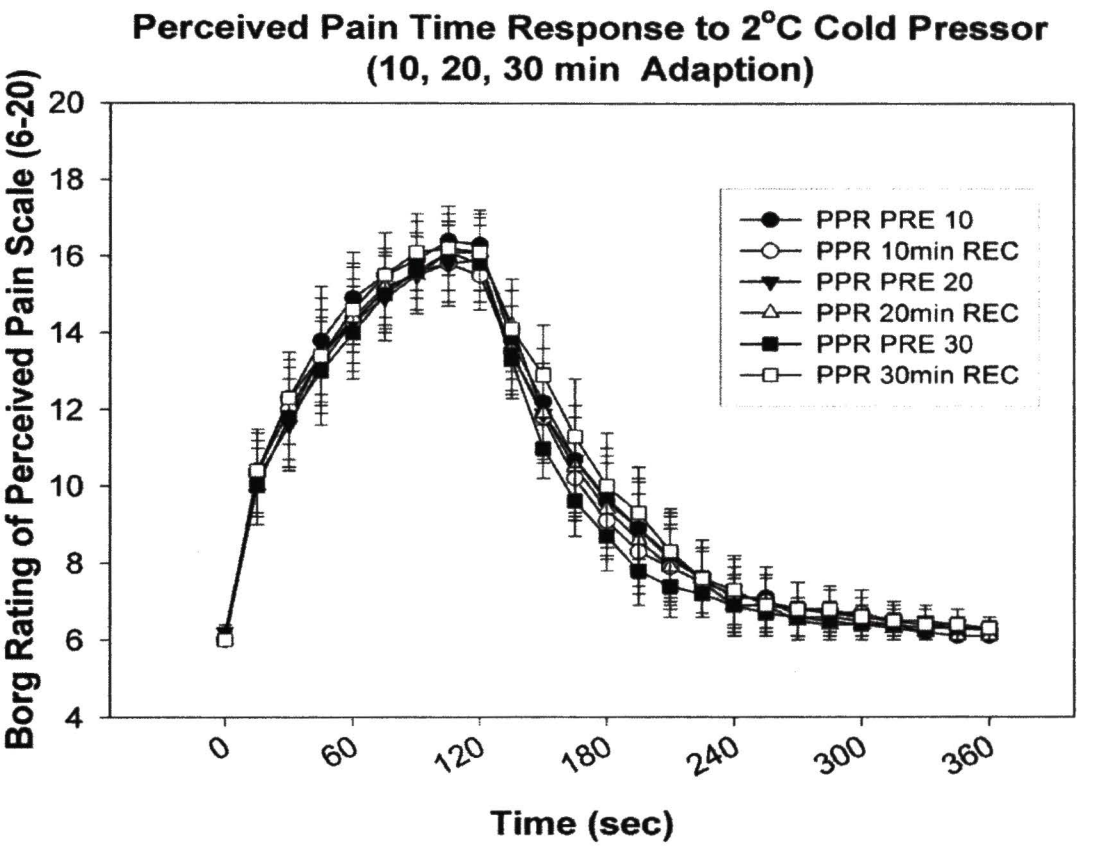

Perceived Pain Time Response to 2°C Cold Pressor
(10, 20, 30 min Adaption)
Borg Rating of Perceived Pain Scale (6-20)
20
18
16
14
12
10
8
6
4
0
60
120
180
240
300
360
Time (sec)
PPR PRE 10
PPR 10min REC
PPR PRE 20
PPR 20min REC
PPR PRE 30
PPR 30min REC

FIGURE 5

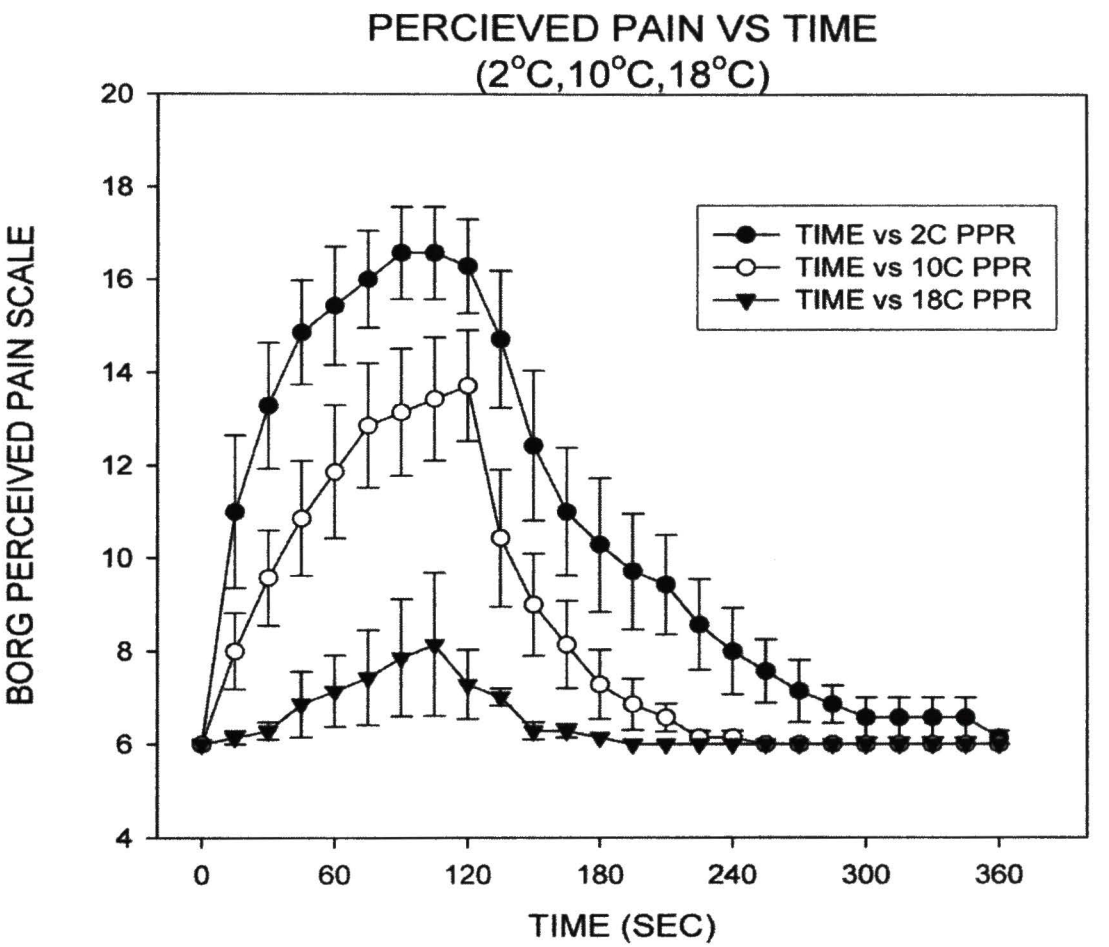

PERCIEVED PAIN VS TIME
(2°C,10°C,18°C)
BORG PERCEIVED PAIN SCALE
TIME (SEC)
TIME vs 2C PPR
TIME vs 10C PPR
TIME vs 18C PPR
20
18
16
14
12
10
8
6
4
0
60
120
180
240
300
360

FIGURE 6

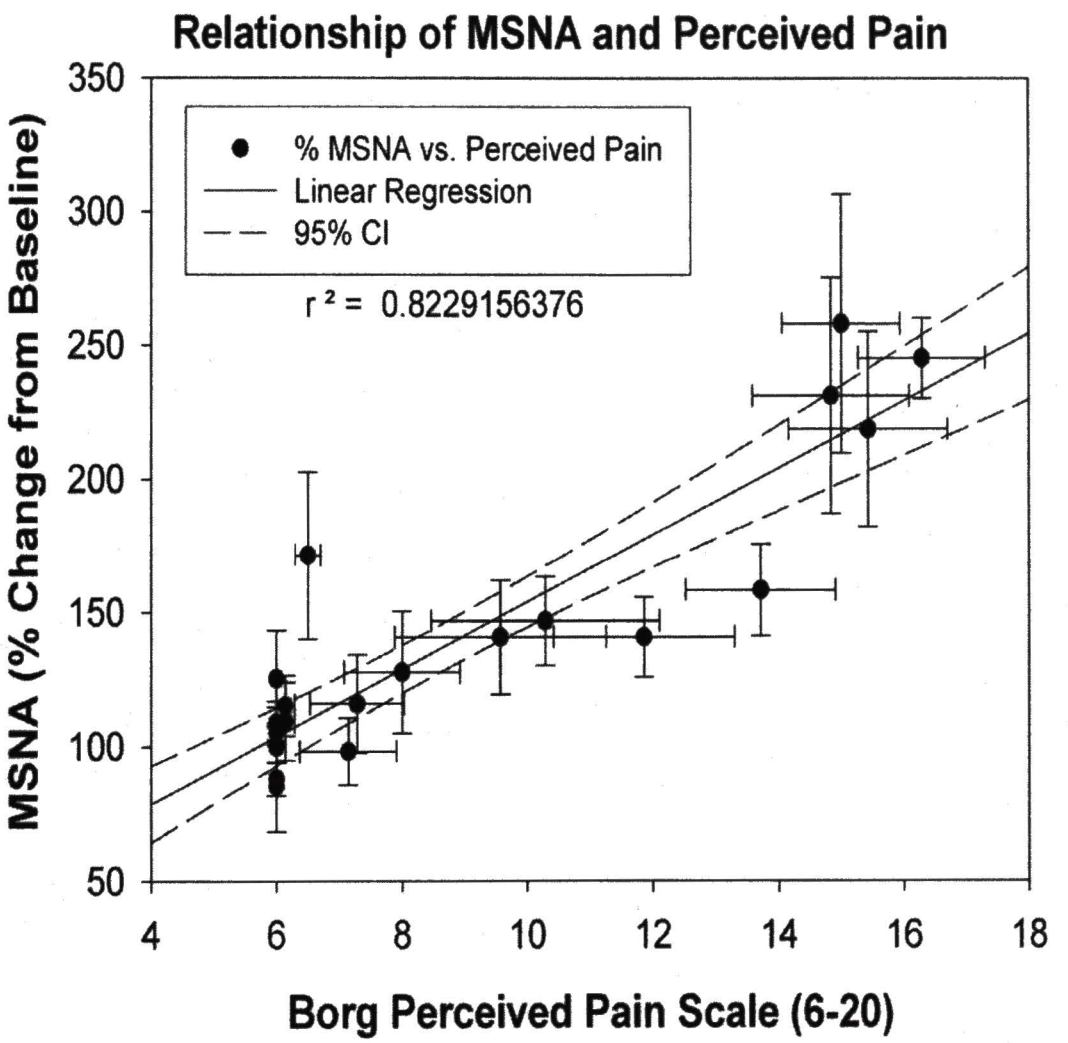

Relationship of MSNA and Perceived Pain
% MSNA vs. Perceived Pain
Linear Regression
95% CI
r² = 0.8229156376
MSNA (% Change from Baseline)
350
300
250
200
150
100
50
4
6
8
10
12
14
16
18
Borg Perceived Pain Scale (6-20)

FIGURE 7

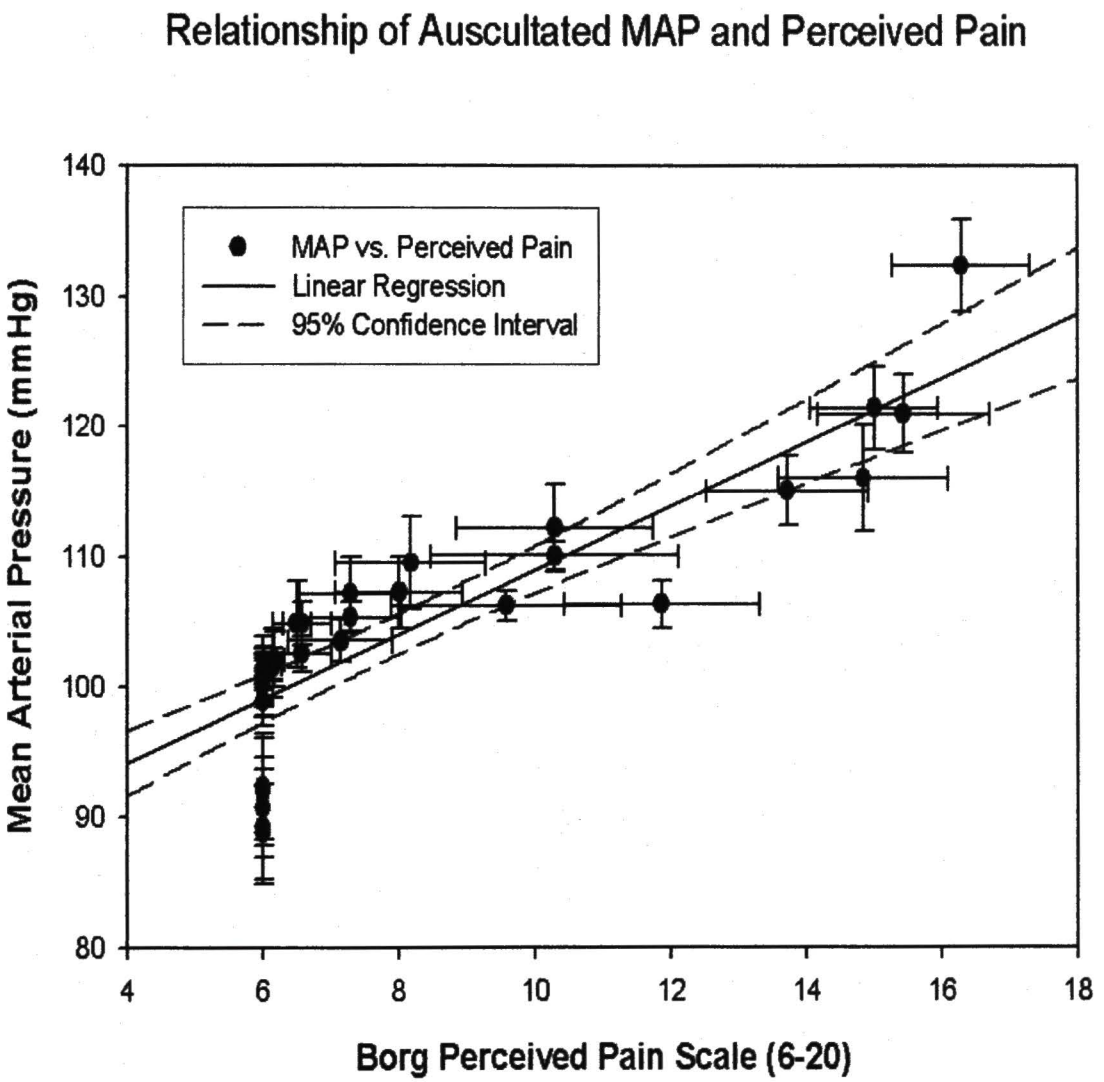
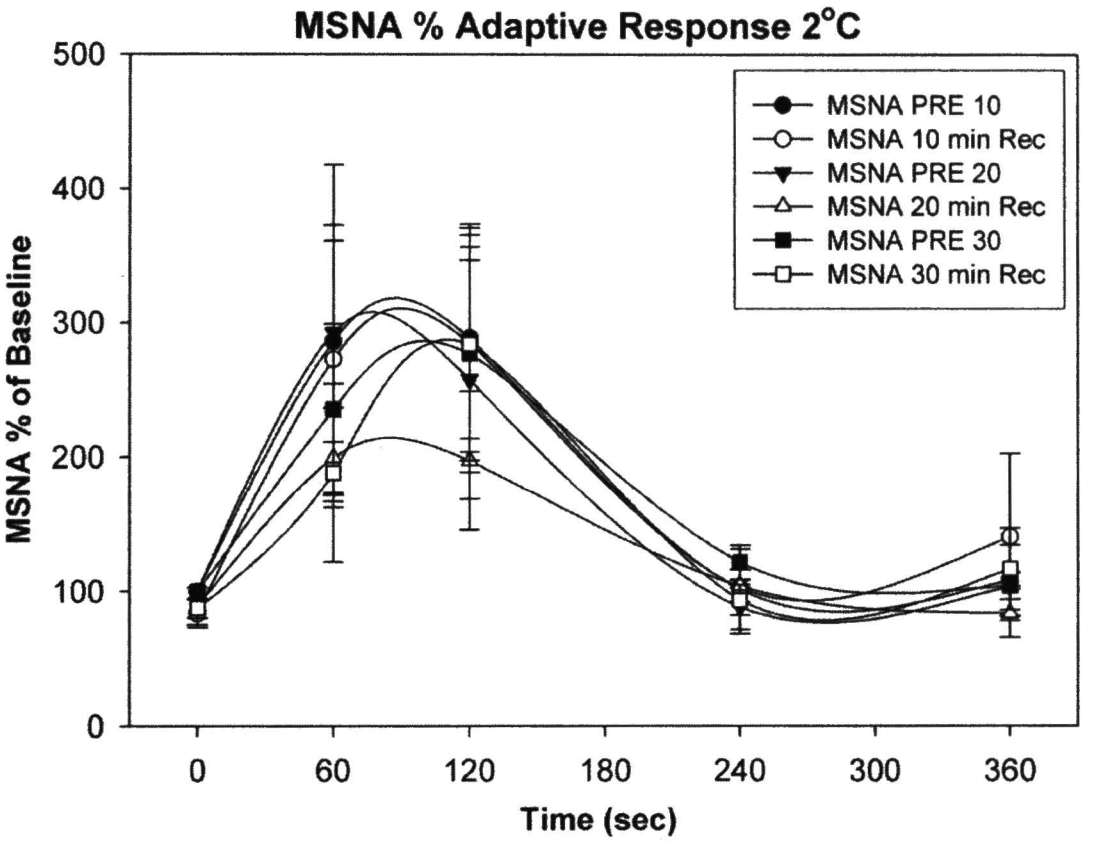

Relationship of Auscultated MAP and Perceived Pain
MAP vs. Perceived Pain
Linear Regression
95% Confidence Interval
Mean Arterial Pressure (mmHg)
140
130
120
110
100
90
80
4
6
8
10
12
14
16
18
Borg Perceived Pain Scale (6-20)

FIGURE 8


MSNA % Adaptive Response 2°C
MSNA % of Baseline
500
400
300
200
100
0
0
60
120
180
240
300
360
Time (sec)
MSNA PRE 10
MSNA 10 min Rec
MSNA PRE 20
MSNA 20 min Rec
MSNA PRE 30
MSNA 30 min Rec

CHAPTER III

BARORECEPTOR MODULATION DURING COLD INDUCED ACUTE PAIN

Raven JS[1], Nguyen J[2], Thakre T[1], Pacchia C[1], Smith M[1]

[1]Department of Integrative Physiology

University of North Texas Health Science Center

3500 Camp Bowie Blvd., Fort Worth, TX. 76107

[2]The University of Texas at Austin

1 University Station, Austin, Texas 78712

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ABSTRACT

Pain leads to increases in muscle sympathetic nerve activity (MSNA) and mean arterial pressure (MAP) suggesting that baroreceptor control of MSNA is altered during pain. This may occur due to resetting and/or shifting the operational point to a new position. The purpose of this investigation was to determine the effect of intermittent cold-pressor induced pain on control of sympathetic nerve activity and baroreceptor resetting in normotensives. Eight subjects were tested for carotid baroreflex (CBR) function using a neck pressure and neck suction technique and MSNA via microneurography during two conditions: control (no intervention) and during a cold pressor test (CPT). Compared to control, the acute pain significantly increased CBR gain (P=0.013). The acute pain stimulus also provoked an upward-rightward resetting and a shift of the operational point toward the lower operating limit of MSNA and to higher operating pressures (p=0.011) of the CBR curve. These findings suggest that during acute pain there is active resetting of the CBR control of MAP, and a modulation of CBR responses leading to a shift of the operational point from the equivalence point (point of highest gain) toward the lower limiting value of the CBR curve. The shift in the operational point may enhance protection against hypotension in settings where pain is associated with a concurrent hypotensive stimulus.

INTRODUCTION

A common experimental method to elicit pain in a human population is the cold pressor test (CPT). The CPT is performed by immersing one's hand into an ice water bath for 2 minutes. This stimulus elicits large elevations in muscle sympathetic nerve activity (MSNA) which in turn leads to frank increases in arterial pressure (13, 22). Victor *et al.* investigated the effects of the CPT on sympathetic outflow and demonstrated a direct relationship between sympathetic nerve discharge, the changes in arterial pressure, and plasma norepinephrine concentration (37). Furthermore, it is important to note that Kregel *et al.* determined sympathetic excitation during hand immersion in cold water occurs only when skin temperature falls to levels that produce a sensation of intense pain (22). Consequently, cold-induced acute pain, like many excitatory stimuli, evokes sympathoexcitation-mediated increases in heart rate (HR), cardiac output (Q), and systemic vascular resistance (SVR), which collectively elevate mean arterial pressure (MAP). (13, 22, 37). Thus, acute pain directly increases arterial pressure in the presence of active baroreceptors. Yet, how the baroreceptors function during acute pain remains relatively unexplored.

A reverse-sigmoid relationship exhists between MSNA and CBR function with MSNA as the response variable. Under resting conditions the operational point located in the region of maximal gain of the sigmoid relationship. This relationship shifts under certain conditions, including exercise and hypertension (15). Under most conditions classical resetting occurs as a rightward shift of the relationship (7, 8). Often resetting is

associated with a shift in the response axis and may sometimes result in a change in position of the operational point within the CBR relationship (8).

Recently Cui et. al., used a pharmacological paradigm to modulate blood pressures and demonstrated a change in the CBR gain in response to acute pain (10). However, our pilot studies suggested that during acute pain, modulation of the CBR function curve gain may also be accompanied by a resetting of the operating point toward the lower limiting MSNA value. This alteration in the operational point would result in higher than expected blood pressures than can be accounted for by a shift in the CBR function curve alone, and may be due to the balance struck between the hypertensive effects of pain, and the hypoalgesic effect of hypertension (9, 14, 18). Therefore, the purpose of this study was to confirm the findings of Cui et al. through the use of a different experimental paradigm and to determine if the change in CBR gain was accompanied by a resetting of the CBR operational point.

METHODS

Subjects: This study was approved by the University of North Texas Health Science Center Institutional Review Board. Eight healthy volunteers (3 females, 5 males, ages 18-32 years) participated in this investigation. All subjects were studied at the same time of day. After providing written, informed consent each subject completed a medical history questionnaire prior to participation in the study. All subjects were non-smokers, reported no personal or familial history of cardiovascular, pulmonary, or neurological disease and were not currently using medications other than oral contraceptives. Female

subjects all tested negative for pregnancy and were not tested during menses to eliminate potential confounding effects on blood volume or cardiovascular function. Subjects were instructed to abstain from alcohol for 24 hours and from caffeine for 12 hours prior to the study.

Cardiovascular Measurements: Heart rate (HR) was measured using a standard 3-limb-lead electrocardiogram (ECG). Arterial beat-to-beat blood pressure (BP), stroke volume (SV), and cardiac output (Q) were measured non-invasively via photoplethysmography at the finger (Finometer, FMS Finapress Medical Systems BV, Amsterdam, the Netherlands) (1, 33). The finometer uses an inverse anti-resonance algorithm to back calculate the aortic pressure wave from the pressure wave measured at the finger. The mathematically reconstructed aortic pressure wave is further modified by a non-linear three-element model of the aortic impedance to produce a final aortic pressure wave. By integrating the calculated aortic waveform per beat, the finometer provides left ventricular SV, when multiplied by HR yields Q. These measures have been previously demonstrated to produce no statistically significant differences from calculation of cardiac output by thermodilution, and to be valid in normotensives and hypertensives (1). MAP was calculated as the mean of the BP values per unit time. Carotid distending pressure (CDP) was calculated as the MAP ± neck pressure or neck suction. This method for estimating CDP has been demonstrated as a reliable measure by Gallagher et al (15). Baroreceptor gain was calculated using the following equation: [$m = (n\sum(xy) - \sum(x)\sum(y))/(n\sum(x^2)- (\sum(x))^2)]$. This method is a common calculation for gain of a first order linear regression, where (m) is the gain, (n) is the number of data points,

(x) is the independent variable and (y) is the dependant variable (11). Finally, the operating point for CBR relationships was determined to be the relationship between MSNA and CDP when no neck suction or pressure was applied during each phase of testing.

Neck Pressure/Neck Suction Stimuli: Neck pressures unload CBR and simulate decreased blood pressure; while neck suctions load CBR and simulate increased blood pressure. A malleable lead collar was placed on the anterior 2/3 of the subject's neck. Neck pressures/suctions were presented to the subjects at +40. +20, 0, -20, -40, -60 mmHg. Neck pressure/suction stimuli consisted of six 10 second cycles of 5 second duration on/off stimuli. During the sham stimulus, the malleable lead collar was placed on the anterior 2/3 of the subject's neck, the equipment used to produce pressures and suctions was then turned on, but no suction or pressure was applied to the subject. The responses to the various hypertensive and hypotensive stimuli were used to determine the gain and operational point of the CBR curve for each subject.

Pain Measurements: The Borg 15-point rating of perceived pain scale was used to rate the subject's perceived pain intensity (2). The Borg pain scale was originally developed as an index of perceived exertion during exercise and has been adapted for perceived pain. It has been used in recent studies to evaluate pain perception during the assessment of cardiovascular performance (38, 39). Using a scale of 6-20, subjects rated their perceived pain just before their hand is placed in the water and every 15 seconds following the immersion of their hand, until the pain rating returned to baseline following the removal of their hand from the water.

Sympathetic Nerve Activity: Postganglionic muscle sympathetic nerve activity (MSNA) was directly measured from the peroneal nerve below the fibular head using standard microneurographic techniques (22). Two sterile tungsten microelectrodes (tip diameter 5-10 μm, 35 mm long, Fredrick Haer and Co., Bowdoinham, ME) were inserted; one, inserted subcutaneously, served as a reference and the other was inserted into the peroneal nerve for measurement of MSNA. Due to their small size, microelectrodes were inserted without the use of local anesthesia to avoid any effect the anesthetic may have on local nerve function. Nerve signals were processed by a preamplifier and an amplifier (nerve traffic analyzer model 662C-3, Department of Bioengineering, University of Iowa, Iowa City, IA) with a total gain of 90,000. Amplified signals were band-pass filtered (700-2,000 Hz), rectified, and discriminated. Finally, a resistance-capacitance circuit with a time constant of 0.1 s integrated raw nerve signals. All signals were sampled at a rate of 1,000 Hz. MSNA recordings were confirmed using the following criteria: 1) pulse-synchronous bursts occurring 1.2-1.4s after the associated QRS complex, 2) reproducible activation during apnea, and 3) no activation following pinch, skin stroking, or startle stimuli (all of which activate skin sympathetic fibers).

Experimental Protocols: All subjects were studied in the supine position. The laboratory was kept at an ambient temperature of 23-24°C. On the day of the experiment, subjects were instrumented for measurement of HR, BP, SV, Q, respiration, and MSNA. All measures were measured continuously at a sample rate of 1,000 Hz. All of the procedures were randomized to reduce possible entrainment of responses. Each subject served as his/her own control. Once the instruments were properly placed, the subjects

were provided a 10-minute quiet period to rest and acclimatize before implementation of the following randomized procedures.

Treatment Protocols: Each subject was exposed to two phases of testing. The stimuli applied during the two phases of testing were randomized to prevent entrainment of responses amongst subjects.

Phase 1 assessed normal baroreceptor function gain for each subject without acute pain. Subjects were instructed to place their right hand in a thermoneutral (25°C) water bath for 2 min. The hand was dried and a 5 min rest period in room air followed to allow a return to baseline HR, BP, SV, Q, and MSNA. The stimulus/rest combination occurred 3 times. Either a hypertensive stimulus at -40 mmHg, a hypotensive stimulus at +40 mmHg, or sham at 0 mmHg, was administered during the second minute of the water bath phase. A complete rest-stimulus cycle totaled 7 minutes. The entire duration of phase 1 was 26 minutes.

Phase 2 assessed baroreceptor gain and operating point during painful stimuli. Subjects were instructed to place their right hand in the CPT ice-water bath (2°C) for 2 min. Hypertensive stimuli (-60 mmHg, -40 mmHg, -20 mmHg), hypotensive stimuli (+40 mmHg, +20 mmHg), or sham (0 mmHg) were then applied during the second minute of the CPT. The hand was dried and a 5 min rest period in room air followed to allow a return to baseline HR, BP, SV, Q, and MSNA. A complete rest-stimulus cycle totaled 7 min. The entire duration of phase 2 was 42 min.

Data Analysis:CDP measurements during control and stimulus time points reflect average blood pressure values ± the neck pressure/suction applied obtained over a 1 minute measurement period. MSNA for all time points are reported as the difference of total activity normalized per 100 heart beats relative to the minute prior to the stimulus. This method for MSNA analysis is performed because normally MSNA total activity cannot be compared between individuals unless a normalization procedure is first applied (37). The 5 s intervals between stimuli were not included in the total activity for a given hypertensive or hypostensive stimuli obtained by neck suction and pressure.

Statistical Analysis: All statistical analyses were performed at a significance level (α) of 0.05. Operational point differences between treatments were analyzed using a paired T-test. MSNA responses to changes in CBR stimulation during the CPT were analyzed using an one-way repeated measures ANOVA. When violations to normality were detected the Kruskal-Wallis one way analysis of variance on ranks was used. Both analyses were then subjected to the Tukey pair-wise multiple comparison of procedures to ascertain significant differences between stimuli. All data are expressed as means ± SEM.

RESULTS

Modulation of CBR gain: Figure 1 depicts representative tracings comparing MSNA during four conditions. These data demonstrate the increased MSNA activity during a CPT, and the entrainment of that activity to CBR activation. The gain response for the CBR to MSNA is a negative relationship. As blood pressure increases, the

response of the baroreflex is to reduce sympathetic outflow to the heart and peripheral vasculature, reducing Q and SVR. The relationship described by Ohm's law demonstrates how the baroreflex operates to reduce MAP. During control conditions the average CBR gain was -4.6 ± 2.1 MSNA units/mmHg. Following the application of the CPT the CBR gain was increased to a significantly more negative value of -18.7 ± 3.6 MSNA units/mmHg (Figure 2) (P=0.013).

Operational point shift: Under normal conditions the operational point of the CBR response may be found at the point of highest gain of the relationship. This point, located centrally on the linear portion of the sigmoid relationship is referred to as the equivalence point. Under experimental-control conditions the operating point for the CBR curve wass (MAP) 94.7 ± 6.6 mmHg, (MSNA) 108.0 ± 176.5 MSNA units (Figure 3). Two changes occurred following the application of the CPT. First was a rightward-upward shift in the response (Figure 3). Second and importantly was the movement of the operational point from the point of highest gain toward the lower limiting value of the CBR curve (Figures 3 and 4). We were able to demonstrate a significant difference between control conditions and CPT conditions for the pressures where the operational points reside (p=0.011). However, there was no significant difference between the MSNA values at the operational points (p =0.378). During cold-induced pain the response to hypertensive stimuli is blunted compared to the response to hypotensive stimuli (Figure 4). Importantly, there was no significant difference in the MSNA values identified for the operational point compared to those for the MSNA values during the 20 mmHg hypertensive stimulus (P=0.082). Furthermore, the response ratio between hypotensive

and hypertensive stimuli of 40 mmHg, during the CPT was significantly greater than that of control conditions ($p = 0.041$) (Figure 5). These data confirm that the location of the operational point has shifted toward the lower limiting value of the CBR curve's reverse sigmoid relationship.

DISCUSSION

The primary novel finding of this study was that under conditions of intense acute pain during which a significant increase in arterial pressure occurs, the operational point of the CBR response is shifted toward the lower limiting value of the reverse sigmoid relationship between blood pressure and MSNA. Moreover, these data demonstrate that the capacity for hypotensive buffering is enhanced, and a higher prevailing arterial pressure is achieved during a painful experience despite enhanced baroreflex gain.

Carotid Baroreceptor Resetting: The CBR function curve is a reverse-sigmoid stimulus-response curve. Changes in blood pressure are detected by the CBR which in turn signal areas of the brain, like the nucleus tractus solatarii (NTS). The NTS acts as an integrating station which modulates MSNA activity to counteract deviations in blood pressure away from the operational point (3, 19, 32). The carotid baroreflex operational point is the position on the CBR function curve of sympathetic tone relative to a carotid distending pressure at rest. Normally this point is situated centrally within the reverse-sigmoid relationship between MSNA and carotid distending pressure, at the median and point of highest gain (25, 26). Baroreceptor resetting of MSNA control consists of altered MSNA relative to specific amount of arterial wall stress. Resetting

frequently shifts the relationship to the right, resulting in elevated mean arterial pressures (MAP) and can occur acutely and/or chronically (8).

Under most physiological conditions, peripheral resetting is a shift in the blood pressure-baroreceptor function curve in the direction of the prevailing level of arterial pressure, such that following elevations in pressure, the baroreceptor activity is reduced at equivalent pressures (7). In turn, there is a parallel shift in the efferent autonomic activity and the associated end organ response. Peripheral resetting occurs during the diastolic phase of a cardiac cycle or after brief exposure to elevated pressures, such as those found in exercise. Acute bouts of peripheral resetting have been shown to be prevented or reduced when the elevations in pressure are pulsatile rather than static (7). However, Smith et al. have shown that vagal baroreflex control of the heart rate resets and shifts rightward following several minutes of exposure to pulsed increases in arterial pressure (34).

During chronic hypertension or when chronic structural changes in the vasculature have occurred, a chronic resetting of the baroreflex is maintained, resulting in the continued elevation in MAP. The result of central baroreflex resetting is increased or decreased afferent baroreceptor nerve activity relative to efferent sympathetic nerve activity. Central resetting can involve neural-humoral interactions and/or altered responsiveness of central structures such as the NTS. Static pressures result in the continuous discharge of the baroreceptors which is followed by a central resetting response. The stereotypical central resetting response is illustrated by elevations in MSNA in the face of increased blood pressure (7).

Pain, exercise, and hypertension have been shown to mediate a resetting of the baroreflex, and thus alter the ability of an individual to compensate for hypotensive and hypertensive stimuli during these stressors (6, 16, 25, 26). Acute and/or chronic hypertension have been clearly shown to produce hypoalgesia (9, 18), a condition of decreased pain sensitivity and increased pain threshold. Yet, hypoalgesia precedes the clinical manifestation of hypertension in individuals with a family history of hypertension (14). While seemingly contradictory, these disparate data support the concept that neural components which mediate baroreceptor sensitivity and pain are integrated within the central nervous system. Recent evidence suggests nociceptive stimulation selectively attenuates the parasympathetic but not the sympathetic limb of the baroreflex (29).

Cui et. al., using a pharmacological paradigm to modulate blood pressures, demonstrated a change in the CBR gain in response to acute pain (10). Through the use of intravenous bolus infusions of sodium nitroprusside and phenylephrine HCl, they increased and decreased blood pressure, respectively. We felt it was necessary to confirm these findings as systemic infusion of an adrenergic agonist may activate alpha(2)-adrenoceptors in the dorsal horn, which are responsible for analgesic gating of pain signals to the brain (27, 30, 40).

Experimental Relevance: Experimental studies, especially those examining the effects of various stimuli on the regulation of blood pressure, may utilize techniques such as arterial or venous catheterization, localized ischemia, thermal effects, and prolonged or intense exercise. Any of these techniques may readily elicit pain stimuli which, if not accounted for, may produce aberrant data and confound analysis. For these

reasons, it is important to take into account the findings of this and other studies which focus on the relationship between pain stimuli and its control over blood pressure regulation. Of particular importance is the finding from this study that during a painful stimulus the CBR operational point resides in a position which favors elevated blood pressure, decreased hypertensive buffering capacity, and increased hypotensive buffering capacity.

Clinical Relevance: Many studies performed have enhanced our understanding of how the baroreceptors regulate blood pressure (12, 20, 21). These investigations were important for describing how the central nervous system integrates various forms of afferent information and produces efferent signals to the heart and vasculature (28). Of particular relevance to this study, Boscan and Paton presented evidence which describe the anatomical structures and central mechanisms used by the NTS to elevate MSNA in response to pain (3-5). While research of cardiovascular control is thorough and expansive, the relationship produced between baroreceptor function and physiological stressors which occur as a component to survival remain a field relatively unexplored. The neural pathways responsible for the shift in the CBR operational point may be the product of an evolutionary advantage. By shifting the operational point toward the lower limiting value of the CBR curve one may support pressures sufficient to maintain cerebral perfusion in the face of substantial blood loss, thus possibly improving chances for survival.

The importance of understanding the functional relationships between CBR function and pain in the clinical setting are highlighted by the recent report published by

the American Heart Association which determined that 65,000,000 of the 71,300,000 adult Americans with at least one form of heart disease have been diagnosed with essential hypertension (36). Future studies in hypertensive populations to investigate pain and its effect on blood pressure regulation is important for the treatment of individuals suffering from trauma, which may be sustained in automotive accidents or military action. The varied and potent responses to pain and changes in blood volume to which hypertensive patients are exposed result in profound pathophysiological modulation of blood pressure control. The shift in operational point described by this paper is functionally important as elevates blood pressure more than would be obtained through a rightward shift in the CBR curve alone. Due to the shift of the operational point, a greater area on the curve of high gain is available to buffer against episodes of hypotension; secondary to blood loss during a painful event. Thus, understanding the mechanisms which contribute to blood pressure modulation within seriously injured normotensive and hypertensive patients is essential. The value of understanding these mechanisms are increasingly important considering hypertensive patients may exhibit a nearly 50% increased risk of mortality following physical trauma (35, 36).

Limitations: Given the complexity of this study we believe it is important to address some potential limitations. Gender differences in pain reporting are well documented in the pain literature (38, 39). For a given stimulus and intensity males report lower pain ratings compared to females (23, 38). Furthermore, racial, socio-economic, and inter-ethnic differences play an important role in pain reporting (17, 24, 38, 39). We attempted to address these issues by utilizing a 2 °C CPT which is a near maximal pain

stimulus for all individuals, by exposing all subjects to the CPT for the same amount of time, by maintaining consistency of the environmental surroundings during experimentation, and by normalizing MSNA responses to baseline and again to heart rate.

Conclusion: These data demonstrate that acute pain shifts the CBR operational point toward the lower limiting value of MSNA. Furthermore, these data confirm the upward-rightward shift and increase in gain of the CBR function curve during painful stimuli. Together these findings highlight the interaction between pain and blood pressure regulatory systems within the central nervous system, and how they operate to buffer against episodes of hypotension during pain. Through its modulation of CBR activity, pain may be viewed as an important survival mechanism for normotensive individuals to cope with hypotensive stimuli during traumatic injury.

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FIGURE LEGENDS

FIGURE 1: Representative tracings comparing MSNA during four conditions. **A**: representative baseline MSNA. **B**: cold pressor test CPT with no neck pressure/suction (operational point); **C**: CPT with +40 mmHg neck pressure (hypotensive stimulus); and **D**: CPT with -40 mmHg (hypertensive stimulus).

FIGURE 2: Plot of mean and individual values of carotid baroreceptor gain (CBR) during control and cold pressor test (CPT) conditions. * $p < 0.05$ Control vs. CPT.

FIGURE 3: Plot of mean values of carotid distending pressure (CDP) versus the change in MSNA from in resting state and during 5 neck pressure/suction stimuli for control and cold pressure test (CPT) conditions. Error bars indicate SEM.

FIGURE 4: Bar graph demonstrating mean change in MSNA from baseline during a cold pressor test (CPT) for no neck pressure and for 5 neck pressure/suction stimuli. Error bars indicate SEM. *$p<0.05$ operating point versus neck pressure/suction.

FIGURE 5: Bar graph of ratio between hypotensive and hypertensive (40 mmHg) MSNA responses between control and cold pressor test (CPT) conditions. * $p < 0.05$ control vs. CPT response ratio.

TABLE 1

Borg Rating of Perceived Pain Scale		
6. No pain	11. Fairly light	16.
7. Very, very light	12.	17. Very intense
8.	13. Somewhat intense	18.
9. Very light	14.	19. Very, very intense
10.	15. Intense	20. Worst pain imaginable

FIGURE 1

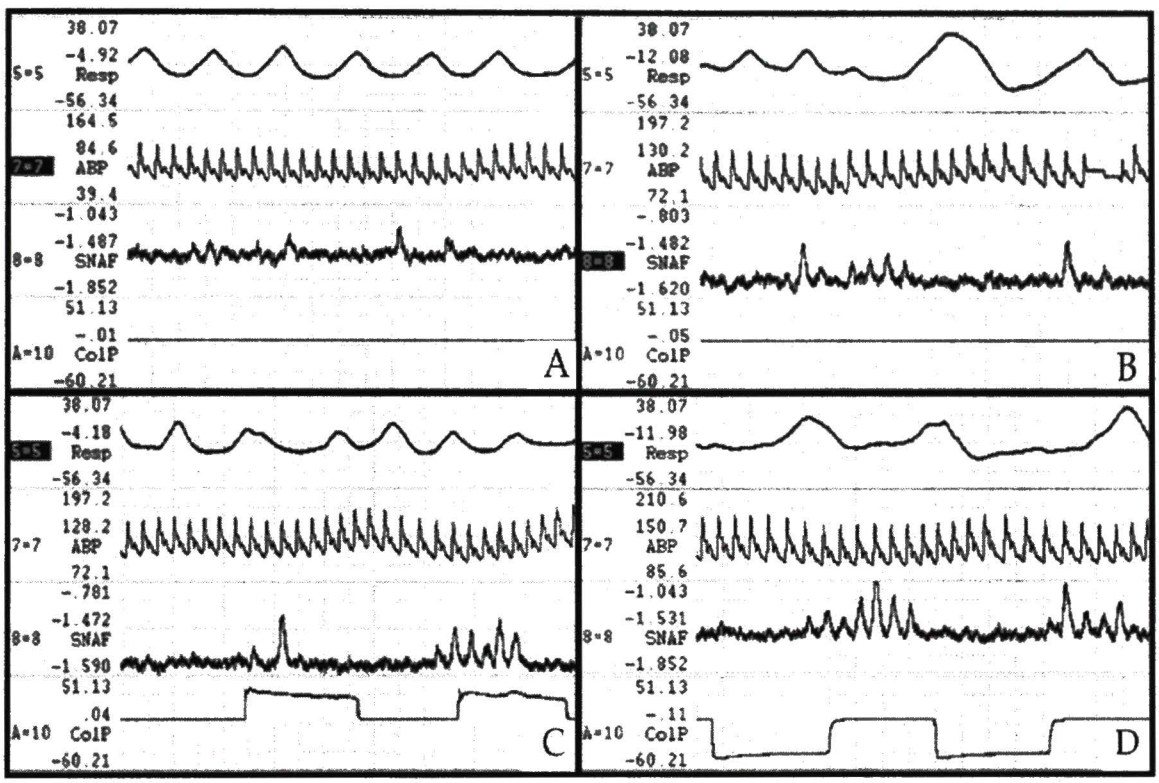

38.07
-4.92
5*5 Resp
-56.34
164.5
84.6
7*7 ABP
39.4
-1.043
-1.487
8*8 SNAF
-1.852
51.13
-.01
A*10 ColP
-60.21
A
38.07
-12.08
5*5 Resp
-56.34
197.2
130.2
7*7 ABP
72.1
-.803
-1.482
8*8 SNAF
-1.620
51.13
-.05
A*10 ColP
-60.21
B
38.07
-4.18
5*5 Resp
-56.34
197.2
128.2
7*7 ABP
72.1
-.781
-1.472
8*8 SNAF
-1.590
51.13
.04
A*10 ColP
-60.21
C
38.07
-11.98
5*5 Resp
-56.34
210.6
150.7
7*7 ABP
85.6
-1.043
-1.531
8*8 SNAF
-1.852
51.13
-.11
A*10 ColP
-60.21
D

FIGURE 2

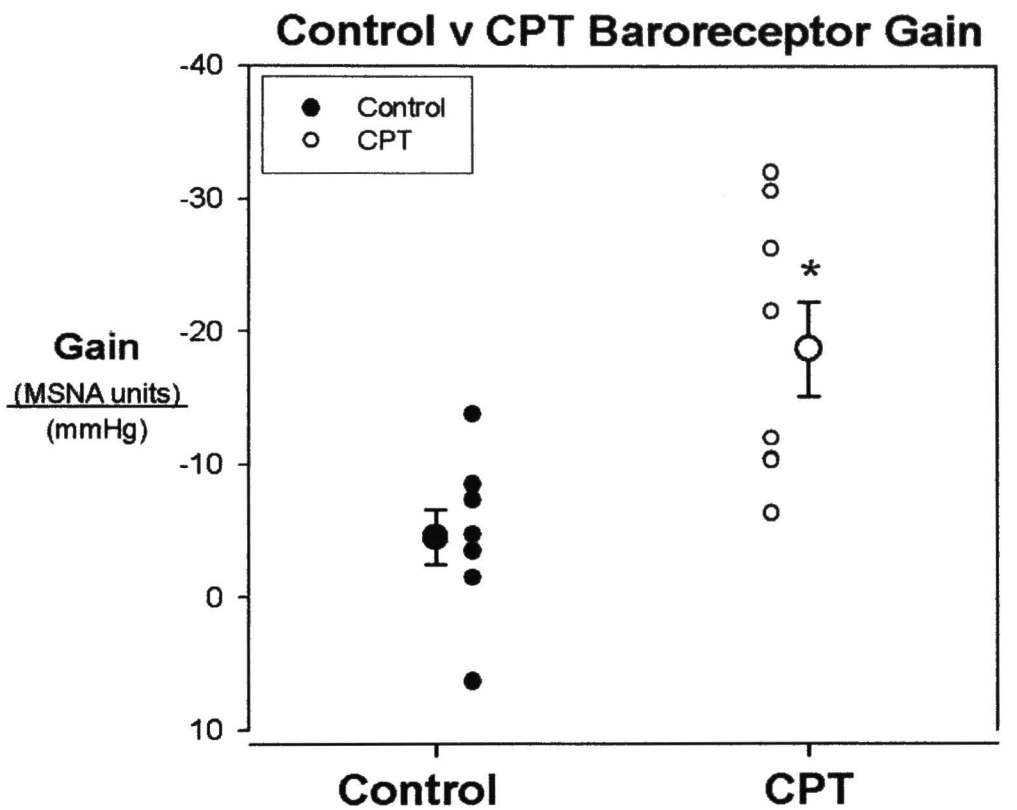

Control v CPT Baroreceptor Gain
Control
CPT
-40
-30
-20
-10
0
10
Gain
(MSNA units)
(mmHg)
*
Control
CPT

FIGURE 3

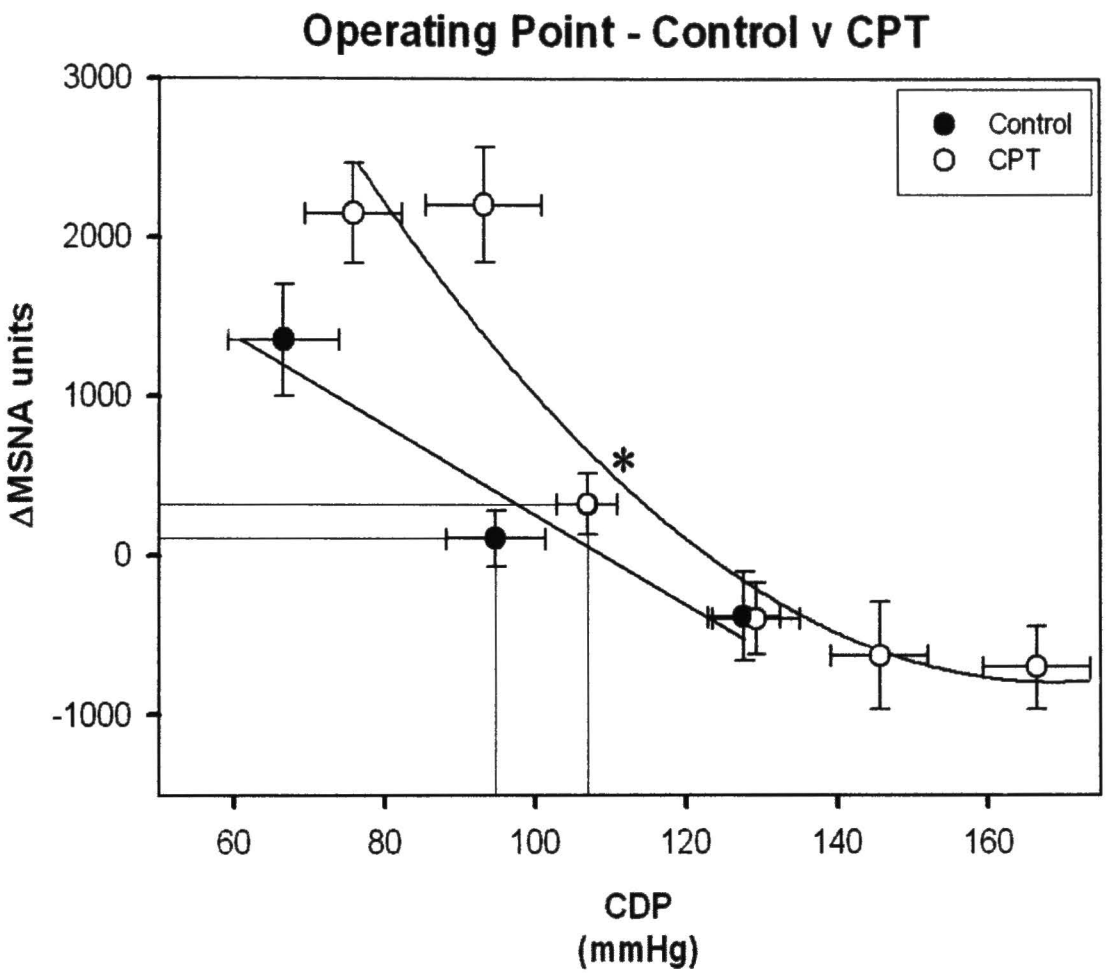

Operating Point - Control v CPT
Control
CPT
ΔMSNA units
3000
2000
1000
0
-1000
60
80
100
120
140
160
CDP
(mmHg)
*

FIGURE 4

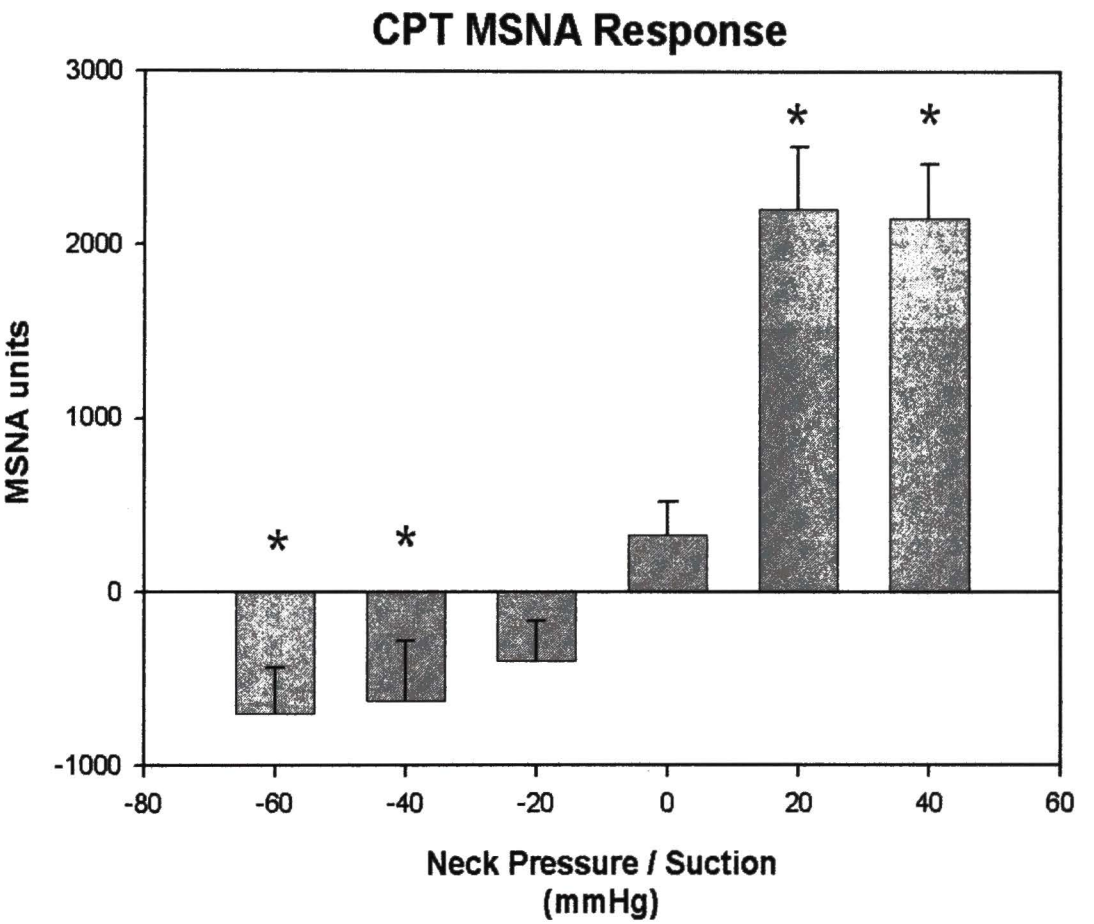
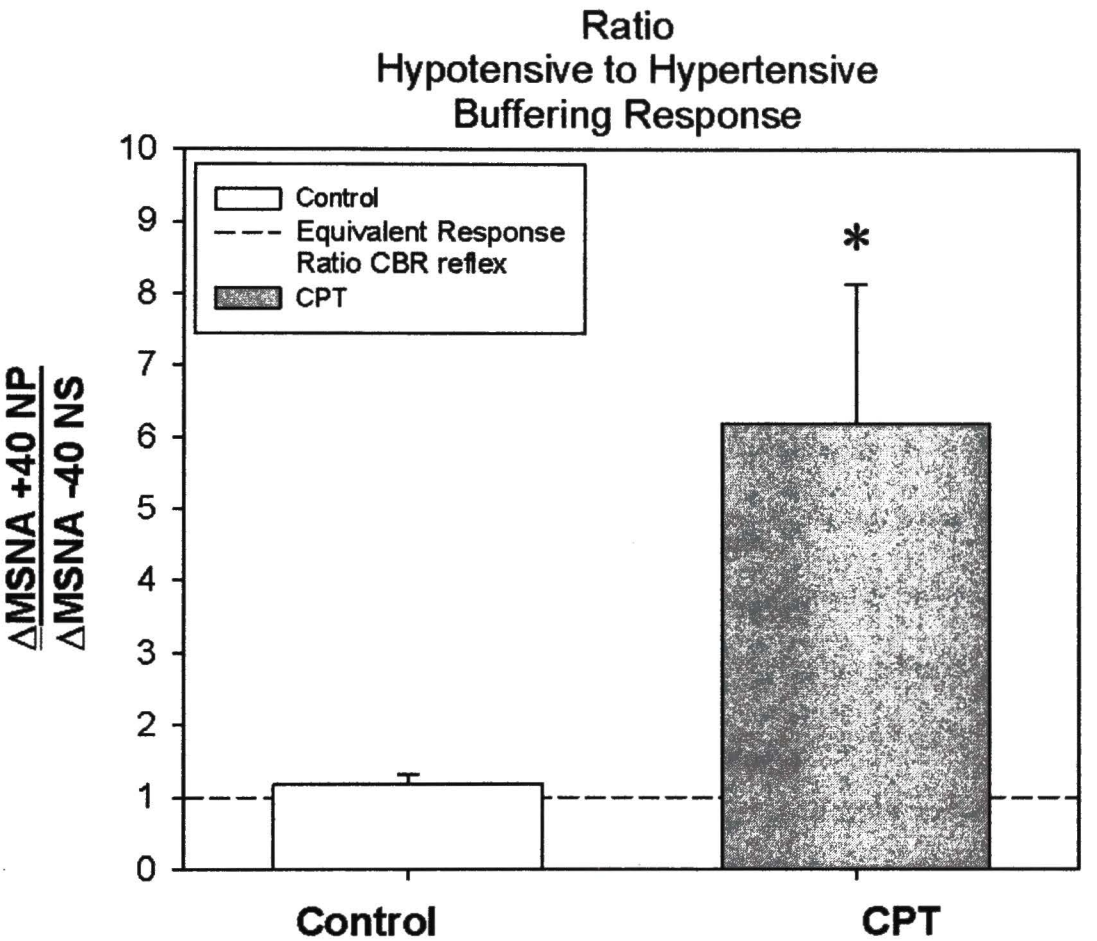

CPT MSNA Response
3000
2000
1000
0
-1000
MSNA units
*
*
*
*
-80
-60
-40
-20
0
20
40
60
Neck Pressure / Suction
(mmHg)

FIGURE 5


Ratio
Hypotensive to Hypertensive
Buffering Response
ΔMSNA +40 NP / ΔMSNA -40 NS
10
9
8
7
6
5
4
3
2
1
0
Control
Equivalent Response
Ratio CBR reflex
CPT
*
Control
CPT

CHAPTER IV

COLD INDUCED PAIN DURING CARDIOPULMONARY BARORECEPTOR UNLOADING IMPROVES MAINTENANCE OF BLOOD PRESSURE

Raven JS[1], Nguyen J[2], Thakre T[1], Pacchia C[1], Smith M[1]

[1]Department of Integrative Physiology

University of North Texas Health Science Center

3500 Camp Bowie Blvd., Fort Worth, TX. 76107

[2]The University of Texas at Austin

1 University Station, Austin, Texas 78712

(Submitted to Experimental Physiology)

ABSTRACT

The purpose of this investigation was to determine the effect of intermittent cold-pressor-induced pain on sympathetic nerve activity and baroreceptor control in normotensive subjects during exposure to central hypovolemic stress. The carotid baroreflex control of muscle sympathetic nerve activity (MSNA) was assessed in 8 healthy subjects using graded neck pressure and neck suction stimuli during the following four conditions: control (no stimuli), cold pressor test (CPT), lower body negative pressure (LBNP), and during simultaneous LBNP and CPT. Compared to CPT alone, LBNP plus CPT induced significant increases in MSNA for a given blood pressure (P=0.015). Furthermore, LBNP plus CPT led to the maintenance of higher blood pressures without significantly increasing MSNA compared to LBNP alone (P=0.014). During simultaneous LBNP and CPT stimuli, the resetting of the carotid baroreceptor (CBR) operational point toward the lower operating limit of MSNA was comparable to the resetting a previous study from our laboratory found to be associated with CPT alone. These findings demonstrate that acute pain induces an elevation in mean arterial pressure (MAP) in the face of decreased venous return. These data also demonstrate that the shift of the operational point from the equivalence point toward the lower limiting value of the CBR curve caused by acute pain remains in spite of cardiopulmonary baroreceptor (CPBR) unloading. Thus, pain leads to a shift in the operational point and to elevations in MAP thereby facilitating the maintenance of blood pressure during central hypovolemic stress.

INTRODUCTION

Physiologically, pain is a neural stimulus which instigates or exacerbates a centrally-mediated stress response. An important part of a pain-mediated stress response is activation of the sympathetic nervous system (*Fagius, Karhuvaara and Sundlof. , 1989, Kregel, Seals and Callister. , 1992, Victor, et al. , 1987*). Evidence suggests that increased nociceptive activity alters the sensitivity of the Nucleus Tractus Solitarii (NTS) to the neural impulses originating from the carotid baroreceptors (CBR) (*Boscan and Paton. , 2001, Boscan, Kasparov and Paton. , 2002, Boscan, Pickering and Paton. , 2002, Bruehl and Chung. , 2004*). Through modulation of NTS sensitivity to CBR afferent input, a resetting of the CBR occurs. Under most conditions, CBR resetting consists of increased systemic MSNA and MAP. Cui *et al.* demonstrated that pain causes an increased gain of the CBR function curve (*Cui, Wilson and Crandall. , 2002*). Furthermore, recent studies from our lab have indicated that pain induces a resetting of the operational point favoring a hypertensive state which accompanies the increase in CBR gain, and thus protects against hypotension.

The purpose of this study was to examine the effect of acute pain stimuli on blood pressure regulation in normotensive individuals during a hypovolemic challenge, by assessing the CBR function curve and operating point. By combining decreased central venous pressure (CVP) and pain, we hope to establish a model of traumatic injury to determine how blood pressure control is altered under conditions of combined pain and hypovolemia. Normally, decreased venous return leads to CPBR unloading precipitating an increase in CBR gain and an upward shift of the CBR function curve (*Chapleau,*

Hajduczok and Abboud. , *1988, Mark and Kerber.* , *1982, Victor and Leimbach.* , *1987*). However, the effect of CPBR unloading during simultaneous cold-induced pain remains unexplored. We hypothesize that the addition of pain to cardiopulmonary baroreceptor (CPBR) unloading will result in elevations in MAP compared to CPBR alone, and facilitate hypovolemic buffering.

Understanding the mechanism of pain and its interaction with blood pressure regulation is critical to the interpretation of blood pressure control studies in which pain or discomfort arises and to the understanding of cardiovascular control in individuals experiencing traumatic injury. In particular, these findings lay the groundwork for future investigation into the responses of hypertensive individuals, who develop hypoalgesia and augmented cardiopulmonary responsiveness to orthostatic stress (*Mark and Kerber.* , *1982*) and who exhibit elevated risk of mortality following physical trauma (*Tamosiunas, et al.* , *2005, Terry, et al.* , *2007*).

METHODS

Subjects: This study was approved by the University of North Texas Health Science Center Institutional Review Board. Eight healthy volunteers (3 women, 5 men, 18-32 years) participated in this study. Subjects were studied during the same time of day. Following written, informed consent each subject completed a medical history questionnaire, before enrollment into the study. All subjects were non-smokers, reported no personal or familial history of pulmonary, cardiovascular, or neurological disease and had not recently used medications (other than oral contraceptives), alcohol, or caffeinated

products for over twelve hours. Female subjects all tested negative for pregnancy and were not tested during menses to eliminate potential confounding effects on blood volume or cardiovascular function.

Cardiovascular Measurements: Arterial beat-to-beat blood pressure (BP) and stroke volume (SV) were measured non-invasively via photoplethysmography at the finger (Finometer, FMS Finapress Medical Systems BV, Amsterdam, the Netherlands). These measures have been previously demonstrated to produce no statistically significant differences in cardiac output compared with cardiac output as determined by thermodilution, and to be valid in normotensives and hypertensive (*Bogert and van Lieshout. , 2005, Schutte, et al. , 2004*). Heart rate (HR) was measured using a standard 3-limb-lead electrocardiogram (ECG) as well as by finger plethysmography.. MAP was calculated as the mean of the BP values per unit time. Carotid distending pressure (CDP) was calculated as the MAP ± neck pressure or neck suction. The method utilized for estimating CDP has been demonstrated as reliable by Gallagher et al (*Gallagher, et al. , 2006*). Baroreceptor gain was calculated using the following equation:

$[m = (n\sum(xy) - \sum(x)\sum(y))/(n\sum(x^2)- (\sum(x))^2)]$. This method is commonly used for assessing gain of first order linear regressions, where (x) is the independent variable and (y) is the dependant variable, (m) is the gain, and (n) is the number of data points (*De Beer, De Beer and Goeyens. , 2007*). The CBR operating points for the various conditions were determined by the activity of MSNA and CDP when no neck suction or pressure was applied.

Lower Body Negative Pressure: Cental hypovolemic challenge was induced by creating a vacuum within a metal cylinder to decrease the pressure -15 mmHg below barometric pressure. Increased venous pooling of blood occurs in response to the lower body negative pressure and decreases CVP, reducing the preload on the heart. These effects have been shown to provoke CPBR unloading and are well-established (*Kimmerly and Shoemaker. , 2003, Victor and Leimbach. , 1987*).

Neck Pressure/Neck Suction Stimuli: Neck pressures decrease transmural pressure across the wall of the carotid sinus, unload CBR, and simulate reduced blood pressure while neck suctions increase trnasmural pressures, load CBR and simulate elevations of blood pressure. A malleable lead collar which covers the anterior 2/3 of a subject's neck was applied 45 sec before each stimulus. Neck pressure/suction stimuli consisted of six 10 second cycles of 5 second duration on/off stimuli at +40. +20, 0, -20, -40, -60 mmHg. During sham stimuli, the malleable lead collar was applied, the equipment was turned on, but no suctions or pressures were applied to the subject's neck. The responses to the hypertensive and hypotensive stimuli determined the CBR gain and operational point for each subject.

Pain Measurements: The Borg 15-point rating of perceived pain scale (Table 1) was used to record the subject's perceived pain (*Borg, 1970*). The Borg scale has recently been adapted for use in studies to evaluate pain perception during the assessment of cardiovascular performance (*Wallbom, et al. , 2002*). Subjects rate their perceived pain on a scale of 6-20. Recording were obtained just before their hand was placed in the water

and every 15 seconds following the immersion of their hand, until the pain rating returned to baseline.

Sympathetic Nerve Activity: Muscle sympathetic nerve activity (MSNA) was directly measured from the peroneal nerve at the fibular head using standard microneurographic techniques (*Kregel, Seals and Callister. , 1992*). Two sterile tungsten microelectrodes (tip diameter 5-10 μm, 35 mm long, Fredrick Haer and Co., Bowdoinham, ME) were inserted into each subject. The first was inserted subcutaneously and served as a reference. The second was inserted into the peroneal nerve to record MSNA. Due to their small size local anesthesics were not used during the insertion process. This was done to avoid any effect the anesthetic may have on local nerve function. Nerve signals were processed by a preamplifier and an amplifier (nerve traffic analyzer model 662C-3, Department of Bioengineering, University of Iowa, Iowa City, IA) with a total gain of 90,000. Amplified signals were band-pass filtered (700-2,000Hz), rectified, and discriminated. All MSNA recordings were sampled at 1,000 Hz. MSNA recordings were confirmed using the following criteria: pulse-synchronous bursts occurring 1.2-1.4 s after the QRS complex, 2) activation during apnea, and 3) no activation following pinch, skin stroking, or startle stimuli (all of which would indicate the activation of skin sympathetic fibers).

Experimental Protocols: All subjects were studied in the supine position at an ambient room temperature of 23-24°C. Subjects were instrumented for measurement of HR, BP, SV, Q, respiration, and MSNA immediately before the experiment. Before subjects were instrumented they were instructed to use the restroom (to reduce elevation

of MSNA caused by viscerovascular reflexes) (*Medda et al. , 1995*). Measures were continuously recorded at a sample rate of 1,000 Hz. To reduce entrainment of responses to the stimuli all procedures were randomized. Each subject served as his/her own control. Each subject underwent a 10-minute quiet period to rest and acclimatize before initiating the following procedures. All phases and stimuli were randomized.

Treatment Protocols: Each subject was exposed to four phases of testing.

Phase 1 was designed to assess normal CBR function. Subjects placed their right hand in a thermoneutral (25°C) water bath for 2 min. The subjects hand was then dried by a towel and a 5 min rest period in room air followed. The stimulus/rest combination occurred 3 times. A hypertensive (-40 mmHg), a hypotensive (+40 mmHg), or sham (0 mmHg) stimuli was administered during the second minute of the water bath. A complete rest-stimulus cycle totaled 7 minutes. The entire duration of phase 1 was 26 minutes.

Phase 2 was designed to assess CBR gain and operating point during painful stimuli. Subjects placed their right hand in a ice-water bath (2°C) for 2 min. Hypertensive (-60 mmHg, -40 mmHg, -20 mmHg), hypotensive (+40 mmHg, +20 mmHg), or sham (0 mmHg) stimuli were applied during the second minute of the CPT. The subjects hand was then dried by a towel and a 5 min rest period in room air followed to allow for a return of sympathetic and cardiovascular variables to return to baseline. A complete rest-stimulus cycle totaled 7 min. The entire duration of phase 2 was 42 min.

Phase 3 assessed baroreceptor function during CPBR unloading. Subjects received 3 min of LBNP. Following the first minute of LBNP the subjects placed their right hand in thermoneutral (25°C) water bath for 2 min. Finally, during the third minute

of LBNP, a single hypotensive stimulus (40 mmHg, 20 mmHg), or sham (0 mmHg) was applied. A 5-min rest period occurred between each stimulus to allow a return to baseline sympathetic activity before another stimulus was applied. A complete rest-stimulus cycle totaled 8 minutes. The cycle was repeated 3 times. The entire duration of phase 3 was 24 min.

Phase 4 assessed baroreceptor function during CPBR unloading combined with painful stimuli. Subjects received 3 min of LBNP (-15 mmHg). After the first minute of LBNP, the subjects were simultaneously exposed to a sympathoexcitatory stimulus, applied via the CPT. During the third minute of LBNP, a single hypotensive (40 mmHg, 20 mmHg), hypertensive (-60 mmHg, -40 mmHg, -20 mmHg), or sham (0 mmHg) condition was applied. A 5 min rest period occurred between each stimulus to allow a return to baseline sympathetic activity, before another stimulus was applied. Figure 1 illustrates a representative tracing of the conditions of baseline, cold pressor water bath plus LBNP, cold pressor water bath plus, LBNP, plus neck pressure, and cold pressor water bath, plus LBNP, plus neck suction. A complete rest-stimulus cycle totaled 8 min. The cycle was repeated 6 times. The entire duration of phase 4 was 48 min.

Data Analysis: CDP measurements during control and stimulus data points are average MAP values recorded over a 1 minute stimulus period ± neck pressure/suction during that stimulus. MSNA for all data are reported as the difference of total activity normalized per 100 heart beats relative to the minute prior to the stimulus. MSNA total activity cannot be compared between individuals unless a normalization procedure is first implemented (*Victor, et al. , 1987*).

Statistical Analysis: All statistical analyses were performed at a significance level (α) of 0.05. Operational point differences between treatments were analyzed using a paired t-test and repeated-measures one-way ANOVA. MSNA responses to changes in CBR stimulation during the CPT plus LBNP were analyzed using a repeated-measures one-way ANOVA. When violations to normality were detected, a Kruskal-Wallis one way analysis of variance on ranks was used. Repeated-measures one-way analyses of variance were subjected to Tukey pair-wise multiple comparisons of procedures to ascertain significant differences between stimuli. All data are expressed as means ± SEM.

RESULTS

Modulation of CBR gain: The gain response for the CBR is a negative relationship. As blood pressure increases the response of the baroreflex is to reduce sympathetic outflow to the heart and peripheral vasculature, reducing Q and systemic vascular resistance. During control conditions the average CBR gain was -4.6 ± 2.1. Following the application of LBNP the CBR gain was increased to a significantly more negative value of -24.8 ± 3.7 (Figure 2) ($p = 0.001$). The gain of the LBNP plus CPT condition was -31.3 ± 5.8. When the gain for the LBNP condition was compared to the CPT plus LBNP condition no significant differences were found (Figure 2) ($p = 0.391$). Finally, when the gain for the CPT condition (-18.7 ± 3.6) was compared to the CPT plus LBNP condition a significant difference was detected (Figure 2) ($p = 0.002$). The combined effect of CPBR unloading and acute pain results in significant increases in CBR gain. While significance was unable to be attained, the comparison of CPBR

unloading to CPBR unloading plus CPT does follow the trend toward increasing the magnitude of the CBR gain.

Operational point shift: Under normal conditions the operational point for the CBR response may be found at the point of highest gain of the relationship. This central location on the linear portion of the sigmoid relationship is referred to as the equivalence point. The operating point of the CBR curve during experimental-control conditions was (MAP) 94.7 ± 6.6 mmHg, (MSNA) 108.0 ± 176.5 MSNA units (figure 3). Following the application of the LBNP there was an upward shift in the CBR reflex curve (Figure 3) leading to an elevation in MSNA activity ($p = 0.035$). A significant rightward shift in the CBR operational point occurred during LBNP plus CPT stimuli when compared to the LBNP stimulus ($p = 0.014$) (Figure 4). Furthermore, the operating point is shifted toward the lower MSNA operating limit of the CBR reflex during LBNP plus CPT conditions (Figure 5). These data are supported by the response ratios between mean MSNA during 40 mmHg hypotensive and hypertensive stimuli amongst conditions (Figure 6). Using paired t-test (figure 6) produced a statistically significant difference between control and CPT conditions ($p = 0.041$). While not statistically significant from control, LBNP+CPT approaches significance ($p = 0.056$) while under-powered. Thus, the lack of significance should be interpreted with caution. Finally, these data demonstrate a statistically significant difference between the MSNA and not the pressures for the operating points during the CPT stimuli compared to the LBNP plus CPT stimuli ($p = 0.015$) (Figure 7).

DISCUSSION

Collectively, these data demonstrated that the shift in the CBR operational point toward the lower limiting value of MSNA which occurs during periods of acute pain is not removed in the face of CPBR unloading. In addition, these data reveal that activation of CPBR in conjunction with acute pain leads to enhanced sympathetic activation compared to pain alone. Finally, these data demonstrate a rightward shift in the CBR function curve relative to the LBNP stimulus when pain accompanies LBNP; thus supporting higher prevailing pressures despite decreased CVP. By shifting the operating point to enhance hypotensive buffering and shifting the CBR reflex curve to the right to produce higher arterial pressure, pain may enhance survival during injuries which produce decreases in CVP. These findings underscore the importance of pain and its interaction with blood pressure regulatory processes. Specifically, this study elucidates how pain facilitates changes in the CBR reflex to buffer against episodes of hypotension regardless of the presence of hypovolemia.

Sympathetic Nervous System and Pain: Various stressors such as exercise, pain, and/or disease elicit strong sympatho-excitatory effects (*Fagius, Karhuvaara and Sundlof. , 1989, Kregel, Seals and Callister. , 1992, Victor, et al. , 1987*). The sympathetic nervous system increases heart rate, systemic vascular resistance, and increases catecholamine levels utilized to enhance survival mechanisms. We examined the concept that pain directly modulates how the central nervous system interprets input from the baroreceptors and attempted to quantify the synergism created between pain, the sympathetic nervous system and blood pressure regulation. Our research has reinforced

the understanding that pain and blood pressure regulation are tightly coupled (*Cui, Wilson and Crandall. , 2002, Randich and Maixner. , 1984*). Previously unknown, this study helped define the combined effect of pain during hypovolemic stress on baroreceptor resetting of MSNA.

Baroreceptor Resetting by Pain: The carotid baroreflex operational point is the position on the CBR function curve of sympathetic activity relative to a carotid distending pressure at rest. Normally this point is situated near the median of the reverse-sigmoid relationship between MSNA and carotid distending pressure, at the point of highest gain (*Mancia, et al. , 1985, McDowall and Dampney. , 2006*).

Baroreceptor resetting consists of altered MSNA relative to a given of arterial wall stress. Resetting frequently results in elevated MAP and can occur acutely and/or chronically (*Chapleau, Hajduczok and Abboud. , 1988*). While hypertension results from changes in the viscoelastic properties of the arterial wall and/or as the result of paracrine and endocrine function, baroreflex resetting must occur in conjunction with these factors to allow for sustained elevations in MAP (*Chapleau, Hajduczok and Abboud. , 1989*). Pain, exercise, and hypertension have been shown to mediate a resetting of the baroreflex, and thus enhance the ability of an individual to compensate for hypotensive and hypertensive stimuli during these stressors (*Bristow, et al. , 1969, Grassi, et al. , 2006, Mancia, et al. , 1978, McDowall and Dampney. , 2006*).

Two classifications of baroreflex resetting exist, peripheral and central. Coleridge et al. assert that under most physiological conditions, peripheral resetting shifts the baroreceptor function curve in the direction of the prevailing arterial pressure, such that

following elevations in pressure the baroreceptor activity is reduced (*Coleridge, et al. , 1984*). Furthermore, during central baroreflex resetting there is increased or decreased afferent baroreceptor nerve activity relative to efferent sympathetic nerve activity. Central resetting can involve neural-humoral interactions such as impaired neuronal prostacyclin synthesis, and/or altered responsiveness of central structures such as the NTS which mediate the baroreflex. This investigation utilized pain to create a central resetting of the CBR reflex controlling MSNA. A stereotypical central resetting response is illustrated by relative elevations in MSNA in the face of increased blood pressure (*Chapleau, Hajduczok and Abboud. , 1988, Chapleau, Hajduczok and Abboud. , 1989*). This type of central resetting was demonstrated by our data and indicates that pain is a potent modulator of the CBR function curve and it operational point. The modulation of the operational point toward the lower limiting value of the CBR function curve between MSNA and CDP is a novel finding. We believe the movement of the operational point facilitates hypotensive buffering when pain is accompanied by losses in blood volume. Of particular importance is the finding that MAP is significantly elevated by pain in the face of decreased venous return.

Carotid Baroreceptor Buffering Capacity: These data elucidate how pain elevates blood pressure. Specifically, the pressor effect of pain occurs in two distinct ways. First, pre-ganglionic sympathetic efferent fibers were activated (*Guyenet, 2006*). Secondly, increased blood pressure was created by resetting the baroreceptors in conjunction with the shift of the operating point to a region within the MSNA-CDP relationship toward the response variable's lower limiting value. The shift in operational

point may be beneficial for buffering central hypovolemia which may precipitate hypotension during a traumatic injury. It is important to point out that the hypertensive buffering capacity is concurrently reduced under these conditions. For this reason we can not accurately predict what the effect on CBR control may be when acute pain and LBNP are experienced by hypertensive populations who experience hypoalgesia and pre-existing CBR resetting (*Bristow et al. , 1969, Chapleau et al. , 1988, Conde-Guzon et al. , 2003*). We would suggest based on these data that some resetting may occur; but to what degree and direction remains to be seen.

Clinical Significance: By elucidating the normal blood pressure response and developing a safe model for pain physiology this study has laid the groundwork for further investigation into baroreceptor modulation by pain and CPBR unloading. Future studies in hypertensive populations should be attempted, as hypertensive individuals have a profound pathophysiology associated with blood pressure regulation. First, hypertension is associated with hypoalgesia, a condition of decreased pain sensitivity (*Guasti, et al. , 2002, Maixner, et al. , 1982*). Hypoalgesia may then reduce the driving force of the resetting that takes place as a result of acute pain. Furthermore, hypertension can not exist without central CBR resetting to have occurred prior to its onset (*France, 1999*). Thus, the response and resetting to pain and decreases in CVP may be altered or impaired. This is evidenced by studies where hypertensive individuals were found to have a nearly 50% increase in risk of mortality following physical trauma (*Tamosiunas, et al. , 2005, Terry, et al. , 2007*).

Acute and/or chronic hypertension has been clearly shown to produce hypoalgesia (*Conde-Guzon, et al. , 2003, Guasti, et al. , 2002).* Yet, hypoalgesia has also been shown to precede the clinical manifestation of hypertension in individuals with a family history of hypertension (*France, 1999*). While seemingly contradictory, one implication from these disparate data is that areas such as the NTS mediate baroreceptor sensitivity and pain modulation. Pickering et al has previously demonstrated pain selectively attenuates the parasympathetic but not the sympathetic limb of the baroreflex (*Pickering, Boscan and Paton. , 2003*). We believe that not only the resetting observed in this experiment, but also the shift in the operational point is the direct result of central resetting within the NTS. The evidence for the interaction between pain and blood pressure regulation suggests that modulation of the CBR function curve and its operating point operates to enhance the hypotensive buffering capacity of the vascular system from a neural standpoint.

Limitations: Given the complexity of this study we believe it is important to address some potential limitations. Gender differences in pain reporting are well documented in the pain literature. For a given stimulus and intensity males report lower pain ratings compared to females (*Lautenbacher and Rollman. , 1993, Weisse, Foster and Fisher. , 2005*). Furthermore, racial, socio-economic, and inter-ethnic differences play importants role in pain reporting (*Greenwald, 1991, Lipton and Marbach. , 1984, Weisse, Foster and Fisher. , 2005, Woodrow, et al. , 1972*). We attempted to address these issues by utilizing a 2 °C CPT which is a near maximal pain stimulus for all individuals, by exposing subjects to the CPT for the same amount of time, by maintaining consistency of

environmental surroundings during experimentation, and by normalizing MSNA responses to baseline and again to heart rate. The negative finding for the comparison between LBNP and LBNP+CPT gain should be interpreted cautiously as it may be attributed to low statistical power.

We observed a slight rightward shift of the LBNP plus CPT CBR reflex curve relative to the CPT CBR reflex curve, but no significance was found. While a negative finding for the rightward shift should be interpreted with caution, the variability of these data suggests that the rightward shift is not the primary physiological outcome of CPBR activation. The contribution of the CPBR when activated by central hypovolemic stimuli does not appear to strongly enhance the nociceptive and CBR afferent signal integration and ensuing modulation of the autonomic control of arterial pressure.

Conclusions: This investigation attempted to produce a novel model for the investigation of CBR modulation during pain associated with CPBR unloading. We believe this investigation demonstrated that the pain induced shift of the operational point for the CBR reflex operates to maintain elevations in MAP during periods of reduced CVP. Pain, by facilitating hypotensive buffering may enhance survival during periods of traumatic injury. Future studies should be conducted in hypertensive individuals as their baroreceptor sensitivity and nociception are subjected to pathological modulation. For these reasons, it is quite likely that maintenance of blood pressure is impaired and contributes to their increased risk of mortality following physical trauma.

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FIGURE LEGENDS

FIGURE 1: Representative tracings comparing MSNA during four conditions. **A**: representative baseline MSNA. **B**: cold pressor test (CPT+LBNP) with no neck pressure/suction (operational point); **C**: CPT+LBNP with +40 mmHg neck pressure (hypotensive stimulus); and **D**: CPT+LBNP with -40 mmHg (hypertensive stimulus).

FIGURE 2: Plot of mean and individual values of carotid baroreceptor (CBR) gain during control cold pressor test (CPT), lower body negative pressure (LBNP), and LBNP+CPT stimuli. * $p < 0.05$ vs control. † $p < 0.05$ vs. CPT.

FIGURE 3: Plot of mean values of carotid distending pressure (CDP) versus the change in MSNA from baseline in resting state and during 2 neck pressure/suction stimuli for control and lower body negative pressure (LBNP) conditions. 1st order linear regressions indicating CBR gain have been included. Drop lines indicate the dependant and independent values for operational points for each condition. Error bars indicate SEM. * $p < 0.05$ operating point LBNP versus control conditions.

FIGURE 4: Plot of mean values of carotid distending pressure (CDP) versus the change in MSNA from baseline at rest, during 2 neck pressure/suction stimuli for lower body negative pressure (LBNP), and during 5 neck pressure/suction stimuli for lower body negative pressure plus cold pressor test (LBNP+CPT) conditions. 1st and

2nd order linear regressions indicating CBR gain have been included. Drop lines indicate the dependant and independent values for operational points for each condition. Error bars indicate SEM. *p<0.05 operating point LBNP+CPT versus LBNP conditions.

FIGURE 5: Bar graph demonstrating mean change in MSNA during simultaneous lower body negative pressure plus cold pressor test (LBNP+CPT) for no change in neck pressure and for 5 neck pressure/suction stimuli. Error bars indicate SEM. *p<0.05 operating point versus neck pressure/suction.

FIGURE 6: Bar graph of mean MSNA response from operational point during 40 mmHg hypotensive vs. hypertensive stimuli. Error bars indicate SEM. * $p < 0.05$ from control ratio.

FIGURE 7: Plot of mean values of carotid distending pressure (CDP) versus the change in MSNA from baseline during 5 neck pressure/suction stimuli for cold pressor test (CPT) conditions, and during lower body negative pressure (LBNP) plus CPT (LBNP+CPT) conditions. 2nd order linear regressions indicating CBR gain have been included. Drop lines indicate the dependant and independent values for operational points for each condition. Error bars indicate SEM. * $p < 0.05$ operating point LBNP+CPT versus CPT conditions.

TABLE 1

Borg Rating of Perceived Pain Scale		
6. No pain	11. Fairly light	16.
7. Very, very light	12.	17. Very intense
8.	13. Somewhat intense	18.
9. Very light	14.	19. Very, very intense
10.	15. Intense	20. Worst pain imaginable

FIGURE 1

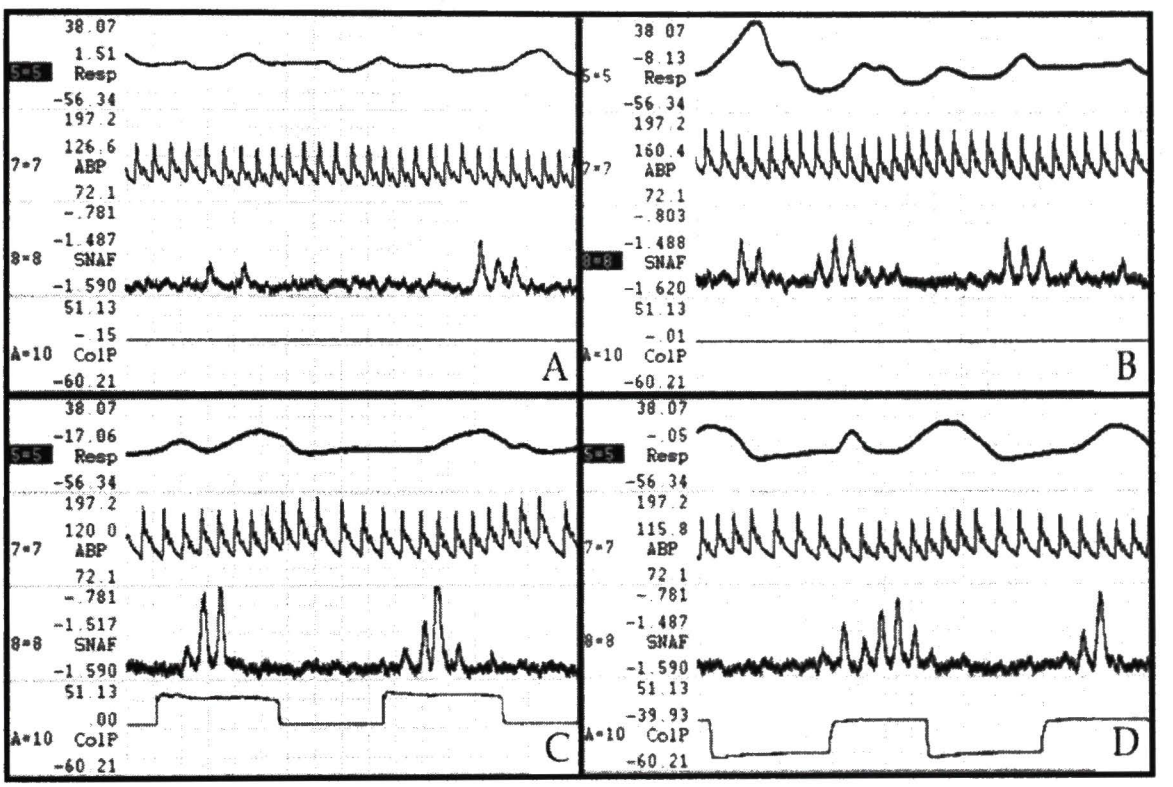

38.07
1.51
S=5 Resp
-56.34
197.2
126.6
7=7 ABP
72.1
-.781
-1.487
8=8 SNAF
-1.590
51.13
-.15
A=10 CoIP
-60.21
A
38.07
-8.13
5=5 Resp
-56.34
197.2
160.4
7=7 ABP
72.1
-.803
-1.488
8=8 SNAF
-1.620
51.13
-.01
A=10 CoIP
-60.21
B
38.07
-17.06
S=5 Resp
-56.34
197.2
120.0
7=7 ABP
72.1
-.781
-1.517
8=8 SNAF
-1.590
51.13
.00
A=10 CoIP
-60.21
C
38.07
-.05
S=5 Resp
-56.34
197.2
115.8
7=7 ABP
72.1
-.781
-1.487
8=8 SNAF
-1.590
51.13
-39.93
A=10 CoIP
-60.21
D

FIGURE 2

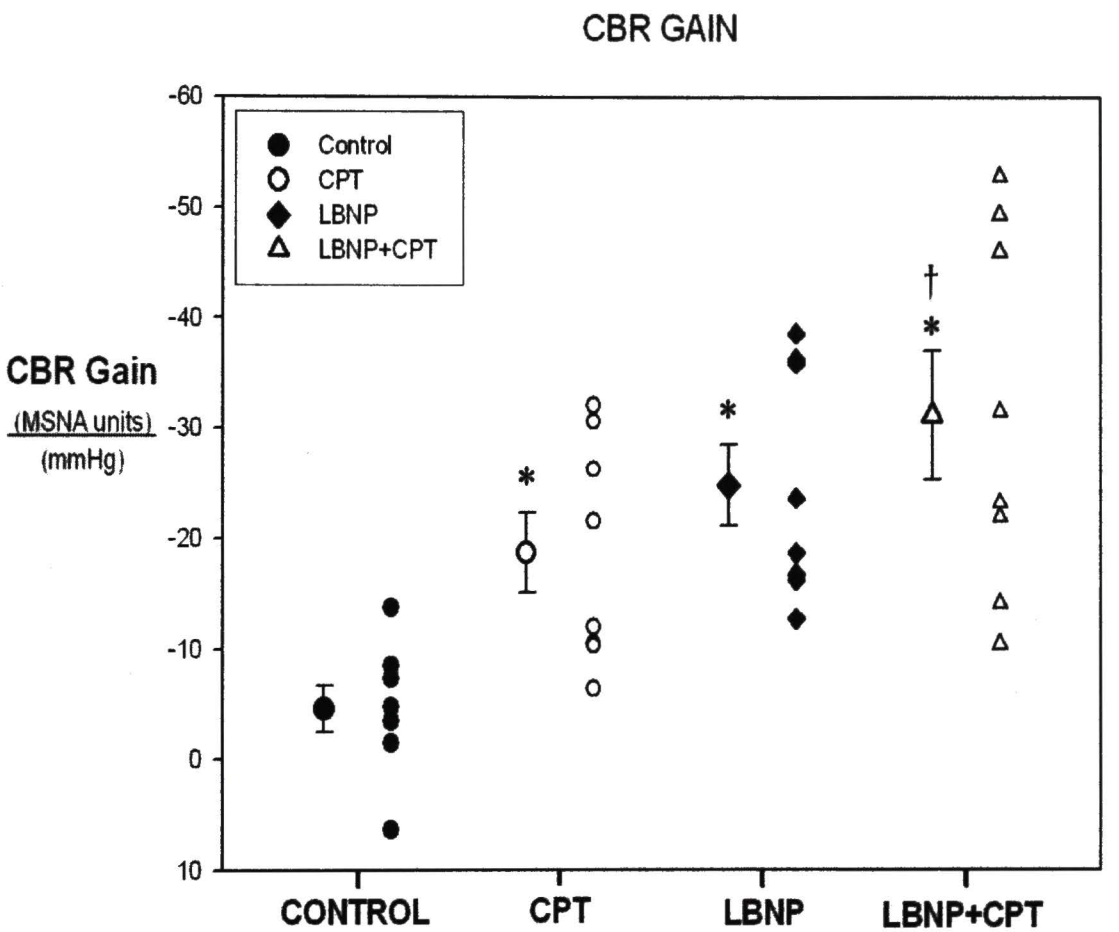

CBR GAIN
CBR Gain
(MSNA units)
(mmHg)
-60
-50
-40
-30
-20
-10
0
10
Control
CPT
LBNP
LBNP+CPT
CONTROL
CPT
LBNP
LBNP+CPT

FIGURE 3

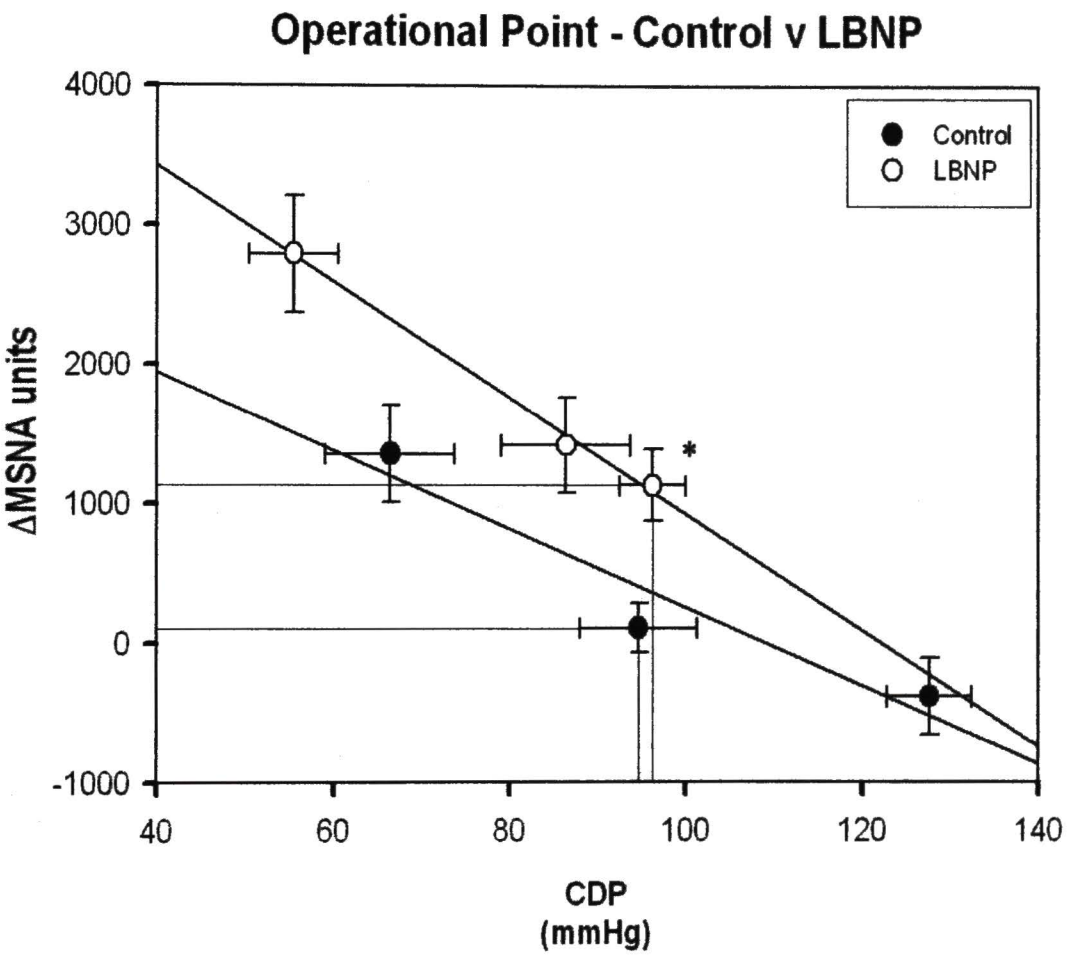

Operational Point - Control v LBNP
Control
LBNP
ΔMSNA units
4000
3000
2000
1000
0
-1000
40
60
80
100
120
140
CDP
(mmHg)
*

FIGURE 4

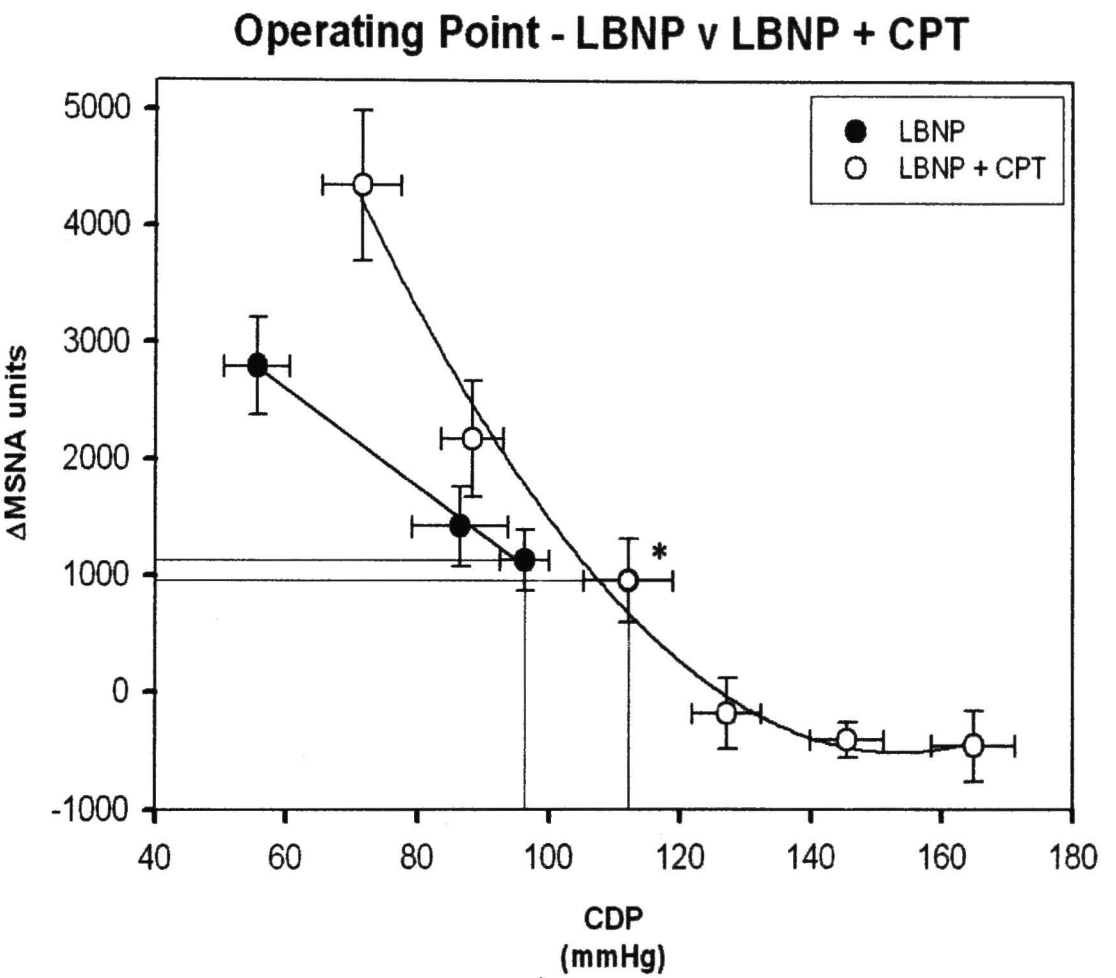

Operating Point - LBNP v LBNP + CPT
LBNP
LBNP + CPT
ΔMSNA units
5000
4000
3000
2000
1000
0
-1000
40
60
80
100
120
140
160
180
CDP
(mmHg)
*

FIGURE 5

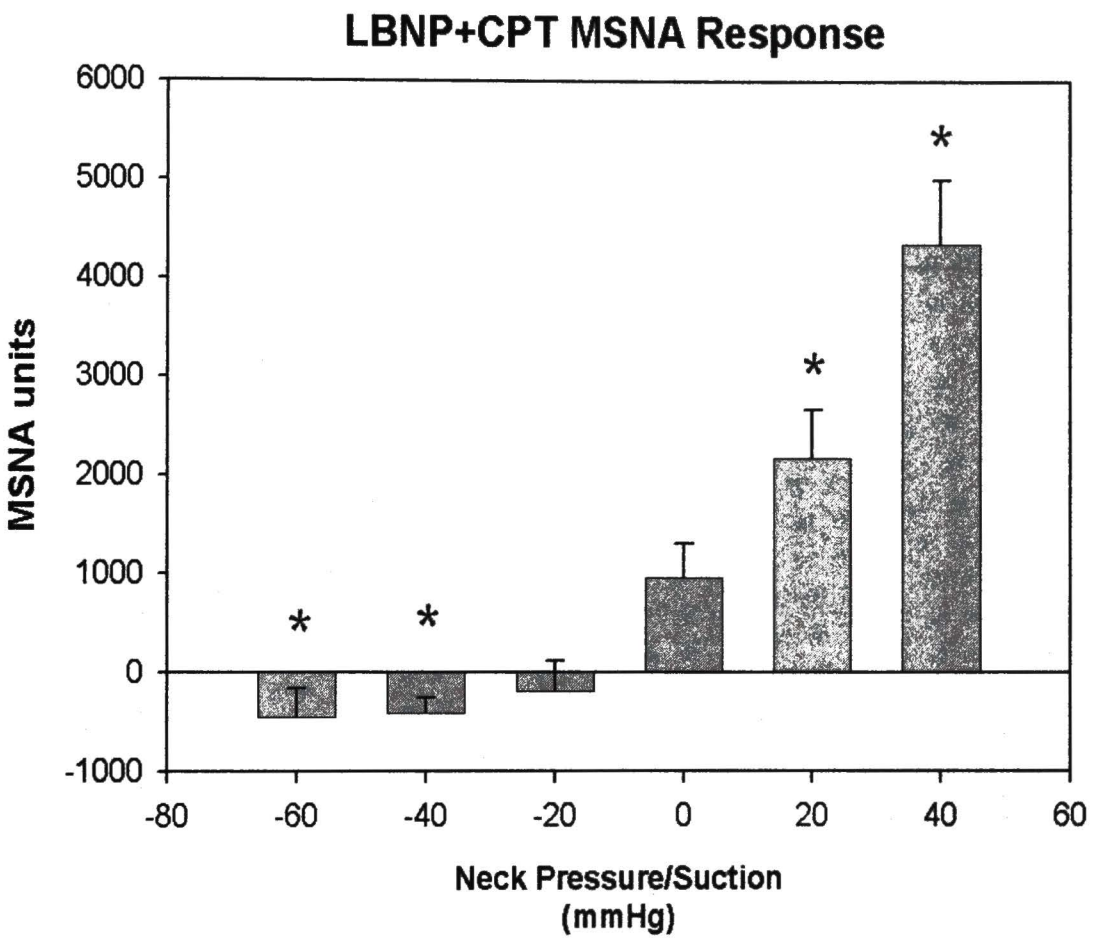

LBNP+CPT MSNA Response
MSNA units
6000
5000
4000
3000
2000
1000
0
-1000
-80
-60
-40
-20
0
20
40
60
*
*
*
*
Neck Pressure/Suction
(mmHg)

FIGURE 6

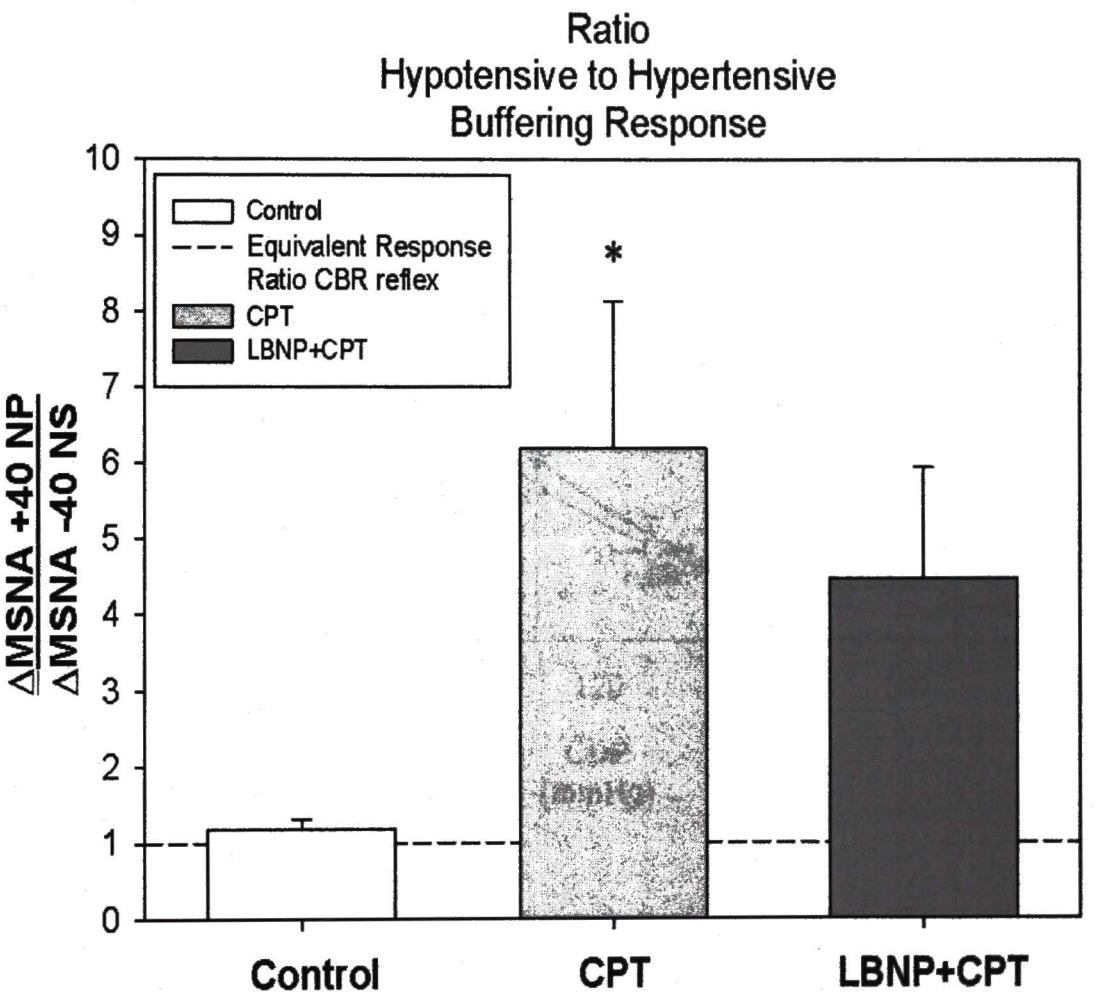
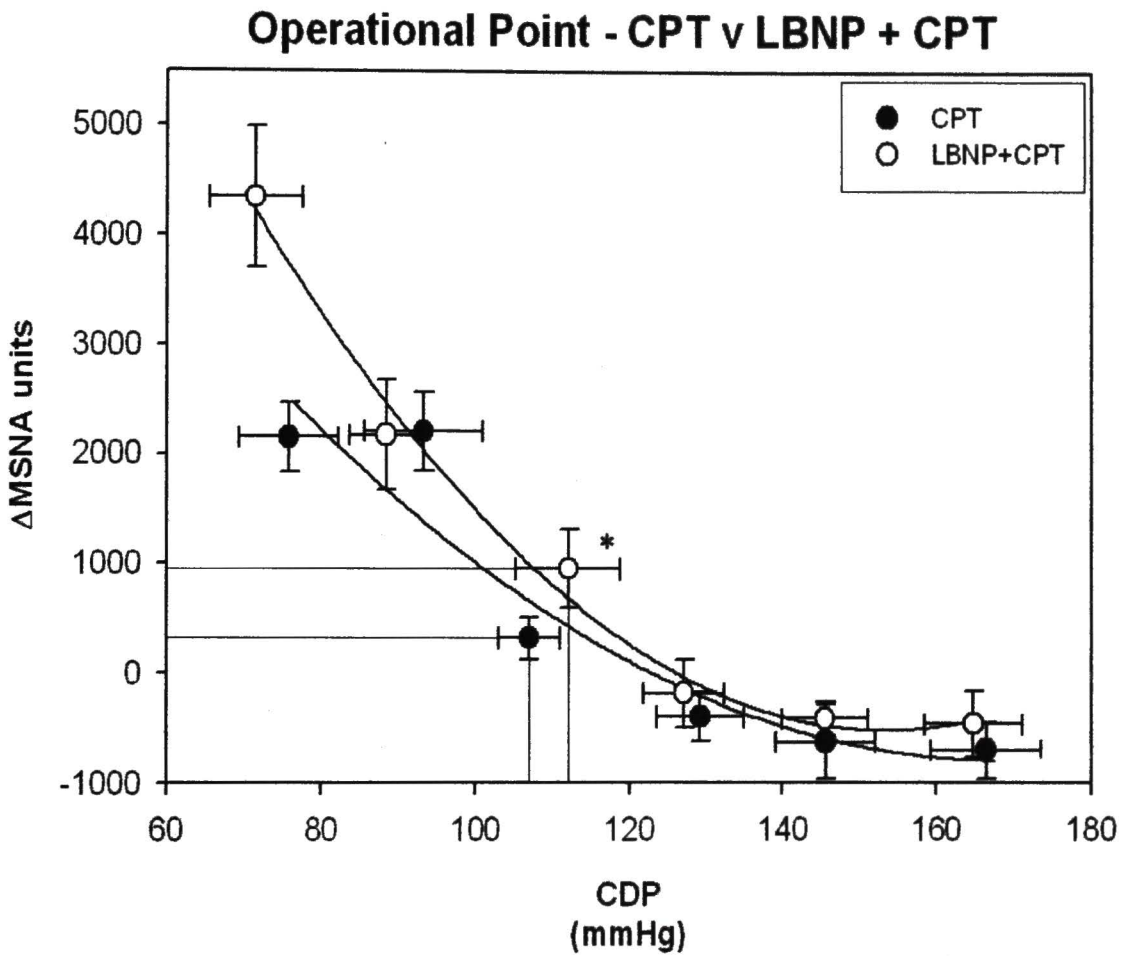

Ratio
Hypotensive to Hypertensive
Buffering Response
Control
Equivalent Response
Ratio CBR reflex
CPT
LBNP+CPT
ΔMSNA +40 NP / ΔMSNA -40 NS
10
9
8
7
6
5
4
3
2
1
0
*
Control
CPT
LBNP+CPT

FIGURE 7


Operational Point - CPT v LBNP + CPT
CPT
LBNP+CPT
ΔMSNA units
5000
4000
3000
2000
1000
0
-1000
60
80
100
120
140
160
180
CDP
(mmHg)
*

CHAPTER V

CONCLUSIONS

The studies described within this dissertation are innovative and constitute the development of an experimental model of trauma through the application of well established methods for assessment of blood pressure and pain, as well as integration of recently developed technologies.

First, to our knowledge the effect of simultaneous LBNP and CPT has never been examined. We anticipate these experiments will lay a foundation for physiological study of trauma in many individuals. One advantage to simultaneous use of these non-invasive procedures is the rapid recovery and reproducibility of the response within subjects.

Second, utilization of finger plethysmography to calculate non-invasively beat-by-beat measures of stroke volume and cardiac output during a pain stimulus is a vast improvement over the pre-existing technologies of acetylene-rebreath and thermodilution techniques. This technology produces little to no discomfort and has been demonstrated to calculate stroke volumes and cardiac outputs significantly close to the measures obtained by thermodilution techniques (Imholtz-1990). Thus, our model is innovative, through both its design and use of technology.

Cold Pressor Testing and Sympathoexcitation

The first investigation demonstrated that perceived pain, MSNA, and blood pressure responses to a given temperature for a CPT are very consistent. Clear graded responses were observed for the physiological variables of MSNA and MAP which directly correlate to the intensity of the pain stimulus.

We were able to determine that a brief rest period of four minutes returns MSNA, perceived pain, and MAP to baseline or near baseline values. While painful/sympathoexcitatory stimuli of greater intensity result in a longer time requirements for a return to baseline values, the recovery phase is similar amongst the various pain intensities. These data demonstrated the reproducibility of the autonomically mediated responses to cold pressor induced pain.

Pain Induces Sympathoexcitation

The first investigation demonstrated the reproducible sympathoexcitation of the CPT is highly correlated to the perceived pain induced by cold pressor stimuli. Thus, individuals experiencing moderate pain are very likely experiencing moderate increases in sympathetic outflow. Physiological responses to pain remain a complicated issue however. To derive specific conclusions regarding an individual's physiological state based simply on pain without considering other factors such as age, race, gender, and cardiovascular health are not recommended. While we did not conduct a meta-analysis for each of the listed factors, the data persuasively demonstrates that within an individual, the intensity of pain experienced will lead to a sympathoexcitation of similar magnitude.

This study is a paradigm which is focused on an acute pain stimulus. Questions regarding longer exposure and chronic pain stimuli can not be readily addressed by these data. Ultimately however, these data support the original hypothesis that increased pain perception evokes increases in MSNA, and that a pain threshold must be achieved or exceeded in order to invoke sympathoexcitation.

Acute Pain Induces CBR Resetting

The results from the first and second investigations established that the sympathetic nervous system is robustly activated in response to pain. The sympathetic nervous system is known as the fight or flight system as it functions to increase heart rate, systemic vascular resistance, and catecholamine levels which enhance survival during periods of stress or injury. Through our investigations we have tested the concept that pain directly modulates how the central nervous system controls blood pressure through the CBR reflex.

During our investigation of CBR function and its response to pain we were able to demonstrate that under conditions of acute pain the operational point of the CBR response is shifted toward the lower limiting value of the reverse sigmoid relationship between blood pressure and MSNA. Furthermore, through the use of a non-invasive experimental paradigm we demonstrated an upward-rightward shift and increase in gain of the CBR function curve induced by acute pain. These findings reveal how pain operates to buffer against episodes of hypotension by modulating the blood pressure regulatory systems within the central nervous system. Through its modulation of CBR

activity, pain may be viewed as an important survival mechanism for normotensive individuals to cope with hypotensive stimuli during traumatic injury.

Acute Pain During CPBR Unloading Enhances Hypotensive Buffering

The third study, demonstrated that the shift in the CBR operational point toward the lower limiting value of MSNA which occurs during periods of acute pain is not removed in the face of CPBR unloading. In addition, we were able to determine that activation of CPBR in conjunction with acute pain enhances sympathetic activation compared to pain alone. Of particular relevance these investigations demonstrated a significant rightward shift in the CBR function curve induced by pain and simultaneous LBNP, relative to LBNP alone. Collectively, the shift of the CBR operating point and the resetting of the CBR reflex curve to the right produce higher arterial pressure and enhance hypotensive buffering; demonstrating that pain may enhance survival during injuries which produce decreases in CVP.

While these data demonstrate that pain increases blood pressure, the neural control of this change most likely occurs via two distinct pathways. First, is the direct activation of pre-ganglionic sympathetic efferent fibers (Guyenet-2006). By bypassing the hypothalamus and NTS and going directly to the rostro-ventro-lateral medualla (RVLM), nociceptive fibers have a direct and unimpeded mechanism to increases sympathetic activity (Guyenet-2006). Secondly, increases in blood pressure are created by the resetting the baroreceptors in conjunction with the movement of the operating point to a region within the MSNA-CDP relationship which is toward the response

variable's lower limiting value. We believe that the shift in the operational point is a beneficial adaptation for buffering against periods of central hypovolemia which may precipitate hypotension during a traumatic injury. However, it is important to point out that the hypertensive buffering capacity is concurrently reduced during this time. For this reason we can not accurately predict what the effect on CBR control may be when acute pain and LBNP are experienced during exercise, or within hypertensive populations (Bristow et al., 1969, *Gallagher et al., 2006)*. We would suggest these data demonstrate that resetting may occur; but to what degree and direction remains to be seen.

Ultimately we believe that these findings underscore the importance of pain and its interaction with blood pressure regulatory processes within the central nervous system and highlight an important role for pain as a facilitator for hypotensive buffering during periods of injury associated with blood loss.

CHAPTER VI

FUTURE DIRECTIONS

We believe that these experiments have presented a safe model for pain and trauma related physiology. Specifically, this study has laid the groundwork for further investigation into baroreceptor modulation by pain and CPBR unloading. Future studies in hypertensive populations should be attempted, as hypertensive individuals have a profound pathophysiology associated with blood pressure regulation. As previously discussed, hypertension is associated with hypoalgesia (Rosa-1994, Conde-Guzon-2003). A result of hypoalgesia may be a reduction in the driving force for the CBR resetting that takes place as a result of acute pain. Furthermore, hypertension cannot exist without central CBR resetting to have occurred prior to its onset (Chapleau-1989). Thus, the CBR response and resetting due to acute pain and decreases in CVP may be altered or impaired within a hypertensive population. A loss in blood pressure regulation associated with pain is suggested by the data which demonstrates increased mortality risk for hypertensive patients who experience physical trauma (Tamosiunas-2005, Terry-2007).

Looking toward other future avenues for research, diabetes stands out as a disease which may exhibit complications similar to those of hypertensives. Yet, advanced diabetic neuropathy, sympathetic dysregulation, obesity, and/or decreased parasympathetic tone are all co-morbidities occurring in diabetes that can directly affect CBR function as well. We propose studies to investigate the hypothesis that

hypertensives have impaired CBR response to pain, leading to a reduced hypovolemic buffering capacity, relative to age matched normotensives. Furthermore, we hypothesize the hypovolemic buffering insufficiency anticipated in hypertensives will be significantly worse in hypertensive diabetics. One considering these studies should proceed with caution, however. While these investigations have demonstrated how normotensive individuals respond to painful stimuli, both hypertensives and diabetics constitute high risk populations. We believe that our methods are safe for use within these individuals. Yet, those individuals who are most likely to suffer from impaired CBR function and response to pain are either stage 2 hypertensives or uncontrolled diabetics. Ultimately, since these at risk patient populations may benefit from research concerning pain induced baroreceptor modulation, future studies associated within academic and clinical settings should be pursued.

CHAPTER VII

INCLUSIVE BIBLIOGRPAHY

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