THE ROLES OF MINERALOCORTICOID RECEPTORS AND GAMMA-AMINOBUTYRIC ACID IN THE PATHOGENESIS OF EXERCISE PRESSOR REFLEX DYSFUNCTION IN HYPERTENSION

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DEDICATION

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Hypertension is a global epidemic. Over 40% of the world population has elevated blood pressures and estimates predict this number to increase 60% in the next 10 years to over 1 billion individuals. While drug therapies are available to a large number of these patients, the treatment that consistently decreases the signs and symptoms of hypertension is exercise. However, exercise in hypertensive patients carries an elevated risk for myocardial ischemia, infarction, cardiac arrest, stroke, and possibly death during and after physical activity due to the exaggerated elevations in blood pressure and sympathetic activity that accompany the physical exertion. Understanding the mechanisms through which hypertension causes a dysregulation in blood pressure management during physical activity is paramount if we are to find safe ways for patients to benefit from exercise without dangerous complications. Our lab has strongly linked hypertension to a dysfunction in the exercise pressor reflex (EPR), a cardiovascular control system that originates within exercising muscle. However, the mechanisms underlying the pathogenesis of this dysfunction remain largely undetermined. To address this gap in knowledge studies were performed to investigate the roles of two logical candidates for the generation of EPR over-activity in hypertension: 1) aldosterone acting via mineralocorticoid receptors, and 2) gamma-aminobutyric acid (GABA) acting within the muscle reflex processing center located in the solitary tract nucleus (NTS) of the brainstem. The role of aldosterone and mineralocorticoid receptors was investigated by feeding normotensive and hypertensive rat a diet containing normal chow, or the mineralocorticoid receptor antagonists spironolactone (SPIR) or eplerenone (EPL). The role of central GABA was investigated in normotensive and hypertensive rats by microdialyzing the GABA synthesis inhibitor (3-mercaptopropionic acid), the $GABA_A$ receptor antagonist (bicuculline), or the $GABA_B$ receptor antagonist (saclofen) into the NTS. The results of the studies demonstrated that: 1) SPIR and EPL can be used as effective treatments to reduce the exaggerated cardiovascular responses to EPR activation in hypertension, and 2) GABA maintains the ability to modulate muscle mechanoreflex activity (a component of the EPR) within the NTS via activation of $GABA_A$ receptors in normotension with this ability being compromised after the development of hypertension.

TABLE OF CONTENTS

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LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE:
INTRODUCTION

HYPERTENSION & THE CARDIOVASCULAR RESPONSE TO EXERCISE

Hypertension

Hypertension is characterized by both an elevated resting arterial blood pressure (BP) and an exaggerated rise in BP during exercise (57, 66, 105, 147, 206). At rest, elevations in BP are commonly defined as a chronic systolic pressure reading of 140 mmHg or greater with a diastolic pressure of 90 mmHg or more (90). Recent surveys of the US population showed the prevalence of hypertension in 2011-2012 to be 29.1% (179). Just three years later, that number is now estimated to be 32.6% of US adults aged 20 or older (170). The cost of treating high blood pressure in the US, which includes health care services, medications, and labor costs due to hypertension-related causes (missed work hours, workers' compensation, health benefits, etc.), totals over $46 billion per year (170). This issue is not limited to the United States. Global prevalence of hypertension has been cited at 26.4% of adults 18 and older as of 2000 (106), and the World Health Organization reported in 2014 that over 40% of the population now has elevated blood pressures (153, 261). Nor is this a problem faced just by adults. Between 2003 and 2006, the US prevalence of prehypertension (defined as 120-139 mmHg systolic pressure, 80-89 mmHg diastolic pressure (90)) was approximately 14% in boys and approximately 6% in

girls among children and adolescents aged 8–17 years. Moreover, the prevalence of hypertension in children within this age range was estimated to be 3–4% in various studies (95, 181). Clearly, the prevention and adequate treatment of hypertension could significantly impact both the general health of the world's population, as well as the global economy.

Exercise & Hypertension

Exercise produces intensity-dependent elevations in heart rate (HR), cardiac output (CO), and blood pressure (BP), which are caused in large part by decreases in parasympathetic nerve activity (PSNA) and increases in sympathetic nerve activity (SNA) (167, 186, 215, 229). These exercise-induced autonomic adjustments are caused by both a central neural drive, termed central command, as well as by a reflex arising in contracting skeletal muscle, termed the exercise pressor reflex (EPR) (26, 120). Additionally, the autonomic adjustments evoked by central command and the EPR are continually modulated by baroreflexes (143).

The EPR has two functional components: the muscle metaboreflex and mechanoreflex. The metaboreflex is activated when chemically-sensitive receptors are slowly stimulated by the metabolic byproducts of muscle contraction such as lactic acid (98, 199), adenosine triphosphate (ATP) (111), and prostaglandin E2 (98, 199). Stimulation of these metaboreceptors predominately activates group IV (C-fibers) sensory afferents innervating skeletal muscle leading to an increase in SNA. The mechanoreflex is activated when mechanically-sensitive

receptors in skeletal muscle respond quickly to deformation of their receptive fields via stretch-activated ion channels (116, 167, 229). Animal studies have shown that these skeletal muscle mechanoreceptors primarily activate thin fiber group III (mechanically-sensitive A-δ fibers) muscle afferents that reflexively increase sympathetic outflow (3, 102, 146, 149, 160).

In normotensive individuals, systolic and mean pressures rise during dynamic exercise as cardiac output and peripheral vasoconstriction in non-active muscles increases. Simultaneously, the vasculature in exercising muscles vasodilates leading to an overall decline in systemic vascular resistance (69). However, in hypertension, the normal physiological decline in systemic vascular resistance during exercise is greatly attenuated or absent (30, 57, 62, 140). This results in an exaggerated increases in BP (30, 32, 140, 257). It has been demonstrated in animal models of human hypertension that the EPR becomes overactive, contributing significantly to the exaggerated increases in BP observed during exercise (114, 169, 229).

The noted increases in peripheral resistance at rest and during exercise in hypertension, along with reduced arterial and left ventricular compliance, can cause asynchronous ventricular wall contraction in the heart leading to left ventricular hypertrophy (39, 164). Further, excessive BP elevation during physical activity can contribute to impaired exercise tolerance in hypertensive patients even in the absence of coronary artery disease or left ventricular dysfunction (39, 135, 147, 180). Combined, these maladaptive responses to hypertension have been shown to be associated with elevated risks for myocardial ischemia, infarction, cardiac arrest, stroke, and

possibly death during and after physical activity (78, 116, 144, 162, 163). These associative adverse affects of exercise in hypertension have been confirmed by numerous epidemiological studies in human patient populations (44, 113, 132, 134, 237).

The Benefits of Exercise

While studies have shown physical activity to have limited success in lowering body mass index (BMI), exercise has been associated with improved body composition. Exercise increases lean mass in most individuals and reduces fat mass in overweight and obese individuals. Resistance exercises may particularly benefit those who are overweight or obese (34). This is particularly important in regards to the current climate of rising obesity rates to epidemic levels in Western civilization (266). Other significant benefits of exercise include increased insulin sensitivity (13), a reduced risk of prostate and breast cancer (129), increased prevention of osteoporosis, retardation of the onset of dementia (123), and improvements in stamina and self-confidence (220). Additionally, exercise has been extensively linked in youth and adults to decrease stress and yield improvements in depression, anxiety, and other mental diseases. (15, 65, 138, 216, 220, 247).

In specific regard to the cardiovascular benefits of light or moderate intensity exercise, it is associated with significant decreases in systolic and diastolic blood pressure in both normotensive and hypertensive individuals (107). Improved blood pressure and cardiovascular

health has been shown to have a linear relationship with decreased risk of morbidity and mortality (141). It has been shown in rats that exercise training reverses the increased sympathetic and decreased parasympathetic nerve activity prevalent at rest. These corrections improve abnormal baroreflex function in hypertension and contribute to lower resting mean BP and HR (122, 159). Vascular resistance is lowered by decreases in arterial stiffness and increases in arterial compliance, both of which have the net effect of lowering blood pressure (95). The overall status of the heart is improved through remodeling during exercise leading to decreases in left ventricular mass, as well as lowered cardiac output and heart rate (245, 254, 270). Exercise has also been shown to better the blood lipid profile (108), leading to improvements in long-term vascular disease.

The amount of exercise required to achieve these benefits has been shown to be relatively modest consisting of about 2 h of exercise at easy to moderate intensities over three bouts per week. Examples highlighted have been brisk walking, easy jogging at a pace of 3-5 mph, or cycling at a pace of 10-12 mph (128, 214). Even lower intensities of exercise show a beneficial prognostic impact compared with completely sedentary lifestyles with reductions of mortality of up to 12-20% being demonstrated (115). Current recommendations for exercise are higher: the American Heart Association and the British Heart Foundation recommend that individuals undertake an exercise program of at least 30 minutes of moderate-intensity aerobic activity at least 5 days per week, and moderate-to-high intensity muscle-strengthening activity at least 2 or more days per week for additional health benefits (7, 14). These benefits cannot be understated.

In terms of longevity of life, individuals who engage in regular exercise live at least 3 years longer than sedentary counterparts (22, 23). When these factors are taken into account, exercise may be considered the most effective, accessible, and cheapest therapy a physician can prescribe.

The potentially adverse affects of exercising with hypertension (up to, and including death) create a serious catch-22. The only treatment that consistently and predictably decreases the signs and symptoms of hypertension is exercise. In fact, the benefits of exercise have been well enumerated, not only in hypertension, but also in almost every other marker of health. Understanding the mechanisms through which hypertension causes a dysregulation in blood pressure management during physical activity is paramount if we are to find safe ways for patients to benefit from exercise without any dangerous complications. Our lab and others have strongly linked hypertension to a exaggeration in the EPR response (102, 125, 167, 173, 229, 231). The current set of studies presented in this dissertation explores one relatively new area of research — the role of mineralocorticoid receptors in aldosterone-related hypertension — and one novel area of hypertension control — the role of GABA signaling — in the function of the EPR in hypertension.

CHAPTER TWO:
REVIEW OF THE LITERATURE

CARDIOVASCULAR CONTROL DURING EXERCISE

Changes in circulatory hemodynamics are modulated by adjustments in autonomic nerve activity during exercise to meet the demands of working skeletal muscle. The autonomic nervous system mediates these cardiovascular responses to exercise by increasing SNA and decreasing parasympathetic nerve activity via three neural mechanisms: central command, the exercise pressor reflex (EPR), and the arterial baroreflex (227). It is worth noting that hemodynamic responses for dynamic and static exercise differ. In dynamic exercise, rapid, moderate increases of BP are modulated by elevations in HR, stroke volume (SV), and cardiac output (CO), while total peripheral resistance is decreased. Combined, these hemodynamic adjustments ensure adequate oxygen and nutrients are delivered to the working muscle (161, 202). Static exercise, on the other hand, is characterized by much larger increases in BP due primarily to HR-mediated increases in CO without concomitant reductions in total peripheral resistance (TPR) (161). It is these autonomically mediated adjustments in the cardiovascular system that allow muscle work to be performed in both forms of exercise.

In 1894, it was first suggested that the cardiovascular responses to exercise were mediated by neural mechanisms (92). These theories were supported in 1913 when Krogh and Linhard

quantified the rapid increases in ventilation and pulse rates at the onset of voluntary exercise that clearly required an operating system of great speed (119). In kind, these studies showed that neural impulses from the motor cortex (termed cortical irradiation) were the likely source for these rapid responses. By 1972, cortical irradiation (renamed central command) had been well established in animal and human studies as a feed-forward neural mechanism that transmitted excitatory impulses to descending motor neurons for locomotion and in a parallel fashion activated cardiovascular control circuits within the medulla oblongata in response to exercise (63). As an example, The ability of increased central motor drive to enhance the cardiovascular response to exercise was demonstrated through the use of partial neuromuscular blockade in which physical activity required a greater central effort (9, 130). However, additional studies emerged that showed central command was only one aspect of a larger, more complicated system.

In 1917, Krogh and Linhard also demonstrated in humans that reflex input from the skeletal muscle itself might be involved in mediating the cardiovascular response to exercise. They observed that increases in HR and ventilation could be induced in response to involuntary leg contraction caused by electrical stimulation; a maneuver which reduced and/or eliminated cortical irradiation (*i.e.* central command) (120). Further support of this came in the late 1930s when Alam and Smirk showed that dynamic exercise-evoked increases in BP and HR were sustained after a bout of exercise when venous outflow from the formerly contracting muscle was prevented (3, 5). Conversely, when increases in cardiac output during exercise were

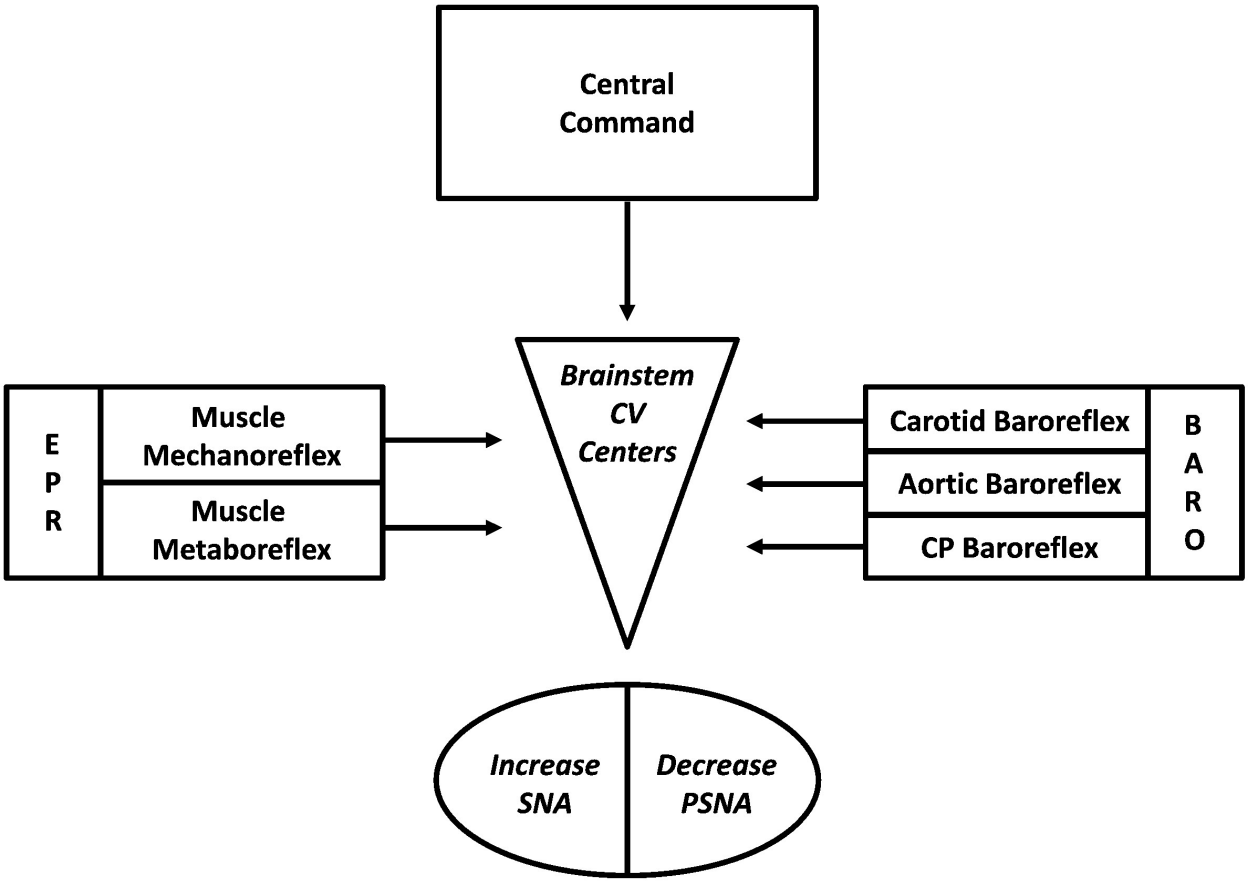

Figure 1: The neural inputs regulating autonomic activity during exercise. Central command and the exercise pressor reflex (EPR) provide input to cardiovascular (CV) centers in the brainstem to increase sympathetic nerve activity (SNA) and decrease parasympathetic nerve activity (PSNA) during exercise. The active carotid and aortic baroreflexes modulate the signals evoked by central command and the EPR. These three inputs are integrated to adjust CV activity to meet the metabolic demands of exercise **(225)**. The muscle mechanoreflex and metaboreflex are active components of the EPR. CP, cardiopulmonary; BARO, baroreflex.

enhanced by digitalis, potentiating leg blood flow, the cardiovascular responses elicited were attenuated (213). This concept of cardiovascular reflexes originating in skeletal muscle was further validated when non-centrally mediated elevations in BP and HR were not present in a patient with a spinal cord lesion (4). This skeletal muscle reflex has now been well studied and documented in the literature and is known as the exercise pressor reflex (EPR) (160).

The arterial baroreflex modulates the circulatory response to exercise induced by central command and the EPR. Very early studies examining the baroreflex first showed the inverse relationship between HR and BP regulated by this neural mechanism (*e.g.* as BP increases, HR decreases and vice versa) (145). Investigations identified the components of the reflex arc, consisting of unencapsulated free nerve endings present at the medial-adventitial border of blood vessels in the carotid sinus bifurcation and aortic arch, now known as the carotid and aortic baroreflexes, respectively (77, 143, 218). The Hering nerve, a branch of the glossopharyngeal nerve, and small vagal branches conduct impulses from the carotid and aortic baroreceptors, respectively. These inputs are integrated in the solitary tract nucleus (*nucleus tractus solitarii*, NTS) in the medulla oblongata (2). The baroreceptors function in a negative feedback loop that modulate beat-to-beat variations in BP by adjusting HR, SV, and TPR (76, 97, 143). While initially thought to be switched off during exercise (142, 188), it has since been shown that the baroreflex remains functional and resets to operate around the higher BPs induced by physical activity (46, 117, 193). Thus, the baroreflex maintains the ability to fine tune and modulate the cardiovascular responses to exercise.

The critical take-away point from this overview is that the three input systems — central command, the EPR and the arterial baroreflex — all contribute significantly to the autonomic regulation of cardiovascular activity during exercise. They each act independently, transmitting neural afferents directly to the medulla oblongata, as well as in concert, modulating the function of one another. This system creates a way for the body to fine-tune and adjust to the metabolic demands of the body during exercise.

THE EXERCISE PRESSOR REFLEX IN HYPERTENSION

EPR and Hypertension

Exercise in hypertension evokes excessive increases in BP from a chronically elevated baseline (189, 190). Compared to normotensive controls, selective activation of the EPR elicits markedly exaggerated increases in pressor and tachycardic responses in several rat models of hypertension (essential hypertension, prenatally programmed hypertension, and angiotensin II-induced hypertension) (114, 169, 229). These elevated cardiovascular responses exist at maximal and submaximal exercise intensities. In addition, the enhanced circulatory responses are accompanied by augmented increases in renal sympathetic nerve activity (rSNA) (167, 229). Exaggerated pressor, tachycardic, and sympathetic responses during muscle contraction in human hypertension have also been demonstrated. For example, augmented increases in BP and SNA were shown in hypertensive adults during low-intensity dynamic handgrip exercise (257).

These studies suggest that the abnormally increased EPR-induced elevations in SNA mediate the exaggerated cardiovascular response to exercise in hypertension. Additionally, these studies have also suggested that in hypertensive patients (32, 64, 209) and rat models of hypertension (127, 168, 207), EPR dysfunction is driven by the pathogenesis of abnormal muscle metaboreflex and muscle mechanoreflex activity (148, 156, 228, 230).

Metaboreflex in Hypertension

The autonomic neural responses to muscle contraction are mediated, in part, by the accumulation of metabolites within skeletal muscle that activate the group IV afferent fibers comprising the sensory arm of the metaboreflex component of the EPR. Activation of the metaboreflex occurs during both dynamic and static exercise. However, blood flow to active muscle during isometric exercise decreases, attenuating the clearance of metabolites produced during contraction which enhances the stimulation of metabolically-sensitive afferent neurons (174). Several metabolites present in exercising muscle, including potassium, lactic acid, bradykinin, byproducts of arachidonic acid metabolism, and diprotonated phosphate, have been identified as being capable of generating pressor and tachycardic responses to muscle contraction (198, 201, 204, 223, 235). These metabolites activate specific receptors, including acid sensing ion channels (ASICs) and transient receptor potential vanilloid 1 (TRPv1) receptors (99, 155, 230, 238), localized on group III and IV afferent sensory fibers within skeletal muscle. Additionally, ATP activates the muscle

metaboreflex (68, 133) via purinergic ligand-gated ion channels (P2X receptors) that have been localized to dorsal root ganglion cells innervating skeletal muscle (19, 25, 131, 136, 259, 262).

In animals models of hypertension, the metaboreflex has been shown to elicit exaggerated pressor and tachycardic responses to electrically induced muscle contraction (168). Introduction of ischemia during muscle contraction in these studies served to "supra" stimulate the metaboreflex by retarding the removal of metabolites from the active skeletal muscle. It was also shown that the augmented cardiovascular responses to supra-stimulation of the metaboreflex were due, at least in part, to increases in SNA (168). These findings were corroborated by additional studies in which selective activation of metabolically sensitive afferent fibers by administration of capsaicin into the hindlimb arteries caused potentiated increases in BP, HR, and rSNA in SHRs compared to WKY rats(127, 168). Taken together, our lab and others have shown that the muscle metaboreflex contributes significantly to the abnormal autonomic responses to skeletal muscle contraction in hypertension.

Investigations designed to examine the exaggerated pressor and tachycardic responses to physical exertion in hypertensive humans support the concept that metaboreflex over-activity activity partially contributes to the altered cardiovascular responses to exercise characteristic of hypertension. For example, studies taking advantage of the fact that decreased blood flow to skeletal muscle during static exercise attenuates the removal of metabolites (32, 64, 209) have demonstrated that hypertensive humans elicit exaggerated increases in BP and muscle SNA

during static handgrip exercise compared to age-matched normotensive adults (32, 209). Importantly, these augmented responses remain after the cessation of handgrip when the circulation to the previously exercising limb is experimentally occluded (*i.e.* during isolated stimulation of the metaboreflex). Similar responses have been shown to also exist in pre-hypertensive young adults (20), as well as older adults (64). Collectively, the evidence in animals and humans demonstrates that muscle metaboreflex over-activity significantly contributes to the exaggerated sympathetic, pressor, and tachycardic responses to exercise in hypertension.

Mechanoreflex in Hypertension

Group III sensory afferent neurons are predominately excited by mechanically-sensitive receptors which are stimulated via distortion of their receptive fields. In attempts to identify areas of the muscle where mechanoreceptors are optimally exposed, lidocaine was injected into the myotendinous junction which abolished the cardiovascular response to mechanoreflex stimulation (175, 176). This finding suggested that the receptive fields of mechanoreceptors are located proximally to tendons within the myotendinous junction near the head of the muscle. While L- and T-type calcium channels and mechanogated channels have yet to be localized to group III afferent nerve endings (67), gadolinium (a trivalent lanthanide that antagonizes these channels) inhibits the pressor and tachycardic responses to selective mechanoreflex activation (72, 73, 167) suggesting these receptors stimulate group III sensory fibers. Similar to metaboreceptor activation, a polymodal sub-population of mechanoreceptors and associated

group III fibers are activated by muscle metabolites such as bradykinin, potassium, byproducts of arachidonic acid, and lactic acid (84, 100, 121, 199, 204, 222). In addition, metabolites are known to sensitize mechanoreceptors to distorition, further enhancing mechanoreflex responsiveness (1, 27, 101). For example, prostaglandins, thromboxanes, and leukotrienes produced by cyclooxygenases (COXs) and lipoxygenases have been demonstrated to stimulate and modulate the firing rates of group III afferent fibers (74, 199, 200). Finally, P2X receptors have been localized on group III and IV fibers, suggesting that ATP may play a role in mechanosensation as well as metabosensation (68, 110, 112). While these studies have told us much concerning the mechanisms of mechanoreflex activation, there are still many questions remaining.

Selective activation of mechanically sensitive afferent fibers through passive muscle stretch produces enhanced elevations in BP, HR, and rSNA in spontaneously hypertensive rats (a model of human essential hypertension) (127, 167). As stated earlier, these abnormally large pressor, tachycardic, and sympathetic responses to mechanoreflex stimulation can be significantly attenuated by blocking skeletal muscle mechanoreceptors gadolinium during both muscle contraction and stretch (167). Additionally, these exaggerations in mechanoreflex function have been demonstrated in adult prenatally programmed hypertensive rats (a model of human maternal dietary protein deprivation induced hypertension) (166, 169). Findings from these animal studies suggest the muscle mechanoreflex significantly contributes to the EPR-mediated exaggerations in cardiovascular responsiveness in hypertension of more than one etiology.

The mechanoreflex is activated during muscle contraction. However, it is difficult to isolate and study in humans because contraction is accompanied by concurrent metabolite production and central command activation. Techniques designed to generate passive exercise, such as low-resistance passive cycling, attempt to stimulate the mechanoreflex without activating the metaboreflex or central command (45, 56, 157). To date, no studies examining the contribution fo the muscle mechanoreflex to the exaggerated cardiovascular response to exercise in hypertension have been reported in humans. As such, there is a strong need to translate the findings delineated from animal research to the human hypertensive patient. Further studies utilizing techniques such as passive cycling may indeed be able to bridge this gap in knowledge.

THE ROLE OF MINERALOCORTICOID RECEPTORS IN CARDIOVASCULAR CONTROL

Aldosterone

The origin and progression of hypertension has been linked in many forms of the disease to over-activation of the sympathetic nervous system. Recent evidence shows that the renin-aldosterone-angiotensin system plays a key role in the generation of hypertension, specifically angiotensin II (Ang II) (37, 217, 221). Angiotensin receptor subtype 1 (AT_1) and subtype II (AT_2) have been identified in many brainstem centers involved in cardiovascular regulation

(187). Blockade of the AT_1 receptor has shown to decrease in BP in cats (236), as well as reduce BP and HR responses to static muscle contraction in rat studies (182). Interestingly, while thiazide-type diuretics decrease hypertension effects through AT_1 receptor blockade, they do not attenuate augmented increases in SNA during exercise in patients with essential hypertension (257).

Despite the reported successful use of AT_1 receptor blockade in treating the symptoms of hypertension, Ang II activation via aldosterone has severe deleterious effects on the cardiovascular system (43, 177, 255) that cannot be effectively prevented by antagonism of AT_1 receptors (12, 244, 268). Studies in rats and humans have shown that treatment with AT_1 receptor antagonists only transiently reduces circulating aldosterone concentrations, returning to pretreatment levels following cessation of blockade (12, 244, 268). This effect is clinically known as aldosterone breakthrough (12, 268) and is associated with increased cardiovascular and renal complications in patients with hypertension, diabetes mellitus, and congestive heart failure (21, 212).

Like Ang II, aldosterone is known to contribute to the pathogenesis of hypertension and target organ complications in humans. Circulating aldosterone penetrates the blood-brain barrier at concentrations that are equal to those in plasma (58, 251, 269), allowing aldosterone to centrally stimulate the sympathetic nervous system (31, 41, 61). Direct infusion of aldosterone into the cerebral ventricles in animal studies have shown sustained increases in SNA and BP (49, 60, 94,

258, 274). Chronic intracerebroventricular (ICV) infusion of aldosterone has also been shown to inhibit arterial baroreflex control of rSNA and HR (80). Increased aldosterone alone is also known to induce cardiomyopathy (18, 70). Patients with primary aldosteronism, a secondary form of hypertension characterized by aldosterone overproduction, exhibit elevated levels in resting SNA that are similar to those shown with patients with essential hypertension. Moreover, this baseline SNA over-activity can be normalized by adrenalectomy in cases where an aldosterone-producing adenoma is present (118).

It has been suggested that the central, sympathetically-mediated pressor action of aldosterone is observed only in the presence of sodium excess or in salt-sensitive animals (80, 258). Additionally, aldosterone administration with salt loading for 4 weeks results in muscle atrophy (18), further contributing to cardiovascular pathogenesis (71). High salt intake increases the concentration of sodium in cerebral spinal fluid and brain tissue, evoking central sympathetic activation through stimulation of epithelial sodium channels (ENaC) (80, 158, 253). Salt intake does not, however, alter vasoconstrictor responses to sympathetic stimulation in rats (86, 96, 184, 263) suggesting the abnormalities evoked are central in origin and do not occur at the vascular level. With regard to SNA and BP regulation during exercise, both excessive salt intake (263) and elevated aldosterone levels (165) have been shown to exacerbate the sympathetic and pressor responses to EPR activation. Despite the suggestions that aldosterone requires excess sodium to have adverse effects, our laboratory has recently demonstrated a strong independent action of aldosterone in the generation of EPR over-activity that is not appreciably affected by increased

salt ingestion (165). These findings suggest that aldosterone maintains the potential to independently affect EPR function.

Mineralocorticoid Receptors

Activation of central mineralocorticoid receptors (MR) may play a key role in the mechanisms underlying aldosterone-related hypertension (49, 50). MRs are expressed in several brain regions involved in cardiovascular regulation, including the hypothalamus, the pariventricular nucleus, and the solitary tract nucleus (*nucleus tractus solitarius*, NTS) (31, 41, 55). While MRs have equal binding affinity for glucocorticoids (210), coexpression of 11β-hydroxysteroid dehydrogenase enzyme type 2 (HSD2) allows aldosterone in humans and corticosterone in some animals, including rodents, to gain access to neurons expressing these receptors (55) by converting local glucocorticoids, such as cortisol, to the inert metabolite cortisone. Cortisone has a lower binding affinity for MR allowing aldosterone to bind the receptor. Both MR and HSD2 are present in the NTS, an important brainstem center involved in cardiovascular control (53, 54). ICV infusion of MR antagonists at doses that have no BP effects when systemically delivered reduce the central pressor action of aldosterone (60). ICV infusion of MR antagonists also reduces BP in rats with normal circulating levels of aldosterone such as Wistar Kyoto (WKY) rats (197), Dahl-salt sensitive rats (81), and spontaneously hypertensive (SHR) rats (196), possibly through reductions of locally produced aldosterone in the brain (59, 81). In addition, central blockade of MR through the antagonist spironolactone may promote natriuresis

and reduce BP without affecting sympathetic outflow in patients with primary hypertension (154). The potential roles of aldosterone and MR in adversely altering autonomic cardiovascular regulation during exercise in hypertension remain largely undetermined.

THE ROLE OF GABA IN CARDIOVASCULAR CONTROL

GABA

Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the central nervous system (CNS) reducing neuronal excitability (260). GABAergic actions are mediated by stimulation of receptors located in presynaptic or postsynaptic loci. Two major types of the GABA receptors exist: 1) the $GABA_A$, which receptor is a pentameric, chloride ionophore that mediates postsynaptic inhibition (150), and 2) the $GABA_B$ receptor, which is a G-protein-coupled receptor that induces reductions in calcium conductance to modulate presynaptic inhibition and enhances potassium conductance to mediate postsynaptic inhibition (252). GABAergic inhibition of the CNS results in pressor or depressor responses in a site-specific manner. For example, pressor responses can result from GABAergic inhibition of neurons that reduce SNA. GABA injections into the CNS also alter vagal cardiac function and levels of vasoactive hormones such as vasopressin and angiotensin, in addition to changes in SNA (11, 16).

The Central Actions of GABA

The NTS, an important autonomic processing center, receives a variety of neural inputs that modulate cardiovascular functions (137, 232). GABA is the primary inhibitory neurotransmitter in the complex intrinsic network of neurons within the NTS (8, 10, 17, 103, 104, 183, 211). GABAergic neurons coordinate the autonomic outflow to cardiovascular organs within this network (10, 35, 103, 104, 192) leading to increases in SNA, HR, and BP when GABA receptors are activated within the NTS (192). However, as the complexity of the NTS networks makes it difficult to study GABAergic neurons in isolation, identifying their role in the regulation of cardiovascular function is an ongoing target of investigation. Studies suggest that the GABA-mediated inhibition of NTS neurons that leads to increases in cardiovascular activity also depends on the actions of excitatory neurons elsewhere in the CNS network. This activity has been studied in the arterial baroreflex and shown to be active in the cortex and other cardiovascular control centers (*e.g.* the rostral ventrolateral medulla, RVLM) (38, 83, 93, 109, 203, 250, 276), while the role of GABA-mediated activity in other cardiovascular control mechanisms such as the EPR remains to be investigated.

Microinjection of $GABA_A$ or $GABA_B$ receptor agonists within caudal regions of the NTS increases arterial pressure due to GABAergic inhibition of NTS neurons (16, 89, 241). Conversely, microinjection of $GABA_A$ or $GABA_B$ receptor antagonists lowers BP (16, 240). The

pressor, tachycardic, and sympathetic responses to GABA receptor agonists are maintained or enhanced in spontaneously hypertensive, DOCA-salt, and renal-wrap hypertensive rats when compared to normotensive controls (36, 249, 256, 267). Studies in renal-wrap hypertensive rats showed that NTS neuron sensitivity is altered in response to inhibition of $GABA_A$ and $GABA_B$ receptors when compared to normotensive controls. Directionally opposite changes in sensitivity occur in response to $GABA_A$ (reduced inhibition) and $GABA_B$ (enhanced inhibition) receptor selective agonists (151).

In electrophysiological preparations using isolated NTS neurons identified through anatomic labeling techniques (152), postsynaptic activation of $GABA_A$ and $GABA_B$ receptors mirrored results seen *in vivo*. The peak response for $GABA_A$-induced currents was significantly greater in hypertensive rats when compared to normotensive controls and that NTS neurons are less sensitive to $GABA_A$ receptor inhibition (10, 104, 248). Similar studies looking at $GABA_B$-evoked responses in NTS neurons showed that postsynaptic responses were attenuated in the presence of $GABA_B$ receptor antagonists compared with responses recorded in untreated hypertensive animals, suggesting an enhanced response in second-order neurons in hypertension (272). Presynaptic antagonism of $GABA_B$ receptor responses may reduce excitatory postsynaptic currents by inhibiting glutamate release in second-order neurons (8, 10, 103, 104, 273). These studies, as well as others, indicate that cardiovascular inputs are modulated, in part, via GABAergic neurons.

The Role of Angiotensin on GABAergic Neurons

As previously discussed, excitability in the medulla is modulated by neurotransmitters such as GABA, but is also regulated by angiotensin II (Ang II). Under hypoxic conditions, increased BP, and rSNA from AT_1 receptor antagonism can be prevented by $GABA_A$ blockade (219). These effects by Ang II depend on the net excitatory and inhibitory effects on sympathetic neurons containing GABA receptors, leading to an effect termed the 'push-pull effect' (29). While other pathways may play a role in inhibition of pressor and SNA effects caused by Ang II (52, 75, 91), GABAergic neurons may also modulate the response to Ang II (79) as projections to the RVLM decrease their output to sympathetic neurons (28). GABAergic activity following BP elevation has also been linked to outward signals from the NTS to the caudal ventrolateral medulla (CVLM) (28). AT_1 receptor expression has been shown on GABAergic neurons in the medulla oblongata, suggesting that Ang II acts both on excitatory neurons in the RVLM, but also on inhibitory neurons (79). Similar to hypoxic conditions, blockade of AT_1 receptors in the RVLM of normotensive anaesthetized rats does not decrease BP and SNA unless combined with the $GABA_A$ antagonist, bicucullin, revealing an excitatory effect when GABAergic input is blocked (243). In conscious rats, AT_1 receptor blockade increases BP, indicating that the mediated inhibition of RVLM neurons is not balanced by tonic excitatory signals (47, 48). AT_1 receptor activation may release glutamate in the RVLM, modulating SNA via GABAergic neurons (242). Likewise, Ang II injection into the RVLM increased glutamate release in the RVLM (275), as

well as GABA release in the hypothalamic PVN (42). While the effects of nitric oxide cannot be discounted for the balance that seems to appear between excitatory and inhibitory effects (271), these separate outcomes are most likely due to distinct mechanisms of AT_1 receptors on pre- and post-synaptic GABAergic neurons that have excitatory and inhibitory consequences, respectively, in cardiovascular control centers in the medulla (219).

Ang II receptor concentrations in some regions of the brain is greater in SHRs than normotensive controls (79, 205, 233). SHRs exhibit more sensitivity to Ang II in the RVLM (79, 171, 264). SHRs and Dahl salt-sensitive hypertensive rats show sustained excitatation in presympathetic neurons in the RVLM to endogenous Ang II when compared to normotensive controls (6, 87, 88). Hypertensive rats also appear to be more sensitive to the central administration of Ang II antagonists causing reductions of systemic MAP (87, 239).

Increases in $GABA_B$ receptor expression in hypertension may be linked to Ang II as well. Ang II application to NTS neuronal cell cultures induce increases in $GABA_B$ receptor expression while having no effect on $GABA_A$ receptor expression (265). Blockade of $GABA_B$ receptors decreases neuronal signaling frequency when combined with Ang II while again having no effect when $GABA_A$ receptors are blocked (265). In animal studies, ICV infusion of Ang II increased $GABA_B$ receptor mRNA and protein levels in the NTS (265). In the Ang II-dependent renal-wrap rat model of hypertension (224), evidence suggests that Ang II may mediate the changes in $GABA_B$ receptor function reported in the NTS (36, 272). Ang II may also increase $GABA_B$

receptor function through signal transduction pathways activated by Ang II and $GABA_B$ receptors co-localized to these neurons.

The Role of GABA in Exercise

It is well recognized that GABA contributes to the regulation of the cardiovascular response to exercise by acting within a number of autonomic nuclei in the brainstem including the NTS, CVLM, and RVLM. For example, evidence suggests that GABAergic mechanisms in the NTS are involved in the exercise-induced resetting of the baroreflex in which the reflex operates around the higher pressures elicited by physical activity (194). An increase in GABA within the RVLM in response to EPR activation has been shown to play an inhibitory role by preventing inappropriate increases in BP (246). Within the CVLM, a decrease in GABA response to exercise has been demonstrated to lead to an increase in both HR and BP (85, 178). Clearly, functional alterations in GABAergic neurons maintain the potential to contribute significantly to the abnormally exaggerated cardiovascular response to exercise that develops with the pathogenesis of hypertension.

SCIENTIFIC RATIONALE FOR CURRENT STUDIES

The background literature highlighted has formed the basis for the studies performed in this dissertation project. Our laboratory recently provided the first direct evidence that experimentally

increasing systemic aldosterone induces EPR over-activity in normotensive rats (165). This finding represented a potential mechanism by which SNA and BP may be inappropriately augmented during exercise after the development of hypertension. That being stated, the results were obtained in normotensive animals. Whether aldosterone and its mineralocorticoid receptors play a role in the genesis of EPR over-activity in hypertension remains to be investigated. As such, the first component of the dissertation research aimed to determine the effect of MR blockade (via the antagonists spironolactone and eplerenone) on the EPR-induced cardiovascular response to muscle contraction in normotensive and spontaneously hypertensive rats. It was hypothesized that antagonizing mineralocorticoid receptors in hypertensive animals would rescue a normal EPR phenotype. Additional mechanisms that do not involve aldosterone or its MR receptors have likewise been shown to contribute to the development of EPR dysfunction in hypertension. For example, our laboratory has recently demonstrated that alterations in the processing of mechanoreflex afferent information within the NTS mediates, in part, the development of muscle reflex dysfunction in hypertension (124-126, 172, 173). Specifically, the studies determined that reductions in the availability of nitric oxide within the NTS led to the irregularities in the central processing of mechanoreflex input. As discussed, GABA is also known to modulate processing circuits within the NTS. Moreover, as previously mentioned, GABA inhibits NTS neurons leading to an increase in SNA, HR and BP. In hypertension, the sympathetically mediated cardiovascular response to mechanoreflex activation is exaggerated. It is logical to suggest that these exaggerated cardiovascular responses may be mediated by abnormal increases in GABA within the NTS. Therefore, the second component of the

dissertation research aimed to determine the contribution of GABA within the NTS to the pathogenesis of mechanoreflex over-activity in hypertension. It was hypothesized that blocking GABA synthesis and/or antagonizing GABA receptors within the NTS would normalize the cardiovascular response to mechanoreflex activation in hypertensive animals. To test this hypothesis, the cardiovascular responses to stimulation of the mechanoreflex were assessed before and after the microdialysis of 3-mercaptopropionic acid (a GABA synthesis inhibitor), bicuculline (a $GABA_A$ antagonist), or saclofen (a $GABA_B$ antagonist) into the NTS.

CHAPTER THREE:
METHODOLOGY

ANIMAL MODELS

Mineralocorticoid Receptor Studies

Experiments for the aldosterone studies were performed in 24 Spontaneously Hypertensive (SHR) and 11 Wistar-Kyoto (WKY) age-matched (15 week) male rats (Harlan Laboratories, Indianapolis, Ind.). SHR were fed (3 weeks) either normal chow (NC, n=8) or a customized diet containing the mineralocorticoid receptor antagonists spironolactone (SPIR, 50 mg/kg/day, n=9) or eplerenone (EPL, 100 mg/kg/day, n=7). WKY were fed either NC (n=6) or SPIR (n=5).

GABA Studies

Experiments for the GABA studies were performed in 21 normotensive (WKY) and 21 hypertensive (SHR) age matched (12-16 week) male rats (Harlan Laboratories, Indianapolis, Ind.). Within the NTS via microdialysis, animals received 3-mercaptopropionic acid (3-MP, 850 μM, 10 μg total; WKY, n = 7; SHR, n = 8), bicuculline (BIC, 88 nM, 5 ng total; WKY, n = 5; SHR, n = 5), or 2-hydroxy saclofen (SAC, 885 μM, 30 ng total; WKY, n=4; SHR, n = 4) for 45 minutes at 2.5 μL/min, as described below.

Ethics and Approvals

All animals were housed in standard caging on 12-hour light-dark cycles and given food and water *ad libitum*, except where otherwise noted. Experimental procedures were approved by the Institutional Animal Care and Research Advisory Committee at the University of Texas at Southwestern Medical Center, as well as conducted in accordance with the United States Department of Health and Human Services National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (24).

EXPERIMENTAL PROCEDURES AND PROTOCOLS

General Surgical Procedures

The following general surgical procedures were performed in all animals.

Rats were anesthetized under 2-3% isoflurane gas in pure oxygen (Isotec3, Ohmeda of Nevada, L.L.C., Sparks, NE). Needle electrodes were placed on the back of the animal to obtain electrocardiograph (ECG) recordings for the measurement of HR. The level of anesthesia was increased if the animal displayed a withdrawal reflex to hindlimb pinching, a corneal reflex was present, and/or HR increased spontaneously. Animals were then intubated and mechanically respirated (Inspira ASVV, Harvard Apparatus, Holliston, MA). A dexamethasone injection (0.1

cc of 4 mg/ml) was administered intramuscularly to the left hindlimb to minimize edema. Both internal common carotid arteries and the left jugular vein were catheterized (Tygon PE-50 tubing, Saint-Gobain, Paris, France) to obtain arterial pressure readings and for fluid administration, respectively. To maintain baseline BP at adequate levels throughout the experimental protocol, a solution (2 ml 1 M $NaHCO_3$ and 10 ml 5% dextrose in 38 ml Ringer solution) (195) was continuously infused into the jugular vein at a rate of 2 mL hr^{-1}. Body temperature was maintained between 37 and 39°C by an isothermal pad (Deltaphase, Braintree Scientific, Inc., Braintree, MA).

Animals were placed into a stereotaxic head frame and spinal unit with the pelvis stabilized with hip spikes (Kopf Instruments, Tujunga, CA). The gastrocnemius and soleus muscles of the right hindlimb were isolated, the calcaneal bone was cut, and the Achilles' tendon sectioned and connected to a force transducer (FT10; Grass Technologies, Middleton, WI) for the measurement of muscle tension. The limb was fixed in one position using clamps attached to the tibial bone. A precollicular decerebration was conducted as described previously (208, 226). Briefly, a bilateral craniotomy was performed and the portion of bone superior to the sagittal sinus was removed. The brain was sectioned immediately rostral to the superior colliculus, and the forebrain was aspirated (Gomco Model 4005, Allied Healthcare Products, St. Louis, MO) rendering the animal insentient. Gas anesthesia was discontinued following decerebration. Oxidized regenerated cellulose (Ethicon, Johnson & Johnson, Somerville, NJ) was placed around the remaining medullary brainstem to minimize hemorrhage and the cranial cavity was packed with cotton.

Animals recovered from surgery for 1 h before beginning any experimental protocol to allow sufficient time for the effects of isoflurane anesthesia to completely dissipate and arterial blood pressure to stabilize.

After the completion of experimental testing, insentient, decerebrated animals were euthanized via intravenous injection of saturated potassium chloride (4 M, 2 ml kg^{-1}). The heart and lungs were harvested and wet weights obtained. Tibial length was also obtained to assess heart mass-to-tibial length ratios.

Specific Surgical and Experimental Procedures

The following specific surgical and experimental procedures were performed as subsequently noted in the protocols for the mineralocorticoid receptor and GABA studies.

Tail-Cuff Blood Pressure Measurements

Non-invasive blood pressure measurements were obtained from animals using tail-cuff measurements from the CODA 8-channel high throughput system (Kent Scientific, Torrington, CT). Following manufacturer recommendations, animals were acclimatized to the protocol with daily measurements for two weeks. Following acclimatization, blood pressure measurements were taken every 2-3 days. Measurements were taken 15 times per animal. The first 5 were

rejected to account for any rises in blood pressure due to the measurement protocol. The remaining 10 measurements were included in a daily mean blood pressure reading if they passed the default CODA software validation criteria. Groups of animals were then binned and averaged to calculate group means.

EPR Testing

A laminectomy was performed from the second to sixth lumbar vertebrae (L_2-L_6) exposing the lower portions of the spinal cord. The dura of the cord was cut and reflected. The L_4 and L_5 ventral roots (housing motor neurons innervating hindlimb skeletal muscle) were separated isolated from dorsal roots (carrying sensory information from hindlimb skeletal muscle). The peripheral ends of the L_4 and L_5 ventral roots were cut and positioned on a custom-made insulated bipolar platinum electrode connected to a square pulse stimulator (S88; Grass Technologies, Middleton, WI). The exposed neural tissue was covered with mineral oil. A schematic of this experimental preparation is presented in Figure 2. To induce EPR stimulation, the gastrocnemius and soleus muscles of the right hindlimb were contracted by electrical stimulation of the L_4 and L_5 ventral roots (constant current stimulation at 3 times motor threshold, pulse duration of 0.1 ms at 40 Hz for 30 s). Before any contraction, muscles were preloaded with 70–100 g of tension. This procedure allowed simultaneous activation of group III and group IV mechanically and metabolically sensitive skeletal muscle afferent fibers.(226)

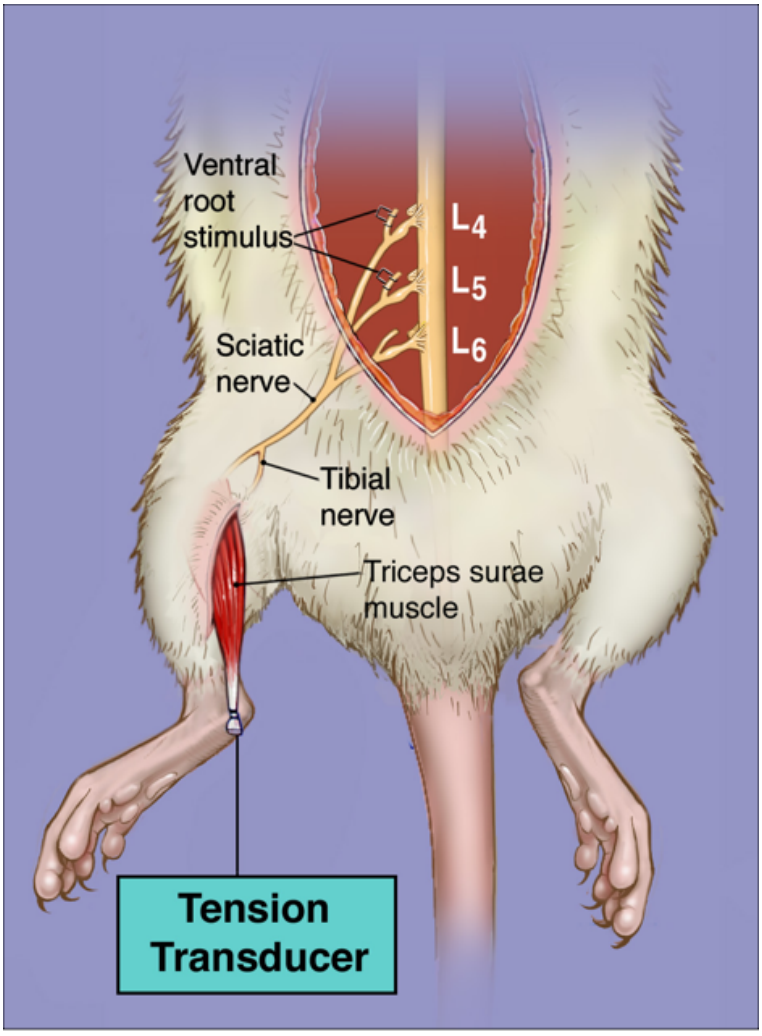

Figure 2: Illustration of the preparation used to activate the EPR via electrical stimulation of the L_4 and L_5 ventral roots as presented in Smith, et al. 2006 (229). Induction of muscle contraction in this manner concomitantly stimulates both the mechanically and metabolically-sensitive components of the EPR. This preparation can also be used to preferentially stimulate the muscle mechanoreflex via passive muscle stretch. Passively stretching the gastrocnemius and soleus muscle selectively activates the group III afferents most closely associate with the mechanically-sensitive component of the reflex.

Mechanoreflex Testing

To selectively activate the skeletal muscle mechanoreflex, the gastrocnemius and soleus muscles of the right hindlimb were passively stretched for either 30 s to 1,000-1,200 g of tension or levels similar to that developed during maximal static contractions using a calibrated 9.5-mm rack and pinion system (Harvard Apparatus, Holliston, MA). Muscles in all experiments were preloaded with 70–100 g of tension prior to performing the stretch maneuver. Stretching skeletal muscle in this manner does not increase muscle metabolism and is often used to preferentially stimulate the mechanically-sensitive afferent fibers associated with the muscle mechanoreflex (234). A schematic of the experimental preparation used to perform passive stretch is presented in Figure 2.

Microdialysis Procedures

A limited occipital craniotomy was performed to expose the dorsal surface of the brainstem. Microdialysis probes (model CMA 11, CMA Microdialysis AB, Holliston, MA) were stereotaxically positioned unilaterally within the NTS of the medulla oblongata in areas known to receive projections from skeletal muscle reflex afferent fibers in rats (coordinates: 0.0mm rostro-caudal to the calamus scriptorius, 0.5 mm lateral to the calamus scriptorius and 0.5mm below the dorsal medullary surface) (51, 172, 185, 191). All probes were placed ipsilateral to the hindlimb to be tested. A schematic diagram demonstrating the location of dialysis probe

placement is presented in **Figure 3**. Once positioned, the probe was continuously perfused at a rate of 2.5 μl min^{-1} with artificial cerebral spinal fluid (aCSF, 0.2 % bovine serum albumin, 0.1% bacitracin and the following ions (mM): 6.2 K^+, 134 Cl^-, 2.4 Ca^{2+}, 150 Na^+, 1.3 P^-, 13 HCO_3^- and 1.3 Mg^{2+}, buffered to a pH of 7.4 with CO_2). After probe insertion, the animal was allowed to stabilize for a minimum of 1 h before beginning experimental procedures. Probe placement was verified by dialyzing five animals with Evans Blue dye and excising the brainstems, which were then fixed in 4% paraformaldehyde, frozen in Tissue-Tek Optimal Cutting Temperature (O.C.T.) Compound (Sakura Finetek USA, Inc., Torrence, CA), sliced into 35-μm sections via a 2800 Frigocut E cryostat (Reichert-Jung, Depew, New York) and analyzed visually.

Experimental Protocol for Mineralocorticoid Receptor Studies

Rats were fed an NC, SPIR, or EPL diet during a 3-week period. During this period, conscious resting blood pressure was assessed periodically using the non-invasive tail-cuff measurement system previously described. Subsequently, acute terminal experiments were performed. All animals underwent the general surgical preparations as described, as well as the surgical procedures required for EPR and mechanoreflex stimulation. Completion of the procedures was followed by 1-hour of recovery. Each animal then had its gastrocnemius and soleus muscles contracted for 30 seconds via electrical ventral root stimulation to establish maximal contraction. The magnitude of passive stretch was then matched to the initial maximal force produced by the animal during contraction. Subsequent contractions or stretches were randomized during the

experiment by asking the Apple Siri virtual digital assistant to ‘flip a coin’ (iOS 7.2-8.3, Apple Inc, Cupertino, CA) where heads was a contraction and tails yielded a passive stretch. This was

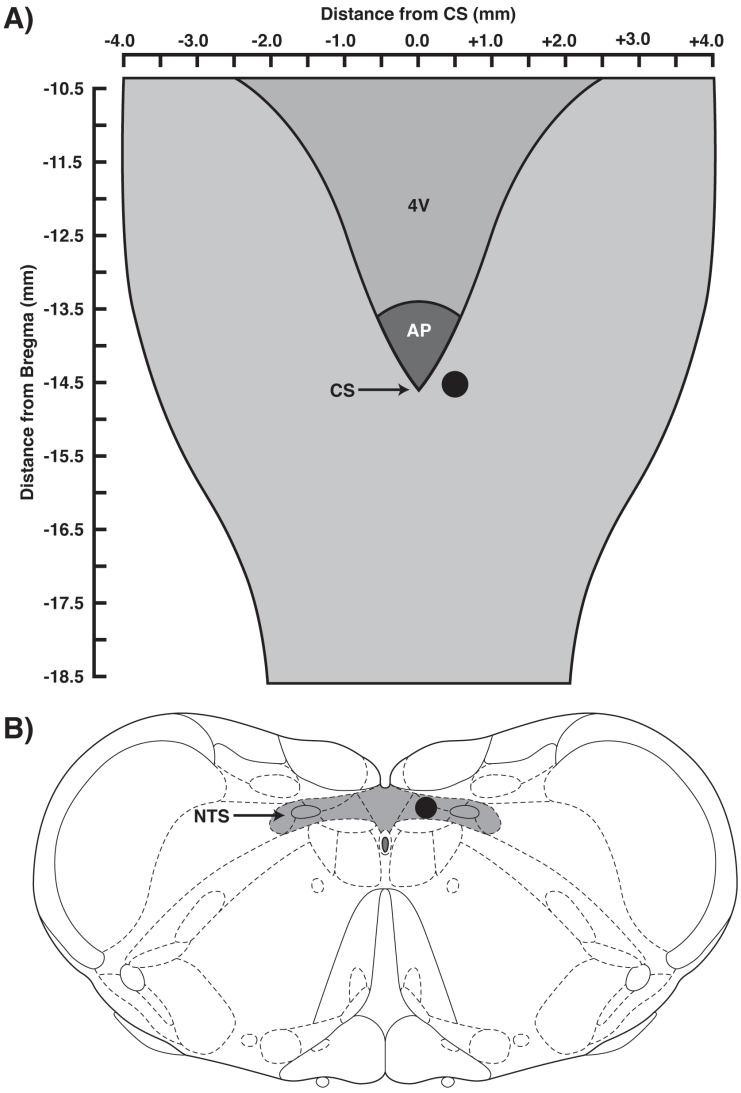

Figure 3: Placement of the microdialysis probe within the medullary brainstem.

A) schematic diagram adapted from Dhruva *et al*. (33) displaying the dorsal surface of the brainstem. The filled circle marks the location at which the dialysis probe was positioned. B) a schematic coronal section of the medulla, modified from Paxinos & Watson (185), depicts the position of the tip of the dialysis probe (filled circle) within the NTS (gray shaded area).

Abbreviations: 4V, fourth ventricle; AP, area postrema; CS, calamus scriptorius; and NTS, solitary tract nucleus (*nucleus tractus solitarii*).

repeated until 3 contractions and 3 passive stretches were obtained. Tissue was harvested and morphological data collected at the termination of the experiment. A schematic of the acute terminal experimental protocol is presented in Figure 4.

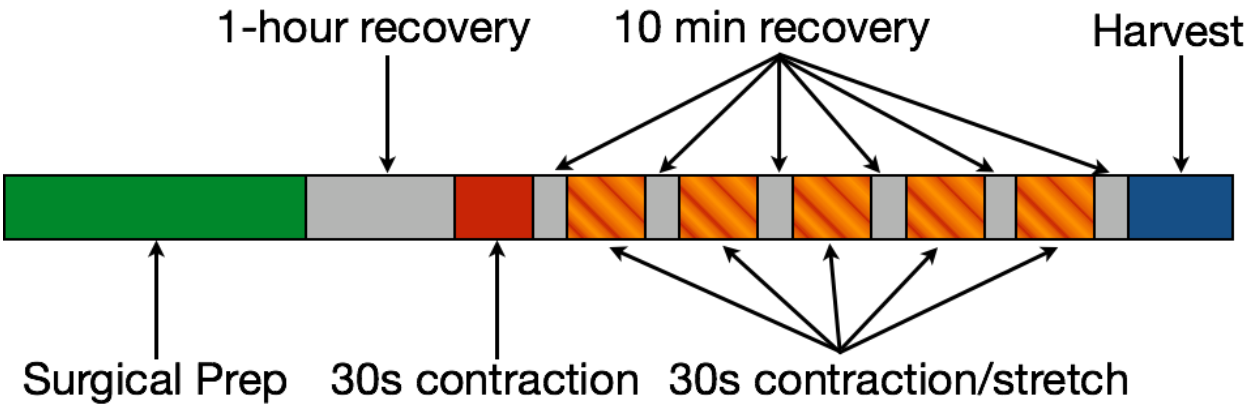

Figure 4: Mineralocorticoid receptor studies general scheme.

Experimental Protocol for GABA Studies

Animals were prepared as described under 'General Surgical Procedures'. Animals underwent additional surgery to allow experimental stimulation of the muscle mechanoreflex, as well as performance of microdialysis procedures. The gastrocnemius and soleus muscles were isolated and attached to a rack-and-pinion system as previously described. Following a 1-hour recovery period, artificial cerebral spinal fluid (aCSF) was microdialyzed into the NTS for 45 minutes.

The leg muscles were passively stretched for 30 seconds (peak developed tension ~ 1200g), followed by 10-minute recovery periods. This was repeated twice. Animals then received microdialyzed of either 3-MP, BIC, or SAC for 45 minutes within the NTS and passive stretches were performed as before. After testing was complete, aCSF was again dialyzed into the NTS to washout the experimental compounds. Following completion of the experimental protocol, morphological data and tissue samples were collected. A schematic of the acute terminal experimental protocol is presented in Figure 5.

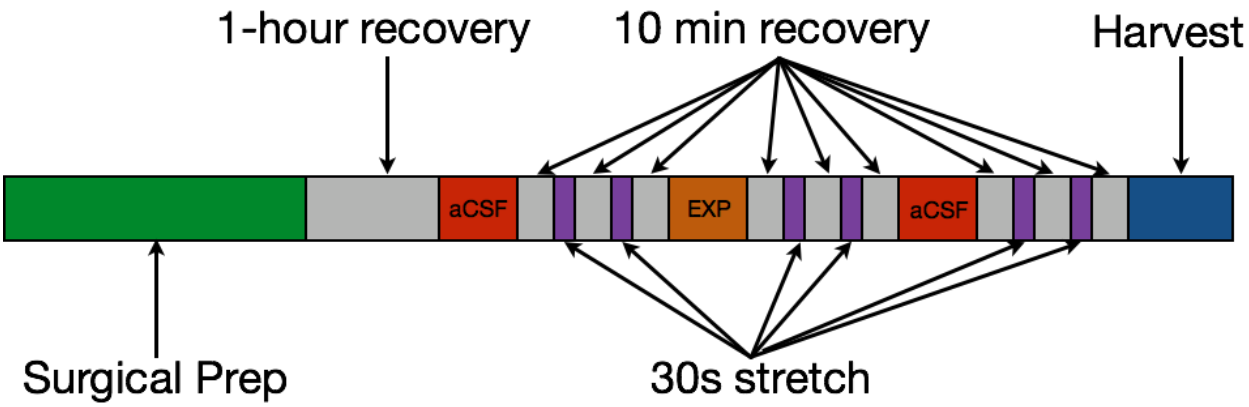

Figure 5: GABA studies general scheme. Animals were dialyzed with aCSF or experimental drug (labled EXP above. 3-MP, BIC, or SAC),

Data Acquisition

Physiological animal data was collected using a Powerlab analog-to-digital converter (ML870, ADInstruments, Dunedin, New Zealand) with the raw signaled filtered through a quad bridge amp (ML224, ADInstruments, Dunedin, New Zealand) set at a sampling rate of at 1-kHz. BP was measured by connecting the arterial catheter to a pressure transducer (MLTO380/D, ADInstruments, Dunedin, New Zealand). MAP was obtained by integrating the arterial pressure signal with a time constant of 1– 4 s. HR was measured from the time between successive R-waves in the ECG using a standard three-lead electrocardiogram attached to an animal bio amp (ML136, ADInstruments, Dunedin, New Zealand). Hindlimb tension was quantified using a force transducer (FT-10; Grass Instruments, Middleton, WI). Data sets of 1-s averages for MAP, HR, and hindlimb tension were analyzed. Baseline values for all variables were determined by evaluating 30 s of recorded data before a muscle contraction or stretch. The peak response of each variable was defined as the greatest change from baseline elicited by contraction or stretch. All data were collected, recorded, and analyzed using commercially available software (LabChart 8; ADInstruments, Dunedin, New Zealand).

STATISTICAL ANALYSIS

Statistics were performed on all data sets using Student's *t*-test or one-way ANOVA with uncorrected Fisher's LSD multiple comparisons or Bonferroni posttests as appropriate to identify differences between specific group means. The significance level was set at $p < 0.05$. All results

are presented as mean ± S.E.M. Analysis were conducted using GraphPad Prism 6 for Mac OS X (GraphPad Software, Inc., La Jolla, CA).

CHAPTER FOUR:
RESULTS

MINERALOCORTICOID RECEPTOR STUDIES

Characterization of Animals on Experimental Diets

Morphometric characteristics and blood chemistry values for each experimental group are presented in **Table 1**. Body weight was lower, but not significantly different, between WKY rats on normal chow and those treated with SPIR. However, SHR rats treated with both mineralocorticoid receptor antagonists, SPIR and EPL, had significantly lower body weights than controls on NC. Heart weight-to-body weight, lung weight-to-body weight, and tibial length-to-body weight ratio differences were all insignificant between groups. Plasma was collected from 5 animals in each experimental group. There were no significant differences between groups for sodium, potassium, or creatinine.

Conscious tail-cuff blood pressure measurements were taken during 3-week treatments of NC, SPIR, or EPL chow. There were no differences between experimental groups in systolic, diastolic, or mean arterial blood pressure. As an example, MAP for all groups over the 3-week treatment is shown in Figure 6. Similarly, heart rate, tail blood volume, and blood flow

measurements from the CODA system were not significantly different between groups (data not shown).

In acute terminal experiments, baseline measurements of HR and BP were taken following 1-hour of recovery post-decerebration. Neither SPIR nor EPL caused a significant change in baseline hemodynamics in either WKY or SHR (Figure 7). As expected, baseline MAP was higher in SHR compared to WKY. These differences between normotensive and hypertensive animals were consistent with previous experiments and values reported in the literature.

Effects of Mineralocorticoid Receptor Antagonist Treatment on the Cardiovascular Response to EPR Activation

In WKY, the MAP and HR responses to EPR activation via muscle contraction were largely unaffected by blockade of mineralocorticoid receptors with SPIR (Figure 8A). In contrast, the pressor and tachycardic responses to stimulation of the EPR during static muscle contraction in SHR were significantly attenuated by SPIR compared to animals fed NC (Figure 8B). EPL treated animals also showed attenuation of responses, albeit not to a significant degree compared to animals fed NC (Figure 8B). Individual responses for each animal are shown in Figure 9. It should be noted that the amount of tension developed during muscle contraction was quite variable. To control for this variability, all cardiovascular data reported have been normalized to the amount of tension developed.

Table 1: Mineralocorticoid receptor animal morphology and characteristics

	WKY		SHR		
	NC	SPIR	NC	SPIR	EPL
	(n=5)	(n=4)	(n=8)	(n=8)	(n=7)
Body weight (g)	332 ± 6	325 ± 4	334 ± 4	321 ± 4*	320 ± 3*
Heart weight/body weight ratio (mg g^{-1})	3.00 ± 0.13	3.40 ± 0.36	3.54 ± 0.20	3.63 ± 0.07	3.39 ± 0.07
Heart weight/tibial length ratio (mg mm^{-1})	26.56 ± 1.33	28.44 ± 2.55	31.22 ± 1.79	34.12 ± 2.45	29.09 ± 0.46
Lung weight/body weight ratio (mg g^{-1})	7.70 ± 0.81	5.43 ± 1.25	8.10 ± 1.01	7.28 ± 0.90	10.05 ± 0.51
Plasma sodium (mmol/L)†	149 ± 5	178 ± 16	172 ± 6	171 ± 9	152 ± 3
Plasma potassium (mmol/L)†	5.5 ± 0.2	5.9 ± 0.7	6.0 ± 0.8	5.8 ± 0.3	5.1 ± 0.3
Plasma creatinine (mg/dL)†	0.40 ± 0.03	0.56 ± 0.09	0.44 ± 0.03	0.50 ± 0.05	0.40 ± 0.01

Values are means ± SEM. †N=5 for plasma sodium, potassium, and creatinine. *$p > 0.05$ compared to SHR_{NC}.

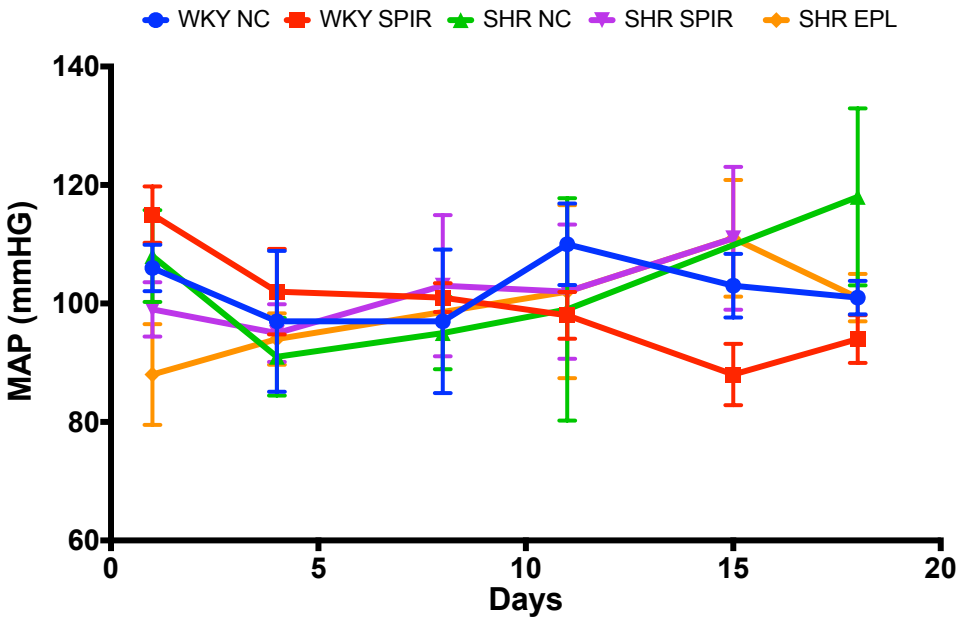

Figure 6: Conscious mean arterial blood pressure (MAP) measured by tail-cuff (n=4 in all groups). NC, normal choiw; SPIR, spironolactone; EPL, eplerenone.

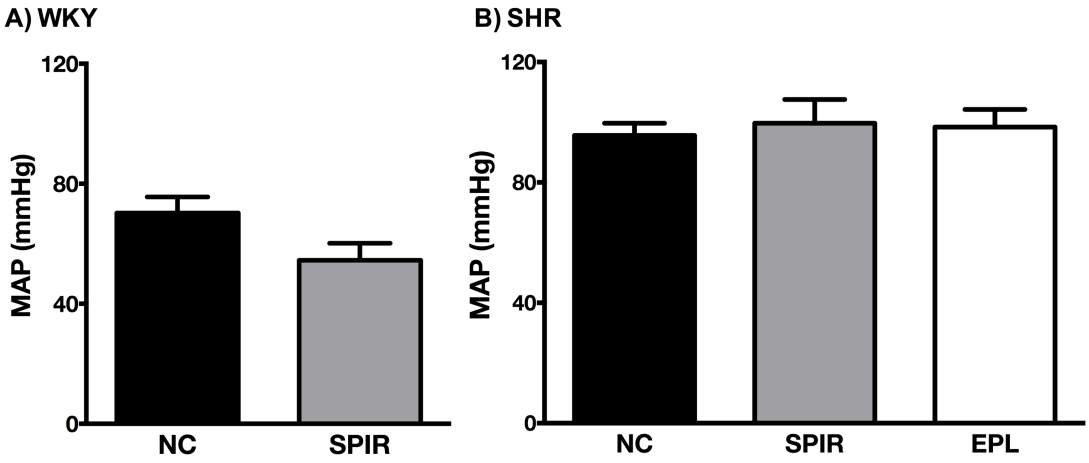

Figure 7: Baseline MAP in A) WKY and B) SHR animals post-decerebration. Dietary treatment with normal chow (NC), spironolactone (SPIR), or eplerenone (EPL) did not alter baseline MAP within either group.

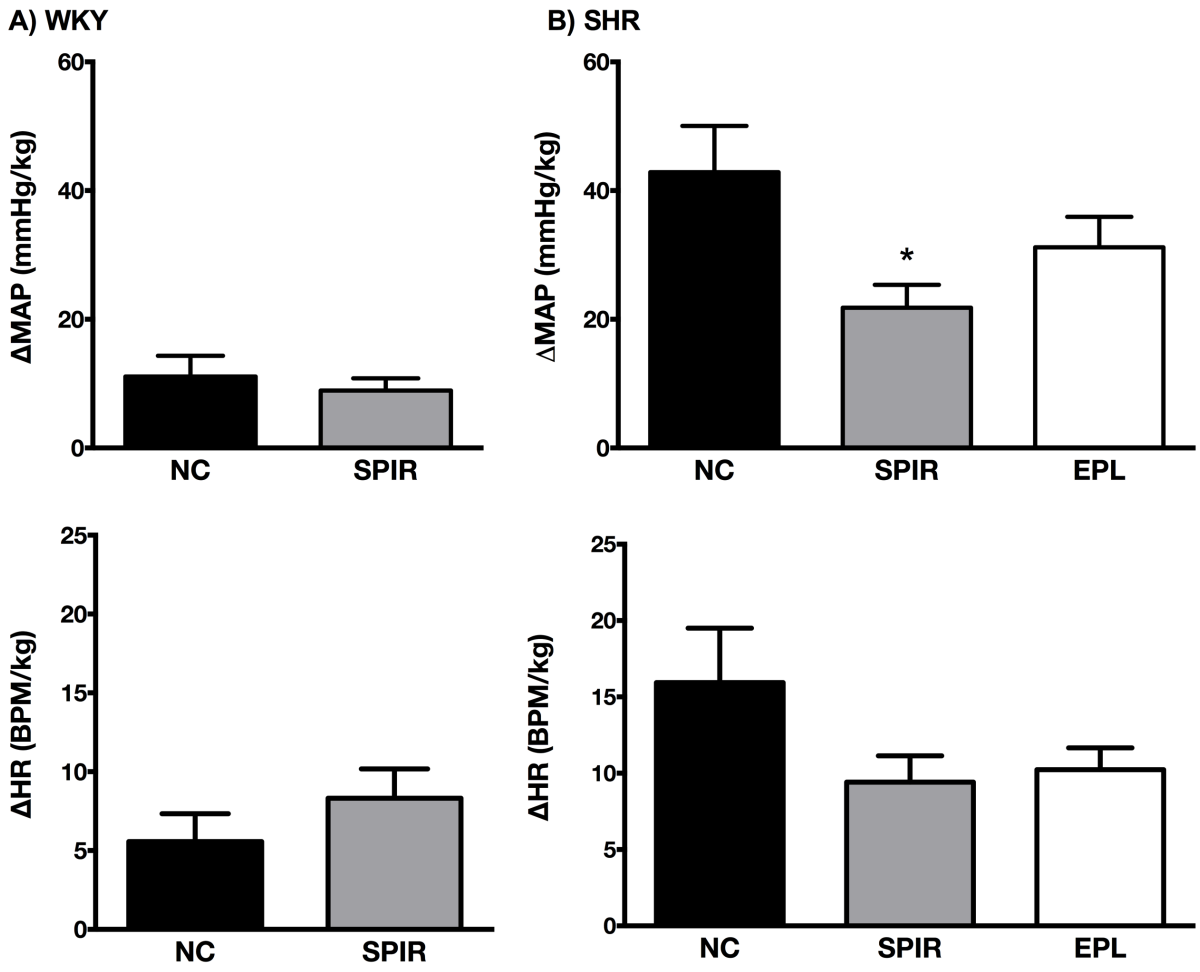

Figure 8: MAP and HR response to activation of the EPR by electrically-induced muscle contraction in A) WKY and B) SHR. * $p < 0.05$ for SPIR vs. NC. Normal chow, NC; spironolactone, SPIR; or eplerenone, EPL.

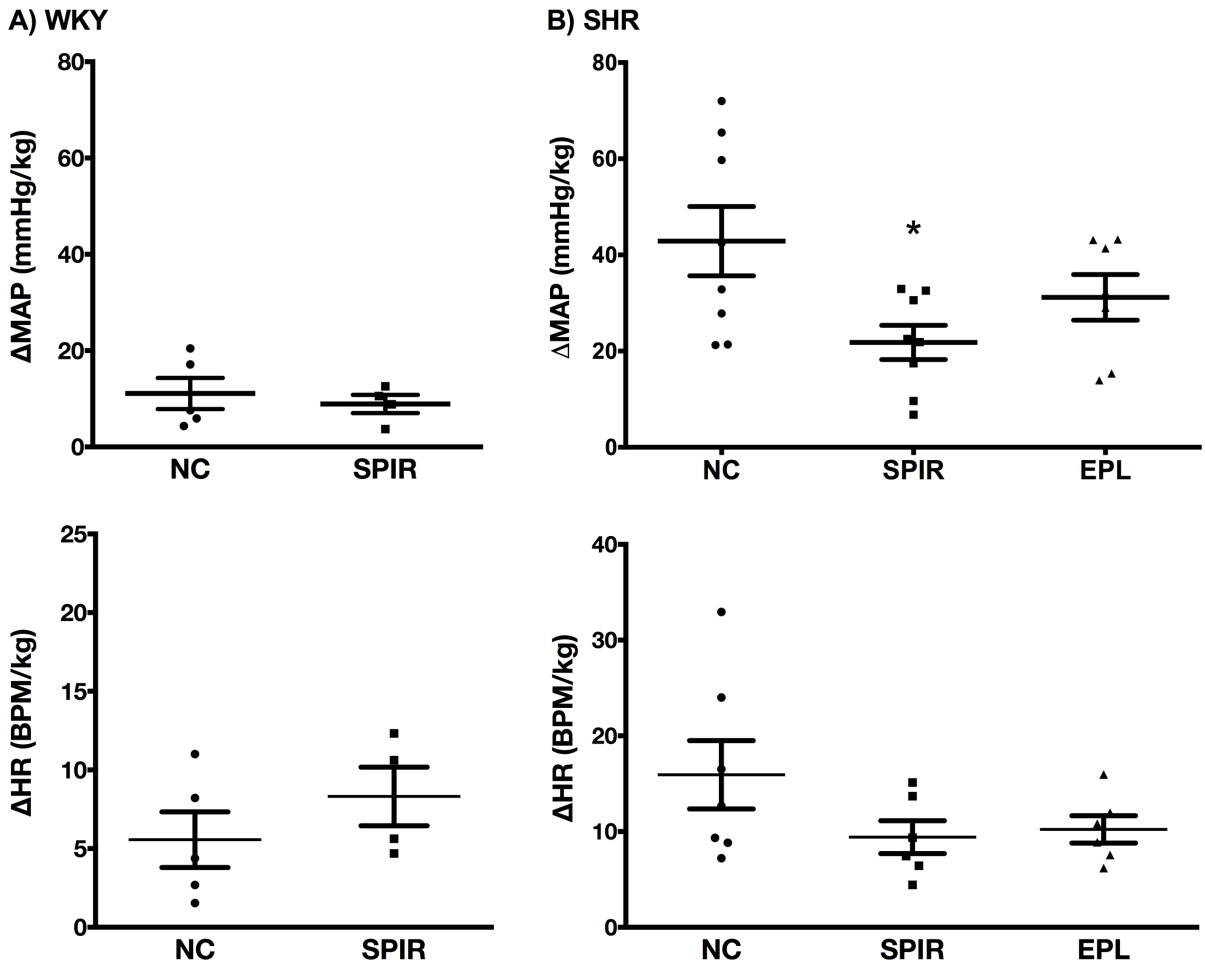

Figure 9: Individual MAP and HR responses to activation of the EPR by electrically-induced muscle contraction in A) WKY and B) SHR. Normal chow, NC; spironolactone, SPIR; or eplerenone, EPL.

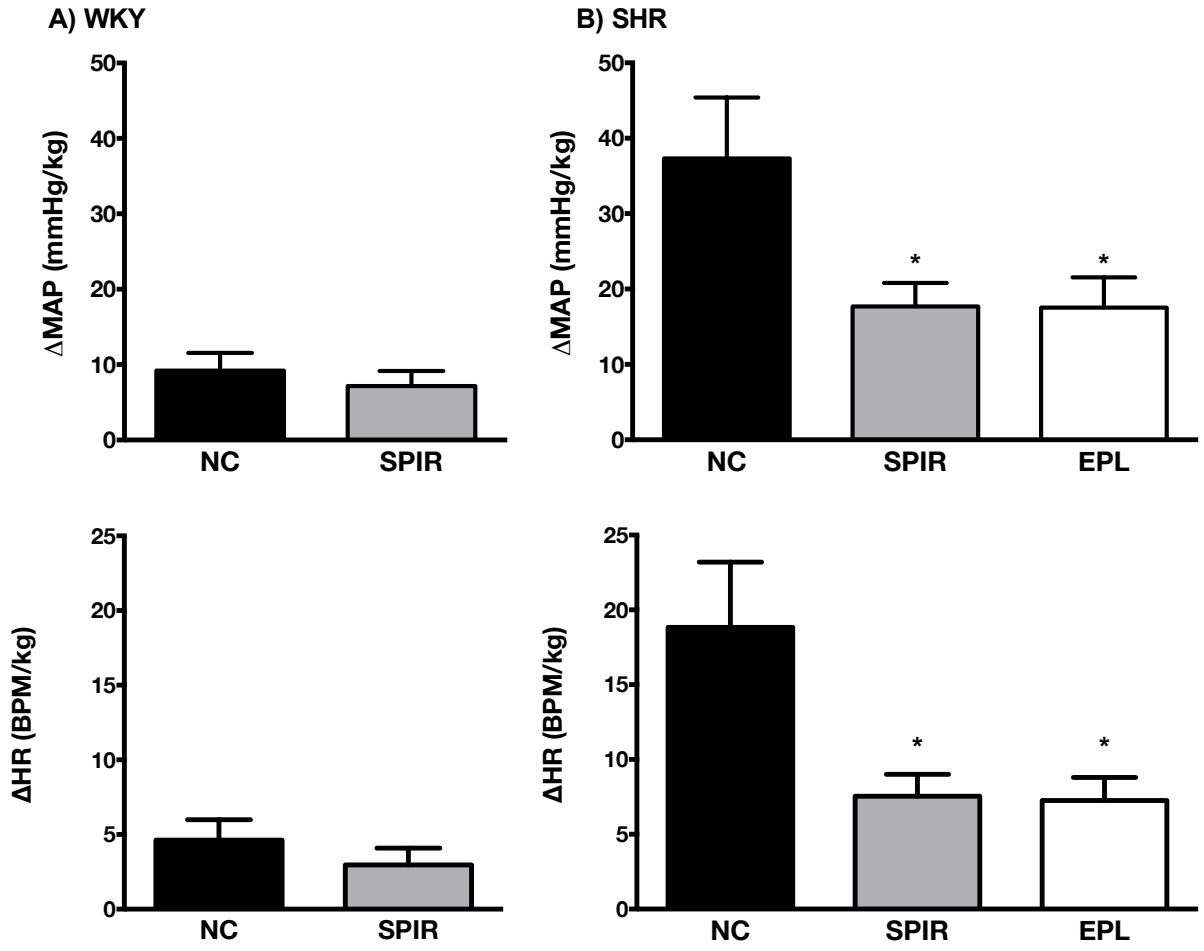

Figure 10: MAP and HR response to activation of the mechanoreflex via passive muscle stretch in A) WKY and B) SHR. * $p < 0.05$ compared to normal chow. Normal chow, NC; spironolactone, SPIR; or eplerenone, EPL.

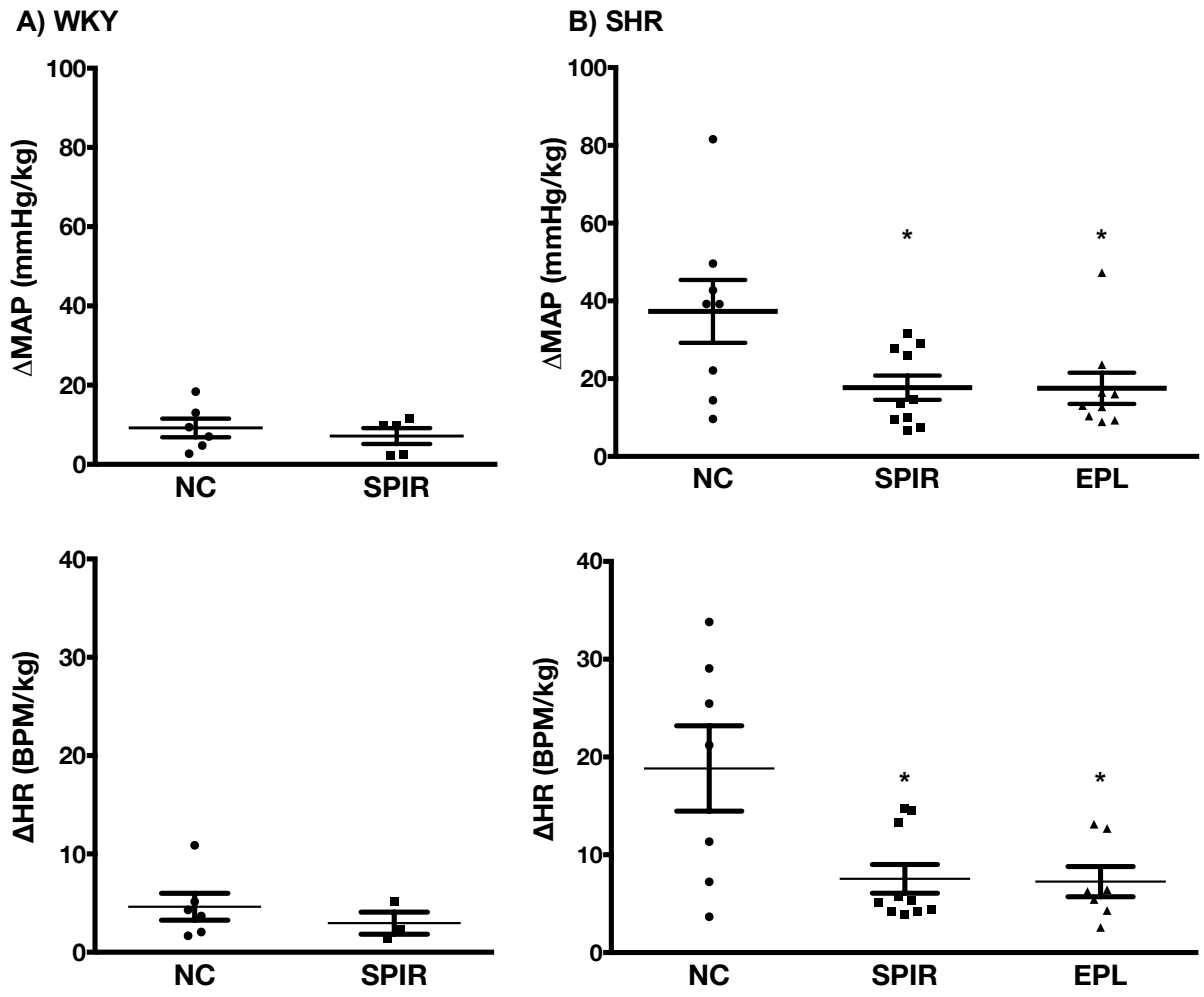

Figure 11: Individual MAP and HR responses to activation of the mechanoreflex via passive muscle stretch in A) WKY and B) SHR. * $p < 0.05$ compared to normal chow. Normal chow, NC; spironolactone, SPIR; or eplerenone, EPL.

Effects of Mineralocorticoid Receptor Antagonist Treatment on the Cardiovascular Response to Mechanoreflex Activation

Mechanically sensitive afferent neurons were preferentially activated by utilizing a passive stretch. Compared to animals fed NC, There was no appreciable difference in the HR and MAP responses to passive muscle stretch in WKY animals receiving SPIR treatment (Figure 10A). In contrast, treatment with both SPIR and EPL induced a significant attenuation in the tachycardic and pressor responses (one-way ANOVA, $p < 0.05$, Figure 10B). In SHR, there was no difference in the reduced effect between SPIR and EPL treatment. While there was no difference in tachycardic responses in WKY animals treated with SPIR (Figure 10A), there was a significant attenuation of response in SHR animals treated with both SPIR and EPL (Figure 10B). Although the amount of tension generated during passive muscle stretch was similar in all experiments, cardiovascular data have been normalized to the amount of tension developed to remain consistent with contraction studies.

GABA STUDIES

Characterization of Animals

Consistent with previous reports, baseline MAP was significantly elevated in all SHR vs. WKY after decerebration (196.7 ± 3.7 mmHg vs. 143.6 ± 7.3 mmHg, respectively; *p < 0.0001).*

Activation of the mechanically-sensitive component of the EPR via passive muscle stretch elicited an exaggerated MAP response in SHR compared to WKY (31.73 ± 3.97 mmHg kg^{-1} vs. 21.08 ± 1.89 mmHg kg^{-1}, $p < 0.05$). Also consistent with previous studies, an exaggerated response in heart rate was observed with passive stretch in SHR vs. WKY (24.95 ± 5.01 BPM kg^{-1} vs. 13.32 ± 1.87 BPM kg^{-1}, respectively; $p < 0.05$). Similarly, SHR vs. WKY comparisons were also consistent with previous studies when looked at by experimental groups (**Table 2**). The data does indicate early signs of heart failure and left ventricular hypertrophy in SHR groups. This is also consistent with characteristics reported in previous reports including SHR animals. It should be noted that although the amount of tension generated during passive muscle stretch was similar in all experiments, cardiovascular data have been normalized to the amount of tension developed to remain consistent with mineralocorticoid receptor studies. Figure 12 shows a representative animal of microdialysis probe placement.

Effects of GABA Synthesis Inhibition (3-Mercaptopropionic Acid) on the Cardiovascular Response to Mechanoreflex Activation

Following an initial 45-minute microdialysis of aCSF and subsequent mechanoreflex testing, a subset of SHR and WKY animals were microdialyzed with 3-mercaptopropionic acid (3-MP), a GABA synthesis inhibitor. There were no significant differences in the pressor or tachycardic responses after 3-MP microdialysis in SHR animals (Figure 13). However, compared to initial aCSF conditions, there was a significant attenuation of the pressor response post 3-MP

microdialysis in WKY animals (Figure 14, one-way ANOVA, $p < 0.05$), although there were no differences between any groups following the aCSF washout period (aCSF: 22.86 ± 1.76 mmHg kg^{-1}, 3-MP: 12.11 ± 2.20 mmHg kg^{-1}, washout: 13.95 ± 4.47 mmHg kg^{-1}). Heart rate responses to mechanical activation of group III afferent fibers in WKYs followed the same trend as observed in the blood pressure response, but did not reach statistical significance.

Effects of a $GABA_A$ Receptor Antagonist (Bicuculline) on the Cardiovascular Response to Mechanoreflex Activation

Treatment of SHR (Figure 15) and WKY (Figure 16) animals with the microdialysis of bicuculline (BIC), a $GABA_A$ receptor antagonist, within the NTS did not produce any significant changes in the pressor or tachycardic response to activation of the muscle mechanoreflex via passive stretch. WKY animals showed an interesting, but statistically insignificant trend towards attenuation following treatment with BIC in the blood pressure response to passive stretch.

Effects of a $GABA_B$ Receptor Antagonist (Saclofen) on the Cardiovascular Response to Mechanoreflex Activation

In a final subset of animals, $GABA_B$ receptor activity was blocked through the microdialysis of saclofen (SAC) within the NTS. Neither SHR (Figure 17) nor WKY (Figure 18) groups showed

any significant difference in the blood pressure or heart rate response to activation of the muscle mechanoreflex following SAC microdialysis compared to aCSF control conditions.

Table 2: GABA Animal morphology and characteristics

	3-MP		BIC		SAC	
	WKY	SHR	WKY	SHR	WKY	SHR
	(n=7)	(n=8)	(n=5)	(n=5)	(n=4)	(n=4)
Body weight (g)	293 ± 7	319 ± 7	322 ± 14	326 ± 4	317 ± 6	314 ± 4
Heart weight/body weight ratio (mg g^{-1})	3.25 ± 0.09	3.76 ± 0.27 *	3.19 ± 0.06	3.46 ± 0.12 *	3.03 ± 0.05	3.52 ± 0.10 *
Heart weight/tibial length ratio (mg mm^{-1})	25.29 ± 0.81	31.02 ± 1.73 *	26.35 ± 0.89	29.77 ± 1.05 *	24.90 ± 0.47	30.34 ± 0.99 *
Lung weight/body weight ratio (mg g^{-1})	9.08 ± 0.91	9.66 ± 0.65 *	7.36 ± 0.27	8.76 ± 0.72 *	8.89 ± 0.95	8.17 ± 0.28
Baseline MAP (mmHg)	160.61 ± 7.40	197.98 ± 4.76 *	147.44 ± 4.64	192.20 ± 8.20 *	98.64 ± 11.75	199.03 ± 1.63 *

Values are ± SEM. * Indicates significantly different from WKY, $p < 0.05$. Wistar-Kyoto, WKY; spontaneously hypertensive, SHR; 3-mercaptopropionic acid, 3-MP; bicuculline, BIC; saclofen, SAC.

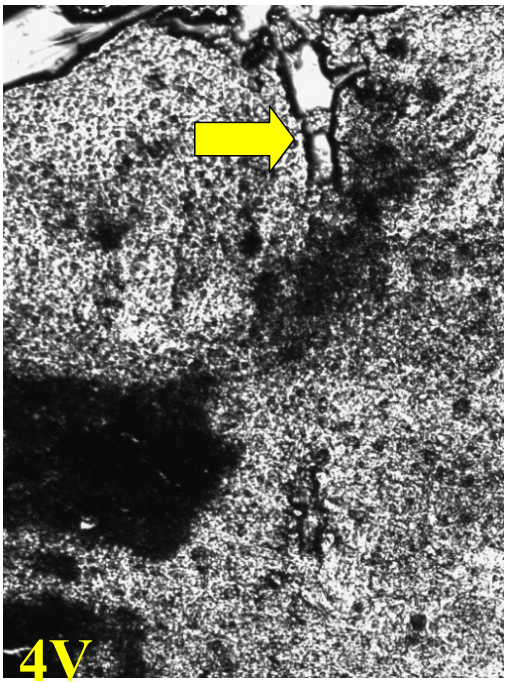

Figure 12: Microdialysis probe placement in one representative animal. Probe was placed 0.5 mm lateral to the fourth ventricle (4V) and 0.5 mm below the dorsal medullary surface. Photomicrograph (4x) shows the probe track within the NTS. Minimal structural damage was caused by placement of the dialysis probe (arrow).

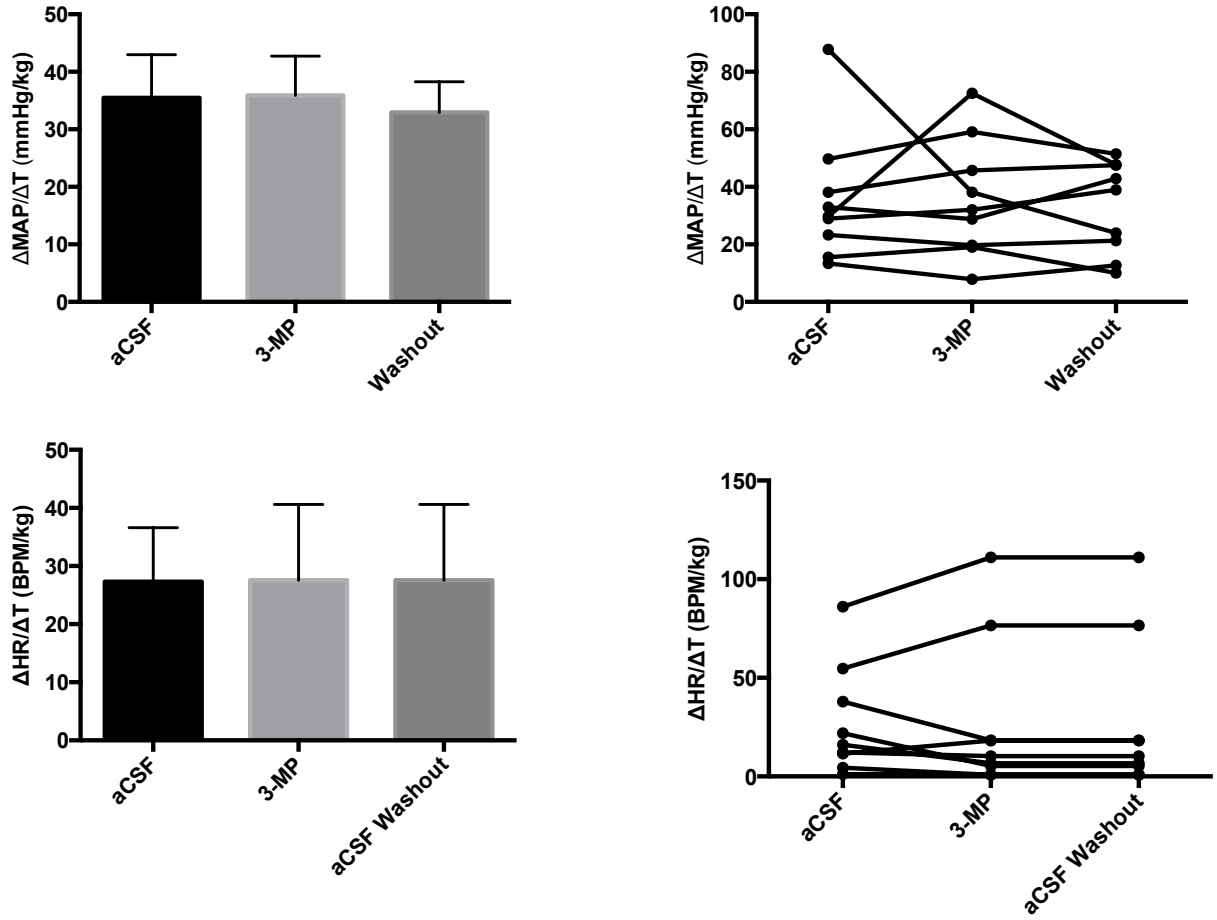

Figure 13: Compared to aCSF control conditions, microdialysis of 3-MP into the NTS did not have any significant effect on the blood pressure nor heart rate response to activation of the muscle mechanoreflex via passive muscle stretch in SHR. Artificial cerebrospinal fluid, aCSF; 3-mercaptopropionic acid, 3-MP. Left panels depict group mean data. Right panels illustrate the responses of each rat.

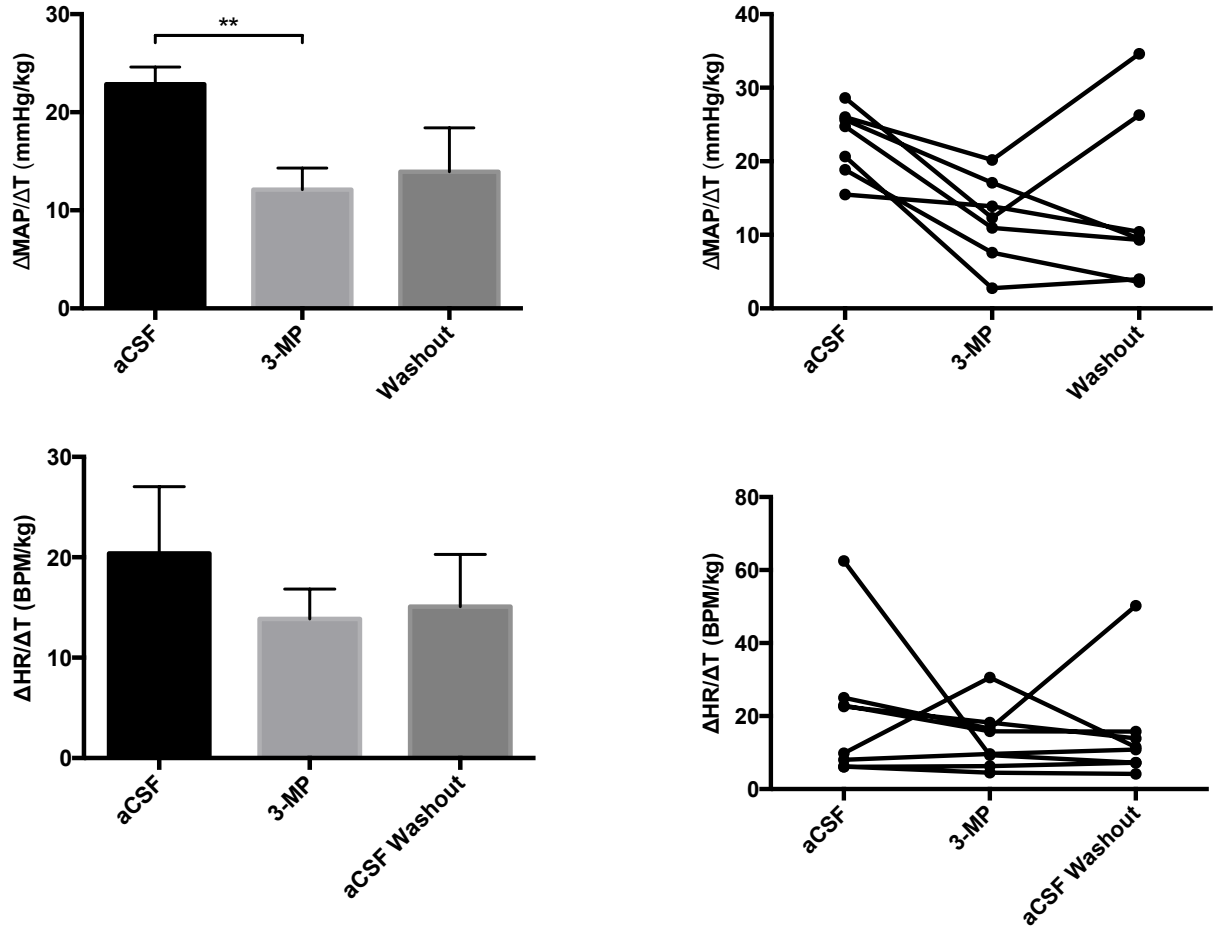

Figure 14: Microdialysis of 3-MP into the NTS of WKY yielded a significant attenuation in the pressor response to passive muscle stretch versus the control aCSF condition. A similar trent was noted in the HR response to mechanoreflex activation, although statistical significance was not reached. ** Indicates significantly different from aCSF condition, $p < 0.05$. Artificial cerebrospinal fluid, aCSF; 3-mercaptopropionic acid, 3-MP. Left panels depict group mean data. Right panels illustrate the responses of each rat.

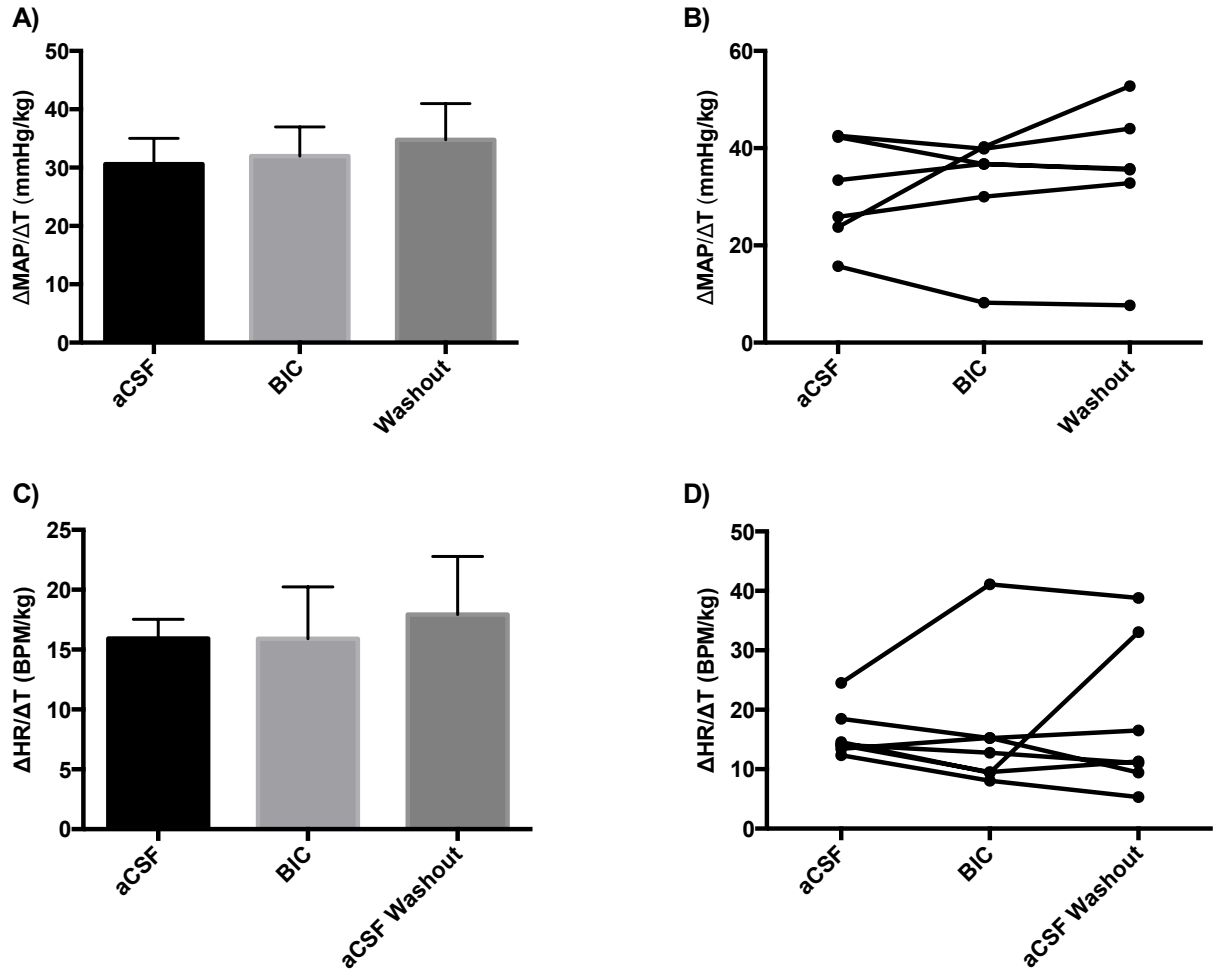

Figure 15: Treatment of SHR animals with the microdialysis of BIC within the NTS showed no significant differences in the blood pressure and heart rate responses to passive muscle stretch compared to aCSF control conditions. Artificial cerebrospinal fluid, aCSF; bicuculline, BIC. Left panels depict group mean data. Right panels illustrate the responses of each rat.

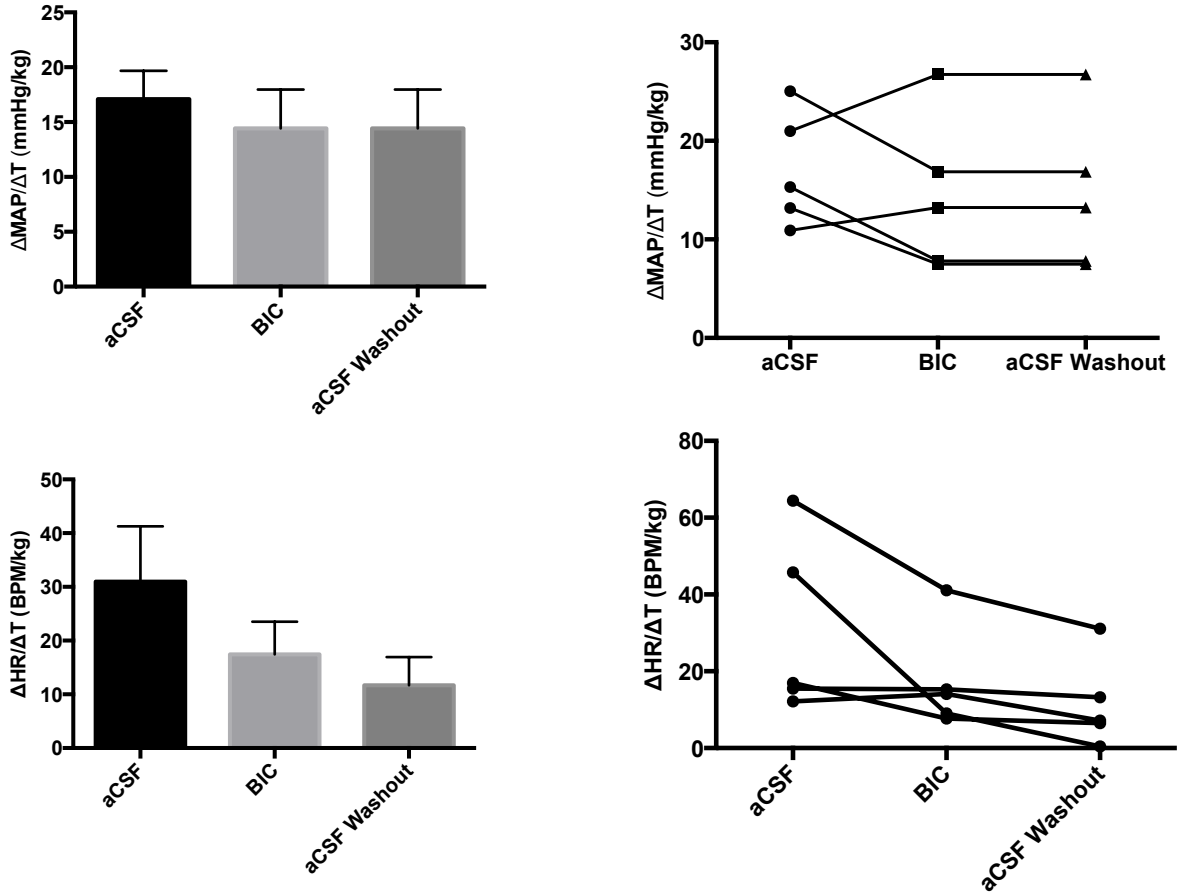

Figure 16: WKY animals treated with the $GABA_A$ inhibitor BIC via microdialysis within the NTS showed no difference in the pressor response to passive muscle stretch compared to aCSF control conditions. A trend towards attenuation in the heart rate response to activation of the muscle mechanoreflex was noted after BIC microdialysis, although differences were statistically insignificant. Artificial cerebrospinal fluid, aCSF; bicuculline, BIC. Left panels depict group mean data. Right panels illustrate the responses of each rat.

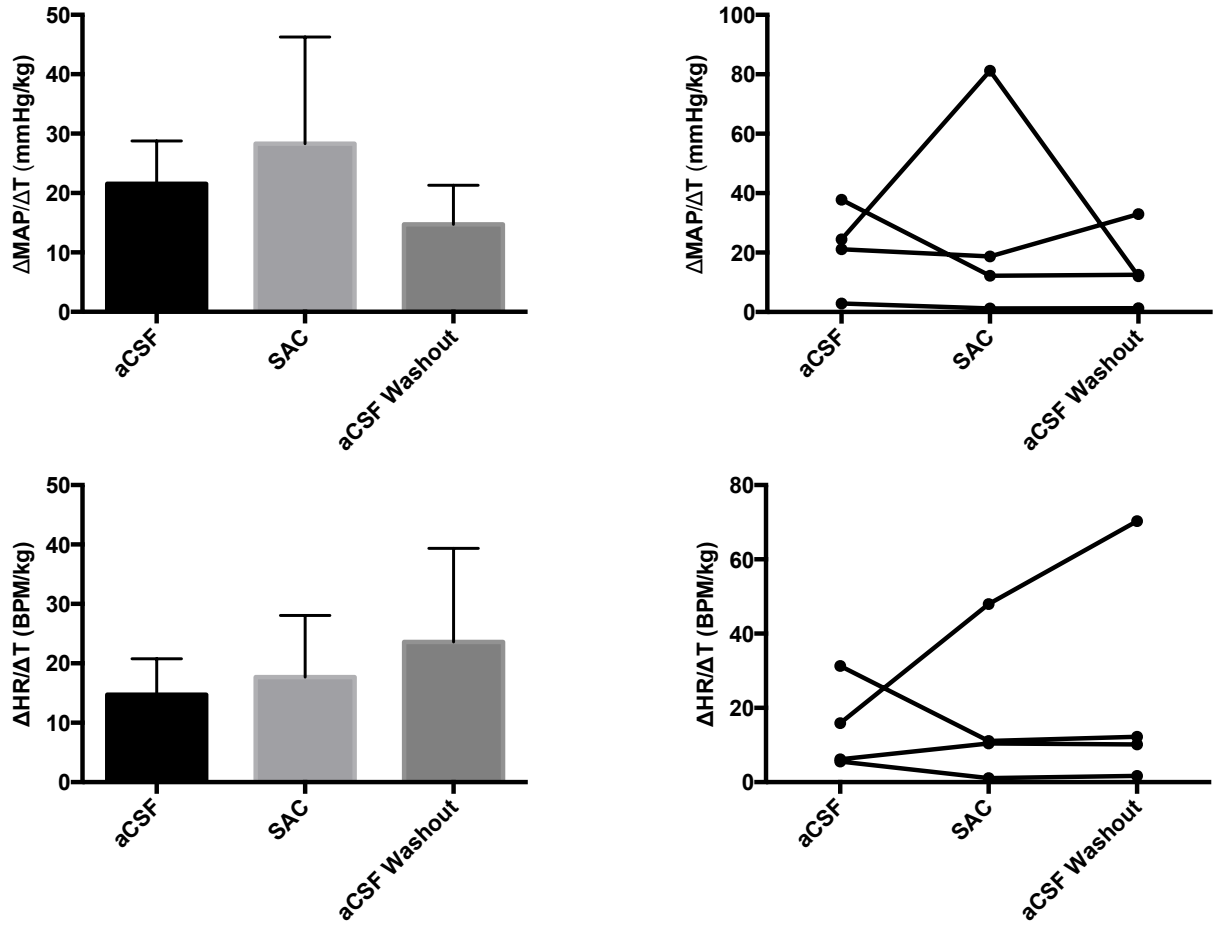

Figure 17: SHR were microdialyzed with SAC within the NTS region. There were no differences in blood pressure or heart rate responses to passive stretch after SAC treatment compared to aCSF control conditions. Artificial cerebrospinal fluid, aCSF; saclofen, SAC. Left panels depict group mean data. Right panels illustrate the responses of each rat.

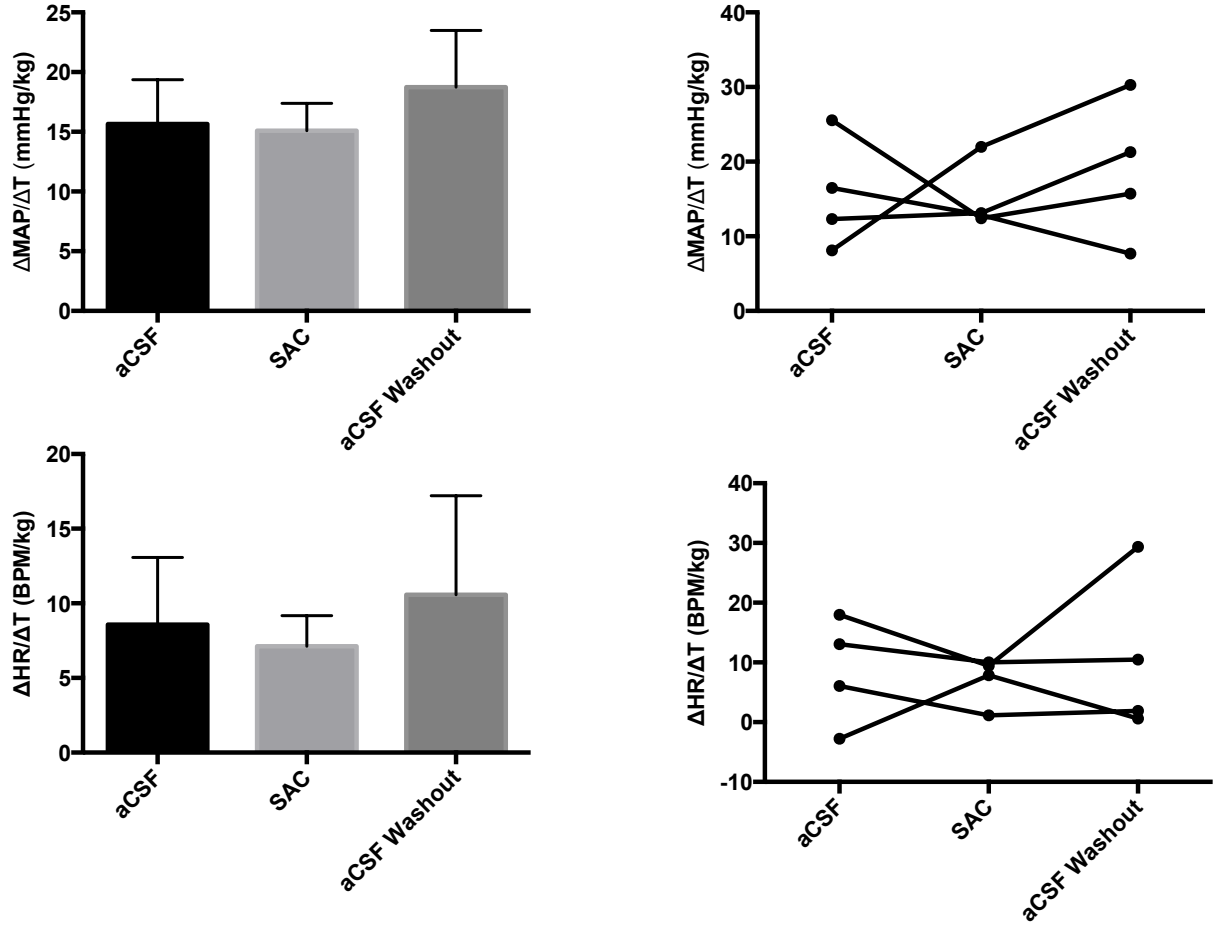

Figure 18: Compared to aCSF control conditions, there were no significant differences in pressor or tachycardic responses to mechanoreflex activation in WKY rats in which SAC was microdialyzed within the NTS. Artificial cerebrospinal fluid, aCSF; saclofen, SAC. Left panels depict group mean data. Right panels illustrate the responses of each rat.

CHAPTER FIVE:
CONCLUSIONS AND RECOMMENDATIONS

MINERALOCORTICOID RECEPTOR STUDIES

Effects of Treatment with Mineralocorticoid Receptor Agonists

There were no significant differences in morphometric measurements of the animals, other than a significant loss of body mass in SHR treated with SPIR or EPL when compared to SHR treated with NC. One of the initial hopes from this data would be that, in SHR, heart weight-to-body weight and heart weight-to-tibial length ratios would show a significant decrease compared to NC treated animals. Increases in these ratios are indicative of the development of cardiomyopathy, such as seen in the development of left ventricular hypertrophy in Sprague-Dawley rats treated with endogenous aldosterone via osmotic mini pumps (165). A significant decrease would be seen as a positive sign of treatment in these animals. Similar conclusions could have been potentially made if changes to the lung weight-to-body weight ratios had been observed. However, the data does not indicate any significant improvement in these measures. This may be due to the relatively short (3 weeks) period of treatment, or due to a lack of a significant effect of SPIR and EPL on these ratios. Plasma samples were drawn from each group of animals in the study. Again, there were no significant differences detected which may also be

due to either the relatively short treatment period or an absence of any significant change due to treatment.

One of the most surprising results from these studies is that SPIR or EPL treatment did not significantly alter baseline blood pressure following three weeks of dietary consumption in either WKY or SHR. It is possible that detection of a true difference in baseline MAP due to treatment was obscured by the relatively low fidelity of the conscious tail-cuff measurement used. Moreover, the design of the acute-terminal experimental protocol may have likewise prevented observation of baseline differences in BP between groups. Animals were decerebrated before the measurement of baseline blood pressures in this protocol. The mechanisms through which SPIR and EPL act centrally have not yet been fully elucidated. By decerebrating the animals, central circuits in portions of the brain that could have been affected by treatment with SPIR and EPL may have been removed.

Mineralocorticoid Receptors and the Exercise Pressor Reflex

Treatment of WKY with SPIR did not have a significant effect on the pressor or tachycardic responses to activation of the EPR compared to animals fed NC. Based on this result, it was determined that treatment with EPL in normotensive WKY animals would not be necessary as there was minimal effect of mineralocorticoid receptor activation on EPR activity. In SHR animals, there was a significant attenuation of the exaggerated pressor response to EPR

activation in SPIR treated animals. Tachycardic responses in these animals showed a strong trend toward attenuation, but did not reach significance. Likewise, responses to EPR activation in animals treated with EPL showed trends towards attenuation of MAP and HR, but also did not reach statistical significance. These measures did not reach statistical significance in HR response in SPIR treated animals and both MAP and HR response in EPL treated animals most likely because of a wide variability in the tensions produced in hindlimb contraction via electrical stimulation in early experiments. This variability was reduced as the protocol was repeated and my surgical technique improved. The significant attenuation in MAP in SPIR treated SHR and the trend towards attenuation in other measures is relevant as it indicates that blocking the actions of aldosterone via antagonizing mineralocorticoid receptors may improve EPR function in hypertension. When treating hypertensive patient populations, a key goal for any anti-hypertensive therapy is to reduce the exaggerated cardiovascular response to physical therapy to a point at which an exercise regimen can safely be introduced as part of they therapy. While the reductions in HR and BP during EPR stimulation after SPIR and EPL treatment may not be statistically significant in this study, they do imply that treatment attenuates EPR over-activity and may reduce some of the risk accompanying exercise in hypertensive patients.

Mineralocorticoid Receptors and the Muscle Mechanoreflex

Following activation of the EPR, the mechanically sensitive arm of the reflex was isolated and stimulated by passive muscle stretch. These data show that SPIR and EPL treatment significantly

attenuated the exaggerated cardiovascular response to mechanoreflex activation in hypertensive animals. As during muscle contraction protocols, blockade of mineralocorticoid receptors with SPIR had no effect on the stretch-induced MAP and HR responses in normotensive WKY. The variability in these experiments was greatly reduced because of an easier surgical protocol and the ability to produce a more consistent stretch between individual experiments. While the pressor responses to mechanoreflex activation were still elevated in SHR after SPIR and EPL treatment when compared to WKY, they were reduced as compared to SHR fed NC. This latter finding shows antagonizing MR with either SPIR or EPL significantly attenuates muscle mechanoreflex over-activity in hypertension.

Recommendations

There are multiple venues for expanding this work in the future. First and foremost in these recommendations is the inclusion of future experiments that look at the chemically-sensitive arm of the EPR (*i.e.* the muscle metaboreflex). Another key area in which the physiological component of these studies can be expanded is the measurement of renal SNA in hypertensive animals treated with MR antagonists. The role for mineralocorticoid receptor activation in cardiovascular regulation can also be further investigated by performing controlled exercise studies in SHR rats receiving SPIR and EPL. While this technique would not isolate the EPR response from central command or arterial baroreceptor reflex, these experiments would validate

further investigations into the EPR and its individual components, the mechano- and metaboreflexes.

In these studies, it was shown that reductions in the pressor and tachycardic response to EPR and mechanoreflex activation in hypertension are mediated in a MR-specific manner. SPIR has been shown to bind with high affinity to mineralocorticoid receptors, androgen receptors, progesterone receptors, and glucocorticoid receptors (40). By using EPL, which has a higher selectivity for mineralocorticoid receptors, we are able to demonstrate that the mechanism of action for SPIR and EPL is most likely conducted through an MR receptor-mediated pathway. The decision between using SPIR or EPL in patients has great clinical relevance. While common side effects of SPIR such urinary frequency, drowsiness, dry skin, and rashes are relatively benign, there are also a number of gender specific effects. Due to interactions of SPIR with the androgen and progesterone receptors, males may experience such side effects as gynecomastia, general feminization, testicular atrophy, reversible infertility, and sexual dysfunction, including loss of libido and erectile dysfunction (139), while females may have menstrual irregularities and breast tenderness and enlargement (82). These undesirable side effects, especially in men, can lead to lowered compliance in adhering to treatment regimens. EPL is not without its downsides either. While EPL does not have the androgenic side effects that SPIR does, EPL is a much more costly drug, often times placing it above the budget of lower-income patients. Physicians must balance these concerns when selecting the best, personalized therapy for patients.

The mechanisms through which activation of mineralocorticoid receptors induce EPR and mechanoreflex over-activity in hypertension have yet to be elucidated, but could either through potential changes in MR receptor expression, or through the downstream effects of enzymes known to associate with MR activity such as HSD2, ENaC, or serine/threonine-protein kinase 1 **(**SGK1).

GABA STUDIES

Central-Acting GABA Synthesis and the Muscle Mechanoreflex

A significant attenuation in the MAP and HR response to passive stretch was observed in WKY animals after 3-MP microdialysis that was not seen in SHR animals. As GABA synthesis is controlled presynaptically, the lack of a decreased effect in hypertensive animals suggests that there may be changes in postsynaptic receptor expression. Alternatively, there may be a change in the sensitivity of postsynaptic GABA receptors that accounts for the difference between normotensive and hypertensive animals. Regardless of the mechanism, the findings suggest that GABA plays a significant role in the central modulation of mechanoreflex function within the NTS of normotensive animals. This modulatory ability appears to be lost after the development of hypertension.

Role of Central $GABA_A$ Receptors in Modulating Mechanoreflex Function

Similar to treatment with 3-MP, antagonism of $GABA_A$ receptors with BIC produced a trend towards a reduction in the cardiovascular response to activation of the mechanoreflex in WKY, albeit statistically insignificant. Also similar to the GABA synthesis experiments, the microdialysis of BIC within the NTS of SHR animals had no appreciable effect on the pressor or tachycardic responses to passive muscle stretch. As the $GABA_A$ receptor resides on the postsynaptic membrane (150), this supports the concept that there is a change in the sensitivity of GABAergic neurons post-synaptically in hypertensive animals. Although not statistically significant, the findings further suggest that the modulatory actions of GABA on mechanoreflex function within the NTS of normotensive rats are mediated through $GABA_A$ receptors.

Role of Central $GABA_B$ Receptor in Modulating Mechanoreflex Function

There were no significant differences in the MAP or HR responses to mechanoreflex activation before or after the microdialysis of SAC within the NTS in either WKY or SHR. This may be due to the fact that the number of animals used in this study was relatively low. Another possible explanation is that $GABA_B$ receptors are present on pre- and postsynaptic neuron terminals and may exhibit differential activity based on their localization. The presence of populations in each location may mask the effects of a subpopulation in this study. In any event, the results suggest

that $GABA_B$ receptors are not involved in the central processing of mechanoreflex afferent information within the NTS of either normotensive or hypertensive animals.

Recommendations

At present, the contribution of GABA to the modulation of mechanoreflex function within the NTS is the only component of the EPR that was studied, largely due to the time constraints required for the protocol. The experimental design takes approximately 6-7 hours to complete, limiting the number of different experiments that can be conducted within one animal while the preparation is still viable. Experiments exploring the role of GABAergic activity in the central processing of metaboreflex sensory input, as well as EPR afferent information within the NTS are still required in both normotensive and hypertensive animals. Additionally, quantifying the expression of the GABA receptor subtypes within the NTS in SHR compared with WKY may provide additional insights into the regulation of muscle reflex activity via GABAergic neurons.

SUMMARY REMARKS

The ultimate goal of all research in hypertension is to find ways to prevent the onset of the disease in individuals who do not have it and to find cures for those who already have chronically elevated blood pressures. Investigations into the mechanisms behind the development and onset of hypertension will ultimately lead to better treatment modalities

towards these ends. The simple truth is that as hypertension rates continue to increase in every age demographic, race, and country in the world, the problem is only going to become more manifest. Exercise is the only treatment that has been consistently shown to have a positive effect in reducing hypertension in patients. The problem is that with exercise in hypertensive patients, there is an increased risk of myocardial ischemia, infarction, cardiac arrest, stroke, and possibly death during and after physical activity (78, 116, 144, 162, 163).

Based on previous studies in our lab and others, understanding the mechanisms underlying the pathogenesis of EPR over-activity in hypertension is key to in making inroads towards the safe prescription of exercise in this disease. The studies in this dissertation advance our knowledge in this area by: 1) demonstrating SPIR and EPL can be used as effective treatments to reduce exaggerated cardiovascular responses produced by EPR activation in hypertension, and 2) GABA maintains the ability to modulate muscle mechanoreflex activity within the NTS via activation of $GABA_A$ receptors in normotension with this ability being compromised after the development of hypertension. The results of these studies support the original hypothesis that antagonizing mineralocorticoid receptors in hypertensive animals reduces EPR over-activity towards a more normal phenotype. Conversely, the experiments do not support the original hypothesis that blocking GABA synthesis and/or antagonizing GABA receptors within the NTS normalizes the cardiovascular response to mechanoreflex activation in hypertensive animals. As is always the case with basic science investigations using animal models, translational studies are

needed before the findings of this research can be applied to the treatment of EPR dysfunction in hypertensive humans.

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