A FUNCTIONAL ANALYSIS OF THE MECHANICALLY AND METABOLICALLY SENSITIVE COMPONENTS OF THE SKELETAL MUSCLE EXERCISE PRESSOR REFLEX IN HYPERTENSION

by

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ABSTRACT

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Background **-** In hypertension, physical activity creates a risk for adverse cardiac events by causing exaggerated increases in mean arterial pressure (MAP) and heart rate (HR). One physiological mechanism responsible for this cardiovascular hyperactivity is the exercise pressor reflex (EPR). The EPR is a neural pathway originating in skeletal muscle that contributes to cardiovascular regulation during exercise. The EPR is composed of two afferent arms: one is comprised of mostly mechanically sensitive, group III afferent fibers that respond to muscle stretch and pressure, and the other is

predominantly metabolically sensitive, group IV afferent fibers that respond to the chemical milieu surrounding working muscle. Knowing that the EPR is overactive in hypertension, it is hypothesized that both the mechanically sensitive and metabolically sensitive components of the EPR are overactive and help drive the exaggerated cardiovascular response to exercise in hypertension. ***Methods and Results –*** To test this hypothesis, an experimental protocol was developed to test the two pathways separately in male normotensive Wistar-Kyoto (WKY) and in male Spontaneously Hypertensive (SHR) rats. The mechanically sensitive component of the EPR was activated by passively stretching the triceps surae muscle. At maximal tension, the increases in MAP and HR were significantly greater in SHR (MAP = 28 ± 5 mmHg; HR = 8 ± 2 bpm) compared to WKY (MAP = 13 ± 2 mmHg; HR = 5 ± 1 bpm) animals. In addition, the cardiovascular response to passive stretch over a range of submaximal intensities was larger in the SHR than in the WKY group. The metabolically sensitive component of the EPR was activated by administering the pungent chemical capsaicin into the rats' hindlimb arterial supply. Capsaicin is known to selectively stimulate the group IV afferent fibers. The cardiovascular responses were greater in SHR animals than in WKY animals. Specifically, the increases in MAP evoked by the capsaicin doses of 0.3 and 1.0 μg/100 μL were significantly larger for SHR (47 ± 7 mmHg and 70 ± 7 mmHg, respectively) rats than for WKY (29 ± 3 mmHg and 43 ± 9 mmHg, respectively) rats. ***Conclusions –*** The abnormal cardiovascular response to exercise that occurs in hypertension is mediated, in part, by the overactivity of the mechanically

and metabolically sensitive components of the exercise pressor reflex. Knowledge about the mechanoreflex and metaboreflex dysfunction in hypertension could lead to therapeutic interventions that would increase exercise tolerance and allow hypertensive individuals to reap the benefits of physical activity without risk for the generation of adverse cardiovascular events.

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CHAPTER 1

INTRODUCTION

1.1 The Cardiovascular System and Hypertension

The definition of hypertension is a diastolic pressure greater than or equal to 90 mmHg or a systolic pressure above or equal to 140 mmHg. In the United States, hypertension poses a major problem as approximately 30% of the population has it at any time and it can lead to stroke and heart disease(36). During the 1990's, the original participants of the Framingham Heart Study, a community-based cohort study begun in the 1940's, and their offspring cohorts, added in the 1970's, were examined at two and four year intervals, respectively. Blood pressure readings and levels of cardiovascular risk factors were taken. Of the 5,296 participants involved, 27% younger than 60, 63% between the ages of 60 and 79, and more than 70% of ages 80 and over suffered from hypertension. In the oldest age group, less than 10% were normotensive and it was found that the absolute risk for cardiovascular events associated with hypertension increased drastically with age(65). In Western societies, blood pressure rises with age. During childhood, it increases with weight and then levels off once growth has ceased. But due to environmental factors or childhood events, a second blood pressure increase occurs late in adult life(58, 71).

Multiple health problems arise from and/or are associated with hypertension: insulin resistance, glucose intolerance, obesity, and elevated sympathoadrenal activity

which causes increased vasoconstrictor sensitivity, thus making blood pressure even more prone to rise(91, 96, 101). Left ventricular hypertrophy is a common effect of chronically elevated blood pressure which may lead to impaired ventricular filling and eventually ventricular dysfunction(132). Left ventricular wall thickness, mass, and mass index have all been shown to increase in hypertension(98). Impaired endothelium-mediated vasodilation is also prevalent in hypertension(21). In the peripheral arteries, smooth muscle hypertrophy, due to sustained elevations in blood pressure among other factors, decreases vessel compliance and together with endothelium dysfunction, causes an even greater increase in vascular resistance and blood pressure(11, 42, 99).

Increased sympathetic nerve activity, along with decreased parasympathetic nerve activity is common in hypertension(90). During the development of hypertension, sympathetic nerve activity causes increased cardiac output, but total peripheral resistance is normal. In established hypertension, when cardiac output and heart rate are normal, total peripheral resistance is elevated(47). The high vascular resistance in conjunction with vascular structural remodeling creates narrow lumen sizes and stiff vessel walls that are hypersensitive to adrenergic vasoconstrictors and not prone to vasodilation(7, 11, 95). Due to changes in the walls of the carotid and aortic arteries, baroreceptor sensitivity is decreased and heart rate is not as easily controlled(13). This perpetuates hypertension and the diminished heart rate response likely adds to left ventricular diastolic dysfunction(49).

1.2 Benefits of Exercise

Exercise is one viable treatment option for people with hypertension. In normotensive adults, aerobic exercise training has been seen to modestly decrease systolic and diastolic pressure by 4 mmHg and 3 mmHg respectively and in hypertensive individuals, decreases of 6 mmHg and 8 mmHg in resting systolic and diastolic pressures have been reported(53). Lowering blood pressure is beneficial as cardiovascular risk (morbidity and mortality) has been shown to increase linearly with increases in resting blood pressure(67).

Regular exercise is known to decrease body fat and improve metabolic processes. In rats, it has been shown that with exercise training, sympathetic nerve activity controlling heart rate is decreased and the parasympathetic vagal control of heart rate is increased. These changes in nerve activity improve the abnormal baroreflex function seen in hypertension and contribute to lower values of resting mean arterial pressure and heart rate(56, 73). Exercise also lowers total vascular resistance by decreasing arterial stiffness and increasing arterial compliance, which in turn lowers blood pressure(49). In experiments with spontaneously hypertensive rats (SHR), low-intensity exercise training has lowered blood pressure by lowering heart rate and cardiac output(126). Exercise training in hypertensive humans has been shown to decrease left ventricular mass and increase peripheral vascular function(122, 133). Specifically, after 20 weeks of low-intensity exercise training for SHR rats, their blood pressure was 60% lower than that of sedentary SHR's and the difference was significant from the

third week of exercise. Also, the exercise-trained hypertensive rats had thinner thoracic artery walls, possibly increasing compliance and lowering total vascular resistance(44).

There is concern, however, about the increased sensitivity to exercise in hypertension.

1.3 Exaggerated Cardiovascular Response to Exercise

In normotension, physical activity causes heart rate and mean arterial pressure to increase for the duration of the activity. Exercise induces an exaggerated cardiovascular response in hypertension. High peripheral resistance leads to decreases in stroke volume, oxygen uptake, and cardiac output(25). While mean arterial pressure is already elevated, systolic and diastolic pressures both rise even more, further increasing the risk for a myocardial event or stroke(39). For the beneficial effects of exercise to be realized in hypertension, physical training must first be proven as a safe, therapeutic option. Therefore, the pathophysiology of exercise must be determined. The cardiovascular response to exercise is mediated by the arterial baroreflex, central command, and the exercise pressor reflex. Recent studies from our lab have provided evidence that the exercise pressor reflex is involved in and mediates the exaggerated cardiovascular response to exercise seen in hypertension.

The exercise pressor reflex is a neural pathway originating in skeletal muscle that is composed of two afferent arms. One arm is comprised of mostly mechanically sensitive afferent fibers that respond to muscle stretch and pressure. The other arm is predominantly metabolically sensitive afferent fibers that respond to the chemical milieu surrounding working muscle(69). In order to better understand how the exercise

pressor reflex modulates the cardiovascular response to exercise in hypertension, it must be determined what component of the reflex is overactive-- mechanically or metabolically sensitive afferents. An experimental protocol was developed to test the pathways separately in normotensive and hypertensive rats. In this study, I tested the hypothesis that both the mechanically and metabolically sensitive components of the exercise pressor reflex are overactive in hypertension, causing an exaggerated cardiovascular response to exercise by producing abnormal increases in mean arterial pressure and heart rate. Knowledge about exercise pressor reflex dysfunction in hypertension could lead to novel treatments that would increase exercise tolerance and allow hypertensive individuals to enjoy the benefits of physical activity without an increased risk for the generation of adverse cardiovascular events.

CHAPTER 2

REVIEW OF RELATED LITERATURE

2.1 Basic Properties of the Cardiovascular System

2.1.1 Cardiac Output

Cardiac output, or the volume of blood pumped out of each ventricle per minute, is the product of stroke volume (the amount of blood pumped out of each ventricle with each contraction) and heart rate (the number of beats per minute)(28-30). Intrinsic and extrinsic changes in either stroke volume, heart rate, or both, cause cardiac output to vary.

Stroke volume is controlled by the ventricular end-diastolic volume, total peripheral resistance, and the contractility of the cardiac muscle(28). The end-diastolic volume, also known as preload, is the volume of blood in the ventricle after ventricular filling and atrial contraction. Stroke volume is proportional to the end-diastolic volume as well as muscle contractility due to an intrinsic property of myocardium, known as the Frank-Starling law of the heart(28, 29). This law describes how an increase in end-diastolic volume stretches myocardia, causing them to contract more forcefully, thus increasing the ventricular force and, in turn, stroke volume(28). Myocardium is made up of a fixed number of individual muscle fibers, called sarcomeres. Each sarcomere is made of thin and thick longitudinally arranged filaments, called actin and myosin, respectively. The myosin filaments consist of globular heads with tails and the actin

filaments attach to the heads to form cross-bridges. Once cross-bridges have formed, the myosin heads hydrolysize ATP, causing the heads to move and this produces the sliding movement of myosin along the actin filament. This sliding motion shortens the sarcomere's length and contracts the heart muscle. When the blood volume inside the ventricle is increased, the sarcomeres are stretched, and the actin and myosin filaments are pulled farther apart, allowing more cross-bridges to form between the two filaments. This produces a greater force of contraction(28, 30, 32)**.** The end-diastolic volume is also affected by the rate of venous return. Total blood volume and venous pressure control the rate at which blood returns to the heart. However, this rate can be altered by changes in vessel compliance, the activity of the skeletal muscle pump (the squeezing of venous vessels during muscle contraction), and the pressure change between the abdominal and thoracic cavities that occurs during respiration(28). Contractility of the heart muscle is controlled extrinsically through the sympathetic and sympathoadrenal systems: both norepinephrine and epinephrine released from sympathetic neurons and the adrenal gland can increase contraction strength by acting on β-1 and -2 adrenergic receptors in the heart. Norepinephrine can also bind to α-1 adrenergic receptors, causing small increases in contraction strength. A decrease in contractility is caused by strong parasympathetic control through cholinergic discharge acting on M_2 muscarinic receptors in the heart(28, 30).

Stroke volume is inversely proportional to total peripheral resistance, which is the sum of all vascular resistance within the systemic circulation. This is also known as afterload(28, 29)**.**

The heart beats in accordance with the autonomic depolarizations of the sinoatrial (SA) node. In the absence of all neural control, the SA node will continue to beat on its own, at its own pace. However, this rhythm can be altered through parasympathetic and sympathetic nerve fiber input that is coordinated by the cardiac control center in the medulla oblongata. An increase in SA node firing frequency is caused by the release of norepinephrine from sympathetic nerve endings working on β-adrenergic receptors and from epinephrine secreted by the adrenal medulla. A decrease occurs when acetylcholine is released from parasympathetic nerve endings derived from the vagus nerve innervating the M_2 muscarinic receptors in the heart(28).

2.1.2 Systemic Blood Flow and Peripheral Resistance

Blood flow in each vascular bed throughout the body is proportional to the change in vascular pressure over vascular resistance and can be modeled by the Poiseuille equation, $Q=\Delta P\pi r^4/8\eta l$. In this equation, Q is volumetric flow rate; ΔP is the change in pressure over the length of the vessel where flow is being measured; r is the vessel radius through which the blood is flowing; η is the blood viscosity; and l is the length of the vessel over which the flow rate is being measured. From this equation it can be seen that flow rate is most dependant on and sensitive to vessel radius. The arterioles are most responsible for the changes in flow rate because their lumen size is easily altered through neural and hormonal control. For this reason, they are known as resistance vessels(28, 29).

Blood flow is regulated by both intrinsic and extrinsic factors. The flow within each vascular bed is regulated intrinsically either myogenically or metabolically. When

there is an increase in vascular pressure, the vessel's smooth muscle responds directly by constricting in order to protect smaller vessels downstream from flow damage. When vascular pressure decreases, the vessels dilate to ensure adequate blood flow to the surrounding tissue. Blood flow can also be affected by the chemical milieu surrounding the vessel. Vasodilation is caused by a decrease in oxygen concentration, an increase in carbon dioxide concentration, release of adenosine or potassium from the surrounding tissue, or a decrease in tissue pH. Blood flow is regulated extrinsically through sympathetic and parasympathetic nerve activity. Sympathetic nerve varicosities innervating the smooth muscle surrounding arterial vessels release norepinephrine which acts on α-adrenergic receptors to cause vasoconstriction. The sympathoadrenal system also causes vasoconstriction by releasing norepinephrine and epinephrine to act on the same receptors. The parasympathetic system causes limited vasodilation in arterioles in the digestive tract, external genitalia, and salivary glands through the release of acetylcholine acting on cholinergic receptors(28).

2.1.3 Arterial Blood Pressure

Arterial blood pressure is determined by the product of cardiac output and total peripheral resistance. Its maintenance is vitally important because it is the mechanism by which blood moves throughout the vascular tree. Under resting conditions, the baroreceptor reflex is an important contributor to the control of blood pressure by regulating cardiac output and total peripheral resistance(28, 29).

Baroreceptors are unencapsulated free nerve endings within the internal blood vessel walls of the carotid arteries, aorta, heart, and lungs that quickly respond to

changes in blood pressure by initiating negative feedback reflexes through autonomic neural activity. They maintain pressure by altering heart rate, stroke volume, and total peripheral resistance. The baroreceptors most sensitive to sudden changes in pressure are located in the aortic arch and carotid sinuses(109). When arterial pressure rises, the vessel walls are stretched and the baroreceptors increase their firing frequency. Their firing inhibits sympathetic outflow and increases parasympathetic outflow from the medulla. This neural activity decreases heart rate, cardiac output, and total peripheral resistance, which lowers the blood pressure. In response to hypotension, the baroreceptors decrease their firing frequency which causes an increase in sympathetic nerve activity and a decrease in parasympathetic nerve activity (Figure 2.1). This leads to increases in heart rate and contractility, causing a higher cardiac output. In addition, vessels are constricted, increasing total peripheral resistance and blood pressure(4, 28). At rest, the baroreflex primarily alters mean arterial pressure by changing total vascular conductance rather than cardiac output(83). Heart rate is usually altered within the first or second beat of a pressure change**,** depending on the latency period of the reflex(59).

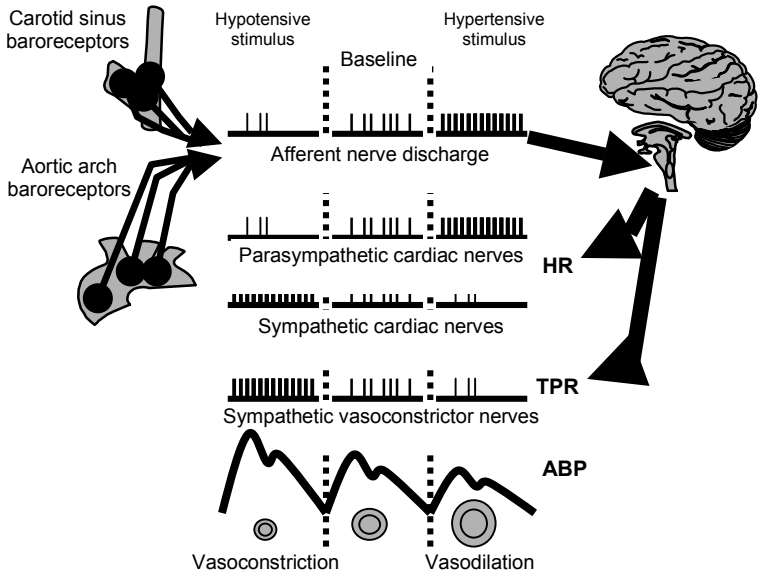

Figure 2.1: The negative feedback baroreflex in response to arterial blood pressure. In response to low blood pressure, the baroreceptors increase their discharge activity in comparison to baseline. This causes an increase in sympathetic nerve activity to the heart and vasculature and a decrease in parasympathetic nerve activity to the heart. These changes result in vasoconstriction to increase total peripheral resistance (TPR) and an increase in heart rate (HR), which both work to raise arterial blood pressure (ABP). Hypertensive stimulus causes the baroreceptors to initiate the opposite response. Source: (23)

Located within the internal wall of the carotid artery are type 1 fibers, which are large, fast fibers that respond to mechanical changes in the vessel wall caused by alterations in pulse pressure, and type 2 fibers, which are slow and unmyelinated and respond to vessel wall deformation caused by fluctuations in mean arterial pressure(107). The afferents travel through the Hering nerve, a branch of the glossopharyngeal, to synapse in the nucleus tractus solitarius of the medulla oblongata within the brainstem(19). From within the medulla, the autonomic nervous system responds to increased pressure sensed by the baroreceptors by inhibiting sympathetic activity and promoting parasympathetic activity, thus attenuating heart rate and causing

vasodilation(4). Efferents from the medulla include the parasympathetic vagus nerve and the sympathetic preganglionic neurons in the thoracic spinal cord. The right vagus nerve primarily controls the SA node while the left vagus nerve acts on the atrio-ventricular (AV) node to control heart rate. Vagal efferents also innervate atrial myocardium and ventricular myocardium, but only sparsely. The sympathetic preganglionic neurons alter heart rate by synapsing with postganglionic neurons that go to cardiac muscle, specifically the SA node and myocytes throughout the atria and ventricles. Vascular resistance is controlled by sympathetic neurons acting on vascular smooth muscle, specifically the arterial and venous adventitia. The net result is that cardiac output and total peripheral resistance are altered, thus regulating blood pressure(2, 28, 118).

There are also stretch receptors located within the atria that respond to increases in venous return. When the atrium distends, this reflex increases sympathetic nerve activity in order to cause tachycardia and lower blood volume by the excretion of larger volumes of urine. When pressure is low, heart rate slows(28). Cardiopulmonary baroreceptors are located in the heart and lungs and are activated by changes in blood volume as well as pressure. Their responses are not as well known as the carotid and aortic baroreceptors', however, they are activated during postural changes and during water immersion(105).

2.2 The Cardiovascular Response to Exercise

2.2.1 Dynamic Exercise

There are two types of exercise that elicit distinct circulatory responses. Dynamic exercise consists of rhythmic contractions that change the muscle length and joint angle. With each contraction, the intramuscular force is increased slightly and blood perfusion is altered(23, 31). During dynamic exercise, sympathetic nerve activity is increased and parasympathetic nerve activity is decreased. These changes in nerve activity acting on the SA node cause heart rate to rise. This increase in heart rate, along with enhanced stroke volume leads to a larger cardiac output. In active skeletal muscle, vascular conductance increases so that more blood perfuses the muscle. This is caused by metabolic vessel dilation; muscle contraction produces metabolic byproducts, such as nitric oxide and hydrogen ions, that cause vessel dilation. In order to maintain blood flow to the heart, brain, and active muscle tissue, blood is shunted away from the viscera, skin, and non-active muscle due to increased vasoconstriction Venous return is augmented by the working skeletal muscle pump, which increases end diastolic volume and activation of the sympathoadrenal system causes an increase in contractility. Both of these events contribute to the larger stroke volume. Systolic blood pressure rises because of the increased volume of blood in the vascular system while diastolic pressure decreases due to local vasodilation of active muscle. Mean arterial pressure, which is calculated by the equation $P_{Diastolic} + 1/3(P_{Systolic} - P_{Diastolic})$, increases slightly as a result of a lower diastolic pressure and a higher systolic pressure(28, 31, 102). As exercise intensity increases, blood flow increases in proportion to the amount of muscle

mass being used, but the requirements can exceed the heart's pumping capacity(104). Once maximum cardiac output is reached, sympathetic nerve activity causes vasoconstriction to occur in skeletal muscle vasculature. This vasoconstriction overpowers metabolic vasodilation and keeps the blood pressure stable(103, 108).

2.2.2 Static Exercise

Static exercise is defined by sustained isometric contractions with no change in muscle length or joint angle, where there are large increases in intramuscular force and pressure and a decrease in muscle perfusion(23). Because there is no time between contractions for blood flow in active muscle to increase and because of the large increase in intramuscular pressure, vascular conductance is reduced. Sympathetic nerve activity causes vasoconstriction and positive chronotropy in order to raise cardiac output in an attempt to keep the active muscle perfused. However, the larger cardiac output is primarily redirected to skin and non-active muscle. Stroke volume varies little, meaning most changes in cardiac output are due to heart rate. Total peripheral resistance remains unaffected, so systolic blood pressure rises. Diastolic pressure remains stable because there is little vasodilation in the active muscle. Due to increased cardiac output, mean arterial pressure rises. Overall, the cardiovascular response to static exercise is directly correlated to the amount of muscle mass being used and the intensity of contraction(31).

These cardiovascular adjustments to exercise are mediated by three neural inputs: the baroreflex, central command, and the exercise pressor reflex (Figure 2.2).

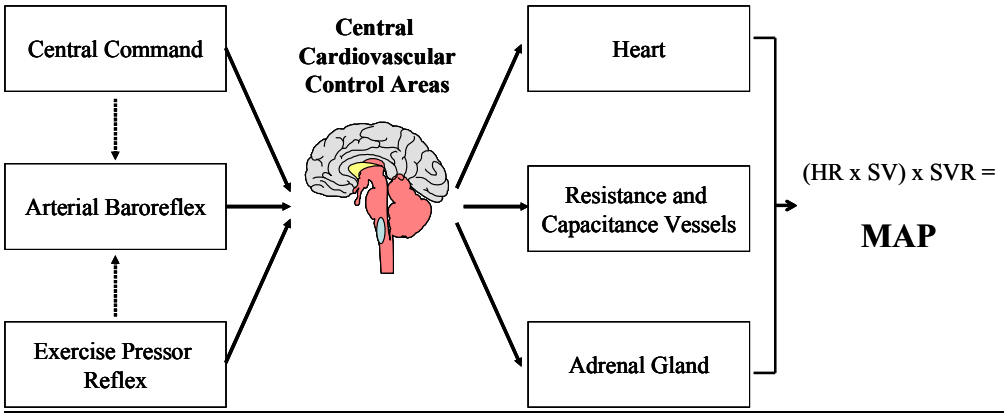

Figure 2.2: The neural inputs that control the cardiovascular response to exercise. Arterial baroreceptors, central command, and the exercise pressor reflex can alter neural activity to the heart, vasculature, and adrenal gland in order to control mean arterial pressure (MAP). The three inputs are integrative and work together to alter heart rate (HR), stroke volume (SV), and systemic vascular resistance (SVR). Source: (23)

2.3 Baroreflex Function during Exercise

At the onset of exercise, when mean arterial pressure and heart rate increase, it would seem as though the arterial baroreflex should increase parasympathetic nerve activity and decrease sympathetic nerve activity to lower heart rate and total peripheral resistance. However heart rate continues to rise. Research has shown that the baroreflex has not been altered or inhibited by exercise. Instead, the baroreceptors have been reset to function around the higher blood pressures invoked by physical activity. They are still capable of buffering changes in mean arterial pressure and heart rate without a change in sensitivity(70). In a human study, it was found that the baroreflex was indeed functional during exercise and that it could still elicit changes in heart rate in response to elevated blood pressure(94).

2.4 Cardiovascular Control by Central Command during Exercise

Central command describes the feed-forward neural pathway originating in motor centers in the brain that activate locomotor neurons in skeletal muscle, and in a parallel fashion, cardiovascular neurons in the brainstem(33, 55). The exact origin of central command is unknown, but animal and human studies have identified the motor cortex, insular cortex, and the mesencephalic and hypothalamic locomotor regions of the brain as possible candidates(129). In different experiments, electrical stimulation of the hypothalamic locomotor region in the cat and electrical stimulation of the mesencephalic locomotor region in the decerebrate rat have been shown to evoke parallel activation of locomotor and cardiovascular areas in the brain(9, 130). At the onset of exercise, central command causes a decrease in parasympathetic nerve activity which releases vagal tone and increases heart rate. Consequently cardiac output rises very quickly(80). In humans, at rest, the parasympathetic nervous system has more control over SA node activity than the sympathetic nervous system(60). Even after exercise has begun, at heart rates up to 100 bpm, the parasympathetic nervous system is the dominant regulator of heart rate. However, at heart rates over 100 bpm, sympathetic nerve activity takes over, causing vasoconstriction and an even greater increase in heart rate. Under these conditions, the increased heart rate is due to both increases in sympathetic and decreases in parasympathetic nerve activity(31, 97). At heart rates greater than 150 bpm, further increases in heart rate are the result of sympathetic nerve activity alone(31). Also at the onset of exercise, central command immediately resets the baroreceptors to operate around higher arterial pressures(94). It is possible that the

interaction between central command and arterial baroreceptors occurs within the nucleus tractus solitarius because projections from the insular cortex that innervate locomotor regions within the hypothalamus and mesencephalon have terminal fields that overlap baroreceptor afferent neurons within this region of the brainstem(93).

2.5 Cardiovascular Control by the Exercise Pressor Reflex during Exercise

The exercise pressor reflex, first described by Alam and Smirk in 1937, is a feedback mechanism originating in skeletal muscle that has the ability to alter arterial pressure and heart rate during exercise(5, 76). The cardiovascular response is mediated by activation of mechanically and chemically sensitive receptors associated with group III and IV afferent neurons, respectively, located within skeletal muscle(69). Group III afferents are thinly myelinated A-δ fibers, most of which respond at the onset of muscle contraction and during passive stretch of the muscle. These receptors and their associated fibers have been termed the skeletal muscle mechanoreflex. Group IV afferents are unmyelinated C fibers that are predominantly activated 4-10 seconds after the onset of contraction and increase their discharge rate steadily until muscle contraction ceases. Further, these fibers have been shown to be activated by a number of chemical substances known to be byproducts of muscle work. These receptors and their associated fibers have been termed the muscle metaboreflex (51, 52, 69). However, there are a small portion of group III fibers that are metabolically activated and a small portion of group IV fibers are mechanically stimulated (Figure 2.3)(51).

A recent experiment in cats showed that both group III and IV afferents respond to low levels of dynamic exercise. 2/3 of group III fibers discharged in sync with

electrically-induced contractions and there was an increase in discharge activity within 2 seconds of the initial contraction. In contrast, 4/5 of group IV afferents discharged with each contraction, however there was an increase in discharge activity as electrical stimulation was maintained and there was no activity in response to passive stretch of the muscle(48). This experiment confirmed that group III afferents have high mechanical sensitivity while group IV afferents do not(1).

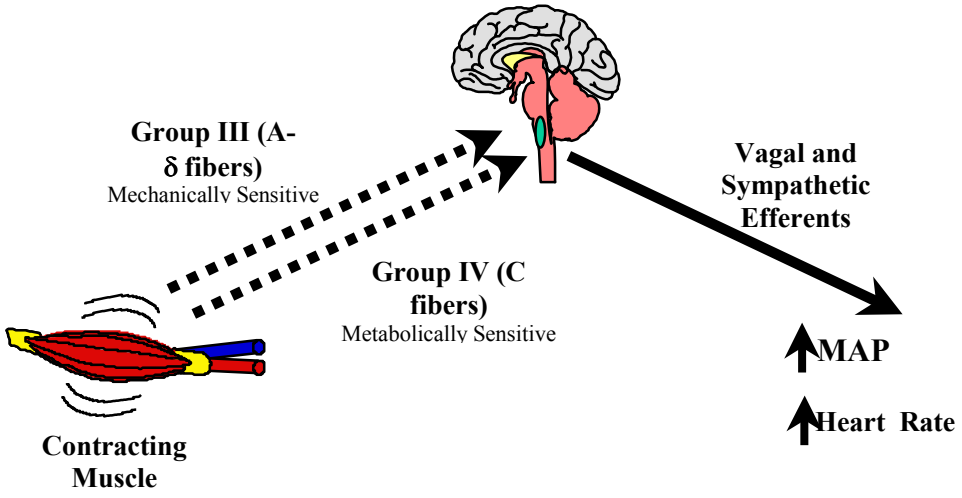

Figure 2.3: The exercise pressor reflex and its circulatory effects. During exercise, muscle contraction stimulates group III and IV afferent neurons, which project to the spinal cord and brainstem. They engage vagal and sympathetic efferents to raise mean arterial pressure (MAP) and heart rate. Source: (23)

The first synapse of most group III and IV afferents occurs within the dorsal horn of the spinal cord, from which they travel to medullary sites within the brainstem(48). Experiments have shown that neurons located in the rostral and caudal ventrolateral medulla and the nucleus tractus solitarius are activated by muscle

contraction(8, 64). Some fibers, however, synapse directly in the nucleus tractus solitarius, decreasing the latency period to stimulate a cardiovascular response(48).

Areas within the brain that have been identified as transmitting neuronal activity along the exercise pressor reflex arc are the nucleus tractus solitarius, the caudal and rostral ventrolateral medulla, the lateral tegmental field, and the ventromedial region of the rostral periaqueductal gray(3, 16, 46, 62). It has been shown that neurons within the ventrolateral medulla can control vasomotor tone in response to activation of the baroreflex and metaboreflex(8, 17). From the brainstem, vagal motor neurons, which are the primary parasympathetic efferents, travel via central preganglionic neurons in the spinal cord to the heart. Here they synapse with postganglionic neurons in or near the muscle walls. Sympathetic efferent neurons project from the brainstem to synapse with sympathetic preganglionic neurons in the spinal cord which then project to paravertebral chain ganglia. These neurons, in turn, innervate postganglionic neurons that travel to the heart and regional vasculature and activate receptors there(23).

The heart is affected both chronotropically and inotropically so that heart rate and contractility increase causing larger cardiac outputs(76). Neural release of norepinephrine on receptors within arterial and venous adventitia produces vasoconstriction. The sympathoadrenal system may also be activated, releasing both epinephrine and norepinephrine into the circulation. This induces systemic vasoconstriction and increases heart rate(28). Vascular beds in both active and inactive skeletal muscle can be vasoconstricted by this efferent pathway. Along with increases in cardiac output, this enhanced vascular tone can lead to elevations in blood

pressure(43, 77). The exercise pressor reflex finely regulates sympathetic nerve activity to ensure that the increased blood flow caused by metabolic vasodilation in active muscle is balanced by the pressor response-mediated vasoconstriction of non-active muscle. This guarantees that the heart is able to adequately pump blood to the exercising muscle(81). The exercise pressor reflex is engaged to a greater extent and more quickly in response to static exercise than to dynamic exercise. This is due to the fact that during static exercise, blood flow to the contracting muscle is decreased, causing a greater accumulation of metabolites. These metabolites increase the discharge activity of group IV afferents. Group III afferents also increase their discharge activity during held contractions, possibly due to the greater development of tension(23).

2.5.1 Group III Afferent Fibers

In animals, the mechanoreflex has been isolated and studied by either passively stretching or applying pressure to the muscle. However, applying pressure to the muscle has not yet been found to increase heart rate(119). During muscle shortening, metabolic byproducts are produced, therefore experiments inducing muscular contraction activate both metaboreceptors and mechanoreceptors and are not conducive to studying the mechanoreflex by itself(23). In an experiment done in anesthetized cats, passive stretch produced rises in mean arterial pressure and heart rate. The increases in mean arterial pressure and heart rate elicited by passively stretching the hindlimb were assumed to be caused by group III afferent fibers only. After denervation by L_5-L_7 and S_1 dorsal root sectioning, the increases in mean arterial pressure and heart rate were

abolished. Dorsal roots in the spinal cord transmit nerve activity from the muscle to the brain. Therefore, the elimination of the cardiovascular response after dorsal root rhizotomy provides evidence that the response was reflex in origin. In addition, the study attempted to quantify the cardiovascular response invoked by the mechanoreflex. The increases in mean arterial pressure and heart rate elicited by passive stretch alone were about half of the total response seen during contraction, which stimulates both group III and IV afferents(119).

In another experiment, it was found that group III afferents fired more during static contraction than during dynamic twitches in anesthesized cats. In this study, 13 of the 17 individual group III afferents discharged while only 10 of the 18 group IV afferent fibers discharged in response to electrically-induced static contraction. In response to rhythmic twitch contractions, 14 of the 17 group III afferents and 9 of the 18 group IV afferents discharged. This data showed the group III afferent fibers discharged more impulses to static contraction than to the rhythmic twitches. Furthermore, the discharge patterns were different for the two types of contraction. For static contraction, most of the group III afferents fired at the onset of contraction and as contraction was held and muscle tension tapered off, so did their discharge activity. During the rhythmic twitches, the group III afferents fired with each separate contraction. Because the group III afferent fibers discharged more during static muscle contraction than during dynamic exercise, the investigators concluded that the mechanoreflex was responsible for the greater exercise pressor response to static contraction(52).

The intensity of the cardiovascular response caused by the mechanically sensitive afferents is dependant on muscle mass, contraction strength, and contraction duration(52, 103). The larger the working muscle and the stronger the contraction strength, the more group III afferent fibers discharge to increase sympathetic nerve activity. This results in a greater cardiovascular response to exercise. It is known that at the onset of exercise, group III-mediated sympathetic activity causes an immediate increase in arterial pressure. At the end of muscle contraction, there is a rapid return of sympathetic nerve activity to its basal level(51, 103). Also, it has been shown that sympathetic and parasympathetic blockade both completely abolish the pressor response to stretch and contraction in decerebrate rats. In a recent experiment, mechanically sensitive afferents were tested by passively stretching the rat hindlimb. When stretch was performed after ganglionic (both sympathetic and parasympathetic nerve activity) and α-adrenergic (sympathetic nerve activity only) blockade, increases in mean arterial pressure and heart rate were eliminated. Given that the cardiovascular response was abolished during α-adrenergic blockade, it was concluded that the group III response to exercise is primarily mediated by the sympathetic nervous system(114).

2.5.2 Group IV Afferent Fibers

In order to isolate and study group IV afferents, muscle contraction and arterial infusion studies have been completed. Contraction studies are used because working muscle produces metabolites that activate group IV afferent fibers. However, contraction of skeletal muscle also activates group III afferent fibers through mechanical deformation. Infusion studies, on the other hand, predominantly activate

group IV afferent fibers because the infusate is injected directly into the arterial supply of the skeletal muscle. Many times, the infusate is injected and the circulation of the limb is occluded to simulate static contraction conditions(23). One common infusate is capsaicin, the main pungent chemical found in hot peppers. While this chemical does not exist naturally in the body, it is known to selectively bind to transient receptor potential vanilloid 1, or TRPv1, receptors which are non-selective calcium channels within the dorsal root ganglia(35, 72). TRPv1 receptors are found exclusively on group IV afferent fibers(117). Thus, using a chemical that binds to TRPv1 receptors is an effective way to isolate the metabolically sensitive component of the exercise pressor reflex.

In an experiment in anesthetized dogs, capsaicin was injected into the vascularly isolated skeletal muscle of the hindlimb. The injections produced cardiovascular responses similar to those of static contraction: increased heart rate, mean arterial pressure, and cardiac output. In addition, the cardiovascular responses to capsaicin were abolished after sectioning of the femoral and sciatic nerves. The femoral and the sciatic nerves contain both group III and IV afferent fibers that innervate skeletal muscle. Therefore, sectioning of them eliminated any contribution they may have provided to the cardiovascular response and ensured that the response was mediated specifically by the group IV afferent fibers(20). In another experiment in anesthesized cats, discharge rates of group III and group IV afferent fibers were recorded in response to hindlimb contraction and injection of capsaicin and bradykinin into the abdominal aorta. After injection of 100-200 μg capsaicin, 5 of 19 group III fibers and 16 of the 19

group IV fibers discharged. Injection of bradykinin stimulated 10 of the 19 group III fibers and 12 of the 19 group IV afferent fibers. Both infusates caused the group IV afferent fibers to fire more. In response to contraction, group IV afferent fibers discharged at an average of 3.8 seconds after the onset of stimulation. This is ample time for metabolites to form in the active muscle. Also, the group IV afferents gradually increased their firing as contraction was held, possibly due to the continued accumulation of metabolites. In addition, the capsaicin injections failed to elicit discharge activity from group III afferents. All of this data led the investigators to conclude that the group IV afferent fibers were metabolically activated. This experimental protocol utilized both contraction and arterial infusion to elicit a pressor response from the metabolically sensitive afferent fibers(51).

Much research has been done to identify metabolites that activate group IV afferents. Substances eliciting a pressor response in humans and animals by arterial infusion include lactic acid, glucose, capsaicin, diprotonated phosphate, potassium, bradykinin, prostaglandins, and changes in pH(26, 27, 85, 100, 110, 111, 117, 119-121, 127). While all of these substances provided evidence of eliciting a pressor response, the physiological metabolites that activate the metaboreflex during exercise are still unknown. It appears, however, that the group IV fibers are responsible for determining whether blood flow to contracting muscle is sufficient and this explains why metabolites, which naturally build up in skeletal muscle when blood flow is occluded, produce pressor responses(75).

During dynamic and static exercise**,** the metaboreflex causes an increase in sympathetic nerve activity to the heart and inactive muscle, which raises heart rate, contractility, and vasoconstrictor tone(80, 128). As muscle contraction is held and blood flow is impeded, metabolites continue to accumulate. Therefore, the cardiovascular response elicited by the metaboreflex is dependant on the muscle mass and length of contraction(75). At rest the metaboreflex plays little role in altering pressure because the group IV afferent fibers do not discharge under free-flow conditions(88)**.** However, when blood flow fails to provide adequate oxygen for a given muscle, the metaboreflex can restore the flow by raising cardiac output through heart rate control. When cardiac output can no longer be increased, the metaboreflex raises arterial blood pressure by sympathetic-induced vasoconstriction of muscle vasculature. The resulting increase in mean arterial pressure helps to maintain muscle perfusion(82).

2.6 The Cardiovascular Response to Exercise in Hypertension

The cardiovascular response to exercise is exaggerated in hypertension. In hypertension, exercise increases systolic, mean, and diastolic pressure, while in normotensive individuals, systolic pressure and mean arterial pressure increase while diastolic pressure decreases or remains stable. In normotensive individuals, systolic pressure rises because cardiac output and peripheral vasoconstriction in nonactive tissue are increased, whereas blood vessels in active skeletal muscle vasodilate, thus preventing a change in diastolic pressure(39)**.** However, in hypertensive individuals, resting peripheral vascular tone is altered and the increased total peripheral resistance

causes an increase in diastolic pressure during exercise(66). In established hypertension, cardiac output and oxygen uptake are decreased during exercise. Peak stroke volume is also reduced due to a larger peripheral resistance(74). The increased peripheral resistance along with reduced arterial and left ventricular compliance causes asynchronous ventricular wall contraction(24, 78). This, along with left ventricular hypertrophy poses a serious risk to physical activity as it can lead to myocardial ischemia, infarction, and possibly death(68).

2.6.1 The Exercise Pressor Reflex and Hypertension

In a previous experiment done in our lab, it was found that the exercise pressor reflex mediates an abnormal cardiovascular response to exercise in hypertension. The circulatory response to electrically-induced static contraction (a maneuver which preferentially activates the exercise pressor reflex) was studied in hypertensive rats (SHR) and normotensive rats (WKY). The change in mean arterial pressure at maximum contraction was 43 ± 6 mmHg in SHR and only 18 ± 3 mmHg in WKY animals. Heart rate increased 12 ± 2 bpm in SHR rats and only 6 ± 1 bpm in WKY animals (Figure 2.4). In addition, the changes in mean arterial pressure were positively correlated to baseline blood pressures in both SHR and WKY groups, such that the greater the resting blood pressure, the greater the cardiovascular response (Figure 2.5). In order to generally assess the neural pathway through which the exercise pressor reflex elicits its cardiovascular response in hypertension, sympathetic blockade was given to SHR rats before and after contraction and was seen to abolish the large increases in mean arterial pressure (Figure 2.6). It was concluded that the exercise

pressor reflex is overactive in hypertension, thus eliciting an exaggerated cardiovascular response to exercise. The responses were greater in SHR than in WKY rats over a wide range of contraction strengths, suggesting that all physical activity, including low-intensity, is more strenuous on the circulatory system in hypertensive individuals. Also, the degree of hypertension was linearly correlated to the magnitude of the response and sympathetic nerve activity is greatly responsible for driving that response(116).

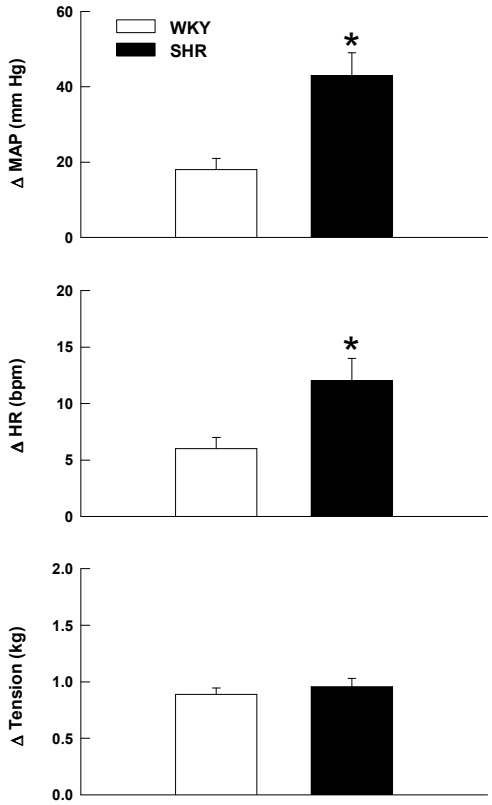

Figure 2.4: Cardiovascular responses to hindlimb contraction in WKY and SHR animals. Changes in mean arterial pressure and heart rate were significantly greater in SHR than WKY rats. *Significance from WKY.

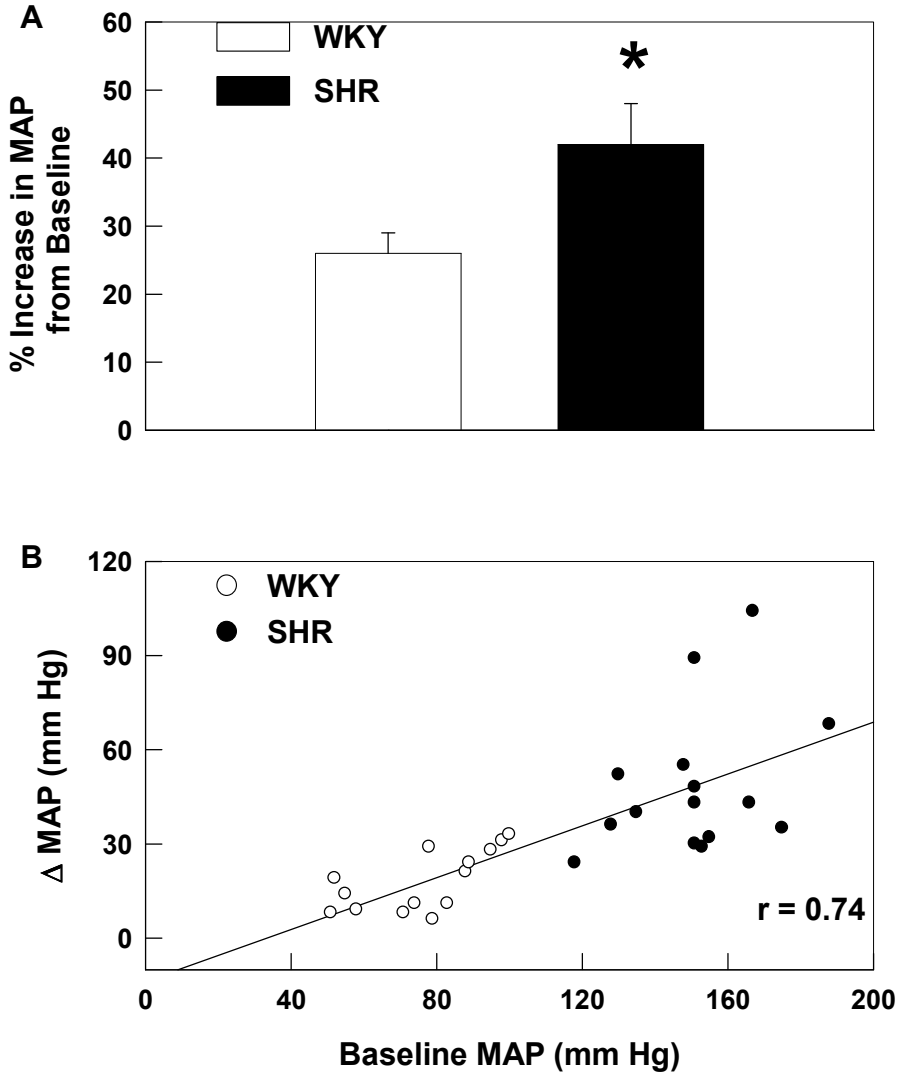

Figure 2.5: The mean arterial pressure response to hindlimb contraction in WKY and SHR rats. A, The percent increase in mean arterial pressure from baseline for SHR animals was significantly greater than that of WKY animals. *Significance from WKY. B, In all normotensive and hypertensive rats, the magnitude of the pressor response was positively correlated to baseline blood pressure.

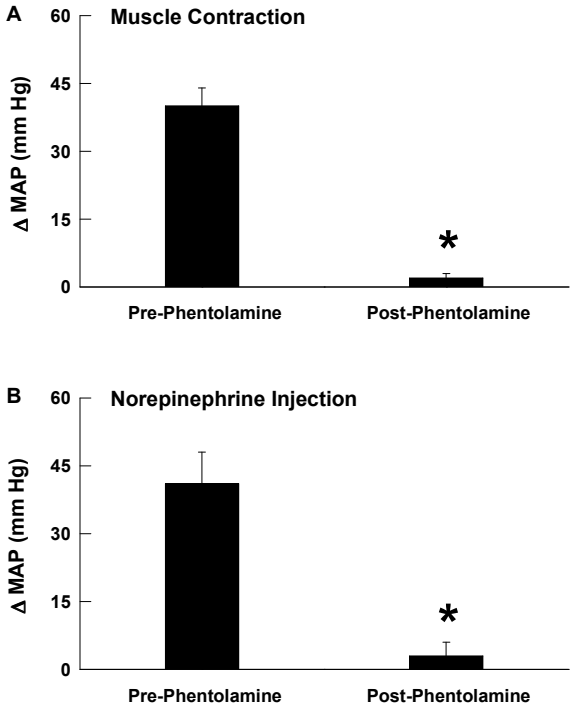

Figure 2.6: The effect of sympathetic blockade on the cardiovascular response to muscle contraction in SHR animals. A, The sympathetic blocker phentolamine abolished the pressor response to hindlimb contraction in SHR animals. B, Phentolamine also attenuated the increase in mean arterial pressure seen in response to intra-venous administration of norepinephrine, validating the efficacy of the sympathetic block. *Significance from pre-phentolamine treatment.

2.6.2 Current Study- Rationale

Knowing that the exercise pressor reflex is overactive in hypertension and that the reflex is composed of metabolically and mechanically activated afferents, it seems reasonable that the exaggerated cardiovascular response is caused by one of both of these components. To test the hypothesis that both afferent groups are overactive in hypertension, the experimental protocols performed tested the muscle mechanoreflex and metaboreflex separately. The mechanically sensitive afferents were tested in

normotensive and hypertensive decerebrate rats by passively stretching the triceps surae muscle. The metabolically sensitive afferent fibers were tested in isolation by infusing capsaicin into the right hindlimb arterial supply and occluding the local circulation for two minutes. Mean arterial pressure and heart rate were monitored to quantify the cardiovascular response. If the mean arterial pressure and heart rate responses produced by both experiments are significantly greater in hypertensive than in normotensive rats, we can conclude that a change or mutation in both the group III and IV afferents is responsible for the increased exercise pressor response in hypertensive individuals.

CHAPTER 3

METHODS

3.1 Subjects

Experiments were performed in 33 Spontaneously Hypertensive (SHR) and 29 Wistar-Kyoto (WKY) age-matched (14-18 weeks) male rats (Harlan, Indianapolis, Ind.). All animals were housed in standard rodent cages on 12 hour light-dark cycles and were given food and water *ad libitum*. All experimental procedures were approved by the Institutional Animal Care and Research Advisory Committee of the University of Texas at Southwestern Medical Center.

3.2 General Surgical Procedures

Rats were anesthetized with isoflurane gas (2-3%) in pure oxygen, intubated and mechanically respirated (Harvard Apparatus) for the duration of the experiment. Also, 0.1 mg dexamethasone was given intramuscularly in the left hindlimb to minimize edema. Both carotid arteries and a jugular vein were catheterized (PE-50, polyethylene tubing) for pressure transducer readings and fluid administration, respectively. To maintain fluid balance and stabilize arterial blood pressure in the animals, 1 M $NaHCO_3$ was continuously infused intravenously at a rate of 2 mL/hr. In addition, arterial blood gases and pH were measured throughout experimentation using an automated blood gas analyzer (Model ABL 5, Radiometer) and kept within normal range (arterial $Po_2 \geq 80$ mmHg, arterial Pco_2 35-45 mmHg, pH 7.3-7.4). Body temperature was maintained

between 36.5 and 38.0°C by a temperature-controlled water-perfused heating pad. For decerebration and the duration of all experimental protocols, animals were held in stereotaxic head units (Kopf Instruments). It has been shown that anesthetized rats have inconsistent exercise pressor responses and that exercise pressor responses after decerebration are more consistent with those of conscious animals therefore, pre-collicular decerebration was executed(40, 113). In order to perform a bilateral craniotomy, holes were drilled into the parietal skull and the bone superior to the central sagittal sinus was removed. The sagittal sinus and dura mater were cut away and the cerebral cortex was aspirated. Once the superior and inferior colliculi were within view, a pre-collicular section was made and the transectioned forebrain was aspirated. To minimize bleeding, small pieces of oxidized regenerated cellulose (Ethicon, Johnson & Johnson) were placed on the internal skull surface and the cranial cavity was packed with cotton. Immediately after pre-collicular transection, anesthesia was discontinued. Upon completion of decerebration, all animals were allowed to stabilize for at least an hour. At the end of experimentation, all animals were euthanized by intravenous injection of saturated potassium chloride and the heart, lungs, right triceps surae, and tibia were harvested for measurement.

3.3 Data Acquisition

A pressure transducer (Model DTX plus-DT-NN12, Ohmeda) measured mean arterial pressure (MAP) by integrating the arterial signal with a time constant of 1-4 seconds. Heart rate (HR) was derived from the same pulse wave with the use of a biotachometer (Gould Instruments). Hindlimb tension was measured by a force

transducer (FT-10, Grass Instruments). An eight channel thermal recorder (Astro-Med, Inc.) directly recorded all data, which was then subjected to A/D conversion (CED micro 1401, Cambridge Electronic Design Ltd) using data acquisition software (Spike 2, version 3, Cambridge Electronic Design, Ltd) on a personal computer.

3.4 Mechanoreflex Testing Procedures

3.4.1 Surgical Preparation

This procedure was performed on 16 SHR and 14 WKY age-matched males. To stimulate the mechanoreflex alone, without the effects of central command or the metaboreflex, the triceps surae muscle was passively stretched after isolating the spinal ventral roots innervating the muscles in the right hindlimb. The triceps surae muscle was stretched at a tension level that was predetermined by electrically-induced contraction of the same hindlimb. A laminectomy was performed to expose the spinal cord and the dura was cut away to reveal the lower lumbar roots (L_2-L_6). The ventral and dorsal roots were separated and the L_4 and L_5 ventral roots, which stimulate the triceps surae muscles, were sectioned in order to control the efferent neural activity to the right hindlimb. Bipolar platinum electrodes were then placed around the cut peripheral ends of the ventral roots and all exposed neural tissue was immersed in warm mineral oil (Figure 3.1). Ventral root stimulation ensures that all cardiovascular responses are the result of selectively activating the skeletal muscle afferent fibers independent of central command. Ventral root stimulation also guarantees that the cardiovascular response is due to the exercise pressor reflex and not from direct activation of afferent fibers.

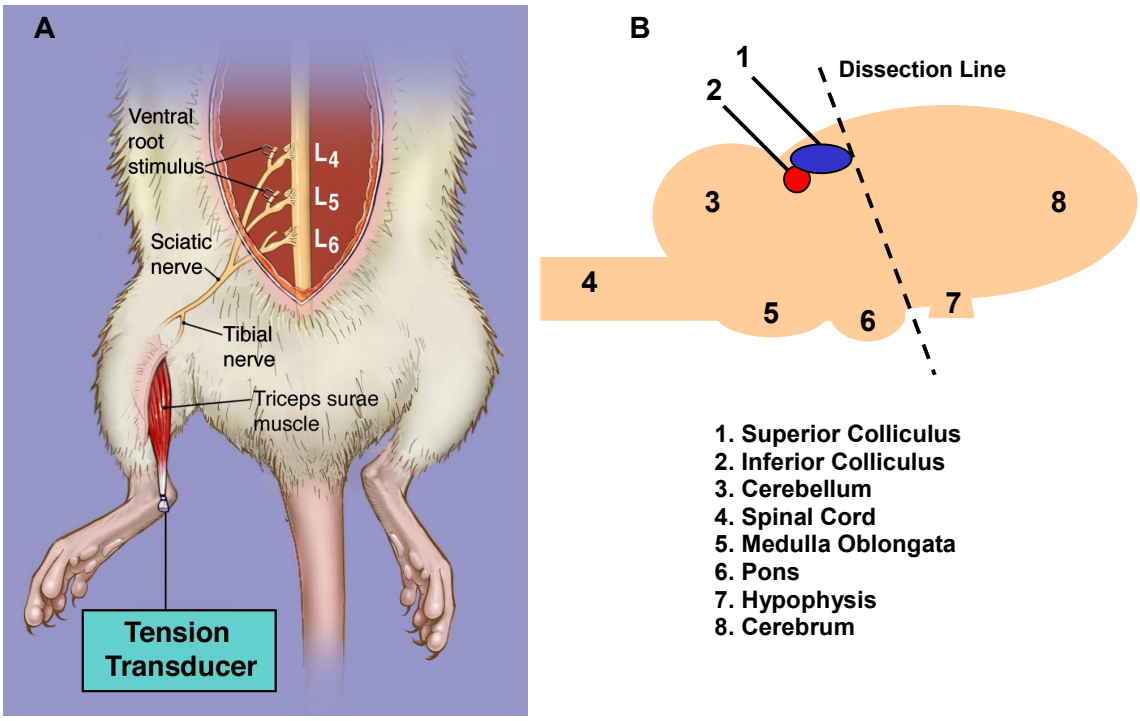

Figure 3.1: Surgical preparation used to activate the exercise pressor reflex in rats. A, Electrical stimulation of the L_4 and L_5 ventral roots induces hindlimb contraction, which activates the exercise pressor reflex. Passive stretch of the triceps surae muscle selectively activates group III afferent neurons. B, Anatomy of the decerebrate procedure. Once the brain is pre-collicularly sectioned and the forebrain has been aspirated, anesthesia is discontinued.

All animals were held stationary in a stereotaxic head unit and spinal frame that utilized rostral lumbar vertebrae clamps. Steel posts stabilized the pelvis and the right hindlimb was fixed with clamps attached to the tibial bone. Next, the right calcaneal bone was cut and the Achilles' tendon was attached to a force transducer. Pre-collicular decerebration was performed and anesthesia was discontinued.

3.4.2 Experimental Protocol

Contraction of the triceps surae muscle was electrically-induced by stimulating the L_4 and L_5 ventral roots with a Grass Instruments S88 stimulator. A constant current (1-3 times motor threshold, 0.1-ms pulse duration, 40 Hz) was applied and the peak force development was recorded. Motor threshold was defined as the minimum current required to produce a muscle twitch, and prior to contraction the muscles were preloaded with 70-100 g of tension. Contraction was performed in order to determine the maximum tension that could be developed by an individual rat's hindlimb muscle. Then, using a calibrated 9.5 mm rack and pinion system (Harvard Apparatus), the right hindlimb was stretched until the maximum tension development was the same as that seen in contraction. In a subset of WKY (n=10) and SHR (n=10) animals, additional passive stretch was performed at randomized submaximal intensities with 15 minutes between each stretch. Subsequently, the dorsal roots from L_4-L_6 were sectioned and maximum intensity stretch was repeated in another subset of animals. All baseline and peak values for mean arterial pressure, heart rate and tension were recorded. Baseline values were taken 30 seconds before contraction or stretch and peak response was measured as the greatest changes from baseline.

3.5 Metaboreflex Testing Procedures

3.5.1 Surgical Preparation

This procedure was performed on 17 SHR and 15 WKY age-matched males in order to study the muscle metaboreflex. To locally administer drugs into the right hindlimb arterial supply and activate group IV afferent fibers, the circulation was

isolated by placing a reversible ligature around the right common iliac vein emptying the leg. Then, the left common iliac artery was catheterized (PE-10, polyethylene tubing) with the tip advanced to the abdominal aorta, so as to allow injections to circulate in the right hindlimb only (Figure 3.2). Finally, pre-collicular decerebration was performed and anesthesia was discontinued.

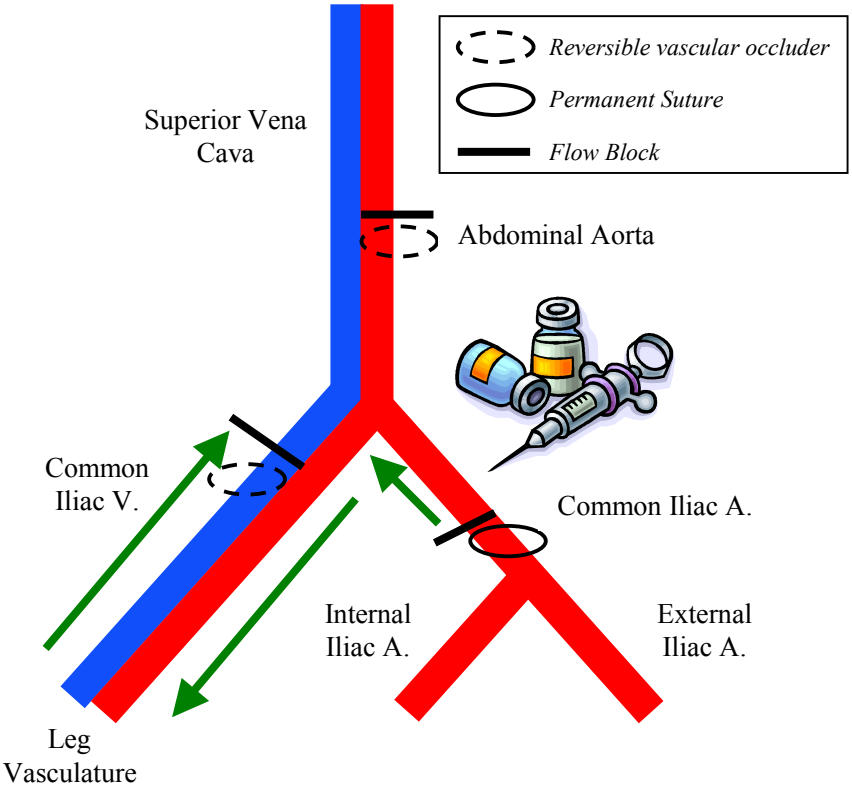

Figure 3.2: Surgical preparation used to activate the metabolically sensitive component of the exercise pressor reflex. Capsaicin administration is limited to the right hindlimb by ligating the left common iliac artery and placing a reversible ligature around the common iliac vein of the right hindlimb.

3.5.2 Experimental Protocol

The pungent chemical capsaicin was used to acutely and selectively stimulate group IV afferents of the right hindlimb by selectively binding to TRPv1 receptors. Upon capsaicin injection into the right common iliac artery, the reversible ligature was pulled for 2 minutes to allow the drug to locally circulate. Saline and vehicle injections were administered first followed by five capsaicin injections in graded doses of 0.01, 0.03, 0.1, 0.3, and 1.0 μg/100 μL. Each capsaicin injection was immediately followed by administration of 200 μL saline to make sure all infusate went into the leg. The capsaicin injections were randomized and there were at least 15 minutes between each injection. A subset of SHR (n=5) and WKY (n=5) rats received injections of capsaicin with the TRPv1 competitive antagonist capsazepine (100 μg/100 μL, Tocris Cookson, Inc) in order to determine what receptor sites capsaicin was acting on. Two minutes before each injection, 150 μL neuromuscular blocker (norcuron) was given intravenously to prohibit muscle twitch and contraction during injection. The peak mean arterial pressure and heart rate responses were recorded. Data recorded 30 seconds before each injection was taken as baseline. The peak response was the value of greatest change from baseline after the injection was given.

To confirm that the infusate was eliciting a response by locally circulating in the hindlimb, 1.0 μg/100μL capsaicin was given intravenously into the jugular vein at the end of experimentation as a control. The mean arterial pressure and heart rate responses of the infusate in systemic circulation were then compared to the responses recorded after local administration.

3.6 Statistics Performed

Statistics were performed on all data sets using linear regression, Student *t* test, or ANOVA with Bonferroni posttests as appropriate. Significance level was set at $P<0.05$. All results are presented as mean ± S.E.M. Analyses were conducted using GraphPad Prism 4 (GraphPad Software Inc).

CHAPTER 4

RESULTS

4.1 Characterization of the Hypertensive Model

Morphometric and hemodynamic baseline data for WKY and SHR animals are presented in Table 4.1. Body masses of both sets of animals were similar. Ratios of heart mass to both body mass and tibial length were significantly greater in SHR than WKY ($P<0.0001$). However, lung weight to body mass was not different between the two groups, confirming that the SHR rats were not in heart failure. Baseline mean arterial pressure was significantly higher in SHR than WKY animals, but baseline heart rate data was not statistically different.

Table 4.1. Morphometric Characteristics and Baseline Hemodynamics. Data are means ± S.E.M. MAP, mean arterial pressure; HR, heart rate. * Significantly different from WKY. $P<0.001$.

	WKY (n=29)	**SHR** (n=33)
Body mass, g	341 ± 9	345 ± 6
Heart mass/Body mass, mg/g	3.2 ± 0.1	4.6 ± 1.0*
Lung weight/Body mass, mg/g	6.4 ± 0.3	7.0 ± 0.4
Heart mass/Tibia length, mg/mm	28 ± 1.0	34 ± 1.0*
Baseline MAP, mmHg	108 ± 8.0	165 ± 8.0*
Baseline HR, bpm	426 ± 11	434 ± 14

4.2 The Mechanically Sensitive Component of the Exercise Pressor Reflex is Overactive in Hypertension

Passive muscle stretch caused significant increases in mean arterial pressure and heart rate from baseline. Typical responses for a WKY and SHR animal can be seen in Figure 4.1.

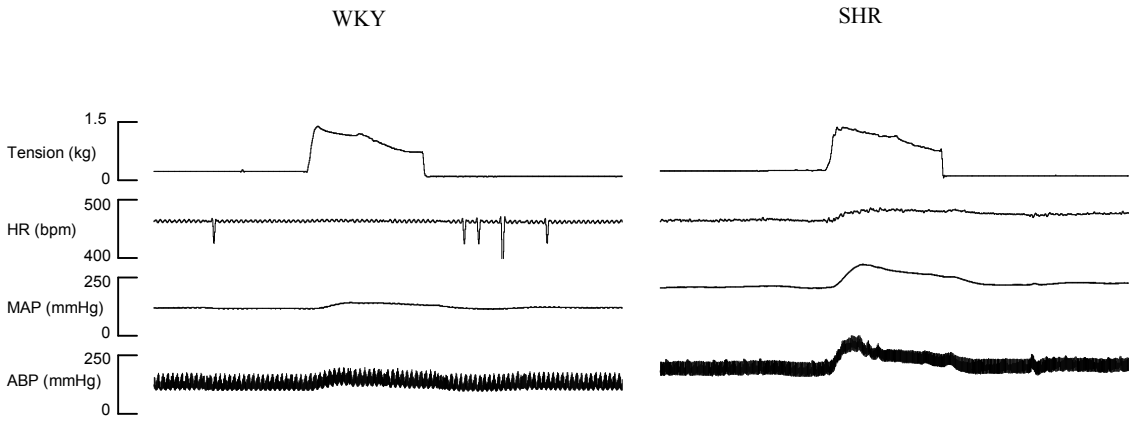

Figure 4.1: Cardiovascular response to activation of the mechanically sensitive component of the exercise pressor reflex. Characteristic response to maximum passive stretch of the hindlimb in a WKY and an SHR animal showing changes in arterial blood pressure (ABP), mean arterial pressure (MAP), heart rate (HR), and tension.

During passive stretch at maximal intensities, the mechanically sensitive component of the exercise pressor reflex caused a greater increase in both mean arterial pressure and heart rate in SHR compared to WKY animals (Figure 4.2). The change in mean arterial pressure in the SHR group (28 ± 5 mmHg) was significantly larger than that seen in the WKY group (13 ± 2 mmHg). The increase in heart rate was also significantly exaggerated in SHR (8 ± 2 bpm) compared to WKY animals (4 ± 1 bpm). The tension

produced by each group was not significantly different, as expected. Moreover, dorsal root sectioning completely abolished the cardiovascular response to stretch in both sets of animals.

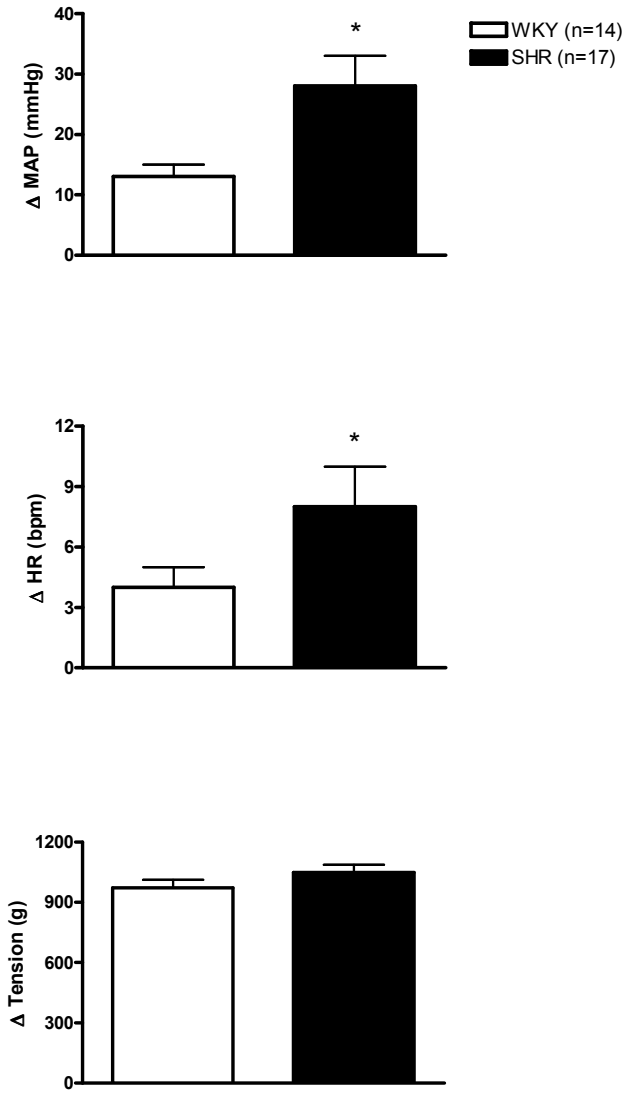

Figure 4.2: Cardiovascular responses to maximal passive stretch during stimulation of the mechanically sensitive component of the exercise pressor reflex. Increases in MAP and HR were significantly greater in SHR animals than in WKY animals. *Significance from WKY. ($P<0.05$)

The cardiovascular responses to graded submaximal levels of passive stretch are shown in Figure 4.3. At submaximal tension levels of 70-100%, the increases in mean arterial pressure were significantly greater in SHR than in WKY animals. At 0-37% of maximal stretch, the average change in MAP was 9 ± 3 mmHg in SHR animals compared to 2 ± 1 mmHg in WKY rats. At 38-69% of maximal stretch, the change in MAP was greater in SHR (19 ± 5 mmHg) than in WKY (7 ± 2 mmHg) and at tension levels ranging from 70-100% of maximal stretch, the changes in MAP were significantly different ($P<0.001$); 27 ± 5 mmHg for SHR and 11 ± 2 mmHg for WKY. Also, changes in heart rate were significantly greater in SHR than in WKY rats at the highest intensities of passive stretch. For intensities between 0 and 37% of maximal stretch, increases in heart rate for SHR were 5 ± 2 bpm compared to 1 ± 1 bpm in WKY. At 38-69% of maximal stretch, the increase in heart rate for SHR was 7 ± 2 bpm and only 3 ± 1 bpm for WKY animals. Finally, at 70-100% of maximal stretch, the changes in heart rate were significantly greater in SHR than in WKY; 9 ± 2 bpm compared to 4 ± 1 bpm.

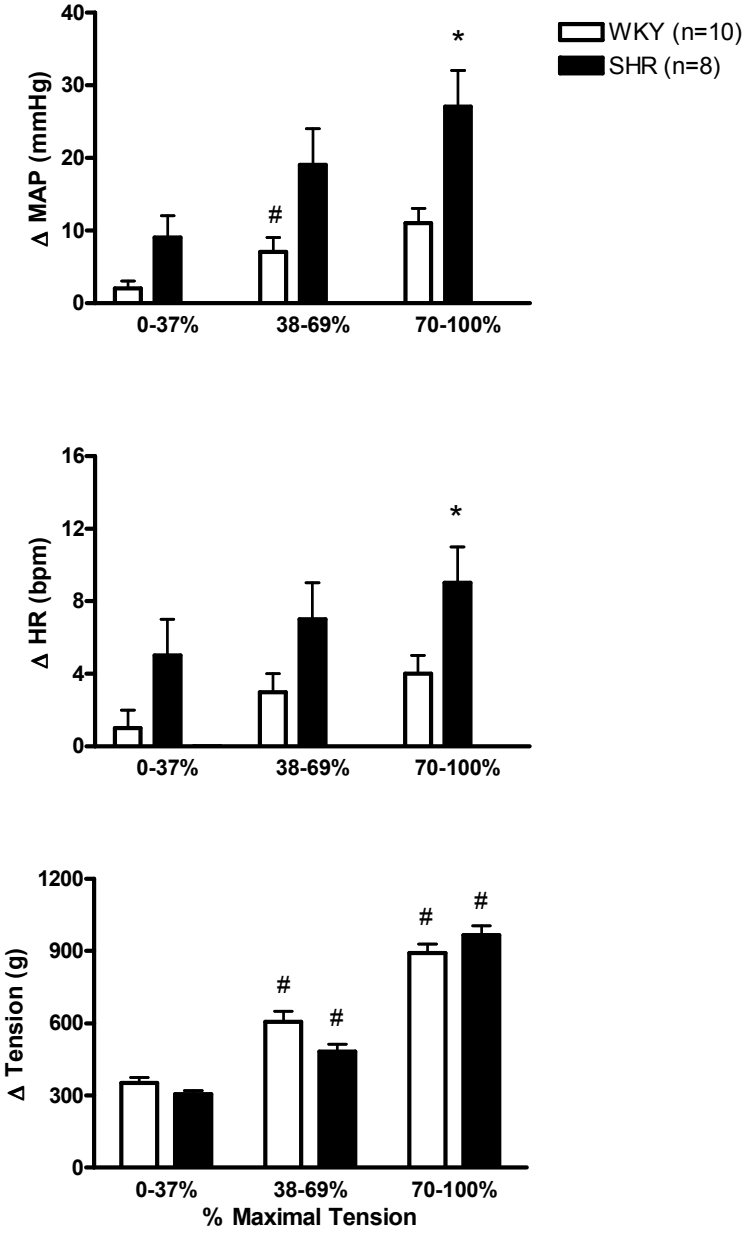

Figure 4.3: The pressor response to passive muscle stretch at submaximal intensities. Changes in mean arterial pressure and heart rate were significantly greater in SHR than WKY rats over a range of stretch tensions. *Significance from WKY. #Response was significantly different from preceding response in the individual WKY and SHR groups. ($P<0.05$)

Changes in developed tension were not significantly different between the two data sets at all intensities of passive stretch. However, the increases in developed tension in both WKY and SHR groups at all intensities were significantly different from that of the preceding stretch intensity. This data supports the idea that the exercise pressor reflex is tension-dependant; for increasing levels of stretch, the cardiovascular response and tension development increased as well.

The changes in mean arterial pressure, heart rate, and tension as a function of tension development at all intensities of passive stretch can be seen in Figure 4.4. The changes in mean arterial pressure and tension-development were positively correlated ($P<0.05$) to stretch intensity in both WKY and SHR animals. Also, the increases in mean arterial pressure and heart rate were greater in SHR than WKY animals, meaning that at all exercise intensities, the exercise pressor reflex elicited an exaggerated response in hypertension. The regression lines for mean arterial pressure and heart rate in WKY animals were $y = 0.111x - 0.525$ and $y = 0.030x + 0.644$, respectively. While in SHR rats the correlation line equations were $y = 0.236x - 0.071$ and $y = 0.037x + 4.41$ for mean arterial pressure and heart rate, respectively. Comparing the slopes of the lines for mean arterial pressure, 0.111 in WKY and 0.236 in SHR, it can be seen that the cardiovascular response is larger in the SHR animals. It is the same with the line slopes for heart rate, 0.030 for WKY and 0.037 for SHR. The regression line equations for tension were $y = 10.4x - 28.2$ for WKY and $y = 7.24x + 150.3$ for SHR.

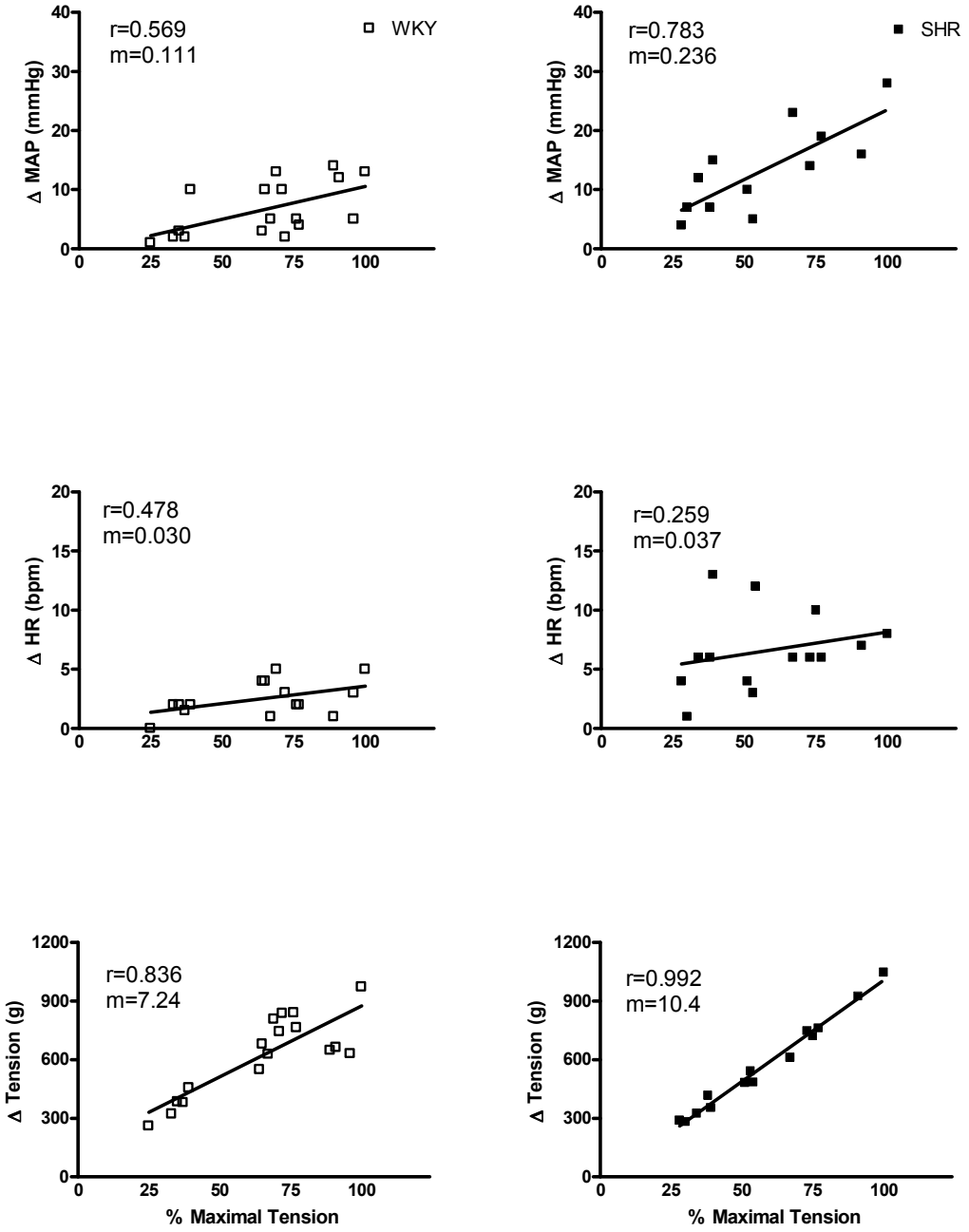

Figure 4.4: Cardiovascular responses to activation of mechanically sensitive skeletal muscle afferents in WKY and SHR rats. Increases in mean arterial pressure and heart rate were greater in SHR than in WKY animals over a range of submaximal work intensities. Also, the slopes (m) of the regression lines for mean arterial pressure and heart rate were higher in SHR than in WKY animals. ($P<0.05$ for all correlations, except heart rate in WKY and SHR groups)

4.3 The Metabolically Sensitive Component of the Exercise Pressor Reflex is Overactive in Hypertension

Examples of the cardiovascular responses elicited by intra-arterial capsaicin administration for both WKY and SHR animals can be seen in Figure 4.5. Figure 4.6 shows the changes in mean arterial pressure and heart rate elicited by activation of the metabolically sensitive afferents of the exercise pressor reflex in response to hindlimb intra-arterial injections of capsaicin at graded concentrations. As the concentration of injected capsaicin increased, so did the changes in mean arterial pressure and heart rate. However, the dose-related increases in mean arterial pressure were significantly greater in SHR animals than in WKY animals at 0.3 and 1.0 μg/100 μL capsaicin. For SHR animals, the increase in mean arterial pressure was 47 ± 7 mmHg compared to 29 ± 3 mmHg in WKY in response to 0.3 μg/100 μL capsaicin. In response to the largest dose of capsaicin given, the MAP increase was significantly higher ($P<0.001$) in SHR than in WKY; 70 ± 7 mmHg compared to 43 ± 9 mmHg. The heart rate responses, however, did not display any trends, nor show any significance between the two animal groups. Injection of saline had no significant effect on mean arterial pressure or heart rate.

In WKY animals, intra-arterial injection of capsaicin plus the competitive TRPv1 antagonist capsazepine effectively blunted the change in mean arterial pressure caused by capsaicin at the highest dose (Figure 4.7). The administration of capsazepine significantly attenuated the increase in mean arterial pressure caused by 1.0 μg/100 μL capsaicin from 43 ± 9 mmHg to 26 ± 4 mmHg. A similar cardiovascular response was

seen in the SHR rats, shown in Figure 4.8. Compared to capsaicin-induced changes in mean arterial pressure, the observed increases in MAP after injection of capsaicin plus capsazepine were significantly lower ($P<0.001$) at doses of 0.3 and 1.0 μg/100 μL. The increase in mean arterial pressure in SHR animals went from 47 ± 7 mmHg to 11 ± 2 mmHg at 0.3 μg/100 μL capsaicin and from 70 ± 7 mmHg to 29 ± 12 mmHg at 1.0 μg/100 μL capsaicin with the administration of the TRPv1 antagonist. Heart rate responses in both WKY and SHR animals, however, were not affected by administration of capsazepine. However, in both sets of rats, the changes in mean arterial pressure ($P<0.0001$) and heart rate ($P=0.0002$) were significantly affected by the concentration of the administered drug.

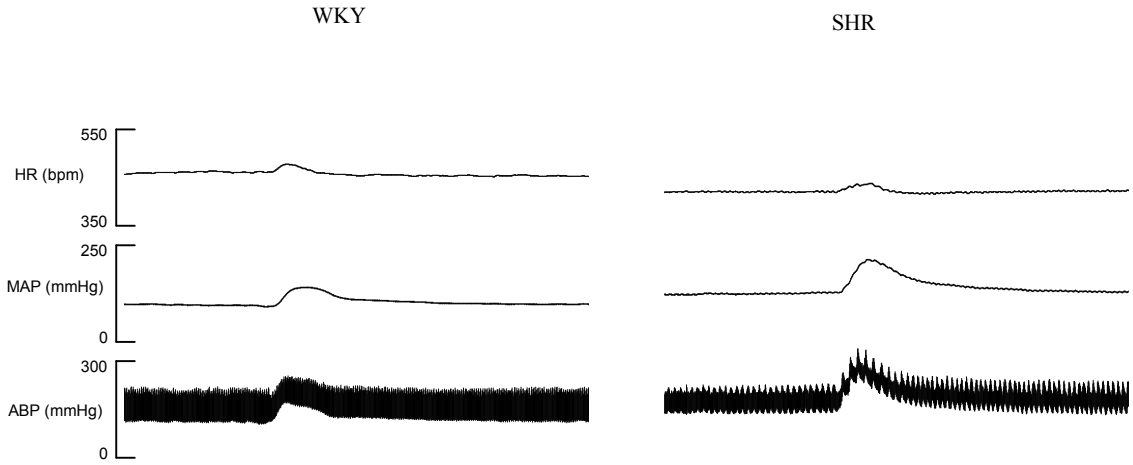

Figure 4.5: Cardiovascular response to activation of the metabolically sensitive component of the exercise pressor reflex. Characteristic response to 1.0 μg/100 μL capsaicin administration in a WKY and an SHR animal showing changes in arterial blood pressure (ABP), mean arterial pressure (MAP), and heart rate (HR).

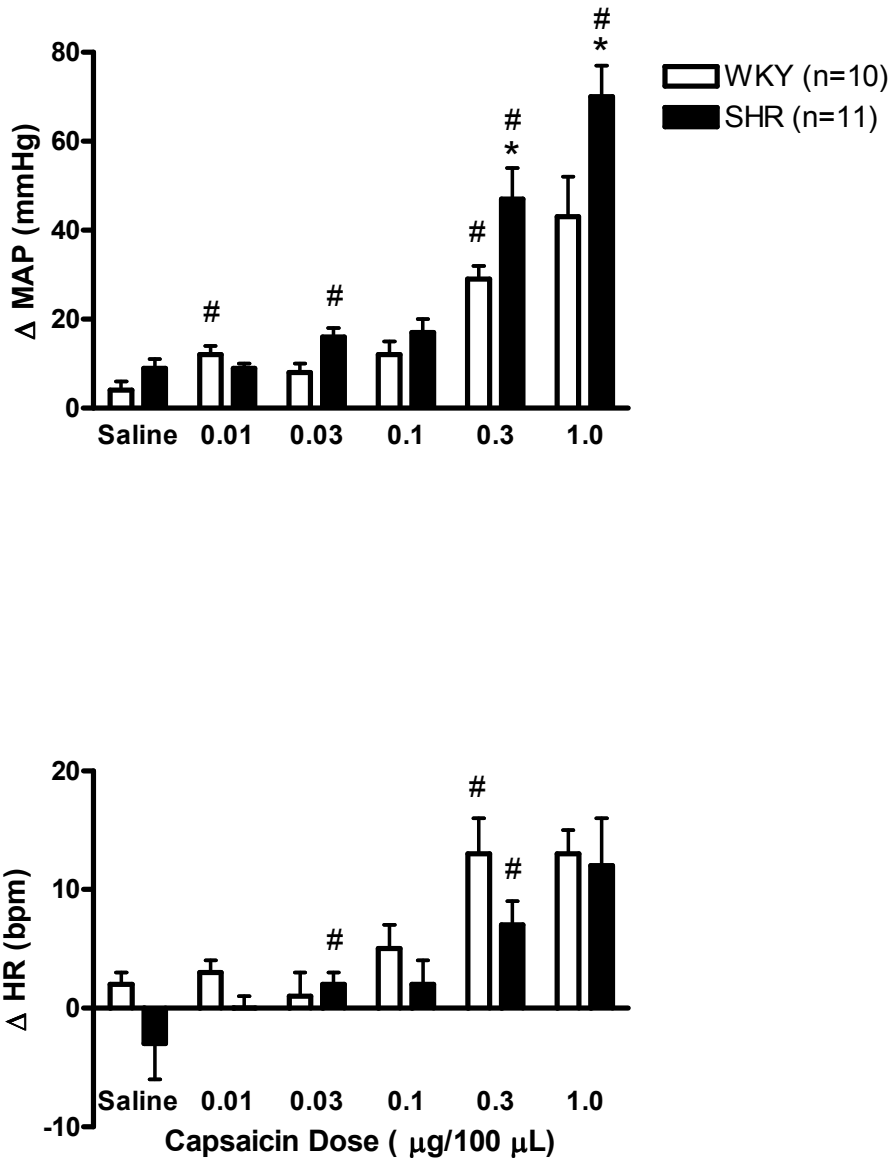

Figure 4.6: Cardiovascular responses to hindlimb intra-arterial injections of capsaicin in SHR and WKY animals. Changes in MAP were greater in SHR than WKY animals, while heart rate responses were more variable. *Significance from WKY. #Response was significantly different from preceding response elicited by capsaicin in the individual WKY and SHR groups. ($P<0.05$)

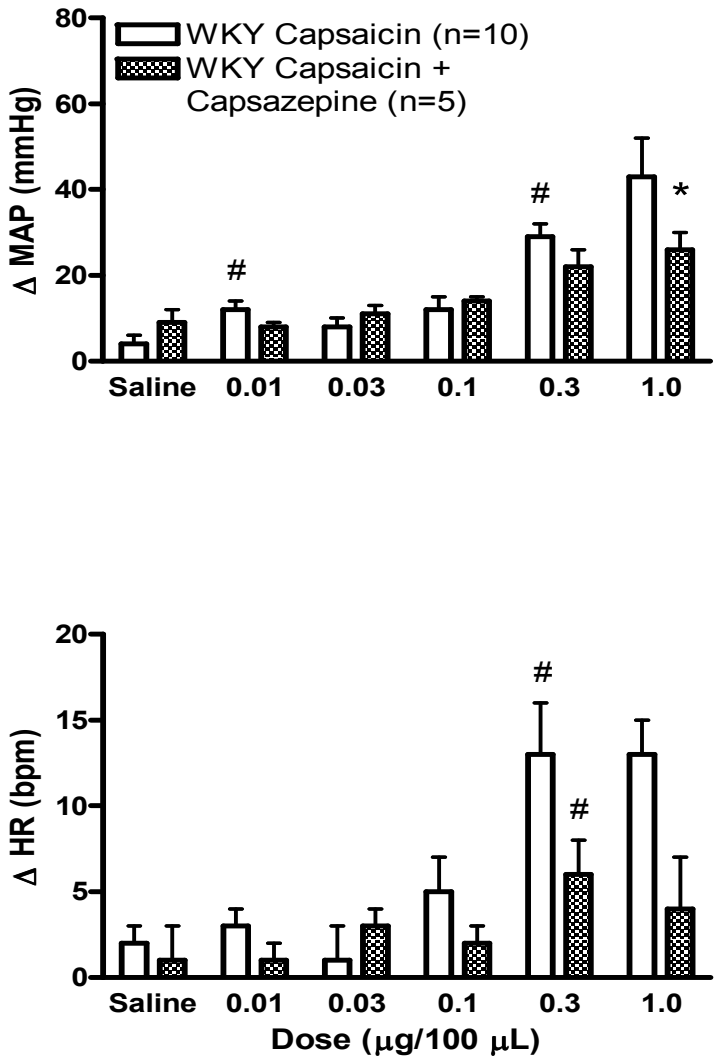

Figure 4.7: Changes in MAP and HR in response to intra-arterial injections of capsaicin and the selective, competitive TRPv1 antagonist capsazepine in WKY rats. Capsazepine effectively reduced the cardiovascular response elicited by the group IV afferent fibers. *Significance from WKY Capsaicin. #Response was significantly different from preceding capsaicin or capsaicin plus capsazepine concentrations for WKY animals. ($P<0.05$)

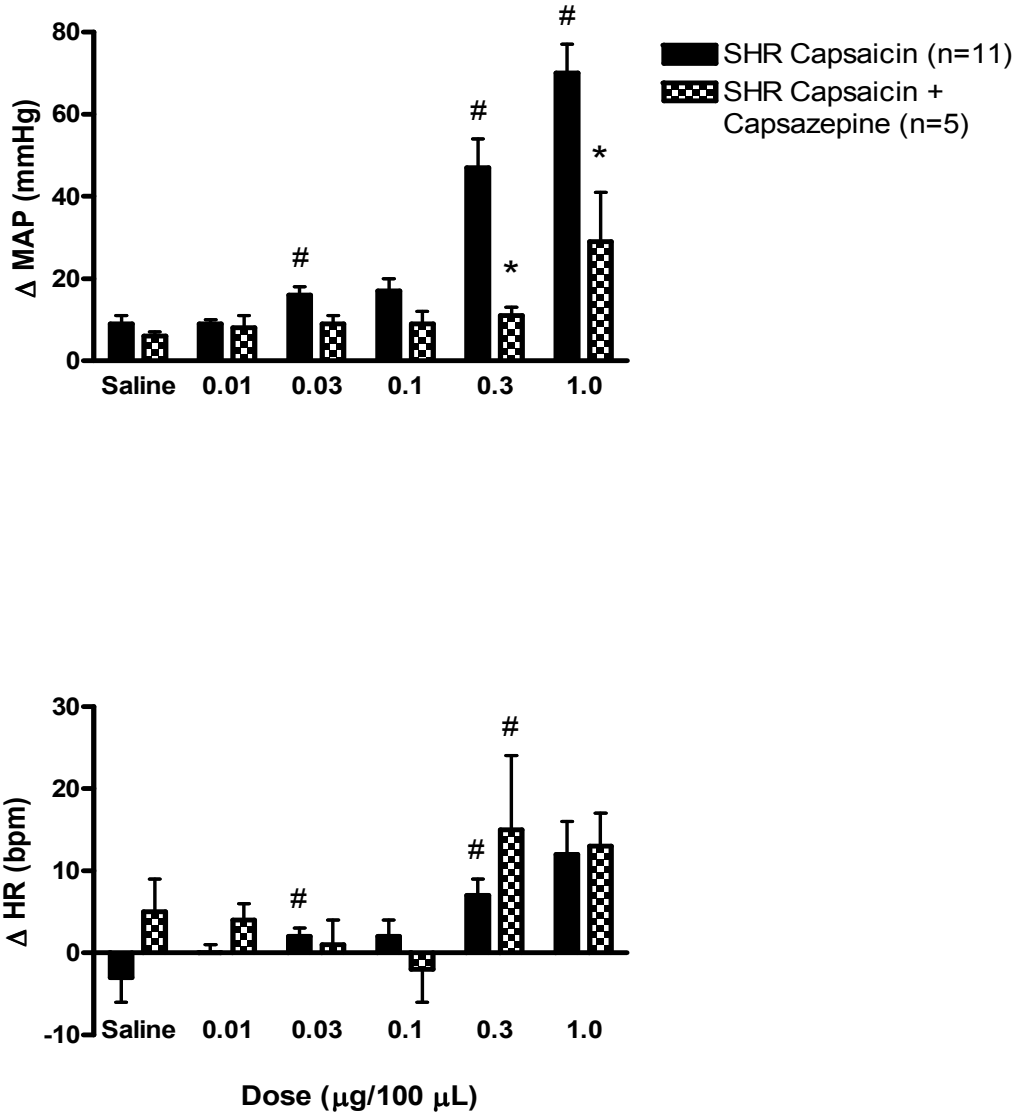

Figure 4.8: Changes in MAP and HR in response to intra-arterial injections of capsaicin and the competitive TRPv1 antagonist capsazepine in SHR rats. Capsazepine administration attenuated the cardiovascular response to capsaicin by blocking the TRPv1 receptors on group IV afferents. *Significance from SHR Capsaicin. #Response was significantly different from preceding capsaicin or capsaicin plus capsazepine concentrations for SHR animals. ($P<0.05$)

As a control procedure, jugular vein injections of 1.0 μg/100 μL capsaicin were given at the end of each experiment. The cardiovascular response to capsaicin in the systemic circulation compared to capsaicin in the occluded hindlimb was significantly different in both sets of animals (Figure 4.9). When capsaicin was administered via the jugular vein instead of the iliac artery, the increases in mean arterial pressure were decreased. In WKY animals, the venous injection produced an increase of 13 ± 7 mmHg, while the arterial injection caused an increase of 43 ± 9 mmHg. And in SHR rats, the venous injection elicited a significant change in mean arterial pressure of 34 ± 10 mmHg and the arterial injection cause an increase of 70 ± 7 mmHg. A decrease in heart rate was seen after intra-venous injection of capsaicin in both WKY (-103 ± 48 bpm) and SHR (-73 ± 30 bpm) animals. These bradycardiac responses were significantly different from the increases in heart rate elicited by activation of the exercise pressor reflex by infusing capsaicin intra-arterially into the hindlimb.

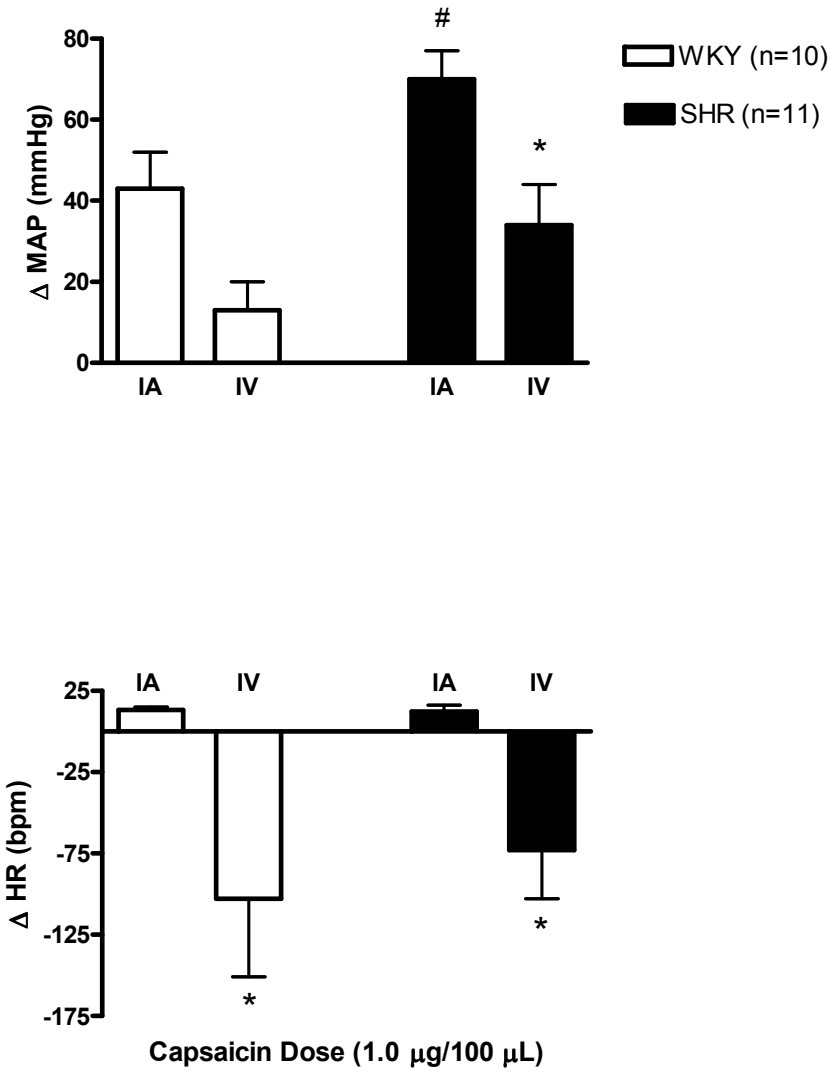

Figure 4.9. Cardiovascular responses to intra-arterial (IA) injections with occlusion and intra-venous (IV) injections without occlusion of 1.0 μg/100 μL capsaicin in WKY and SHR animals. Capsaicin causes an attenuated increase in mean arterial pressure and a decrease in heart rate when introduced into the systemic circulation. *Significance from intra-arterial injections. . #Response was significantly different between WKY and SHR animals. ($P<0.05$)

CHAPTER 5

DISCUSSION

5.1 Important New Findings

The cardiovascular response to exercise in hypertension is exaggerated(116, 117). Previous studies in our lab have shown that these augmented increases in mean arterial pressure and heart rate are mediated, in part, by activation of the exercise pressor reflex(116). Prior to this study, however, it was unknown as to what component of the exercise pressor reflex (the mechanoreflex or the metaboreflex) was causing the exaggerated response. Through these sets of experiments, it can be concluded that i) the mechanically sensitive component of the exercise pressor reflex is overactive in hypertensive rats and ii) the metabolically sensitive afferents known to contribute to the exercise pressor reflex are overactive in hypertensive rats. One corollary finding was that the mechanically sensitive afferents elicit increases in mean arterial pressure and heart rate in response to passive stretch that are positively correlated to the stretch intensity. Also, the changes in mean arterial pressure and heart rate seen in response to hindlimb capsaicin injections were indeed caused by activation of group IV afferent neurons because the introduction of capsaicin into the systemic circulation produced different cardiovascular results.

5.2 The Muscle Mechanoreflex in Hypertension

The exaggerated cardiovascular response to passive stretch demonstrated that the mechanically sensitive component of the exercise pressor reflex is overactive in SHR rats. Increases in both mean arterial pressure and heart rate were greater in hypertensive rats than in normotensive rats in response to selective activation of mechanically sensitive afferent fibers. In addition, the changes in mean arterial pressure and heart rate were positively correlated to the tension developed by the triceps surae muscle.

One important fact about the exercise pressor reflex is that it produces cardiovascular responses that are tension-dependant. The working muscle's tension development is proportional to the resulting increases in mean arterial pressure and heart rate(113). This tension-dependant property of the exercise pressor reflex was evident in the results of both normotensive and hypertensive rats. The regression lines in Figure 4.4 show that the cardiovascular response increased as the muscle worked harder in both WKY and SHR rats. However, for the same amount of developed tension, the changes in mean arterial pressure and heart rate in response to activation of the muscle mechanoreflex were greater in hypertension. Importantly, for each individual rat, the maximum tension developed during stretch was predetermined by the maximal amount of tension the animal could develop during physiological contraction of the hindlimb skeletal muscle. This allowed stimulation of the muscle mechanoreflex at maximal and submaximal intensities within the physiologic range of tension developed during normal muscle contraction. Thus, mechanoreflex function was

probed under conditions each individual rat could experience in a natural situation, as opposed to having the same maximal stretch intensity for all animals.

The data in Figure 4.3 shows the changes in mean arterial pressure and heart rate elicited by the mechanoreflex in response to passive stretch at submaximal intensities were significantly greater in hypertensive rats. Moreover, the linear regression lines in Figure 4.4 show the changes in mean arterial pressure and heart rate in response to passive stretch were positively correlated to stretch intensities in both WKY and SHR rats. However, the steeper slopes for the SHR animals provide evidence that the mechanically sensitive afferent fibers are overactive in hypertension because for a given tension, the cardiovascular responses are enhanced. Since both sets of animals had cardiovascular responses to passive stretch that were linearly related to stretch intensity, it might seem difficult to access which set had the abnormal response to exercise. However, it has been well established that the Wistar-Kyoto strain of rats is the genetic control for Spontaneously Hypertensive Rats(84). Also, the changes in mean arterial pressure and heart rate are in line with results of previous studies and are taken to be normal(114, 116). Therefore, it can be concluded that the steeper slopes seen in the regression lines for SHR rats are due to the hyperactivity of the mechanically sensitive afferents. The changes in magnitude of both mean arterial pressure and heart rate were greater in animals that had higher baseline blood pressures.

To be sure that the circulatory responses seen were due to the mechanically sensitive component of the exercise pressor reflex, the dorsal roots transmitting sensory information from the stretched hindlimb were sectioned. This maneuver abolished the

cardiovascular response to stretch, indicating the changes elicited were reflex in origin. Further, as the animals were decerebrated, input from central command was eliminated because central command has been shown to originate in cortical areas(14, 22). However, it should be noted that the baroreceptors were left intact. In hypertension, baroreflex function is attenuated. The sensitivity with which the baroreflex controls heart rate is attenuated, possibly due to dysfunction of its vagal component(73). It has also been suggested that the baroreflex dysfunction present in hypertension is partly responsible for the increased sympathetic nerve activity(56). Since the baroreflex is known to inhibit the exercise pressor reflex (including the mechanoreflex), the reduced function of the baroreflex could release that inhibition and account for some of the cardiovascular changes seen in response to passive stretch in the SHR rats(92).

Another limitation is that passive stretch is not static exercise. The triceps surae muscle was manipulated in a manner that was different from contraction and the exercise pressor reflex is known to be stimulated during contraction. Therefore, the mechanically sensitive component of the exercise pressor reflex needs to be examined under more physiological conditions. One possible method is to block the mechanically sensitive afferent fiber receptors during muscle contraction and compare the cardiovascular response with that elicited by contraction with no blockade.

5.3 The Muscle Metaboreflex in Hypertension

In response to intra-arterial capsaicin injections in the hindlimb, the increases in mean arterial pressure were greater in SHR than in WKY rats, especially at the two highest concentrations. However, the changes in heart rate were much more variable.

While the metabolically sensitive afferents are overactive in their control of mean arterial pressure, they seem to play little role in the exaggerated increases in heart rate previously seen during activation of the exercise pressor reflex in hypertensive rats(117). It could be that the group IV afferents do not control heart rate as well as the group III afferent fibers. In the previous study on the exercise pressor reflex and hypertension, it was shown that increases in heart rate were larger in SHR than in WKY rats. However, these cardiovascular responses were in response to static contraction, when both group III and group IV afferent fibers were activated. It could be that the group III afferent fibers had more control over these heart rate changes than did the group IV afferent fibers.

The greater control of heart rate by mechanically sensitive group III afferents could also explain why the changes in heart rate produced by the metabolically sensitive group IV afferents were not significantly different from the changes seen in WKY animals. However, the results did show increases in heart rate in response to higher concentrations of capsaicin that were significantly different than those seen after injection of saline. Therefore, it can be concluded that the group IV afferent fibers do cause increases in heart rate in both WKY and SHR animals. However, the changes in heart rate in each group of animals were similar.

One limitation of these experiments is that capsaicin is not a naturally-occurring chemical in the body and it is not a metabolic byproduct of working muscle. However, it is well established that capsaicin selectively binds to TRPv1 receptors which are located predominantly on group IV afferent fibers(117). Therefore, it can be assumed

that the cardiovascular responses were due to the activation of group IV afferent fibers. To substantiate this, capsazepine, a chemical known to be a selective, competitive antagonist to the TRPv1 receptors was given. The results in Figures 4.7 and 4.8 demonstrate that capsazepine acted on the same receptor sites as capsaicin because the group IV afferent fiber-mediated increases in mean arterial pressure were effectively reduced when capsazepine was given simultaneously with capsaicin(12). In both WKY and SHR animals, the introduction of capsazepine into the circulation of the hindlimb significantly reduced the mean arterial pressure response to the highest dose of capsaicin. Capsazepine also caused diminished heart rate responses in WKY rats. While the heart rate response was not significantly affected by capsazepine in WKY or SHR animals, this fits with the previous data that heart rate response to activation of the metaboreflex is variable and difficult to characterize.

The firing of metabolically sensitive afferent fibers was not completely blocked by the administration of capsazepine. It could be that the cardiovascular responses were not completely abolished because there was not enough capsazepine to compete with the capsaicin molecules. It is also possible that the polymorphic property of the group III and group IV afferents allowed some metabolically sensitive group III fibers to fire in response to capsaicin. Although TRPv1 receptors are not known to be found on group III fibers, capsaicin could activate receptors that have yet to be identified. These unidentified receptors could exist on both group III and group IV fibers and account for some of the cardiovascular response.

To be sure that the cardiovascular responses were due to activation of group IV afferent fibers in the hindlimb only, capsaicin was also administered intra-venously at the end of experimentation. The results from this jugular injection showed that capsaicin elicits a blunted increase in mean arterial pressure and a decrease in heart rate when in the systemic circulation. This response was vastly different from that seen when capsaicin was injected intra-arterially and only allowed to circulate in the hindlimb. Therefore it can be concluded that the cardiovascular responses were caused by the metabolically sensitive fibers of the exercise pressor reflex in the right triceps surae muscle. Additionally, the bradycardia and small increase in mean arterial pressure are consistent with the effect of capsaicin on receptors in the pulmonary circulation(18).

The volume of fluid injected with each capsaicin dose did not affect the cardiovascular responses. This was verified by the slight increases in mean arterial pressure and heart rate in response to saline. In addition, randomization of the capsaicin injections was performed throughout the study with no appreciable change in the cardiovascular response elicited by each individual injection. This showed that the effects of the injected capsaicin were not additive; the allotted 15 minutes between injections was enough time to eliminate the effects of the circulating capsaicin from the previous injections.

An important limitation of the present study is that capsaicin was used to activate metabolically sensitive afferent fibers in a manner that is not consistent with their activation during normal muscle contraction. Intra-arterial capsaicin

administration does not mimic static exercise, but instead causes graded activation of group IV afferent neurons(117). This could have an affect on the cardiovascular response. Due to decerebration, input from central command was eliminated. However, the baroreceptors were left intact. Again, the reduced sensitivity of the baroreflex could release inhibition on the metaboreflex and account for some of the exaggerated cardiovascular response in the SHR rats(92). However, the use of capsaicin to isolate group IV afferent fibers is well accepted and the changes in mean arterial pressure and heart rate in the WKY animals were consistent with previous literature(50, 114, 116).

5.4 Possible Mechanisms of Exercise Pressor Reflex Hyperactivity

The results from this study have shown that the mechanically sensitive group III and metabolically sensitive group IV afferent fibers, which contribute to the exercise pressor reflex, are overactive in hypertension. Several possibilities exist as to the mechanism of dysfunction exhibited by each component of the exercise pressor reflex. Alterations in the peripheral afferent arm of the exercise pressor reflex, such as changes in the chemical stimuli and receptors or sensitivity of the mechanically sensitive receptors, could lead to the exaggerated cardiovascular response. Lactic acid, hydrogen ions, bradykinin, potassium ions, diprotonated phosphate, prostaglandins, and ATP analogs have all been shown to activate afferent fibers in muscle and blood vessels(26, 27, 38, 85, 111, 119, 127). A change in the release or amount of any of these could augment the exercise pressor reflex. Also, the $P2X_3$ receptor in cats and the TRPv1 receptor in rats and humans have been shown to be primarily localized to group

IV afferents(38, 117). These receptors are currently being studied to see if they mediate the metaboreflex contribution to the exercise pressor reflex. A change in the conformation or regulation of these receptors or others could contribute to exercise pressor reflex dysfunction. In addition to the afferent arm of the exercise pressor reflex being overactive, hypertension could cause alterations in the spinal cord and brainstem as well as the efferent arm of the reflex, leading to further exaggerations in the cardiovascular response to exercise.

The changes seen in the exercise pressor response could be due alterations in the spinal cord and/or brainstem. Many of the group III and group IV afferent fibers synapse in the dorsal horn of the spinal cord, specifically to Rexed's laminae I, II, V, and X(48, 86). From the spinal cord, the afferents project to areas within the brainstem such as the nucleus tractus solitarius, the caudal and rostral ventrolateral medulla, the caudal hypothalamus, and the periaqueductal gray(3, 16, 46, 62). Neural activity within the spinal cord and brainstem involves neurotransmitters and neuromodulators such as glutamate, substance P, and nitric oxide(37, 131). Alterations in the amount, release, or function of any of these chemicals or their receptors in the spinal cord or brainstem could affect the cardiovascular response to exercise in hypertension(54).

The exaggerated response could also be caused by changes in the sympathetic efferent arm of the reflex. It has been established that basal sympathetic nerve activity is increased in hypertension, and it is known that the mechanoreflex and metaboreflex increase sympathetic nerve activity. Activation of either of these reflexes during exercise in hypertension may further exaggerate sympathetic nerve activity, eliciting

large increases in blood pressure and heart rate. It is also possible that morphological and/or functional changes in the peripheral vasculature in response to sustained, high blood pressure could cause the exaggerated pressor response.

5.5 Interactions between the Arterial Baroreceptors, Central Command, and the Exercise Pressor Reflex

While there is little concern that central command and the arterial baroreceptors affected the results of these experiments, interactions between the three mechanisms that alter the cardiovascular response to exercise exist.

The arterial baroreflex buffers changes in mean arterial pressure and heart rate. In order to preserve the sensitivity of the baroreflex, the receptors are reset to operate around higher arterial pressures during exercise(70). During dynamic and static exercise, both central command and the exercise pressor reflex are involved in the baroreflex resetting(87, 88). These three mechanisms work together to control the cardiovascular response and maintain blood pressure during physical activity. However, when any one of these blood pressure regulators is altered, the baroreflex function could be affected and the cardiovascular response to exercise potentiated. It has been established that the exercise pressor reflex is overactive and that baroreflex sensitivity is reduced in hypertension(73, 92, 116). While little is known about the activity of central command in hypertension, it is possible that neural changes occur there as well. Any and all of these changes in the arterial baroreceptors, central command, and the exercise pressor reflex could magnify abnormalities in any of the

other pressure-regulating neural mechanisms and thus contribute to the exaggerated cardiovascular response elicited by exercise in hypertension.

5.6 The Exercise Pressor Reflex in Heart Failure

The cardiovascular response to exercise is abnormal in heart failure, as well as hypertension. Studies in cardiomyopathic rats have shown that the exercise pressor reflex mediates an exaggerated cardiovascular response to static muscle contraction(112). Further, it has been observed that the selective activation of the group IV afferent fibers by capsaicin administration produced a diminished cardiovascular response. Therefore, it is thought that the mechanically sensitive afferent fibers are overactive in order to compensate for the abnormal function of the group IV afferent fibers(117). In the current study, it was found that both the mechanically sensitive and metabolically sensitive afferent fibers were overactive in hypertension. This could account for the higher increases in mean arterial pressure observed in response to electrically-induced hindlimb contraction in hypertensive rats compared to cardiomyopathic rats (43 ± 6 mmHg compared to 32 ± 2 mmHg)(112, 116). Therefore, the pathophysiology of the exercise pressor reflex seems to be different in heart failure compared to hypertension.

5.7 Future Studies

To clarify the contribution of the mechanically sensitive exercise pressor component to the exaggerated cardiovascular response seen in hypertension, the mechanoreflex needs to be tested during contraction. As mentioned, passive stretch does not precisely mimic dynamic or static exercise, therefore the mechanoreflex-

mediated cardiovascular response to contraction would be more similar to a native response to exercise. Further analysis of the group III afferents could be performed by administering the trivalent lanthanide gadolinium, a known blocker of mechanically sensitive skeletal muscle receptors during electrically-induced contraction(41). Administration of gadolinium during hindlimb contraction would allow the effects of group III afferent fibers to be quantified. The changes in mean arterial pressure and heart rate in response to contraction after injection of gadolinium could be compared with the cardiovascular response to contraction without the pharmacologic blocker. This comparison would show what magnitude of the total exercise pressor response is due to the mechanically sensitive afferent fibers only.

To test the group IV afferent fibers in more detail, endogenous substances released by the skeletal myocytes should be injected into the hindlimb arterial supply. Capsaicin is not a naturally occurring chemical in the body, therefore the group IV afferent fibers should be stimulated by metabolites that are naturally produced by working muscle. These include adenosine, potassium ions, hydrogen ions, and lactic acid among others(28). The TRPv1 receptor should continue to be targeted since it is found exclusively on group IV afferent fibers(117). In addition to capsaicin, heat, acid, and various lipids bind to the TRPv1 receptor and induce opening, leading to membrane depolarization and propagation of an action potential(15). Once the group IV fiber-mediated cardiovascular response to endogenous substances has been determined, antagonists to the same substances should be given during hindlimb muscle contraction. By pharmacologically blocking the group IV afferent fibers during muscle contraction,

the cardiovascular response without input from group IV afferents could be measured. Then, the results could be compared to the response elicited by both components of the exercise pressor reflex to contraction. This would allow the contribution of the group IV neurons to the exercise pressor reflex to be quantified.

In order to study the exercise pressor reflex without the affects of the arterial baroreflex, the two previous sets of experiments should be repeated in barodenervated rats. While decerebration eliminates the input from central command, the baroreceptors are still functional. Therefore, the experiments should be performed in rats in which the arterial baroreceptors have been denervated. Barodenervation of the carotid sinus and aortic arch would ensure that all cardiovascular responses seen after muscle contraction were due to the mechanically and metabolically sensitive components of the exercise pressor reflex.

In hypertension, the afferent arm of the exercise pressor reflex is augmented by changes in peripheral skeletal muscle and blood vessels. Alterations of neural transduction in the spinal cord and brainstem, as well as increases in sympathetic nerve activity from the efferent arm of the reflex arc affect the cardiovascular response to exercise in hypertension. Central processing in the brainstem is of particular interest because current research has established the regulatory role of nitric oxide within the brainstem on exercise pressor reflex function(63, 115). To determine what actually causes the exaggerated response of group III and group IV afferents in hypertension, greater research will have to be done in identifying the neural activity that occurs within the nucleus tractus solitarius of the medulla oblongata. The nucleus tractus solitarius is

an area of interest because most of the group III and IV afferent fibers synapse in the spinal cord and then project there. Neuroanatomical evidence suggests that the nucleus of the solitary tract is a crucial site for sensory processing of exercise pressor reflex input(48, 89, 123, 124). Neural activity from central command and the arterial baroreceptors may also occur in that area (Figure 5.1)(93). Also, within the nucleus tractus solitarius, there are many neurotransmitters and neuromodulators involved in the exercise pressor reflex arc that are known to be altered in hypertension. The nitric oxide pathway is of particular interest because the activity of medullary neurons that receive and process sensory information from group III and IV afferents can be modulated by its endogenous production(61, 106, 125). Within the nucleus tractus solitarius, nitric oxide and L-citrulline are produced through oxidation of L-arginine by an enzymatic reaction mediated by nitric oxide synthase(79). Centrally derived nitric oxide has been marked as an important inhibitory component of the signal transduction pathway that controls sympathetic outflow from the medullary brainstem(6, 57). A previous study in our lab showed that increasing the production of nitric oxide in the nucleus tractus solitarius attenuated the increases in mean arterial pressure caused by the exercise pressor reflex(115).

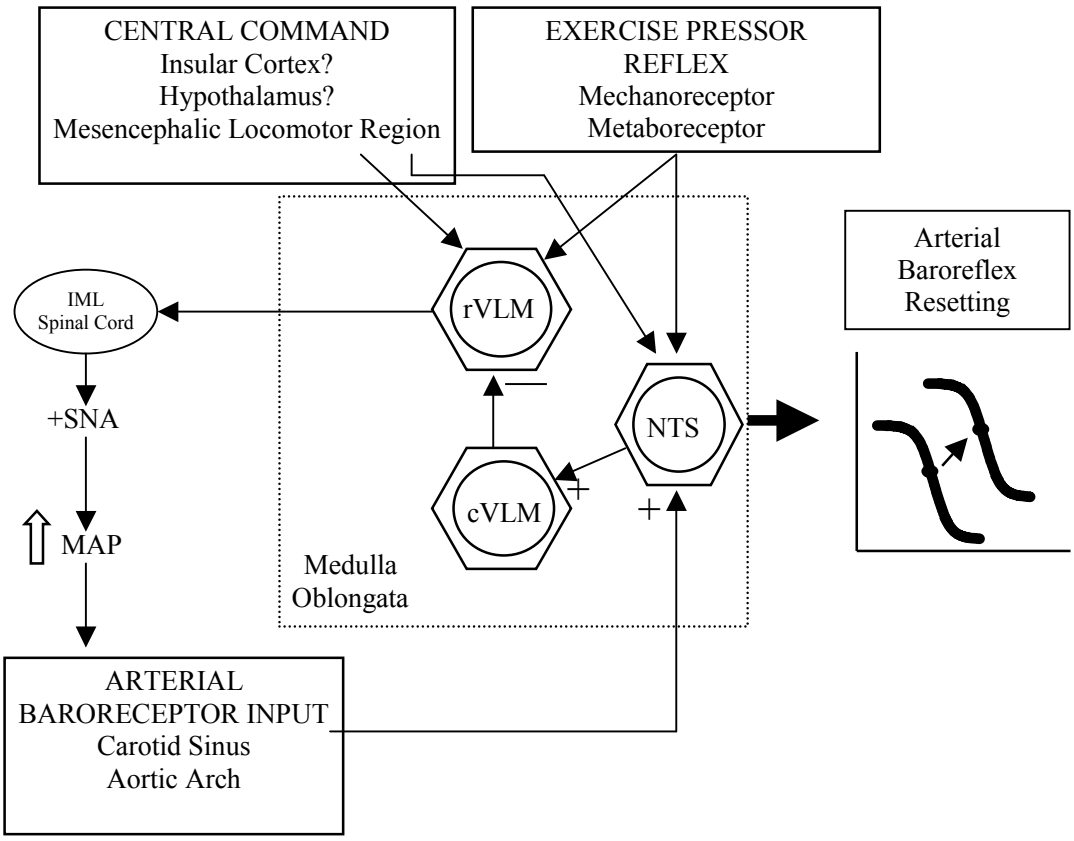

Figure 5.1: Diagram of plausible neural pathways between arterial baroreceptors, central command, and the exercise pressor reflex during exercise. Arterial baroreceptor afferents project to the nucleus tractus solitarius (NTS), where exercise pressor reflex afferents also synapse. The exercise pressor reflex also may alter neuronal function within the caudal and rostral ventrolateral medullar (cVLM and rVLM) to control cardiovascular responses. Evidence suggests that central command afferents synapse within the rVLM and NTS. During exercise, neurons from the NTS project to the cVLM and then on to the rVLM. From the rVLM, efferents synapse in the intermediolateral cell columns (IML) of the spinal cord and then travel with sympathetic neurons to raise sympathetic nerve activity (SNA) and mean arterial pressure (MAP). Source: (23)

In hypertension, it is possible that alterations in the nitric oxide pathway are responsible for the exaggerations in sympathetic nerve activity seen in response to exercise. It has been shown that methylated arginines, which are elevated in

hypertension, can pharmacologically inhibit nitric oxide synthase(10, 45). Also, the superoxide anion NAD(P)H (nicotinamide-adenine dinucleotide phosphate) is known to inactivate nitric oxide. Moreover, NAD(P)H oxidase activity is increased in hypertensive individuals due to the increased physical stress and/or the presence of angiotensin II(34). This presents the possibility that decreased levels of nitric oxide in hypertension are responsible for the elevations in sympathetic nerve activity.

To test this hypothesis, it will be necessary to perform microdialysis during the same passive stretch and capsaicin infusion protocols that were used in this study. To better understand how nitric oxide is involved in the exercise pressor arc, various nitric oxide precursors (L-arginine), donors (diethylamine nonoate), and nitric oxide synthase inhibitors (N^G-nitro-L-arginine methyl ester hydrochloride and N^G -monomethyl-L-arginine monoacetate) will be delivered to the nucleus tractus solitarius in SHR and WKY rats, while the group III and group IV afferent fibers are activated. This will allow the bioavailability of nitric oxide to be experimentally controlled in conjunction with the distinct activation of the mechanically and metabolically sensitive skeletal muscle afferents. More importantly, it will provide a biochemical basis for the abnormal cardiovascular response to exercise seen in hypertension.

5.8 Conclusions and Clinical Significance

From these results, it is concluded that both the mechanically sensitive and metabolically sensitive components of the exercise pressor reflex are overactive in hypertension. In response to passive stretch, the mechanically sensitive afferents elicited increases in mean arterial pressure and heart rate that were greater in SHR

animals than in WKY animals and the increases were linearly correlated to the tension development. Also, activation of the metabolically sensitive afferents by capsaicin administration produced exaggerated increases in mean arterial pressure in hypertensive rats.

The effects of hypertension are complicated and widespread throughout the circulatory system. Enhanced basal sympathetic nerve activity along with the vascular remodeling due to sustained, long-term exposure to high blood pressure causes changes in the cardiovascular system at rest and during exercise(21, 90). The exaggerated response to exercise seen in hypertension is characterized by increases in systolic pressure, diastolic pressure, and vascular resistance(25, 39). Cardiac output, oxygen uptake, and stroke volume are all reduced(25).

Prolonged exposure to hypertension can lead to stroke and heart disease(49). Therefore it is imperative that blood pressure be lowered in affected individuals. Regular exercise can decrease body fat, improve metabolic processes, lower basal sympathetic nerve activity, as well as normalize pressor and baroreceptor responses(56, 67). Exercise can also decrease arterial stiffness and increase compliance, which in turn lowers total vascular resistance (49). The result of all these changes is lower arterial blood pressure. Unfortunately, exercise training is often accompanied with adverse effects in hypertensive patients. There exists risk of stroke or other cardiac events during the execution of physical activity. Exaggerated cardiovascular responses to exercise mediated by the exercise pressor reflex contribute to the enhanced risk. Therefore, increasing our understanding of the mechanoreflex and metaboreflex

dysfunction in this disease may lead to the development of therapeutic interventions designed to make exercise a safer modality of treatment. Further, by dissecting the neural mechanisms of the exercise pressor reflex, specifically the neuronal activity within the nucleus tractus solitarius, it will be possible to qualitatively and quantitatively describe the cause of the abnormal circulatory response. This may lead to the development of therapeutic strategies that prevent physical activity from contributing to cardiovascular risk in hypertension.

APPENDIX A

MECHANOREFLEX DATA

wky n		**1**
file name		WKY 2
weight (g)		330

	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	nd			nd					
Resting/DCR	78			409					
Passive stretch	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
100%	68	88	20	406	411	5	86	815	729
91%	83	95	12	413	413	0	71	734	663
89%	78	92	14	414	415	1	81	729	648
Avg. Baseline	76.75			410.5			79.33333		

Wet Spec.	
heart (g)	1.23
lungs (g)	nd
triceps surae (g)	2.19
tibia length (cm)	3.55
heart/body	0.003727
heart/tibia	0.346479
lung/body	#VALUE!

wky n		**2**
file name		WKY 3
weight (g)		315

	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	nd			nd					
Resting/DCR	71			406					
Passive stretch	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
100%	44	46	2	399	399	0	76	734	658
96%	42	47	5	411	414	3	77	708	631
64%	42	43	1	nd	nd	#VALUE!	60	481	421
Avg. Baseline	49.75			405.3333			71		

Wet Spec.	
heart (g)	1.29
lungs (g)	nd
triceps surae (g)	1.92
tibia length (cm)	3.75
heart/body	0.004095
heart/tibia	0.344
lung/body	#VALUE!

wky n			**3**						
file name			WKY 5						
weight (g)			312						
	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	nd			nd					
Resting/DCR	83			457					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	78	85	7	445	448	3	88	1077	989
77%	84	88	4	458	460	2	72	836	764
67%	82	84	2	446	446	0	97	761	664
37%	79	81	2	468	468	0	72	437	365
Avg. Baseline	81.2			454.8			82.25		
Wet Spec.									
heart (g)	1.04								
lungs (g)	nd								
triceps surae (g)	2.03								
tibia length (cm)	3.544								
heart/body	0.003333								
heart/tibia	0.293454								
lung/body	#VALUE!								

wky n			**4**						
file name			WKY 6						
weight (g)			330						
	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	nd			nd					
Resting/DCR	88			478					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	72	98	26	449	462	13	69	1116	1047
71%	77	87	10	438	445	7	78	822	744
65%	79	89	10	432	436	4	78	757	679
25%	74	75	1	458	458	0	78	339	261
Avg. Baseline	78			451			75.75		
Wet Spec.									
heart (g)	1.17								
lungs (g)	nd								
triceps surae (g)	1.97								
tibia length (cm)	3.572								
heart/body	0.003545								
heart/tibia	0.327548								
lung/body	#VALUE!								

wky n			**5**						
file name			WKY 7						
weight (g)			341						
	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	nd			nd					
Resting/DCR	52			367					
Passive stretch	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
100%	51	75	24	368	373	5	93	980	887
67%	55	63	8	371	373	2	87	678	591
Avg. Baseline	52.66667			368.6667			90		
Wet Spec.									
heart (g)	1.37								
lungs (g)	nd								
triceps surae (g)	2.09								
tibia length (cm)	3.549								
heart/body	0.004018								
heart/tibia	0.386024								
lung/body	#VALUE!								

wky n			**6**						
file name			WKY 9						
weight (g)			346						
	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	nd			nd					
Resting/DCR	55			346					
Passive stretch	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
100%	57	65	8	407	410	3	83	1192	1109
76%	57	62	5	410	412	2	68	908	840
35%	59	62	3	413	415	2	58	442	384
Avg. Baseline	57			394			69.66667		
Wet Spec.									
heart (g)	0.98								
lungs (g)	nd								
triceps surae (g)	2.2								
tibia length (cm)	3.635								
heart/body	0.002832								
heart/tibia	0.269601								
lung/body	#VALUE!								

wky n			**7**						
file name			WKY 12						
weight (g)			312						
	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	102			311					
Resting/DCR	109			452					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	87	106	19	447	450	3	69	1009	940
64%	54	60	6	364	367	3	96	693	597
37%	48	51	3	nd	nd	#VALUE!	77	425	348
Avg. Baseline	74.5			421			80.66667		
Wet Spec.									
heart (g)	0.95								
lungs (g)	2.43								
triceps surae (g)	1.91								
tibia length (cm)	3.731								
heart/body	0.003045								
heart/tibia	0.254623								
lung/body	0.007788								

wky n			**8**						
file name			WKY 13						
weight (g)			309						
	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	77			346					
Resting/DCR	51			340					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	44	47	3	324	326	2	84	1251	1167
72%	45	47	2	294	297	3	75	911	836
37%	43	44	1	291	294	3	77	505	428
Avg. Baseline	45.75			312.25			78.66667		
Wet Spec.									
heart (g)	0.88								
lungs (g)	1.9								
triceps surae (g)	1.92								
tibia length (cm)	3.438								
heart/body	0.002848								
heart/tibia	0.255963								
lung/body	0.006149								

wky n	**9**	
file name	WKY 14	
weight (g)	323	

	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	91			268					
Resting/DCR	89			444					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	66	80	14	395	398	3	95	1260	1165
69%	69	82	13	416	421	5	91	899	808
39%	68	78	10	393	395	2	61	516	455
Avg. Baseline	73			412			82.33333		

Wet Spec.	
heart (g)	0.99
lungs (g)	1.58
triceps surae (g)	1.92
tibia length (cm)	3.489
heart/body	0.003065
heart/tibia	0.283749
lung/body	0.004892

wky n	**10**	
file name	WKY 15	
weight (g)	327	

	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	94			287					
Resting/DCR	98			382					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	119	136	17	359	366	7	84	1064	980
64%	97	99	2	350	355	5	71	701	630
33%	112	114	2	341	343	2	80	402	322
Avg. Baseline	106.5			358			78.33333		

Wet Spec.	
heart (g)	1.04
lungs (g)	2.46
triceps surae (g)	1.92
tibia length (cm)	3.556
heart/body	0.00318
heart/tibia	0.292463
lung/body	0.007523

wky n			**11**						
file name			Age1WKY						
weight (g)			397						
	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	107			314					
Resting/DCR	58			406					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	45	54	9	391	401	10	81	1095	1014
Avg. Baseline	51.5			398.5			81		
Wet Spec.									
heart (g)	1.24								
lungs (g)	2.04								
triceps surae (g)	2.15								
tibia length (cm)	nd								
heart/body	0.003123								
heart/tibia	#VALUE!								
lung/body	0.005139								

wky n			**12**						
file name			Age2WKY						
weight (g)			403						
	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	105			238					
Resting/DCR	79			387					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	65	70	5	395	401	6	69	1114	1045
Avg. Baseline	72			391			69		
Wet Spec.									
heart (g)	1.18								
lungs (g)	1.98								
triceps surae (g)	2.26								
tibia length (cm)	nd								
heart/body	0.002928								
heart/tibia	#VALUE!								
lung/body	0.004913								

wky n			**13**						
file name			WKY 16						
weight (g)			348						
	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	112			314					
Resting/DCR	100			361					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	89	107	18	342	346	4	79	1035	956
Avg. Baseline	94.5			351.5			79		
Wet Spec.									
heart (g)	1.1								
lungs (g)	1.98								
triceps surae (g)	2.09								
tibia length (cm)	3.871								
heart/body	0.003161								
heart/tibia	0.284164								
lung/body	0.00569								

wky n			**14**						
file name			WKY 17						
weight (g)			403						
	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	124			327					
Resting/DCR	74			337					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	69	76	7	331	335	4	72	988	916
Avg. Baseline	71.5			334			72		
Wet Spec.									
heart (g)	0.98								
lungs (g)	3.16								
triceps surae (g)	2								
tibia length (cm)	3.909								
heart/body	0.002432								
heart/tibia	0.250704								
lung/body	0.007841								

shr n	**1**	
file name	SHR 2	
weight (g)	320	

	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	nd			nd					
Resting/DCR	175			320					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	159	204	45	322	328	6	82	989	907
67%	176	199	23	318	324	6	88	700	612
39%	191	206	15	318	331	13	76	430	354
Avg. Baseline	175.25			319.5			82		
Wet Spec.									
heart (g)	1.38								
lungs (g)	nd								
triceps surae (g)	1.79								
tibia length (cm)	3.34								
heart/body	0.004313								
heart/tibia	0.413174								
lung/body	#VALUE!								

shr n	**2**	
file name	SHR 3	
weight (g)	327	

	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	nd			nd					
Resting/DCR	188			450					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	104	170	66	425	453	28	85	986	901
75%	133	205	72	412	439	27	85	759	674
54%	102	137	35	411	439	28	68	557	489
Avg. Baseline	131.75			424.5			79.33333		
Wet Spec.									
heart (g)	1.32								
lungs (g)	nd								
triceps surae (g)	1.73								
tibia length (cm)	3.408								
heart/body	0.004037								
heart/tibia	0.387324								
lung/body	#VALUE!								

shr n	**3**	
file name	SHR 4	
weight (g)	327	

	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	nd			nd					
Resting/DCR	nd			nd					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	74	91	17	342	347	5	87	1107	1020
91%	73	89	16	324	331	7	81	1006	925
73%	71	84	13	315	322	7	70	819	749
28%	75	79	4	340	344	4	76	366	290
Avg. Baseline	73.25			330.25			78.5		

Wet Spec.	
heart (g)	1.26
lungs (g)	nd
triceps surae (g)	1.76
tibia length (cm)	3.456
heart/body	0.003853
heart/tibia	0.364583
lung/body	#VALUE!

shr n	**4**	
file name	SHR 5	
weight (g)	340	

	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	nd			nd					
Resting/DCR	nd			nd					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	84	154	70	323	339	16	91	988	897
54%	79	119	40	321	333	12	72	554	482
34%	76	94	18	315	325	10	78	382	304
Avg. Baseline	79.66667			319.6667			80.33333		

Wet Spec.	
heart (g)	1.26
lungs (g)	nd
triceps surae (g)	1.8
tibia length (cm)	3.394
heart/body	0.003706
heart/tibia	0.371243
lung/body	#VALUE!

shr n	**5**	
file name	SHR 6	
weight (g)	340	

	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	nd			nd					
Resting/DCR	nd			nd					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	91	117	26	317	330	13	73	1095	1022
77%	87	109	22	316	323	7	83	872	789
75%	80	107	27	317	327	10	78	845	767
34%	95	100	5	320	325	5	86	431	345
Avg. Baseline	88.25			317.5			80		

Wet Spec.	
heart (g)	1.47
lungs (g)	nd
triceps surae (g)	2.09
tibia length (cm)	3.487
heart/body	0.004324
heart/tibia	0.421566
lung/body	#VALUE!

shr n	**6**	
file name	SHR 10	
weight (g)	372	

	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	168			396					
Resting/DCR	nd			nd					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	98	125	27	358	374	16	90	1039	949
77%	108	124	16	356	360	4	80	812	732
51%	112	122	10	364	368	4	63	545	482
30%	109	116	7	363	364	1	78	360	282
Avg. Baseline	106.75			360.25			77.75		

Wet Spec.	
heart (g)	1.39
lungs (g)	3.2
triceps surae (g)	2.28
tibia length (cm)	3.63
heart/body	0.003737
heart/tibia	0.38292
lung/body	0.008602

shr n			**7**						
file name			SHR 17						
weight (g)			287						
	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	169			393					
Resting/DCR	130			402					
Passive stretch	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
100%	121	145	24	400	406	6	76	1100	1024
73%	116	131	15	400	404	4	75	819	744
53%	109	114	5	409	412	3	89	630	541
Avg. Baseline	119			402.75			80		
Wet Spec.									
heart (g)	0.92								
lungs (g)	1.9								
triceps surae (g)	1.56								
tibia length (cm)	3.589								
heart/body	0.003206								
heart/tibia	0.256339								
lung/body	0.00662								

shr n			**8**						
file name			SHR 20						
weight (g)			310						
	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	181			370					
Resting/DCR	118			421					
Passive stretch	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
100%	101	120	19	348	357	9	79	1171	1092
38%	101	108	7	303	309	6	89	505	416
Avg. Baseline	106.6667			357.3333			84		
Wet Spec.									
heart (g)	1.12								
lungs (g)	2.7								
triceps surae (g)	1.77								
tibia length (cm)	3.736								
heart/body	0.003613								
heart/tibia	0.299786								
lung/body	0.00871								

shr n	**9**	
file name	SHR 22	
weight (g)	303	

	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	206			392					
Resting/DCR	148			403					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	138	176	38	400	404	4	75	786	711
Avg. Baseline	143			401.5			75		

Wet Spec.	
heart (g)	1.04
lungs (g)	1.97
triceps surae (g)	1.77
tibia length (cm)	3.603
heart/body	0.003432
heart/tibia	0.288648
lung/body	0.006502

shr n	**10**	
file name	Age1shr	
weight (g)	295	

	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	140			nd					
Resting/DCR	158			342					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	110	128	18	336	338	2	93	1400	1307
Avg. Baseline	134			339			93		

Wet Spec.	
heart (g)	1.08
lungs (g)	2.46
triceps surae (g)	1.2
tibia length (cm)	nd
heart/body	0.003661
heart/tibia	#VALUE!
lung/body	0.008339

shr n	**11**	
file name	Age2shr	
weight (g)	397	

	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	183			366					
Resting/DCR	151			391					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	84	93	9	344	351	7	70	1285	1215

Avg. Baseline	117.5			367.5			70		

Wet Spec.	
heart (g)	1.555
lungs (g)	3.72
triceps surae (g)	2.06
tibia length (cm)	nd
heart/body	0.003917
heart/tibia	#VALUE!
lung/body	0.00937

shr n	**12**	
file name	Age3shr	
weight (g)	378	

	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	200			nd					
Resting/DCR	135			366					
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**
100%	137	174	37	366	369	3	79	1109	1030

Avg. Baseline	136			366			79		

Wet Spec.	
heart (g)	1.6
lungs (g)	4.13
triceps surae (g)	1.79
tibia length (cm)	nd
heart/body	0.004233
heart/tibia	#VALUE!
lung/body	0.010926

shr n			**13**							
file name			Age4shr							
weight (g)			375							
	MAP (mmHg)			**HR (bpm)**			**Tension (g)**			
Resting/2%	185			342						
Resting/DCR	152			386						
Passive stretch	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ	
100%	157	193	36	nd	nd	#VALUE!	89	1035	946	
Avg. Baseline	154.5			386			89			
Wet Spec.										
heart (g)	1.45									
lungs (g)	3.42									
triceps surae (g)	1.91									
tibia length (cm)	nd									
heart/body	0.003867									
heart/tibia	#VALUE!									
lung/body	0.00912									

shr n			**14**							
file name			SHR 23							
weight (g)			328							
	MAP (mmHg)			**HR (bpm)**			**Tension (g)**			
Resting/2%	188			345						
Resting/DCR	128			304						
Passive stretch	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ	
100%	128	140	12	304	307	3	102	1378	1276	
Avg. Baseline	128			304			102			
Wet Spec.										
heart (g)	1.02									
lungs (g)	2.08									
triceps surae (g)	1.94									
tibia length (cm)	3.777									
heart/body	0.00311									
heart/tibia	0.270056									
lung/body	0.006341									

shr n			**15**							
file name			SHR 24							
weight (g)			316							
	MAP (mmHg)			**HR (bpm)**			**Tension (g)**			
Resting/2%	183			357						
Resting/DCR	166			317						
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	
100%	165	177	12	309	313	4	99	1202	1103	
Avg. Baseline	165.5			313			99			
Wet Spec.										
heart (g)	1.09									
lungs (g)	1.96									
triceps surae (g)	1.83									
tibia length (cm)	3.482									
heart/body	0.003449									
heart/tibia	0.313038									
lung/body	0.006203									
shr n			16							
file name			SHR 25							
weight (g)			354							
	MAP (mmHg)			**HR (bpm)**			**Tension (g)**			
Resting/2%	120			314						
Resting/DCR	nd			nd						
Passive stretch	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	**Baseline**	**Peak**	**Δ**	
100%	67	76	9	235	239	4	78	1345	1267	
Avg. Baseline	67			235			78			
Wet Spec.										
heart (g)	1.12									
lungs (g)	3.06									
triceps surae (g)	1.86									
tibia length (cm)	3.774									
heart/body	0.003164									
heart/tibia	0.296767									
lung/body	0.008644									

shr n	**17**	
file name	SHR 26	
weight (g)	340	

	MAP (mmHg)			**HR (bpm)**			**Tension (g)**		
Resting/2%	180			361					
Resting/DCR	153			300					
Passive stretch	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
100%	133	146	13	nd	nd	#VALUE!	83	1209	1126

Avg. Baseline	143			300			83		

Wet Spec.	
heart (g)	1.15
lungs (g)	1.94
triceps surae (g)	1.87
tibia length (cm)	3.746
heart/body	0.003382
heart/tibia	0.306994
lung/body	0.005706

APPENDIX B

METABOREFLEX CAPSAICIN DATA

page #	11
wky n	**1**
file name	aklwkyc2cap
weight (g)	464

	MAP (mmHg)			**HR (bpm)**		
Resting/2%	66			329		
Resting/DCR	46			475		
Cap Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	46	47	1	476	476	0
vehicle	46	70	24	469	483	14
0.01µg/100µL	45	48	3	447	447	0
0.03µg/100µL	47	51	4	445	445	0
0.1µg/100µL	50	55	5	440	444	4
0.3µg/100µL	53	84	31	438	456	18
1µg/100µL	54	118	64	439	454	15
Avg. Baseline	48.375			453.625		
Jugular Inj.			Δ			Δ
1µg/100µL	nd	nd	#VALUE!	nd	nd	#VALUE!

Wet Spec.	
heart (g)	1.56
lungs (g)	1.87
triceps surae (g)	nd
tibia length (cm)	4.123
heart/body	0.003362
heart/tibia	0.378365
lung/body	0.00403

page #	25
wky n	**2**
file name	aklwky04cap
weight (g)	379

	MAP (mmHg)			**HR (bpm)**		
Resting/2%	80			261		
Resting/DCR	159			377		
Cap Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	158	169	11	388	386	-2
vehicle	170	172	2	390	386	-4
0.01µg/100µL	173	192	19	386	390	4
0.03µg/100µL	nd	nd	#VALUE!	nd	nd	#VALUE!
0.1µg/100µL	165	175	10	348	348	0
0.3µg/100µL	nd	nd	#VALUE!	nd	nd	#VALUE!
1µg/100µL	nd	nd	#VALUE!	nd	nd	#VALUE!
Avg. Baseline	165			377.8		
Jugular Inj.			Δ			Δ
1µg/100µL	nd	nd	#VALUE!	nd	nd	#VALUE!

Wet Spec.	
heart (g)	1.41
lungs (g)	2.53
triceps surae (g)	2.6
tibia length (cm)	3.97
heart/body	0.00372
heart/tibia	0.355164
lung/body	0.006675

page #	28
wky n	**3**
file name	aklwky05cap
weight (g)	363

	MAP (mmHg)			**HR (bpm)**		
Resting/2%	65			345		
Resting/DCR	138			526		
Cap Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	179	186	7	546	555	9
vehicle	nd	nd	#VALUE!	nd	nd	#VALUE!
0.01µg/100µL	157	169	12	568	563	-5
0.03µg/100µL	108	114	6	585	592	7
0.1µg/100µL	62	94	32	560	585	25
0.3µg/100µL	66	91	25	539	556	17
1µg/100µL	58	68	10	551	568	17
Avg. Baseline	109.7143			553.5714		
Jugular Inj.			Δ			Δ
1µg/100µL	nd	nd	#VALUE!	nd	nd	#VALUE!

Wet Spec.	
heart (g)	1.04
lungs (g)	1.96
triceps surae (g)	2.13
tibia length (cm)	3.867
heart/body	0.002865
heart/tibia	0.268942
lung/body	0.005399

page #	32
wky n	**4**
file name	aklwky07cap
weight (g)	298

	MAP (mmHg)			**HR (bpm)**		
Resting/2%	59			265		
Resting/DCR	104			416		
Cap Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	90	92	2	420	428	8
vehicle	76	87	11	451	469	18
0.01µg/100µL	77	85	8	460	466	6
0.03µg/100µL	73	77	4	454	460	6
0.1µg/100µL	84	96	12	426	432	6
0.3µg/100µL	nd	nd	#VALUE!	416	429	13
1µg/100µL	74	117	43	465	484	19
Avg. Baseline	84			437.8333		
Jugular Inj.			Δ			Δ
1µg/100µL	76	106	30	423	373	-50

Wet Spec.	
heart (g)	0.92
lungs (g)	2.49
triceps surae (g)	1.75
tibia length (cm)	3.732
heart/body	0.003087
heart/tibia	0.246517
lung/body	0.008356

page #	32
wky n	**5**
file name	aklwky08cap
weight (g)	286

	MAP (mmHg)			**HR (bpm)**		
Resting/2%	108			303		
Resting/DCR	149			459		
Cap Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	162	167	5	483	478	-5
vehicle	183	206	23	495	474	-21
0.01µg/100µL	207	222	15	492	492	0
0.03µg/100µL	137	143	6	510	510	0
0.1µg/100µL	133	145	12	411	414	3
0.3µg/100µL	153	171	18	408	408	0
1µg/100µL	137	178	41	378	390	12
Avg. Baseline	157.625			454.5		
Jugular Inj.			Δ			Δ
1µg/100µL	111	222	111	434	122	-312

Wet Spec.	
heart (g)	0.9
lungs (g)	2.18
triceps surae (g)	1.55
tibia length (cm)	3.67
heart/body	0.003147
heart/tibia	0.245232
lung/body	0.007622

page #	37
wky n	**6**
file name	aklwky09cap
weight (g)	291

	MAP (mmHg)			**HR (bpm)**		
Resting/2%	101			213		
Resting/DCR	149			359		
Cap Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	146	140	-6	366	366	0
vehicle	103	117	14	250	303	53
0.01µg/100µL	168	180	12	379	383	4
0.03µg/100µL	194	214	20	385	390	5
0.1µg/100µL	196	213	17	383	387	4
0.3µg/100µL	194	222	28	341	350	9
1µg/100µL	156	215	59	nd	nd	#VALUE!
Avg. Baseline	163.25			351.8571		
Jugular Inj.			Δ			Δ
1µg/100µL	234	217	-17	374	354	-20

Wet Spec.	
heart (g)	0.85
lungs (g)	1.36
triceps surae (g)	2.06
tibia length (cm)	3.717
heart/body	0.002921
heart/tibia	0.228679
lung/body	0.004674

Date	12/16/2004
wky n	**7**
file name	wky 2*
weight (g)	370

	MAP (mmHg)			**HR (bpm)**		
Resting/2%	52			298		
Resting/DCR	132			566		
Cap Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	124	131	7	567	565	-2
vehicle	127	135	8	563	565	2
0.01µg/100µL	86	98	12	498	507	9
0.03µg/100µL	49	53	4	411	401	-10
0.1µg/100µL	101	104	3	394	395	1
0.3µg/100µL	74	111	37	350	380	30
1µg/100µL	63	78	15	378	386	8
Avg. Baseline	94.5			465.875		
Jugular Inj.			Δ			Δ
1µg/100µL	52	83	31	nd	nd	#VALUE!

Wet Spec.	
heart (g)	1.31
lungs (g)	2.65
triceps surae (g)	nd
tibia length (cm)	nd
heart/body	0.003541
heart/tibia	#VALUE!
lung/body	0.007162

Date	12/13/2004
wky n	**8**
file name	wky1*
weight (g)	370

	MAP (mmHg)			**HR (bpm)**		
Resting/2%	69			289		
Resting/DCR	155			445		
Cap Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	152	156	4	454	456	2
vehicle	161	167	6	469	469	0
0.01µg/100µL	158	160	2	464	466	2
0.03µg/100µL	150	153	3	472	474	2
0.1µg/100µL	152	158	6	476	479	3
0.3µg/100µL	155	173	18	nd	nd	#VALUE!
1µg/100µL	132	141	9	nd	nd	#VALUE!
Avg. Baseline	151.875			463.3333		
Jugular Inj.			Δ			Δ
1µg/100µL	125	130	5	493	323	-170

Wet Spec.	
heart (g)	1.21
lungs (g)	2.39
triceps surae (g)	nd
tibia length (cm)	nd
heart/body	0.00327
heart/tibia	#VALUE!
lung/body	0.006459

Date			2/11/2005				**Date**			2/24/2005					
wky n			**9**				**wky n**			**10**					
file name			wkyiacap1				**file name**			wkyiacap2					
weight (g)			384				**weight (g)**			452					
	MAP (mmHg)			**HR (bpm)**				**MAP (mmHg)**			**HR (bpm)**		
Resting/2%	78			349			Resting/2%	62			288		
Resting/DCR	90			504			Resting/DCR	140			470		
Cap Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ	**Cap Inj.**	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	95	98	3	507	508	1	saline	130	135	5	468	472	4
vehicle	101	111	10	506	506	0	vehicle	92	116	24	479	482	3
0.01µg/100µL	97	112	15	494	501	7	0.01µg/100µL	75	93	18	473	473	0
0.03µg/100µL	106	122	16	482	485	3	0.03µg/100µL	72	85	13	462	462	0
0.1µg/100µL	104	118	14	475	479	4	0.1µg/100µL	64	69	5	468	471	3
0.3µg/100µL	108	145	37	461	467	6	0.3µg/100µL	62	102	40	457	465	8
1µg/100µL	100	180	80	452	458	6	1µg/100µL	60	130	70	447	458	11
Avg. Baseline	100.125			485.125			**Avg. Baseline**	86.875			465.5		
Jugular Inj.			Δ			Δ	**Jugular Inj.**			Δ			Δ
1µg/100µL	71	79	8	444	420	-24	1µg/100µL	60	80	20	438	395	-43
Wet Spec.							**Wet Spec.**						
heart (g)	1.27						heart (g)	1.3					
lungs (g)	2.88						lungs (g)	3.5					
triceps surae (g)	2.36						triceps surae (g)	2.71					
tibia length (cm)	3.991						tibia length (cm)	4.04					
heart/body	0.003307						heart/body	0.002876					
heart/tibia	0.318216						heart/tibia	0.321782					
lung/body	0.0075						lung/body	0.007743					

page #			13				**page #**			16					
shr n			**1**				**shr n**			**2**					
file name			aklshr03cap				**file name**			aklshr05cap					
weight (g)			337				**weight (g)**			367					
	MAP (mmHg)			**HR (bpm)**				**MAP (mmHg)**			**HR (bpm)**		
Resting/2%	80			348			Resting/2%	97			314		
Resting/DCR	116			404			Resting/DCR	120			453		
Cap Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ	**Cap Inj.**	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	138	133	-5	404	373	-31	saline	111	118	7	448	451	3
vehicle	130	138	8	406	411	5	vehicle	109	114	5	461	459	-2
0.01µg/100µL	155	163	8	424	422	-2	0.01µg/100µL	92	112	20	449	450	1
0.03µg/100µL	150	165	15	435	432	-3	0.03µg/100µL	104	115	11	439	441	2
0.1µg/100µL	150	163	13	423	423	0	0.1µg/100µL	nd	nd	#VALUE!	436	445	9
0.3µg/100µL	148	200	52	410	430	20	0.3µg/100µL	110	174	64	428	440	12
1µg/100µL	169	277	108	405	435	30	1µg/100µL	102	182	80	431	447	16
Avg. Baseline	144.5			413.875			**Avg. Baseline**	106.8571			443.125		
Jugular Inj.			Δ			Δ	**Jugular Inj.**			Δ			Δ
1µg/100µL	nd	nd	#VALUE!	nd	nd	#VALUE!	1µg/100µL	103	127	24	424	417	-7
Wet Spec.							**Wet Spec.**						
heart (g)	1.37						heart (g)	1.42					
lungs (g)	1.72						lungs (g)	2.66					
triceps surae (g)	nd						triceps surae (g)	nd					
tibia length (cm)	3.718						tibia length (cm)	3.763					
heart/body	0.004065						heart/body	0.003869					
heart/tibia	0.368478						heart/tibia	0.377358					
lung/body	0.005104						lung/body	0.007248					

page #			17					**page #**			20					
shr n			**3**					**shr n**			**4**					
file name			aklshr06cap					**file name**			aklshr08cap					
weight (g)			373					**weight (g)**			386					
	MAP (mmHg)			**HR (bpm)**					**MAP (mmHg)**			**HR (bpm)**				
Resting/2%	70			277				Resting/2%	198			351				
Resting/DCR	162			487				Resting/DCR	121			472				
Cap Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ		**Cap Inj.**	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ		
saline	162	176	14	522	520	-2		saline	123	132	9	478	478	0		
vehicle	191	237	46	553	556	3		vehicle	128	137	9	499	500	1		
0.01µg/100µL	170	176	6	573	569	-4		0.01µg/100µL	139	146	7	513	513	0		
0.03µg/100µL	165	188	23	572	571	-1		0.03µg/100µL	112	127	15	539	540	1		
0.1µg/100µL	95	100	5	572	574	2		0.1µg/100µL	127	139	12	548	547	-1		
0.3µg/100µL	89	112	23	587	592	5		0.3µg/100µL	100	160	60	554	559	5		
1µg/100µL	100	142	42	566	581	15		1µg/100µL	110	185	75	549	556	7		
Avg. Baseline	141.75			554				**Avg. Baseline**	120			519				
Jugular Inj.			Δ			Δ		**Jugular Inj.**			Δ			Δ		
1µg/100µL	78	95	17	570	530	-40		1µg/100µL	123	133	10	544	489	-55		
Wet Spec.								**Wet Spec.**								
heart (g)	1.4							heart (g)	1.6							
lungs (g)	2.54							lungs (g)	nd							
triceps surae (g)	nd							triceps surae (g)	2.56							
tibia length (cm)	3.842							tibia length (cm)	3.938							
heart/body	0.003753							heart/body	0.004145							
heart/tibia	0.364394							heart/tibia	0.406298							
lung/body	0.00681							lung/body	#VALUE!							

page #			27					**page #**			30					
shr n			**5**					**shr n**			**6**					
file name			aklshr11cap					**file name**			aklshr12cap					
weight (g)			360					**weight (g)**			381					
	MAP (mmHg)			**HR (bpm)**					**MAP (mmHg)**			**HR (bpm)**				
Resting/2%	61			365				Resting/2%	148			345				
Resting/DCR	160			458				Resting/DCR	222			471				
Cap Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ		**Cap Inj.**	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ		
saline	180	191	11	470	475	5		saline	209	214	5	471	468	-3		
vehicle	182	231	49	477	486	9		vehicle	126	139	13	423	429	6		
0.01µg/100µL	176	187	11	496	500	4		0.01µg/100µL	119	124	5	423	425	2		
0.03µg/100µL	138	164	26	516	528	12		0.03µg/100µL	153	160	7	503	507	4		
0.1µg/100µL	143	163	20	528	528	0		0.1µg/100µL	126	142	16	456	466	10		
0.3µg/100µL	123	215	92	522	540	18		0.3µg/100µL	80	101	21	408	410	2		
1µg/100µL	116	206	90	501	528	27		1µg/100µL	142	224	82	nd	nd	#VALUE!		
Avg. Baseline	152.25			496				**Avg. Baseline**	147.125			450.7143				
Jugular Inj.			Δ			Δ		**Jugular Inj.**			Δ			Δ		
1µg/100µL	97	130	33	481	401	-80		1µg/100µL	119	131	12	410	131	-279		
Wet Spec.								**Wet Spec.**								
heart (g)	1.2							heart (g)	1.23							
lungs (g)	2.4							lungs (g)	3.53							
triceps surae (g)	1.29							triceps surae (g)	nd							
tibia length (cm)	3.834							tibia length (cm)	3.876							
heart/body	0.003333							heart/body	0.003228							
heart/tibia	0.312989							heart/tibia	0.317337							
lung/body	0.006667							lung/body	0.009265							

page #	38
shr n	**7**
file name	aklshr13cap
weight (g)	283

	MAP (mmHg)			HR (bpm)		
Resting/2%	85			364		
Resting/DCR	231			518		
Cap Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	241	254	13	520	512	-8
vehicle	244	275	31	537	533	-4
0.01µg/100µL	140	148	8	480	474	-6
0.03µg/100µL	157	169	12	563	565	2
0.1µg/100µL	173	198	25	553	554	1
0.3µg/100µL	157	190	33	537	538	1
1µg/100µL	144	185	41	520	517	-3
Avg. Baseline	185.875			528.5		
Jugular Inj.			Δ			Δ
1µg/100µL	148	165	17	462	429	-33

Wet Spec.	
heart (g)	0.9
lungs (g)	1.61
triceps surae (g)	1.52
tibia length (cm)	3.534
heart/body	0.00318
heart/tibia	0.254669
lung/body	0.005689

page #	40
shr n	**8**
file name	aklshr15cap
weight (g)	308

	MAP (mmHg)			HR (bpm)		
Resting/2%	89			278		
Resting/DCR	164			452		
Cap Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	175	182	7	447	447	0
vehicle	178	193	15	443	443	0
0.01µg/100µL	182	193	11	436	434	-2
0.03µg/100µL	181	200	19	421	425	4
0.1µg/100µL	228	259	31	408	408	0
0.3µg/100µL	200	242	42	366	366	0
1µg/100µL	nd	nd	#VALUE!	nd	nd	#VALUE!
Avg. Baseline	186.8571			424.7143		
Jugular Inj.			Δ			Δ
1µg/100µL	nd	nd	#VALUE!	nd	nd	#VALUE!

Wet Spec.	
heart (g)	1.21
lungs (g)	1.58
triceps surae (g)	1.71
tibia length (cm)	3.638
heart/body	0.003929
heart/tibia	0.3326
lung/body	0.00513

page #	40
shr n	**9**
file name	aklshr16cap
weight (g)	297

	MAP (mmHg)			HR (bpm)		
Resting/2%	116			430		
Resting/DCR	193			566		
Cap Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	213	238	25	569	566	-3
vehicle	248	262	14	580	579	-1
0.01µg/100µL	118	131	13	563	560	-3
0.03µg/100µL	150	170	20	549	553	4
0.1µg/100µL	151	171	20	561	561	0
0.3µg/100µL	162	197	35	587	587	0
1µg/100µL	242	317	75	577	581	4
Avg. Baseline	184.625			569		
Jugular Inj.			Δ			Δ
1µg/100µL	173	250	77	524	473	-51

Wet Spec.	
heart (g)	0.96
lungs (g)	2.58
triceps surae (g)	1.83
tibia length (cm)	3.594
heart/body	0.003232
heart/tibia	0.267112
lung/body	0.008687

Date	12/14/2004
shr n	**10**
file name	shr1*
weight (g)	406

	MAP (mmHg)			HR (bpm)		
Resting/2%	90			323		
Resting/DCR	324			535		
Cap Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	303	303	0	543	545	2
vehicle	288	292	4	545	548	3
0.01µg/100µL	268	276	8	552	555	3
0.03µg/100µL	235	255	20	547	547	0
0.1µg/100µL	197	220	23	565	565	0
0.3µg/100µL	184	242	58	537	544	7
1µg/100µL	187	256	69	550	559	9
Avg. Baseline	248.25			546.75		
Jugular Inj.			Δ			Δ
1µg/100µL	149	227	78	544	502	-42

Wet Spec.	
heart (g)	1.35
lungs (g)	2.32
triceps surae (g)	nd
tibia length (cm)	nd
heart/body	0.003325
heart/tibia	#VALUE!
lung/body	0.005714

Date			12/17/2004			
shr n			**11**			
file name			shr 2*			
weight (g)			420			
	MAP (mmHg)			**HR (bpm)**		
Resting/2%	98			344		
Resting/DCR	157			553		
Cap Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	156	167	11	553	557	4
vehicle	nd	nd	#VALUE!	nd	nd	#VALUE!
0.01µg/100µL	133	138	5	572	575	3
0.03µg/100µL	110	116	6	583	585	2
0.1µg/100µL	99	105	6	577	580	3
0.3µg/100µL	72	104	32	542	547	5
1µg/100µL	86	128	42	565	565	0
Avg. Baseline	116.1429			563.5714		
Jugular Inj.			Δ			Δ
1µg/100µL	nd	nd	#VALUE!	nd	nd	#VALUE!
Wet Spec.						
heart (g)	1.43					
lungs (g)	2.36					
triceps surae (g)	nd					
tibia length (cm)	3.962					
heart/body	0.003405					
heart/tibia	0.360929					
lung/body	0.005619					

APPENDIX C

METABOREFLEX CAPSAICIN PLUS CAPSAZEPINE DATA

page #	34
wky n	**1**
file name	aklwky02capz
weight (g)	286

	MAP (mmHg)	HR (bpm)
Resting/2%	80	314
Resting/DCR	94	481

Capz Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	96	102	6	478	475	-3
vehicle	90	111	21	481	488	7
0.01µg/100µL	115	124	9	501	501	0
0.03µg/100µL	93	102	9	495	497	2
0.1µg/100µL	71	80	9	504	504	0
0.3µg/100µL	72	108	36	477	487	10
1µg/100µL	82	108	26	509	513	4
Avg. Baseline	89.125			490.75		
Jugular Inj.			Δ			Δ
1µg/100µL	96	134	38	472	427	-45

Wet Spec.	
heart (g)	0.91
lungs (g)	2.75
triceps surae (g)	1.83
tibia length (cm)	3.659
heart/body	0.003182
heart/tibia	0.248702
lung/body	0.009615

page #	34
wky n	**2**
file name	aklwky03capz
weight (g)	282

	MAP (mmHg)	HR (bpm)
Resting/2%	99	317
Resting/DCR	223	483

Capz Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	207	218	11	492	498	6
vehicle	222	206	-16	504	510	6
0.01µg/100µL	183	190	7	498	498	0
0.03µg/100µL	187	197	10	510	516	6
0.1µg/100µL	183	200	17	528	534	6
0.3µg/100µL	179	202	23	522	522	0
1µg/100µL	210	240	30	522	528	6
Avg. Baseline	199.25			507.375		
Jugular Inj.			Δ			Δ
1µg/100µL	nd	nd	#VALUE!	nd	nd	#VALUE!

Wet Spec.	
heart (g)	0.79
lungs (g)	2.05
triceps surae (g)	1.74
tibia length (cm)	3.561
heart/body	0.002801
heart/tibia	0.221848
lung/body	0.00727

page #	35
wky n	**3**
file name	aklwky04capz
weight (g)	264

	MAP (mmHg)	HR (bpm)
Resting/2%	98	286
Resting/DCR	137	456

Capz Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	122	125	3	472	467	-5
vehicle	128	136	8	408	408	0
0.01µg/100µL	145	154	9	336	342	6
0.03µg/100µL	163	173	10	342	348	6
0.1µg/100µL	154	167	13	360	360	0
0.3µg/100µL	145	156	11	324	330	6
1µg/100µL	140	156	16	402	396	-6
Avg. Baseline	141.75			387.5		
Jugular Inj.			Δ			Δ
1µg/100µL	144	165	21	324	324	0

Wet Spec.	
heart (g)	0.79
lungs (g)	1.3
triceps surae (g)	1.78
tibia length (cm)	3.649
heart/body	0.002992
heart/tibia	0.216498
lung/body	0.004924

page #	36
wky n	**4**
file name	aklwky05capz
weight (g)	299

	MAP (mmHg)	HR (bpm)
Resting/2%	110	283
Resting/DCR	176	370

Capz Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	191	208	17	365	363	-2
vehicle	173	193	20	360	360	0
0.01µg/100µL	210	218	8	366	366	0
0.03µg/100µL	191	211	20	364	364	0
0.1µg/100µL	216	233	17	365	368	3
0.3µg/100µL	204	223	19	359	368	9
1µg/100µL	209	227	18	360	367	7
Avg. Baseline	196.25			363.625		
Jugular Inj.			Δ			Δ
1µg/100µL	153	189	36	360	306	-54

Wet Spec.	
heart (g)	0.87
lungs (g)	1.62
triceps surae (g)	1.86
tibia length (cm)	3.735
heart/body	0.00291
heart/tibia	0.232932
lung/body	0.005418

page #			36					**page #**			22					
wky n			**5**					**shr n**			**1**					
file name			akIwky06capz					**file name**			akIshr10capz					
weight (g)			317					**weight (g)**			342					
	MAP (mmHg)			**HR (bpm)**					**MAP (mmHg)**			**HR (bpm)**				
Resting/2%	94			248				Resting/2%	198			311				
Resting/DCR	143			407				Resting/DCR	215			444				
Capz Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ		**Capz Inj.**	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ		
saline	nd	nd	#VALUE!	408	414	6		Saline	236	244	8	410	427	17		
vehicle	119	128	9	432	432	0		Vehicle	183	185	2	384	390	6		
0.01µg/100µL	79	84	5	352	350	-2		0.01µg/100µL	203	203	0	384	396	12		
0.03µg/100µL	81	86	5	361	363	2		0.03µg/100µL	166	169	3	348	348	0		
0.1µg/100µL	86	100	14	385	385	0		0.1µg/100µL	185	185	0	408	396	-12		
0.3µg/100µL	80	100	20	405	412	7		0.3µg/100µL	192	202	10	348	372	24		
1µg/100µL	82	123	41	427	436	9		1µg/100µL	171	179	8	366	384	18		
Avg. Baseline	95.71429			397.125				**Avg. Baseline**	193.875			386.5				
Jugular Inj.			Δ			Δ		**Jugular Inj.**			Δ			Δ		
1µg/100µL	76	88	12	343	292	-51		1µg/100µL	nd	nd	#VALUE!	nd	nd	#VALUE!		
Wet Spec.								**Wet Spec.**								
heart (g)	0.9							heart (g)	nd							
lungs (g)	1.06							lungs (g)	nd							
triceps surae (g)	2.01							triceps surae (g)	nd							
tibia length (cm)	3.781							tibia length (cm)	nd							
heart/body	0.002839							heart/body	#VALUE!							
heart/tibia	0.238032							heart/tibia	#VALUE!							
lung/body	0.003344							lung/body	#VALUE!							

page #			24					**page #**			25					
shr n			**2**					**shr n**			**3**					
file name			akIshr03capz					**file name**			akIshr04capz					
weight (g)			344					**weight (g)**			348					
	MAP (mmHg)			**HR (bpm)**					**MAP (mmHg)**			**HR (bpm)**				
Resting/2%	161			416				Resting/2%	141			332				
Resting/DCR	151			474				Resting/DCR	135			474				
Capz Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ		**Capz Inj.**	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ		
saline	152	156	4	489	487	-2		Saline	165	170	5	545	545	0		
vehicle	232	239	7	509	522	13		Vehicle	156	166	10	511	504	-7		
0.01µg/100µL	152	163	11	523	527	4		0.01µg/100µL	138	141	3	552	552	0		
0.03µg/100µL	132	143	11	534	534	0		0.03µg/100µL	98	103	5	554	549	-5		
0.1µg/100µL	126	138	12	540	540	0		0.1µg/100µL	107	111	4	551	542	-9		
0.3µg/100µL	107	121	14	534	540	6		0.3µg/100µL	nd	nd	#VALUE!	nd	nd	#VALUE!		
1µg/100µL	109	138	29	546	552	6		1µg/100µL	nd	nd	#VALUE!	nd	nd	#VALUE!		
Avg. Baseline	145.125			518.625				**Avg. Baseline**	133.1667			531.1667				
Jugular Inj.			Δ			Δ		**Jugular Inj.**			Δ			Δ		
1µg/100µL	nd	nd	#VALUE!	nd	nd	#VALUE!		1µg/100µL	nd	nd	#VALUE!	nd	nd	#VALUE!		
Wet Spec.								**Wet Spec.**								
heart (g)	1.41							heart (g)	1.72							
lungs (g)	2.06							lungs (g)	nd							
triceps surae (g)	3.54							triceps surae (g)	2.31							
tibia length (cm)	3.737							tibia length (cm)	nd							
heart/body	0.004099							heart/body	0.004943							
heart/tibia	0.377308							heart/tibia	#VALUE!							
lung/body	0.005988							lung/body	#VALUE!							

page #	30
shr n	**4**
file name	aklshr05capz
weight (g)	366

	MAP (mmHg)			**HR (bpm)**		
Resting/2%	77			370		
Resting/DCR	159			485		
Capz Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
saline	120	126	6	419	429	10
vehicle	80	85	5	450	438	-12
0.01µg/100µL	103	115	12	345	350	5
0.03µg/100µL	85	101	16	361	373	12
0.1µg/100µL	138	148	10	329	336	7
0.3µg/100µL	nd	nd	#VALUE!	nd	Nd	#VALUE!
1µg/100µL	nd	nd	#VALUE!	nd	Nd	#VALUE!
Avg. Baseline	114.1667			398.1667		
Jugular Inj.			Δ			Δ
1µg/100µL	160	275	115	420	243	-177

Wet Spec.	
heart (g)	1.27
lungs (g)	1.66
triceps surae (g)	2.17
tibia length (cm)	3.897
heart/body	0.00347
heart/tibia	0.325892
lung/body	0.004536

page #	31
shr n	**5**
file name	aklshr06capz
weight (g)	346

	MAP (mmHg)			**HR (bpm)**		
Resting/2%	75			318		
Resting/DCR	176			502		
Capz Inj.	**Baseline**	**Peak**	Δ	**Baseline**	**Peak**	Δ
Saline	195	201	6	502	500	-2
Vehicle	232	269	37	505	480	-25
0.01µg/100µL	203	217	14	508	508	0
0.03µg/100µL	189	198	9	513	513	0
0.1µg/100µL	192	210	18	485	489	4
0.3µg/100µL	235	244	9	nd	nd	#VALUE!
1µg/100µL	235	285	50	423	439	16
Avg. Baseline	207.125			491.1429		
Jugular Inj.			Δ			Δ
1µg/100µL	213	108	-105	411	496	85

Wet Spec.	
heart (g)	nd
lungs (g)	1.25
triceps surae (g)	1.94
tibia length (cm)	3.779
heart/body	#VALUE!
heart/tibia	#VALUE!
lung/body	0.003613

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