2019

The Effect of Dorsal Rhizotomy on Renal Sodium Excretion Following Dorsal Spinal Stimulation

Tanko Tijani Ahmed
Minnesota State University, Mankato

THE EFFECT OF DORSAL RHIZOTOMY ON RENAL SODIUM EXCRETION FOLLOWING DORSAL SPINAL STIMULATION

By

Tanko Tijani Ahmed

A Thesis Submitted In Partial Fulfillment

Of The Requirement for the Degree of

Masters of Science

In

Biology

Minnesota State University, Mankato

Mankato, Minnesota U.S.A

(December, 2019)

November 25, 2019

The Effect of Dorsal Rhizotomy on Renal Sodium Excretion Following Dorsal Spinal Stimulation

Tanko Tijani Ahmed

This thesis has been examined and approved by the following members of the student's committee.

_____

Dr. Penny Knoblich

_____

Dr. Michael Bentley

_____

Dr. Rachel Cohen

ABSTRACT

Hypertension is one of the most common risk factors in the development of heart disease, stroke and end stage renal failure. Sympathetic overactivity is believed to be one of the main mechanism behind resistant hypertension. Renal denervation has been used to treat resistant hypertension, although this procedure is an invasive one.

Spinal cord stimulation (SCS) is a known modality to treat ischemic pain, angina pectoris, and peripheral vascular diseases. In previous studies in this laboratory, unilateral spinal cord stimulation in spontaneously hypertensive rat (SHR) at 67% of the motor threshold increased urinary sodium and water excretion significantly, without affecting mean arterial pressure (MAP) or renal blood flow. Bilateral spinal cord stimulation in spontaneously hypertensive rats (SHR) at 67% of the motor threshold at the level of T11 – T12 increased urinary sodium and water excretion significantly, while complete renal denervation eliminated the response.

To further understand the mechanism by which dorsal spinal stimulation increases urinary sodium and water excretion, spinal stimulation was used in four groups of SHR, two intact and two with dorsal rhizotomy of the renal nerve. SCS was applied at 67% of motor threshold at the level of T11 – T12, to one group under each condition, intact or dorsal rhizotomized rats.

SCS produced a significant increase from baseline in urinary sodium excretion in rats with intact renal nerves only. A similar increase was also observed when urine volume was analyzed. Dorsal rhizotomy alone produced a significant increase in urine volume, and a decrease in MAP in the rats also subjected to SCS.

These results indicates that the natriuretic effect of SCS is dependent on the antidromic transmission of the electrical signal on the renal afferent nerves back to the kidney.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude and appreciation to my advisor, Professor Penny Knoblich, Biology Department, Minnesota State University, Mankato, (MNSU), U.S.A. for her patience, support, encouragement, and guiding me in every step of this research.

I would like to thank Professor Michael Bentley and Professor Rachel Cohen, members of research committee and faculties of Biology Department of MNSU for their vast support, comment and input in my research.

I would like to acknowledge Mrs. Kate Voight, former Administrative Assistant for the Biology Department, MNSU for her input and coordination, and Mr. Brent Pearson in animal care facility for setting up rats.

I am grateful to the Department of Biological science for the funding resources that allowed me to conduct this research.

Finally, I must express my profound gratitude to my family and friends for their support and motivation throughout my research.

TABLE OF CONTENTS

LIST OF ABBREVIATION

5-HT	5-hydroxytryptamine
ACE	Angiotensin Converting Enzyme
ADH	Antidiuretic Hormone
AT1R	Angiotensin type 1 receptor
AT2R	Angiotensin type 2 receptor
CAMP-PKA	Cyclic Adenosine Mono Phosphate – Protein Kinase A
CAPs	Compound Action Potential
CGRP	Calcitonin Gene Related Peptide
CNS	Control Non Stimulated
CO	Cardiac output
COX 2	Cyclooxygenase 2
CS	Control Stimulated
DNS	Dorsal Rhizotomy Non Stimulated
DS	Dorsal Rhizotomy Stimulated
GABA	Gamma Amino Butyric Acid
L1	1st Lumber Vertebra
NOS	Nitric Oxide Synthase
OSA	Obstructive Sleep Apnea
PGE 2	Prostaglandin E2
SCS	Spinal Cord Stimulation

T11	11th Thoracic Vertebra
T12	12th Thoracic Vertebra
T9	9th Thoracic Vertebra
TPR	Total Peripheral Resistance
TRPV1	Transient Receptor Potential Cation Channel Subfamily V Member 1

List of Tables

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Hypertension is defined as a medical condition that occurs when the force exerted by the blood against the wall of the blood vessels is higher than normal (Thambar, 2015). According to medical guidelines, hypertension is defined as a systolic blood pressure of ≥ 140 mmHg or diastolic blood pressure ≥90mmHg. An increased blood pressure is a leading risk factor of mortality and the third leading risk factor of disease globally (Murray et al., 2002).

Hypertension is one of the most common risk factors for the development of heart disease (Thomas and Allison 2019). Hypertension is common in the community especially in elderly patients, and is a contributing factor for heart disease, stroke, end stage renal failure, and myocardial infarction. Approximately 68 million (31%) adults in the United States aged 18 years and older had hypertension in 2015 (Thomas and Allison 2019). Of this adult population, 48 million were receiving pharmacological treatment but only 31 million had their blood pressure controlled (Thomas and Allison 2019). The prevalence of hypertension is higher in men than women and in African Americans (42.7%) when compared with Caucasians (27.8%) and Mexican Americans (27.8%) (Murray et al., 2002).

The heart and the kidneys interact with each other to maintain homeostasis, using neurohumoral regulatory mechanisms under normal condition. These regulatory mechanisms become impaired during congestive heart failure, which results in renal dysfunction (Whelton et al., 2005). Congestive heart failure results in the activation of the renal sympathetic efferent nerve which causes the release of renin, sodium and water retention, and reduced renal blood

flow (Whelton et al., 2005). Technological advancements have led to the development of renal nerve ablation (destruction of the renal nerve) in the treatment of drug resistant hypertension in humans. Several studies have been conducted to investigate the mechanism underlying the effect of renal nerve denervation on sodium and water excretion and blood pressure; results from these studies showed an increase in urine volume and natriuresis, increased mean renal blood flow and decreased renal vascular resistance (Whelton et al., 2005). It is unclear if the antihypertensive effect of renal nerve denervation is due to the selective ablation of the renal afferent nerve or the renal efferent nerve, or a combination.

Spinal cord stimulation is an underutilized therapy which involves electrical stimulation on the dorsal dura of the spinal cord, most commonly used to relieve chronic neuropathic pain. Spinal cord stimulation was first proposed in 1967 as a treatment for pain (Shearly et al., 1967). Spinal cord stimulation (SCS), also called neuro-stimulation, produces direct mild electric pulses that interfere with pain stimuli reaching the brain. This has been used, not only in the treatment of chronic pain, but also in a multitude of other disorders, such as angina, gastrointestinal and urological diseases, epilepsy, psychiatric diseases and movement disorders (Foss and Osborn, 2016).

Spinal cord stimulation is also effective in improving blood flow, which is necessary for relieving ischemic pain. Weak to moderate epidural stimulation of the spinal cord improves the ischemic conditions by suppressing sympathetic activity to the effector organs. A study was performed on anaesthetized rats to record the peripheral changes in the microcirculation of the hind limbs during dorsal spinal stimulation. The stimulation parameters used in this study were similar to the ones used in the clinic (50Hz, 0.2msec with a stimulation intensity that is 2/3 of that

evoking muscle contraction in the lower abdomen and legs). According to this study, spinal cord stimulation increased blood flow to the skin and muscles of the hind limb after the electrode was placed epidurally above the second lumber segment. This increased blood flow effect was abolished when the ventral root innervating the paw of the hind limb was transected (Linderoth et al., 1991, Linderoth and Foreman, 1999).

Previous studies in the Knoblich laboratory at Minnesota State University Mankato, showed that unilateral dorsal spinal stimulation on the left dorsal spine at T11-T12 (the point in which the renal sensory nerves enter the spine) increased urinary sodium excretion significantly, without affecting renal blood flow or mean arterial pressure (Merger and Knoblich, 2003), (Stearns and Knoblich 2007). Subsequently, it was demonstrated that renal denervation eliminated the renal response to dorsal spinal stimulation. However, since renal nerve ablation eliminates both afferent and efferent inputs to the kidney, it is unclear if the stimulation is acting through inhibition of the renal efferent nerves, or retrograde transmission on the renal afferent nerves. To selectively study the contribution of the renal afferent nerve to the natriuresis response, a procedure termed dorsal rhizotomy was used (Schlaich et., 2012). This procedure disrupts the afferent neural pathway from the kidney to the spinal cord by sectioning the dorsal root just at the point it enters into the spinal cord at the level of T11-T12.

The proposed study will selectively ablate the renal afferent nerves in order to determine the contribution of the renal sensory nerves to the increase in sodium and water excretion that results from dorsal spinal stimulation.

BACKGROUND

Physiology

The cardiovascular system consists of the heart, blood vessels, and blood. The heart and the blood vessels help to transport vital nutrients throughout the body as well as remove metabolic waste. The blood helps to protect the body against foreign microbes and toxins, as well as regulate body temperature. Mean arterial blood pressure is a function of cardiac output (CO), and total peripheral resistance (TPR) (Levy and Pappano, 2007). (BP=CO×TPR). Cardiac output is a function of heart rate and stroke volume. Stroke volume is the volume of blood pumped from the left ventricle per beat. The total peripheral resistance is regulated dynamically by vasoconstriction or vasodilation in arteries and arterioles, which have abundant smooth muscles in their walls. There are three groups of mechanisms that control the tone of these arterial blood vessels which are; neuro-humoral, endothelial, and myogenic mechanism.

Neuro-humoral factors such as vasopressin and the sympathetic nervous system can raise the blood pressure through vasoconstriction, which increases total peripheral resistance (Mohrman and Heller, 2014). The myocyte and endothelial factors that maintain tonic arterial constriction or tone, can be studied in isolated, cannulated small arteries. These arteries can develop spontaneous myogenic tone when the lumen is pressurized (M.A Hill Et al. 2001). According to the author studies they demonstrated that the arterioles can exhibit a state of partial contraction or myogenic tone, which is dependent on the level of intraluminal pressure. This means that an increase in pressure will result in vasoconstriction, whereas a decrease in pressure leads to vasodilation. The physiological significance of this vasomotor response relates

to its participation in local blood flow autoregulation, setting of basal peripheral vascular resistance and regulation of capillary hydrostatic pressure (M.A Hill Et al. 2001).

Blood volume

The blood volume is determined by the amount of water and sodium ingested, excreted by the kidney into the urine, and lost into the gastrointestinal tract, lungs, and skin. In order to maintain the blood volume within a normal range, the kidney regulates the amount of water and sodium that is lost in the urine. For example, if excessive sodium and water are ingested, the kidney will excrete more sodium and water in the urine. The human kidneys are paired organs, positioned bilaterally within the retroperitoneal space of the abdominal cavity at the level of T12 to L3. The kidneys are supplied with blood by paired renal arteries that enter the renal hilum. The blood flows out of the kidneys through the paired renal veins which drains into the inferior vena cava and back into the cardiovascular circulation. The kidneys main functions are the regulation of water balance electrolyte and osmolarity, maintenance of pH homeostasis, and hormone secretion. Among these numerous functions the kidney is also involved in the modulation of blood pressure in the cardiovascular system (Winklewski et., 2017).

Blood flowing to the kidney is filtered at the glomerulus. These filtrates contain sodium, water and other substances. As the filtrate travels through the proximal tubule, loop of Henle, distal and collecting tubules, the concentration of sodium is altered as sodium is transported across the tubular wall and into the renal interstitium and ultimately into the blood (Matthew and Victor, 1999). Some of the renal tubules are permeable to water, so water leaves those tubular regions along with sodium.

Renin-Angiotensin-Aldosterone System (RAAS)

The renin-angiotensin-aldosterone system modifies blood pressure through a variety of effects in different tissues, including alteration in vascular tone, augmentation of the activity of the sympathetic nervous system, changes in the structure and function of the cardiovascular beds, and renal salt and water homeostasis (Matthew and Victor, 1999). This system begins with the production of renin in the kidney, specifically by the macula densa of the juxta-glomerular apparatus. Renin release is stimulated by a decrease in effective arterial volume, renal perfusion pressure or glomerular filtration rate, and by an increase in sympathetic activity as a result of stimulation of the β1 adrenergic receptors (Matthew and Victor, 1999). Once in circulation, renin catalyzes the conversion of angiotensinogen, which is released by the liver, to angiotensin I. Angiotensin I which is an inactive form, comes in contact with angiotensin converting enzyme (ACE), which sits on the surface of the vascular endothelium. This enzyme cleaves angiotensin 1 (inactive) to produce the active angiotensin II moiety. Angiotensin II activates G-protein coupled receptors type 1 and 2 (AT_1R, AT_2R) (Mohrman and Heller, 2014).

Angiotensin II increases sodium transport along different sites of the proximal tubules, thick ascending limb of the loop of Henle, distal and collecting tubules, thereby leading to greater sodium retention. Another hormone known as aldosterone stimulates sodium transport from the tubular fluid into the interstitium. Together these two hormones, angiotensin II and aldosterone, provide a powerful mechanism for increasing sodium retention and fluid volume in the body. A third hormone called anti-diuretic hormone (ADH), increases water permeability in the late distal tubules and collecting tubules. Water follows sodium and the net effect of angiotensin II and aldosterone is increased blood volume and pressure (O' Callaghan Et al., 2013).

Renal sympathetic nervous system

There are two ways in which the renal sympathetic nervous system affects blood pressure. First it supplies the kidney with a rich network of efferent sympathetic fibers which is located in the adventitia of the renal artery. The efferent sympathetic fibers are exclusively noradrenergic. Secondly, kidney signals are returned to the central nervous system through afferent sympathetic fibers, also located in the adventitia of the renal arteries. Afferent signals from end organ sensors and baroreceptors as well as from the hypothalamus, cortex, and limbic system are received and integrated by the autonomic centers in the medulla oblongata and midbrain. Efferent signals are then transmitted to sympathetic pre-ganglionic neurons in the inter-mediolateral column of the spinal cord. Fibers from neurons in the intermediolateral column (T_{10} - T_{12}, L_1 - L_2) extend through splanchnic nerves to post-ganglionic neurons located in pre-vertebral ganglia. The post-ganglionic neuron then extends its fibers to the kidney through the adventitia of the renal arteries (Bertog and Sievert, 2012).

Renal efferent sympathetic activity

The renal tubular cells of the kidney (Müller and Barajas, 1972), juxtaglomerular apparatus (Barajas, 1964) and renal vasculatures (Ljunggvist and Wagermark, 1970, Barajas and Wang, 1979) are supplied by the efferent sympathetic fibers, which also supply every aspect of the kidney. The stimulation of the efferent fibers of the kidney results in the activation of the adluminal basolateral Na/K adenosine triphosphatase (Aperia et al. 1992), thus promoting sodium and water retention, renin secretion through the juxtaglomerular apparatus (Skott and Jensen, 1993) and vasoconstriction of renal arterioles, thus, resulting in a general increase in mean arterial pressure.

Renin release stimulates the production of angiotensin II, thereby stimulating the release of mineralocorticoids (aldosterone). Aldosterone, being the primary endogenous mineralocorticoid produced in the zona glomerulosa of the cortex of the adrenal gland, acts on the kidney to promote the active reabsorption of sodium and passive reabsorption of water, as well as mediating vasoconstriction. There appears to be a graded response depending on the intensity of the sympathetic signal, such that with low frequency stimulation, renin secretion is first affected, followed by tubular reabsorption and renal vascular tone at higher frequencies (Koepke and Dibona, 1985).

According to Dibona, at any given renal perfusion pressure, renal sympathetic denervation shifts the diuresis and natriuretic curve to the left i.e. an increase in water and sodium excretion in denervated animals compared to the intact animal (Dibona GF, 1989). Thus, it is assumed that an abrogation or disruption of the renal sympathetic efferent nerve constitutes a therapeutic target in the management of hypertension (Dibona GF, 1989).

Renal afferent sympathetic activity

The kidney transmits signals via afferent sympathetic fibers, to neurons of the posterior grey column of the ipsilateral spinal cord (Ciriello and Calaresu, 1983, Rosas-Arellano et al., 1999). The cell bodies of the afferent sympathetic fibers are located in the dorsal root ganglia. Studies using rat models have demonstrated that the cell bodies of the renal afferent sympathetic are located at the level of T9- L1 (Kopp et al., 2002). The signals are then relayed from the afferent fibers to the autonomic centers of the central nervous system as well as to the contralateral kidney. The afferent fiber endings are found in all parts of the kidney; with the richest network located in the renal pelvis.

Two receptors types are responsible for the transmission of these signals, the mechanoreceptors and the chemoreceptors. The mechanosensitive receptor relays information regarding hydrostatic renal pelvic pressure, as well as renal arterial and venous pressure. The chemosensitive receptors are activated by renal ischemia and changes in the chemical milieu of the renal interstitium (Ciriello and de Oliveira, 2002). Afferent sympathetic neurons (dorsal root) transmit signals to the central nervous system and communicate with sympathetic centers in the central nervous system, thus regulating the overall sympathetic tone. This finding was demonstrated in a study using animal model whose kidneys were injured by phenol injection. The authors found that blood pressure rises after phenol injection, but the blood pressure rise was prevented by prior dorsal rhizotomy (ye et al., 2002). The authors concluded that hypertension due to renal insufficiency in rats can be prevented by dorsal rhizotomy (Campese and Kogosov, 1995).

Experiments using rat models have shown that the kidney afferent sensory nerve is unmyelinated with an average diameter of 1.3 micrometer and is located in the renal pelvic wall where afferent neurons are sensitive to stretch (Hausberg et al., 1974). An increase in renal pelvic pressure stretches the renal pelvic wall, and activates the renal mechanoreceptors, leading to afferent renal neurotransmission. Thus enhanced diuresis increases renal pelvic pressure, which activates the renal mechanosensors, increasing renal afferent activation (Dagmara and Pawel, 2017). Another factor that affects renal nerve responsiveness is increased dietary sodium intake. High sodium diets activate renal mechanoreceptors which inhibit renorenal reflexes, resulting in the suppression of the renal sympathetic efferent nervous system, thereby minimizing sodium reabsorption and increasing urinary sodium excretion (Hausberg et al., 1974).

The sympathetic nervous system has a profound effect on the kidney's ability to regulate blood pressure, and, vice versa, the kidney has an important effect on the overall sympathetic tone (Bertog and Sievert, 2012).

Renal denervation

In the animal model, renal nerve disruption Is achieved via various methods such as bilateral dorsal rhizotomy, chemical sympathectomy (repeated daily subcutaneous injection of guanethidine) or directly by physical stripping of the renal arteries followed by application of phenol in alcohol (separation of the renal arteries from renal nerve with an insertion of thread saturated with 10% phenol in 95% ethanol around the renal artery), systemic administration of transient receptor potential (TRP) V1 receptor agonist, capsaicin or peri-axonal application of capsaicin for a more selective ablation of the afferent renal nerve (Jason D et al. 2014).

Resection of the renal nerve was first proposed in the early 1920s for the treatment of nephralgia and pain induced by hydronephrosis in humans. Denervation of the renal afferent sympathetic nerve from the thoraco-lumber section, produced a marked improvement in patients with hypertension (Papin and Ambard, 1924). Bilateral nephrectomy has been shown to normalize sympathetic activity (Smithwick, 1951) because the procedure eliminated the renal afferent nerve endings.

As technologies advanced, safe and less invasive renal denervation techniques were developed which include: radiofrequency energy delivered into the lumen of the renal artery via catheter based electrodes (single or multiple), externally focused ultrasounds, and chemical infusion of low doses of alcohol into the renal artery.

Out of these three proposed techniques, the most widely used is the radiofrequency energy delivered into the lumen of the renal artery via catheter-based electrodes, although there is currently no biomarker of successful denervation available. These demonstrate a 47% decrease in neurotransmitters released from the kidney, thus decreasing sympathetic renal activity (Winklewski et., al 2017). Complications of this procedure include vasospasm, renal artery stenosis, and anesthetic issues such as transient bradycardia have been recorded due to fluctuations of systemic blood pressure. Another method used to disrupt afferent renal nerve signaling is systemic administration of the transient receptor potential (TRP) V1 receptor agonist otherwise known as capsaicin. Jason et al., demonstrated that capsaicin not only ablates small unmyelinated C-fibers, but also destroys afferent neurons in the kidney; however, it is not specific for renal afferents since systemic administration of capsaicin has been shown to cause degeneration of transient receptor potential V1 + sensory fibers throughout the body (Jason et al., 2014). Jason et al., went further to look at the selective role of renal afferent signaling in the regulation of renal and cardiovascular function. In order to go about their study, they needed to come up with a technique that selectively denervated the renal afferent nerve, thus leaving the efferent renal nerve intact. The approach they used was peri-axonal application of capsaicin to the renal nerve, because the majority of the renal afferent nerves are unmyelinated, sensitive to capsaicin and TPRV1 receptors are localized along the axons of the sensory fibers as well as nerve terminals. Capsaicin is able to bind to these receptors and selectively denervate the nerve (Jason et al., 2014).

Renorenal Reflex (Renal Mechano-Sensory Nerve)

An increase in the activity of the renal efferent sympathetic nerve, increases renal afferent nerve activity, which in turn decreases renal efferent sympathetic nerve activity through the activation of a negative feedback mechanism (Inhibitory Renorenal Reflex) (Kopp et al., 2007). Increased dietary sodium intake, increases renal pelvic pressure, which activates the renal mechanosensory nerves in the renal pelvis (Kopp et al., 2002). Due to the activation of the renorenal reflex in high salt conditions, increased renal afferent activity suppresses renal efferent sympathetic nerve activity which results in an increase in sodium excretion.

The importance of the reno-renal reflex is to maintain sodium and water homeostasis. It is underlined by the low activation threshold which is approximately 3mmHg pressure in the renal pelvis, and its abolition results in salt-sensitive hypertension. In the absence of an intact renal afferent nerve, rats on a diet high in sodium become hypertensive. Previous studies have shown that afferent renal nerve activity and urinary sodium excretion responses to increased renal pelvic pressure are enhanced by a high sodium diet and suppressed by a low sodium diet. The mechanism involved in the activation of the renal mechanosensory nerves as a result of stretching the renal pelvic wall involves the induction of the cyclooxygenase-2 (COX-2) which leads to the increase in renal pelvic synthesis of prostaglandin E2 (PGE2). Prostaglandin E2 (PGE2) will in turn increase the release of substance P, through the activation of cyclic adenosine monophosphate protein kinase A (cAMP-PKA) signal transduction pathway. Substance P activates the afferent renal nerves by stimulating neurokinin-1 receptors in the renal pelvic area (Kopp et al., 2008).

Types of Hypertension

Primary Hypertension

Primary hypertension, otherwise known as essential hypertension, is an increased blood pressure that does not have a known secondary cause. Genetic factors are known to play a role in this kind of hypertension, accounting for 30-50% of individual cases. Other factors responsible for primary hypertension are certain environmental and lifestyle changes (Shradha et al, 2018). Commonly implicated medications and foodstuffs that increase blood pressure are non-steroidal anti-inflammatory medications, corticosteroids, calcineurin inhibitors, stimulant sympathomimetics, female hormone replacements, hormonal oral contraceptives, excess sodium diet, alcohol and illicit drugs. Life style choices like a sedentary lifestyle and a diet poor in fruits and vegetables, as well as carbohydrate rich diets that lead to obesity, also contribute to increases in blood pressure (Shradha et al, 2018).

Dysregulation of the renin angiotensin aldosterone system has been associated with essential hypertension. Genetic changes or modifications in the angiotensinogen locus produces extra copies of angiotensinogen, which in turn increase the plasma angiotensinogen level and subsequently angiotensin II. This mechanism has been associated with hypertension in transgenic mice as well as in humans with essential hypertension. In spontaneously hypertensive rats (SHR), blockade of the central renin angiotensin aldosterone system with the intraventricular administration of an angiotensin type 1 receptor blocker has successfully prevented hypertension in pre-hypertensive SHR. According to the study, the authors concluded that over activity of the brain renin angiotensin aldosterone system was implicated in hypertension in spontaneously hypertensive rats (O'Callaghan et al, 2013).

Secondary hypertension

Secondary hypertension is an increase in blood pressure that occurs as a result of secondary identifiable causes such as kidney diseases, or conditions that affect the arteries, heart, and endocrine system. This can also occur during pregnancy. This kind of hypertension accounts for 5-10% of hypertensive patients. Proper treatment can control both the underlying condition and the high blood pressure, which can reduce the risk of serious complications, including heart disease, kidney failure and strokes.

The prevalence of secondary hypertension increases to 20-60% in young patients (<30 years of age) and patients with resistant hypertension. The most common secondary causes found in resistant hypertension are obstructive sleep apnoea (OSA), which results in sympathetic overdrive, and hyperaldosteronism (Conn's adenoma or bilateral adrenal hyperplasia causing mineralocorticoid excess) (Shradha et al, 2018).

Blood pressure measurement

Blood pressure is an unstable biological variable, which is subject to seasonal, circadian, hormonal and immediate external influences. As such, a single point measurement is unlikely to be a representation of an individual's usual blood pressure (National Institute for Health and Care Excellence, 2011, Galderisi et al, 2013). Thus it is important to remember this when diagnosing and treating patients with this condition. Multiple blood pressure readings should be taken in one sitting, as well as repeat blood pressure measurement over several clinical encounters. This practice is important in order for patients to obtain readings that more closely resemble the usual blood pressure readings (Galderisi et al, 2013).

Treatment of Hypertension

The pathophysiological progression of hypertension is important in terms of guiding treatment regimens (Fisher and Paton, 2012). There has been a remarkable improvement in the treatment of hypertension over the past 50 years. Despite these advances, it is estimated worldwide that 1 billion people still remain hypertensive. The global prevalence is projected to exceed 1.15 billion by 2025 (Mittal and Singh, 2010). This results from multiple factors, including under-diagnosis of the condition and treatment noncompliance.

There are three different strategies used in lowering blood pressure, which are; traditional pharmacological therapies, alternate therapeutic strategies and surgical therapeutic strategies

Traditional pharmacological therapies

The success of a treatment paradigm in lowering blood pressure in patients with resistant hypertension, with evidence of cardiovascular damage varying from slight to marked, has been achieved via targeting the sympathetic nervous system activity. Traditional therapy involves the use of pharmacological drugs to lower blood pressure. At present, angiotensin II converting enzyme (ACE) inhibitors, angiotensin II type 1 (AT1) receptors antagonists, β receptor blockers, calcium channel blockers, and diuretics represent the primary pharmacological treatment options in patients with hypertension (Chobanian, 2009). The therapeutic agents could be prescribed as either a monotherapy or in combination.

In general, in patients with uncomplicated essential hypertension, minimal alteration in muscle sympathetic nervous activity has been reported in studies examining the effects of long term administration of traditional antihypertensive agents such as angiotensin converting

enzymes inhibitors (ACE) (Grassi et al., 1998) or angiotensin 1 receptor antagonists (Krum et al., 2006). β-adrenergic blockers have also been demonstrated to have a neutral effect on central sympathetic outflow, particularly when reductions in heart rate are accounted for (Wallin et al., 1984). It is interesting to know that chronic administration of some traditionally used antihypertensive compounds such as diuretics and dihydropyridine calcium channel blockers, can actually stimulate central sympathetic outflow (Grassi, 2004). Thus the sympathetic effects of traditional blood pressure lowering drugs need to be carefully considered. (Fu et al., 2005) demonstrated that successful blood pressure lowering with combined angiotensin 1 receptor antagonists and diuretics, in a group of newly diagnosed patients with moderate essential hypertension, was associated with chronic exacerbation of muscle sympathetic nervous activity, possibly due to baroreflex unloading (lowered blood pressure sensed by the baroreflex). These studies identify the inadequacies of traditional hypertensive drug treatments in treating excessive central sympathetic outflow in hypertension.

Pathogenesis of resistant hypertension

The sympathetic nervous system has been implicated to be the cause of resistant hypertension (Katholi et al., 2010, Grassi, 2009, Shultz et al., 2007). Resistant hypertension is defined as a persistent increase in blood pressure, despite taking two or more medications of different classes, including ACE inhibitors combined with diuretics and calcium channel blockers (Fisher and Paton, 2012). The contribution of the renal sympathetic efferent and afferent nerves to resistant hypertension and chronic renal failure have been recognized. In the case of resistant essential hypertension, the renal vascular bed receives greater sympathetic activation than any other vascular beds. Increased stimulation of the renal sympathetic results in increased renal

vascular resistance, which in turn causes an increase in plasma renin activity. The increased renin, via angiotensin II and aldosterone, facilitates sodium and water retention, and decreases renal blood flow (Katholi et al., 1977, Katholi et al. 2010).

The reno-renal inhibitory reflex is attenuated when renal function is impaired, for example in ischemic kidney disorders. Increased afferent renal nerve activity contributes to the increased sympathetic nerve activity observed in patients with resistant hypertension. This is to say that an increase in renal afferent nervous activity will cause a positive feedback increase in renal efferent sympathetic activity, as hypertension worsens and becomes resistant to treatment. This concept was supported in a study with five patients that couldn't undergo renal denervation in their initial trial due to short renal arteries or dual renal arteries. This allowed their hypertension to worsen over the years despite optimal treatment being offered to them. In view of these sympathetic mechanisms, denervation of both the afferent and efferent renal nerves should result in long term attenuation of resistant hypertension.

According to this article (Katholi et al., 2010), therapeutic denervation of the sympathetic renal nerve in patient with resistant hypertension will result in a 14mmHg decrease in systolic blood pressure at 1 month, which can be improved to a 27mmHg decrease in systolic blood pressure by 12 months (Krum et al., 2009). The article further suggested that the response seen was due to the destruction of the afferent renal nerves (Fletcher, 2001). Studies done on animals have shown that selective denervation of the renal afferent sensory nerves by dorsal rhizotomy not only lowered blood pressure but also reduced end organ damage caused by excessive sympathetic nervous system activity (Campese et al., 1995, Hausberg et al., 2002, Dibona, 2003).

Several studies have provided evidence that the kidney is a sensory organ (Katholi et al, 1984, Ye et al, 2002, Katholi et al, 2010). The renal nerves contain multiple afferent unmyelinated fibers and some thinly myelinated fibers that carry impulses to the contralateral kidney, as well as centrally (Katholi et al, 2010). The renal afferent nerve has been shown to be involved in both renal-renal regulation and cardiovascular regulation. Animal studies have shown the involvement of renal afferent nerve activity in renovascular or renal failure hypertension (Katholi et al, 1983, Katholi et al, 1984, Kopp et al, 2003, Schlaich et al, 2009), and that selective renal denervation will attenuate this hypertensive effect. According to these studies, since the renal afferent nerve is likely the cause of resistant hypertension, there must be a signal that is being sent continuously from the renal afferent nerve that enhances the central sympathetic nervous system activity. The authors believed the signal to be adenosine and the receptor to likely be a chemoreceptor. During increased metabolic activity, adenosine is known to be released by the renal proximal tubular cells into the tubular fluid, where it stimulates chemoreceptive nerve endings in patients with hypertension and heart failure (Katholi et al, 2010).

Studies in animals have shown that stimulation of adenosine-sensitive nerve endings within or near the renal pelvis activates central sympathetic nervous activity through the afferent renal nerve, resulting in hypertension (Katholi et al, 1983, Katholi et al, 1984). Intrarenal adenosine has also been found to be elevated in patient with a stenosed renal artery and patients with metabolically stressed kidneys (Katholi et al, 1995).

Alternate therapeutic strategies

Central sympatholytic

Stimulation of α2 or imidazoline receptors within the central nervous system, directly reduces excessive central sympathetic outflow in hypertension (Fisher and Paul, 2010). Over the years the oral administration of either clonidine which is α2 and imidazoline receptor agonist, or moxonidine which is a selective imidazoline receptor agonist, has been shown to effectively reduce sympathetic nervous system activity and blood pressure in essential hypertension (Esler et al., 1997, Wenzel et al., 1998). However, both of these two medications have been reported to have an unpleasant side effect such as drowsiness, dizziness and orthostatic intolerance. The side effects of these drugs may to some extent be circumvented with effective dosing. A second generation imidazoline binding agent such as rilmentidine, has been reported to be more promising and beneficial in reducing sympathetic nervous system activity and blood pressure (Esler et al., 2004). It has been reported to be well tolerated by patients and it is effective in reducing left ventricular hypertrophy in patients with essential hypertension (Koldas et al., 2003).

Central nitric oxide

Several studies have shown that nitric oxide may be a therapeutic target to arrest excessive central sympathetic activation in hypertension. Nitric oxide is a signaling molecule that is synthesized from the conversion of amino acid L-arginine to L-citrulline by the enzyme nitric oxide synthase (NOS). There are three isoforms of nitric oxide synthase, namely the endothelial (eNOS), neuronal (nNOS) and inducible (iNOS) nitric oxide synthase. Endothelial and neuronal nitric oxide synthase are both expressed in mammalian cells.

In mammals, the endothelial isoform is a primary signal generator in the control of vascular tone, insulin secretion, and airway tone. It is involved in the regulation of cardiac function and angiogenesis (growth of new blood vessels). The neuronal isoform is involved in nervous system development. It functions as a retrograde neurotransmitter involved in long term potentiation, thus is important in learning and memory (Fisher and Paul, 2010).

All nitric oxide isoforms are expressed in the central and peripheral nervous system. Several studies have suggested that nitric oxide is a key signaling molecule involved in the tonic restraint of sympathetic outflow from the brain stem (Thomas et al., 2001). According to these studies, systemic infusion of a competitive nitric oxide synthase inhibitor in healthy humans causes sympathetic activation and marked elevations in blood pressure (Lepori et al., 1998, Sander at al., 1999, Young et al., 2009).

Aerobic exercise training

Aerobic exercise training is one of the non-pharmacological approaches to reducing blood pressure in patients with hypertension. The mechanism behind this likely involves an alteration in both neuro-humoral and neural cardiovascular control.

Laterza et al. demonstrated that 4 months of moderate intensity aerobic exercise training in never treated hypertensive patients significantly reduced muscle sympathetic nervous system activity and increased arterial baroreflex regulation of sympathetic nervous system activity (Laterza et al., 2007). Exercise has been shown to elicit an increase in peripheral and central nitric oxide synthase activity (NOS) (Mueller, 2007). This is associated with an upregulation of central antioxidants and downregulation of central pro-oxidants (Zucker et al., 2004).

Weight loss

Over the years there has been a strong association between weight gain and hypertension (Brown et al., 2000). Grassi et al. demonstrated that hypertension due to obesity results in an elevation in muscle sympathetic nervous system activity and renal nor-adrenaline spillover (Grassi et al., 2000). Weight loss following caloric restriction alone, or in combination with exercise training, has been associated with reductions in muscle sympathetic nervous system activity (Straznicky et al., 2005). The most effective therapeutic strategy for obesity related hypertension, particularly in the western world, is weight loss, which will in turn reduce sympathetic nervous system activity and lower blood pressure.

Reduction in psychosocial stress

Stress generally has been implicated in the pathogenesis of essential hypertension (Esler et al., 2008). Although work related stress may result in a daytime ambulatory blood pressure increase, the mechanism linking stress to hypertension seems to be complex and multifactorial, although the sympathetic nervous system is known to play an important role (Esler et al., 2008).

Elevated stress has been associated with an increased risk of atherosclerosis and acute cardiovascular events (Rosengren et al., 2004). Thus, stress reduction may be beneficial in lowering sympathetic nervous system activity and blood pressure (Rozanski et al., 1999).

Surgical therapeutic strategies

Due to the prevalence of resistant hypertension, more invasive approaches to reducing blood pressure were developed. These include renal sympathetic denervation and carotid sinus baroreflex stimulation.

Surgical renal denervation has been used over the years to treat resistant hypertension even before the availability of antihypertensive medications. Due to the fact that the procedure may result in several complications and had high rates of postoperative morbidity and mortality, it became less utilized. Renal denervation is done by either a surgical method or via catheter ablation. In the catheter-based method, radiofrequency energy is delivered to the renal artery wall by the insertion of an intravascular catheter through the common femoral artery. In 2009, Krum and his colleagues performed a trial of catheter-based renal sympathetic denervations in patients with resistant hypertension. The authors demonstrated a marked reduction in blood pressure (-27mmHg systolic/-17mmHg diastolic) a year after the procedure (Krum et al., 2009). The benefits of the catheter-based approach in comparison to surgical sympathectomy, are that it is brief with a median procedure time of 38 min, has no deleterious effect on renal function, and can be performed without long term complications (Fisher and Paul, 2010).

Renal denervation interrupts both afferent and efferent renal sympathetic nerves (DiBona et al., 2013). Renal efferent denervation decreases blood pressure through reduced renal vasoconstriction, renin secretion and sodium retention, and increased renal blood flow. Renal afferent denervation suppresses sympathetic nervous activity systemically and also lowers muscle sympathetic nervous activity by 66% (Fisher and Paton, 2012).

The surgical implantation of a device that elicits stimulation of the carotid baroreflex, has been used to target the sympathetic nervous system in resistant hypertension. Brauwald et al., demonstrated that a carotid nerve stimulator implanted in patients for the treatment of intractable angina pectoris elicited consistent reductions in blood pressure and symptomatic relief of angina (Braunwald et al., 1970). The complication with technological procedures such as

battery life and current leakage, at the time limited the widespread use of the device (Fisher and Paton, 2012, Uppuluri et al., 2009). Chronic baroreflex activation by a device decreases blood pressure, glomerular filtration rate, plasma renin activity, norepinephrine level, and sodium reabsorption. This is known to be more beneficial in obesity related hypertension (DiBona et al., 2013). This procedure involves the implantation of an electrode around the carotid sinus adventitia, such that only the baroreceptors are stimulated, without stimulating the chemoreceptors and mechanoreceptors (Fisher and Paton, 2012).

Deep Brain Stimulation

Electrical stimulation of the periaqueductal grey matter area, elicited a sustained reduction in blood pressure in a 58 year old man treated for neuropathic facial pain (Fisher and Paul, 2009). The reduction in blood pressure was followed by a reduction in pain. Chronic stimulation of the periaqueductal grey area can improve hypertension to the point that medication can be discontinued (Fisher and Paton, 2012).

Spinal cord stimulation

Spinal cord stimulation is achieved by placing an electrode in the epidural space over the dorsal column, a few levels above the affected spinal segments. Mild to moderate electrical pulses at various frequencies, usually 50-60 Hz, are delivered to the spinal cord to elicit paresthesia in the painful region.

Spinal cord stimulation dating back to the 1960's, has become part of routine pain therapy; it is estimated that over 30,000 spinal cord stimulation systems have been implanted every year worldwide (Linderoth and Meyerson, 2010). Guan et al. studied the effect of spinal cord stimulation on pain inhibition, along with with Dr. Srinavasa Raja, who was a senior

researcher at Johns Hopkins University, Baltimore, Maryland. They looked at the physiological basis for the pain suppressive effect of spinal cord stimulation, which was a widely used pain inhibition therapy at the time (Guan et al, 2010). The authors designed an extensive electrophysiological study, using animal models with neuropathic pain. They later went on to demonstrate that spinal cord stimulation (SCS) may markedly attenuate neuronal responses in the spinal dorsal horn to both natural innocuous pain and electrical noxious stimuli applied to a nerve injured hind paw (Guan et al, 2010).

Spinal cord stimulation is an outgrowth of the well-known gate control theory postulated by Melzack and Wall in 1965 (Melzack and Wall, 1965). They proposed that a mechanism in the dorsal horn of the spinal cord acts like a gate that inhibits or facilitates transmission from the body to the brain on the basis of the diameters of the active peripheral fibers, as well as the dynamic actions of brain processes. Electrical current activates large afferent fibers that close the gate of pain transmission, while noxious stimuli activates small sized afferent nerves that send messages to the brain. The gate control theory serves as the basic foundation for explaining the mechanism of spinal cord stimulation, even though details of the theory still remain controversial and require additional studies.

In the 1970's and 1980's, a number of experimental studies appeared with the aim of investigating the mode of action of spinal cord stimulation. The limitation of these studies is that they were performed on anaesthetized normal healthy animals, subjected to acute, noxious stimuli, thus their relevance for investigating the effect of spinal cord stimulation on neuropathic pain was questionable. It was not until 1994 that spinal cord stimulation was experimentally studied in an animal model of neuropathic pain (Meyerson et al, 1994, Linderoth et al, 1995).

The hyperexcitability demonstrated by multimodal wide dynamic range cells in the dorsal horn seems to be related to increased basal release of glutamate, which is an excitatory amino acid, and an inhibition of gamma aminobutyric acid (GABA) which is an inhibitory neurotransmitter (Stiller et al, 1996, Cui et al 1997).

Spinal cord stimulation (SCS), may suppress the enhanced responsiveness of this multimodal wide-dynamic range cells to innocuous peripheral stimuli. Guan et al, reported that dorsal column stimulation did suppress the wide-dynamic range neuronal response to peripheral electrical activation of C-fibers, although the effect was also observed in the sham operated rats. The authors later suggested that there was no clear evidence that spinal cord stimulation is helpful for alleviating acute nociceptive pain. This finding contradicts with the gate control theory by Melzack and Wall in 1965, from which spinal cord stimulation emerged (Melzack and Wall, 1965). In 1974, it was demonstrated that spinal cord stimulation in patients did not influence pain perception, but effectively attenuated tactile and pressure allodynia (Lindblom and Meyerson 1975). A study in 1995 showed that there was no effect of dorsal column stimulation on the C-fiber component of the flexor reflex in nerve injured rats.

Spinal Neurochemical Mechanisms

Gamma Amino Butyric Acid (GABA)

Aβ fiber input and GABA release activates GABAergic inhibitory interneurons in the superficial laminae of the dorsal horn (Daniel and MacDermott, 2009, Schoffnegger et al, 2006). GABA is an important inhibitory neurotransmitter in the gate control mechanism. In models with neuropathic pain, spinal cord stimulation increases the spinal release of GABA in animals that responded well to the analgesic effect of spinal cord stimulation, thus subsequently resulting in

a decrease in glutamate and aspartate release (Linderoth et al, 1996). The authors suggested that the GABAb receptor is responsible for the aforementioned effect, and it plays a more important role than the GABAa receptor in mediating the inhibitory effect (Meyerson et al, 1997).

The inhibition of the wide dynamic range neuronal hyperexcitability and animal pain behavior was closely associated with the time course of elevated GABA levels in the dorsal horn after spinal cord stimulation. Shealy et al. proposed a mechanism through which spinal cord stimulation acts to abate pain stimuli in rats (Shealy et al., 1967). The release of an inhibitory neurotransmitter Gamma Amino Butyric-Acid (GABA) leads to a decrease in extracellular glutamate concentration in the dorsal horn. Even though there is clear evidence of successful outcomes with this technique, the mechanism of action of spinal cord stimulation is still not completely understood (Wolter, 2014).

Linderoth et al, demonstrated that intrathecal administration of sub-effective doses of baclofen enhanced spinal cord stimulation analgesia in both human patients and animal models (Lindroth et al, 2008). Baclofen is an agonist of gamma amino butyric acid, and it is used as a muscle relaxant and an antispasmodic agent in treating muscle symptoms such as spasm, pain, and stiffness caused by multiple sclerosis, spinal cord injury or other spinal cord disorders (Richard D, et al., 1989). Baclofen can be administered to the patient either orally (by mouth) or intrathecally (directly into the spinal cord). Interestingly, the duration of time that the extracellular GABA level remained elevated exceeded the duration of spinal cord stimulation (Meyerson, 1997). The findings by Meyerson may indicate a dysfunctional GABAergic reuptake mechanism after nerve injury.

Joosten et al, demonstrated that during the early phase of neuropathic pain, the intracellular GABA content of the dorsal horn neurons decreases, but increases in the later phase (Joosten et al, 2011). Thus, the involvement of the GABAergic mechanism in the analgesic effect of spinal cord stimulation may change during the progress of neuropathic pain.

Thus further studies need to be done to look into other mechanisms involved in the release of GABA during spinal cord stimulation in neuropathic pain.

Serotonin (5-HT)

Spinal cord stimulation also stimulates other neurotransmitters systems in the spinal cord. Linderoth et al. demonstrated that spinal cord stimulation induces serotonin release in the spinal dorsal horn of cats (Linderoth et al., 1992). They further showed that increased endogenous serotonin content after spinal cord stimulation may involve local GABAergic circuitry (Song et al, 2009). There are several serotonin (5-HT) receptor subtypes (5-HT 1-7), usually seven, (7) that exert diverse effects on spinal pain processing. Nerve injury will change the expression and function of these receptor subtypes (Liu et al, 2010, and Suzuki et al, 2005). Song et al., (2011) expanded the understanding of the role the different spinal serotonin receptors play in spinal cord stimulation analgesia during neuropathic pain. The authors showed that activation of 5-HT2A, 5-HT3, and 5-HT4 receptors in the dorsal horn may contribute to the spinal cord stimulation induced decrease in pain transmission. Interestingly, 5-HT3 is known to mediate a fast excitatory response and plays a role in pain facilitation, but surprisingly, according to Song et al., (2011) activation of the 5-HT3 receptor results in spinal cord stimulation analgesia.

Nerve injury changes 5-HT3 receptor activity. When this occurs, spinal cord stimulation analgesia is partially mediated through activation of spinal GABAergic interneurons that express

5-HT3 receptors (Song et al, 2011). Increased release of serotonin increases the expression and synthesis of dynorphin, enkephalin and GABA within the spinal cord (Li et al., 2003). This allows for the delayed and prolong analgesic actions of spinal cord stimulation.

Enkephalin and dynorphin are opioid peptides that play an important role in pain modulation. They are produced in the central nervous system.

Muscarinic and Adrenergic Mechanism

The other two important mechanisms that play an important role in spinal cord stimulation analgesia involve cholinergic and adrenergic neurotransmission. According to in vivo micro-dialysis studies, spinal cord stimulation induces the release of both acetylcholine and noradrenaline in the spinal cord (Schechtmann et al, 2008). In neuropathic rats that responded to spinal cord stimulation with analgesia, acetylcholine content was significantly elevated in the dorsal horn of the responding rats alone. The release of acetylcholine is unaffected in nonresponsive rats (Schechtmann et al, 2008 and Song et al, 2008). Behavioral studies showed that administering sub-effective intrathecal doses of a muscarinic agonist could transform non responding animals to responding animals (Song et al, 2008, Linderoth et al, 2013)

Spinal stimulation-induced pain inhibition was completely blocked by intra-thecally administered atropine and a muscarinic M4 receptor antagonist, and partially attenuated by M1 and M2 antagonists. Thus inhibition of neuropathic mechanical hypersensitivity by spinal cord stimulation is associated partially with an increased release of acetylcholine that activates spinal muscarinic receptors (Schechtmann et al., 2008). Radhakrishnan and Sluka (2003) demonstrated that transcutaneous electric nerve stimulation (TENS), which is another treatment modality based on the gate theory, also activates a spinal cholinergic mechanism to achieve pain inhibition.

Acetylcholine and noradrenaline are also capable of exciting spinal GABAergic interneurons by binding to muscarinic receptors and α1-adrenoceptors, found on GABAergic interneurons, to produce analgesia after spinal cord stimulation (Chen and Pan, 2004, Zang et al, 2009). Thus, noradrenaline and acetylcholine may excite spinal GABAergic interneurons to produce analgesia after spinal cord stimulation (Song et al., 2008).

Antidromic mechanism of the renal afferent nerve

The idea of an antidromic mechanism for SCS effects was initially proposed by Bayliss (Bayliss, 1901). Bayliss observed that dorsal root stimulation at high intensity induced peripheral vasodilation mediated by thin fibers. This finding was confirmed by the observation that a high intensity stimulation of the dorsal roots provoked an increase in muscle blood flow (Hilton and Marshall, 1980). Hilton and Marshall, (1980) studies demonstrated the antidromic effect of the dorsal roots L6-S1 on blood flow through the gastrocnemius muscle in anaesthetized cats. The authors showed that stimulation of the peripheral ends of the ligated dorsal roots with current pulses of 0.3-0.5msec duration and at intensities most effective in activating the smaller afferent fibers, for periods of 15-20 sec, produced a 50-60% increase in muscle vascular conductance which was slow in onset and long outlasted the stimulus.

Foreman et al., 2003 investigated the fiber types responsible for spinal cord stimulation-induced vasodilation. The purpose of this study was to determine if SCS produces cutaneous vasodilation via antidromic activation of the unmyelinated C-fiber or the small myelinated fibers. The authors recorded antidromic compound action potential (CAPs) in the tibial nerve in response to spinal cord stimulation at L2-L3 spinal level. CAPs of small and large myelinated afferent fibers were observed in response to spinal cord stimulation at all intensities. The authors

concluded that SCS-induced vasodilation at ≤60% of the motor-threshold may be mediated via only the myelinated fibers, whereas vasodilation at ≥90% of motor-threshold may also involve antidromic activation of some unmyelinated C-fibers (Foreman et al., 2003).

Wu et al., 2006, studies investigated the contribution of vanilloid receptor type 1 (VR-1) containing fibers to spinal cord stimulation induced vasodilation. This study demonstrated that several vasodilators are contained in sensory nerve endings and are released during SCS. CGRP (a potent vasodilator) is co-localized in TRPV1 sensory containing terminals. SCS activates TPRV-1 containing sensory fibers in the unmyelinated C-fiber or myelinated Aδ sensory fibers. Activation of TRPV-1 causes the release of CGRP that binds to CGRP-1 receptors in the vascular endothelium, which initiates the synthesis and release of nitric oxide (NO) from the vascular endothelium. NO (a vasodilator) binds to the vascular smooth muscle which induces vasodilation of the vascular smooth muscle. In conclusion SCS-induced vasodilation is predominately mediated via VR-1 containing sensor fibers (Wu et al., 2006)

Uses of Spinal cord stimulation

Spinal cord stimulation has been used successfully for the treatment of ischemic pain, angina pectoris and peripheral vascular diseases. Ischemic pain occurs when there is an imbalance between the oxygen supply and demand. This can also be defined as the pain that occurs when the peripheral blood flow to tissues is decreased, thus resulting in inadequate delivery of oxygen (Simpson et al., 2009). Spinal cord stimulation is beneficial for restoring this balance between oxygen demand and supply through several different mechanisms (Linderoth and Foreman, 1999).

In the case of the ischemic pain of refractory angina pectoris, which is very severe and unresponsive to anti-ischemic therapies, relief can best be achieved when spinal cord stimulation is applied at the T_1-T_2 level, inducing paresthesia covering the precordial chest (Jessurun et al., 1996). Aside from placement of the electrode at the thoracic level, it should also be noted that placement at a higher cervical level may provide efficient pain relief as well (Gonzalez et al., 1998). Several studies have supported the anti-anginal effect of spinal stimulation, which resulted in a decrease in the frequency of angina attacks. The mechanism of action behind the anti-anginal effect of stimulation is not clearly understood and is still debated among researchers. Chandler et al. (2003) focused on the mechanism of cardiac pain suppression by spinal cord stimulation. The authors could not determine if the main anti-anginal effect was due to direct inhibition of nociceptive transmission, or mediated by a local redistribution of blood flow and a decrease in coronary oxygen demand (Linderoth and Foreman, 1999). According to their study, they believed that local redistribution of blood flow and a decrease in coronary oxygen demand could be the result of cardiac sympathetic depression resulting in the anti-anginal effect. Other studies have debunked Chandler's views and proposed that endogenous opioids are released into the cardiac circulation during spinal cord stimulation. This could be the reason for local sympathetic suppression (Eliasson et al., 1998).

Location

Easily accessible large diameter afferent fibers are located at the dorsal column of the spinal cord. Hence, this makes the dorsal column an ideal location for applying electrical stimulation (Linderoth and Foreman, 1999). When an electrode (cathode) carrying current is placed on the dorsal column, it produces an electric field that stimulates spinal sensory fibers.

This stimulation recruits large diameter low threshold fibers located in the dorsal root first, before closing the gates to the dorsal horn. For pain control it is recommended to keep the electrode near the midline (Oakley J.C., 2002).

The specific spinal segment selected for stimulation in humans and animals is an important determinant of clinical results. The stimulating electrode is usually placed superficially on the spinal cord ranging from approximately T_7-T_8 (Meglio M, et al., 1981) to T_{10} (Jacobs et al., 1990) or to lower regions of the thoracic spinal cord (Broseta J, et al., 1986).

John et al., compared the effect of dorsal column stimulation at different stimulation sites; which were T_{11}, T_{13} and L_2. The stimulation parameters used at each of the three sites were 0.2 mA at either 25 or 50 Hz, or 0.6 mA at either 25 or 50 Hz, at a pulse duration of 0.2 ms^{-1}.

Their results demonstrated that the largest changes in blood flow and vascular resistance to the legs occurred during dorsal column stimulation at the L_2 spinal segment, using a stimulus intensity of 0.6 mA at either 25 or 50 Hz (John E et al., 1996).

To increase cutaneous blood flow to the lower extremities, the spinal segment selected for stimulation is at the level of L_2-L_3. Stimulation at T_{10} and above reduces peripheral blood flow to the lower extremities. Studies have shown that the vasodilation effect of L_2-L_3 spinal stimulation is abolished, when the spine is transected at T_{13} or muscimol (A psychoactive drug, which is a potent $GABA_a$ agonist, when administered it alters neuronal activities in multiple regions including the cerebral cortex, hippocampus and cerebellum) is applied topically on the dorsal surface of the spinal cord (Wu et al., 2008). Linderoth et al. using rat models, placed the electrode on the spinal cord at the level of T_{12}-L_1 level (Linderoth B et al., 1994). Thus, dorsal column stimulation in either human or animal studies can be applied anywhere from the lower

thoracic region (T_{12}) to the upper lumber region (L_2-L_3) of the spinal cord to increase blood flow to the lower extremities.

Electrical Parameters

All spinal cord stimulation parameters (amplitude, pulse width, frequency) influence the interaction of stimulation with the nervous system and impact the delivery of charge. The pulse is the basic unit of electrical stimulation in neuromodulation. It consists of a sustained delivery of a specific amount of current amplitude, which is measured in volts (V), for a specific amount of time measured in microseconds (μs). Each pulse is followed by an equal flow of current in the opposite direction to balance the charge. This is also a safeguard against electrode-tissue damage due to chemical reactions that might result from charge buildup (J.P Miller, et al., 2016).

Amplitude and pulse width

In amplitude controls, two types of systems are involved: a voltage-controlled system and a current-controlled system. With regards to the voltage-controlled system, amplitude is prescribed as the potential difference (V) applied to the electrode surface. In this case, the actual flow of current is dependent upon impedance at the electrode-tissue interface. The current-controlled system delivers a prescribed current, thus allowing the voltage to vary with impedance. As long as the impedance is stable over time, there would not be a clinical difference between the constant voltage and constant current systems (Schade et al., 2010). Another factor that has a great impact on the stimulation amplitude required is the spinal cord movement effect and cerebral spinal fluid thickness. Pulse width refers to the length of time each pulse of current is applied. A narrow pulse width will require a high amplitude to activate a neuron or axon when strength duration is considered, while a wider pulse width will require a lower amplitude

(Jonathan P et al., 2016). Therefore, the threshold for the generation of an action potential will typically follow a hyperbolic curve.

Frequency

The frequency parameter in spinal cord stimulation is defined as the number of pulses per second or hertz. This parameter can be adjusted to be effective. The frequency most commonly used in clinical practice ranges from 40-100 Hz (North et al., 1993). Several studies on spinal cord stimulation have demonstrated that the frequency can be an important determinant of activating specific pain relieving mechanisms. For example, in transcutaneous electrical stimulation and electro-acupuncture, low rate frequency (2-10Hz) activates μ-opioid receptor pathways, whereas high rate therapy (100 Hz) activates endogenous d_s-opioid systems (Ulett et al., 1998). When frequencies around 50 Hz are used, the dorsal horn GABAergic neurons are activated. Spinal cord stimulation (SCS) also activates interneurons that use other transmitters such as acetylcholine and adenosine (Linderoth et al., 2008), serotonergic cells in the rostroventromedial medulla (Song et al., 2011), and nuclei in the locus coeruleus region containing norepinephrine (Song et al., 2013). Frequencies at higher rates induce greater blood flow than frequencies at lower rates.

In humans during spinal cord stimulation, the perception threshold is the voltage at which the patient starts to experience paresthesia. The discomfort threshold is the voltage at which the patient cannot withstand the paresthesia anymore. The usage range is the difference between the perception threshold and the discomfort threshold (Oakley J.C., 2002).

In animal models, the motor threshold is used to calculate the stimulation intensity or voltage. The motor threshold is determined by gradually increasing the stimulation voltage from

zero to the point where muscle contraction is visible. The contraction observed is as a result of stimulating the dorsal column afferent sensory fibers. Stimulation of the afferent sensory fibers produces a reflex response that activates the motor neurons, which results in muscle contraction (Wu et al., 2008). The parameters used in conventional spinal cord stimulation include a frequency of 50 Hz, at a pulse duration of 0.2ms. The stimulation voltage is determined individually, usually at 40-60% of the motor threshold. (Song et al., 2014).

CHAPTER II

CURRENT STUDY

Purpose of the study

The purpose of this study is to understand the mechanism behind which dorsal spinal stimulation increases renal sodium and water excretion. This research answered the following.

Does the renal afferent nerve affect renal changes resulting from spinal cord stimulation, by retrograde transmission of the electrical impulse?

Do renal changes resulting from spinal cord stimulation, occur as a result of a modification of the renal efferent nerve output?

This work will help determine effective therapies for the treatment of resistant hypertension. Spinal cord stimulation has been used for more than 30 years, and is effective in managing severe conditions like chronic pain, and ischemic pain, due to its ability to increase peripheral blood flow. Previous studies in the Knoblich laboratory have investigated the effect of dorsal column spinal stimulation on urinary sodium excretion, and showed that unilateral spinal cord stimulation at T_{11}-T_{12}, with a stimulation voltage of 67% of the motor threshold, a frequency of 50 Hz, and a duration of 0.2 ms^{-1}, is effective in increasing urinary sodium and water excretion in the spontaneously hypertensive rat (SHR). (Mager and Knoblich 2003).

Additional studies in this lab have also shown that the dorsal spinal cord stimulation induction of urinary sodium and water excretion is not the result of an increase in renal blood

flow or a change in mean arterial pressure. Furthermore, the renal response was eliminated by renal denervation, which destroys both the afferent and efferent components of the renal nerve.

The current study will determine the role of the sensory (afferent) portion of the renal nerve in the renal response to spinal stimulation. For this project, we will use a spontaneously hypertensive rat that will undergo dorsal rhizotomy of the renal nerves, which is selective destruction of only the afferent portion of the renal nerve.

Hypothesis

The renal afferent nerve is responsible for transmitting sensory stimuli from the kidney to the spinal cord. Electrical stimulation of the dura of the spinal cord at the level in which these nerves enter the spine, could elicit a reciprocal or retrograde transmission on the afferent renal nerve that would travel back to the kidneys, thus affecting renal changes. We hypothesize that electrical stimulation of the dorsal column of the spinal cord affects renal changes by retrograde transmission of renal stimuli from the spinal cord back to the kidney. This hypothesis will be tested by selectively ablating (severance of) the renal afferent nerve alone, while keeping the renal efferent nerve intact.

Hypothesis 1: The renal afferent nerve is responsible for transmitting a retrograde signal to the kidney, during dorsal spinal simulation that affects renal sodium excretion

Hypothesis 2: Severance of the dorsal (afferent) root of the renal nerve, just distal to the dorsal root ganglion, will eliminate the effect of dorsal spinal stimulation on renal sodium excretion.

Animal model

Spontaneously hypertensive rats (SHR strain) are an excellent model of primary hypertension and have been used to study cardiovascular disease (Pinto et al., 1998). Okomoto and colleagues obtained this rat strain in the 1960s by selective inbreeding Wistar-Kyoto rats with high blood pressure (Okamoto and Aoki, 1963). Hypertension begins at 5-6 weeks of age, with systolic pressure between 180 and 200mmHg in the adult (Conrad et al., 1995).

Hypertensive development in spontaneously hypertensive rats is somewhat related to the kidney. Kidneys transplanted from the SHR strain to a normotensive Wistar rat increased blood pressure in the recipient. Conversely transferring a kidney from a Wistar strain to SHR normalized blood pressure in the recipient (Kawabe et al., 1978). This is only effective if transplantation occurs at a young age, before hypertension develops in the donor. Thus, this supports a role for the kidney in the development of hypertension in the SHR.

CHAPTER III

MATERIALS AND METHODS

Animal Care

A total of thirty two (32) male spontaneously hypertensive rats (SHR), ranging from 14 to 20 weeks of age, were raised in the animal colony at Minnesota State University, Mankato. The rats were allowed to reach a minimum age of 14 weeks to ensure that hypertension was well established. The rats were housed in standard rat cages, given standard rat chow (Lab Diet 5001 Rodent Diet; PMI Nutrition International LLC, Brentwood, MO) and water *ad libitum*. The rats were at a controlled temperature of 21 ± 2°C, and were kept on a 12-hour day/night light cycle. The rats were randomly assigned to one of the following study groups.

Control Not-Simulated (CNS) - Intact nerves/no stimulation (8 rats)

Control Stimulated (CS) - Intact nerves/ bilateral dorsal spinal stimulation (8 rats)

Denervated Not-Stimulated (DNS) - Dorsal rhizotomy/no stimulation (8 rats)

Denervated Stimulated (DS) - Dorsal rhizotomy/ bilateral dorsal spinal stimulation (8 rats)

All rats were subjected to all surgical procedures except in the intact rats, the dorsal root was not transected, and in the unstimulated rats, no stimulation was applied.

Exclusion criteria include; incidental death of rats, inability to collect urine, or a sudden drop in mean arterial pressure below 100 mmHg.

Surgical Preparation

The date of the surgery, the rat's date of birth, weight, anesthetic dose, volume of saline delivered, and duration of surgery were recorded. The weight of the rat was measured in

grams, using a weighing balance (A&D Weighing EK-3000EP intrinsically safe portable balance 3000g × 0.1g). The rat was initially anesthetized with isoflurane in O_2, (3%, 1.0 L/minute) using a vaporizer (Model 100 Vaporizer; Surgi Vet,Waukesha, WI) and an anesthetic chamber.

Anesthetic gas was delivered until the rat was no longer responsive. Isoflurane was used initially to ease the stress of the injection of the primary anesthetic, which was Inactin (thiobutabarbital sodium) 100 mg/kg i.p. Inactin was chosen for its long lasting anesthetic effect, and minimal effect on the cardiovascular system. Response tests were conducted, such as the blink reflex and response to tail pinching to determine proper anesthetic depth. Once the rat reached sufficient anesthetic depth, the incision sites (neck, pelvic region, and back) were shaved with a clipper (Oster model Golden A5, McMinnville, TN). After shaving, the rat was placed in dorsal recumbency on a heating pad (Heating Pad Ultraheat; Sunbeam, Boca Raton, FL), which was adjusted to maintain rectal temperature between 36-37 °C. Temperature was monitored by inserting a rectal thermometer (Traceable Digital Thermometer; VWR Scientific) coated with lubricant (Surgilube; Fougera, Melville, NY) into the rectum, and taped to the tail.

A ventral midline incision with a sterile surgical blade (#10 Henry Schein; Southall, England) was made in the neck region of the rat. The trachea was exposed via blunt dissection, and a cut was made with a small surgical scissors (105 mm micro dissecting scissors HSB 010-10; Hammacher Instrument, Solingen, Germany) just below the larynx for the insertion of an endotracheal tube (PE 240; Becton Dickinson) to aid respiration.

Placement of the Jugular Vein Catheter for Saline Infusion

The jugular vein was cannulated to allow the infusion of saline (0.9% sodium chloride, USP; Baxter Healthcare Corp.) to maintain adequate hydration during the experiment.

Using an infusion pump (PHD 2000 Programmable; Harvard Apparatus, Holliston, MA), saline was infused at a rate of 15 ml/kg/hr. The jugular vein was isolated on the left side through the same midline incision used to isolate the trachea. Once isolated the jugular vein was ligated with suture at the distal end, to prevent bleeding upon cutting. With the aid of magnifying lenses (Optivisor; Doneqan Optical Co.), a small cut was made in the lumen of the jugular vein with a micro dissecting scissors (3", straight, tip 0.3mm RS-5610; Roboz Surgical Instrument). Care was taken to avoid complete transection of the vein. A section of saline filled tubing (PE 50; Becton Dickinson) connected to a three-way stopcock (Baxter Healthcare Corp., 2C6240) via a tubing adaptor (Becton Dickinson, 23G) was inserted into the vein, and secured using nylon suture. To ensure proper insertion into the lumen of the jugular vein, a saline filled syringe was attached to the stopcock, the system was opened and a negative pressure was applied until blood was observed flowing into the catheter. The blood was re-infused by placing positive pressure on the syringe and then the system was closed. The stopcock was attached to tubing which was attached to a 30 ml luer lok syringe (Becton Dickinson) that was loaded onto the infusion pump. A flow rate of 15ml/kg/hr was entered onto the pump controls and infused for the duration of the procedure, to keep the rat hydrated and able to produce urine.

Placement of the Carotid Artery Catheter for Measurement of Blood Pressure

The same midline incision that was used to isolate both the trachea and jugular vein, was used to isolate approximately a one centimeter section of the left carotid artery. The distal end of the carotid artery was ligated with nylon suture. On the proximal end of the carotid artery, a micro aneurism clip was placed to prevent blood loss. With the use of magnifying lenses,

an incision was made into the carotid artery with microscissors, which was followed by the insertion into the vessel of a heparinized saline (0.3 ml heparin per 50 ml saline) filled tube (PE 50, Becton Dickinson), connected to a three-way stopcock with a tubing adaptor. Once the catheter was inserted, it was secured with suture, and tested for correct positioning. This was done by removing the aneurism clip, turning the stopcock to open the system, and observing for blood flow into the tubing. The blood was returned to the vascular system by the infusion of heparinized saline. The stopcock was attached to a pressure transducer connected to Biopac hardware (Biopac Systems Inc.), and a computer. Arterial blood pressure and heart rate were recorded, and stored on the computer for later analysis.

Placement of the Bladder Catheter for the Collection of Urine

The bladder was exposed via a small scalpel incision on the ventral midline, just anterior to the pelvic bone. The cranial edge of the bladder was exteriorized. Then, a small purse-string suture was placed in the cranio-ventral wall of the bladder, using a curved needle (Regular Surgeon's Round Bodied ½ circle taper point needle; Miltex Instruments, Bethpage, NY) and nylon suture. A small cut was made in the center of the purse string, using a small scissors (105 mm microscopic scissors HSB 010-10; Hammacher Instrumente, Solingen, Germany). A tube (PE 240, Becton Dickinson), flamed at one end to create a lip, was inserted. The purse-string was tightened around the tube, just beneath the lip to secure it into the bladder. Any active bleeding on the bladder was alleviated by surgical cautery (Martin Electrosurgical Unit MD 62; KLS Martin, Tuttlingen, Germany). The tube was allowed to exit the abdomen ventrally, and the abdomen and skin was closed around the tube with a simple interrupted suture pattern, using nylon suture and a needle (Regular Surgeon's surgical needle 3/8 circle reverse cutting edge; Miltex

Instruments, Bethpage, NY). This ensured the collection of all urine produced over the duration of the procedure.

Procedure for Laminectomy

All rats underwent laminectomy and exposure of the dorsal roots. The rat was placed on its ventral surface, taking care to preserve the placement of the catheters, particularly the bladder catheter. The posterior of the rat was supported by a board with an opening in it, through which the bladder catheter protruded, which allowed urine to be collected unimpeded. The dorsal portion of the spine was palpated to locate the tall tenth thoracic vertebrae (T10), and the skin was marked with a marking pen.

A skin incision was made with a #10 scalpel to expose the spine from T9-L1. The dorsolateral portions of the spinal cord at T9-T13 junction was exposed by scraping off the overlying muscle with a scalpel. Once sufficient exposure was obtained, the rat was immobilized for the laminectomy, using a stereotaxic device (Lab Standard Stereotaxic Single unit 56100 with spinal cord surgery adaptor). The rat was secured to the device bilaterally, using the articulating processes of T9 and T13. Once the rat was in place, a surgical microscope (Stereo Zoom 4; Leica Microsystems) was used to aid in the laminectomy, which was performed by gently clipping the bone at T11-T12 away with a fine bone ronguer. Bone at T11-T12 was carefully clipped away until enough spinal cord was exposed to be able to successfully place the electrodes bilaterally on the dura of the dorsal surface of the spine between T11 and T12.

Procedure for Dorsal Rhizotomy

Additional bone removal was required to expose the dorsal roots of T11 and T12. The dorsal root ganglion was carefully exposed bilaterally at the intervertebral foramen by gently

clipping away the bone. Great care was taken to ensure no damage to neurological tissue occurred. On each side, the dorsal roots of T11 and T12 were followed proximally from the ganglia to the point at which they entered the spine. Using a surgical microscope, each dorsal root was identified and cut proximal to the dorsal root ganglia using a 20-gauge beveled needle (Kendall; 20 G 1 ¼) with the tip bent at a 90°angle. Dorsal root was transected in the denervated groups only. Extreme care was taken to preserve the ventral roots.

Collection Periods

A 45-minute equilibration period followed the surgical setup. Following the equilibration was six, fifteen-minute collection periods: one pre-stimulation period (Baseline), one period during-stimulation (period 1), and the remaining four periods (periods 2 – 4) post-stimulation. During these periods, urine was collected along with recordings of blood pressure (mmHg), and heart rate (beats/min). Urine was aspirated from the tubing and bladder using a 1 ml syringe (Becton Dickinson) with tubing (PE 50, Becton Dickinson) attached, and urine was placed into a graduated 1-ml syringe (Becton Dickinson) with the tip cut off. Urine volume was recorded to the nearest tenth of a milliliter, the syringe tip was covered with para-film (Pechiney Plastic Packaging Company; Chicago, Ill.), and urine was stored in the refrigerator (at 10°C) for later sodium analysis by flame photometer.

Motor Threshold Determination and Spinal Stimulation

In the two groups of rats receiving stimulation (CS and DS), the motor threshold was determined just prior to the second collection period (stimulation period). This was ascertained by placing two copper wires (Cathode-positive) with the tip wrapped around like a

ring, one on each side of the dorsal spinal column at T11-12. A needle (Anode-negative) was placed one on each side, in the paravertebral muscles.

The cathode (red) and anode (black) was connected to the positive (+) and negative (-) poles of the stimulator (Grass), respectively. Following precise placement, a small amount of conductive electrolyte gel (Signa Gel, Parker Laboratories Inc., Fairfield, NJ), was coated over the cathode to ensure proper conduction at low voltage. The stimulator was set at 2 pulse per seconds (hertz), and 0.2 seconds duration. The voltage was gradually increased until the paravertebral muscles began to twitch. This voltge was recorded as the motor threshold. The intact nerve and dorsal rhizotomy, no-stimulation groups (CNS and DNS) all underwent an identical procedure as the stimulated groups, with the same time delay in calculating the motor threshold between baseline and period 1 in the stimulated groups, but did not receive any electrical stimulation. Dorsal spinal stimulation occurred during period 1 with the parameters of 50 pulses per second, 0.2 seconds duration, and 0.66% of the motor threshold voltage.

Urine Collection and Sodium Determination by Flame Photometry

The urine samples collected during six 15-minute collection periods were analyzed for sodium content using a flame photometer. Any sample less than 0.30 ml was diluted to 0.3 volume with distilled water, to ensure an adequate volume for the analysis. The total sodium excreted was calculated by multiplying the sodium concentration by the final volume in the sample. Sodium concentration was determined by following the calibration and operation procedures of the flame photometer outlined in the instrument guide and instruction manual. The final sodium concentration (mmol/l) was divided by 1000 to get the sodium concentration per ml, and this value was then multiplied by the final diluted volume, producing the total sodium

excreted in the 15-minute period. This value was divided by the rat's weight (kg) and multiplied by 1000 to get a final value in μmol/kg/15min.

Measurement of Blood Pressure and Heart Rate:

Computer recordings of mean arterial blood pressure and heart rate were analyzed using the Biopac software. Mean values were measured for each 15-minute period and transferred to a Microsoft Excel worksheet.

Data Analysis

Heart rates (bpm), urine volume (μL/kg), blood pressure MAP (mmHg) and urinary sodium excretion rates (U_{Na}, μmol/kg/15min) were averaged for each group/period and reported as mean ± standard error. The change-in data for each rat was calculated by subtracting the measured data of each of the periods 1 through 5, from the baseline value, and this was expressed as ΔMAP, ΔU_v, and ΔU_{Na}.

Sigma plot 12.5 program was used for statistical analysis. U_{Na}, MAP, HR, and U_v were compared within and between groups using a two-way ANOVA with repeated measures. Also, the change in data of period 1 through 5 from baseline which was expressed as ΔMAP, ΔUV, ΔUNa, were compared within and between groups using two-way ANOVA with repeated measures. The above data were compared between each treatment groups (II AND IV) and their control groups (I and III) using one-way ANOVA. Mean baseline and mean sum of excretion data for period 1-5 were compared among groups using a one way ANOVA. Statistical significance was accepted when $p<0.05$. Group II and IV were compared with group I and III respectively, to determine the effect of dorsal spinal stimulation on sodium excretion in intact and denervated

rats. Groups III and IV were compared to groups I and II respectively to determine the baseline effects of denervation.

CHAPTER III

RESULTS

General information:

In this study thirty two (32) male spontaneously hypertensive rats (SHR) were randomly assigned into four (4) groups. The Control Not-Stimulated (CNS) rats, considered a control group, in which the renal afferent nerve was intact and rats received no dorsal column stimulation, was compared with the Control-Stimulated (CS) rats, which underwent dorsal column stimulation. In the Denervated-Not-Stimulated (DNS) rats, renal afferent denervation was done without bilateral dorsal column stimulation, and this was used as a control for the Denervated-Stimulated (DS) group, which went through both renal afferent denervation and bilateral dorsal column stimulation. In order to determine the effects of renal afferent denervation on urinary sodium excretion, the DNS and DS groups were compared to the CNS and CS groups, respectively.

Mean age, body weight, saline infusion rate, stimulation intensities and surgery times are shown in Table 1 for all groups. One way anova was used to compare 5 parameter among the four (4) groups. Statistical significance was accepted when $P<0.05$. No difference among the groups was found in any of these parameters.

Urinary Excretion

Bilateral Dorsal Spinal Cord Stimulation in SHR with Intact Renal Nerves

The mean arterial pressure, heart rate and urine sodium excretion was measured in six 15-minute increments. The pre-stimulation period is considered as baseline period for comparison of the data.

The stimulation period is considered as the period following baseline in which stimulation was done for the entire 15 minutes (Period 1). Stimulation was removed, and 4 additional collection periods followed (periods 2-4).

Urinary sodium excretion is shown in Figure 1A. Although stimulation during period I caused an increase in urinary sodium excretion, when compared with baseline (57.93 vs 31.53 µmol/kg/15min), this was not statistically significant P=0.071. In the CNS group, which received no stimulation, urinary sodium excretion during periods 1 through 5 did not change significantly from baseline.

Comparison between like periods in the CS and CNS groups showed that there was no significant difference (Figure 1A). However, when the data was normalized as the change from baseline (Figure 1B), a statistically significant difference was found (P=0.02), (F=3.177) concerning treatment x time, when CS was compared with CNS. However there was no statistically significant difference in any individual period when like periods were compared between groups.

Mean baseline and mean sum of sodium excretion for period 1-5 in each of the 4 groups showed that there was no significant difference (P=0.105 and P=0.561 respectively) (Fig 1C). Mean sum of increase in sodium excretion over baseline for period 1-5 in each of the 4 groups showed that there was no statistical difference (P=0.703) (Fig 1D).

Urine volume for each period is shown in Figure 2A. A significant difference was found in treatment x period when CS was compared to CNS (P= 0.016). Comparison of like periods between CS and CNS showed a significantly higher urine volume in the CS group than the CNS group during baseline (P=0.013), Period 1 (P=0.003) and period 2 (P=0.027). In the CNS group,

urine volume was increased significantly over baseline during period 5. When data was normalized as the change from baseline, the difference in treatment x time remained between the CS and CNS groups.

Mean baseline and mean sum of urine volume for period 1-5 in each of the 4 groups is shown in (Fig 2C). A statistical significant difference was found within mean baseline when CS group was compared with CNS group (P=0.009). Mean sum of urine volume for period 1-5 in each of the 4 groups showed no statistical significant difference (P=0.561)

Mean sum for change in urine volume for period 1-5 showed no statistical significant difference (P=0.224) (Fig 2D).

Bilateral Renal Denervation

DNS and DS went through bilateral renal afferent denervation before collecting the data (urine sodium excretion, mean arterial pressure and heart rate). In these groups baseline is the first data collection period post denervation, (directly following the equilibration period). No statistically significant differences were found in sodium excretion between the DS and the DNS rats during any individual period, or in treatment x time (Figure 1A). The same was observed when CNS was compared with DNS, in that the results showed no statistical significant difference.

When sodium excretion was normalized as the change from baseline, there was no significant difference when DNS was compared with DS during any time periods, or when treatment x time was evaluated. Furthermore, no differences were found when the denervated groups were compared to the intact groups.

Urine volume was not different between the DS and DNS groups during any individual period, or when treatment x time was compared (Figure 2A). Urine volume did not change from baseline during any period in either group. When urine volume was normalized as change from baseline, and treatment x time was compared, no difference was found between the two denervated groups (Figure 2B). When denervated rats were compared to intact rats, a treatment x time difference was noted between the DNS and the CNS groups, both with raw urine volumes, and when data was normalized as change from baseline. There was a significance difference during period 5 when compared to baseline in DNS group

DNS and DS did not show any significant difference when both groups were compared. The same was observed when CS and DS was compared, result showed no significant difference.

Mean Arterial Pressure (MAP)

Since urinary sodium excretion is affected by mean arterial pressure, the mean arterial pressure (MAP) was monitored for each rats. There was no statistical difference in mean arterial pressure between any of the groups, or any of the periods (Figure 3A). A trend of lower pressure was observed in the denervated groups, and this was significant only when the DS was compared to the CS group ($P = 0.037$). In all groups, mean arterial pressure gradually declined during the course of the acute experiment, but values were not significantly lower than baseline. When MAP was normalized as the change from baseline (Figure 6), no differences were found between the groups.

Heart Rate

There was a statistical significant difference in heart rate when treatment x time was compared between the CS and CNS groups (Figure 4A). The CNS group showed a trend of a gradual decrease in heart rate in period 1, through period 3, which then increased in period 4 and 5 but not to the level of the baseline (difference not statistically significant).

The CS group showed a gradual increase in period I from baseline, which then maintain a fairly constant heart rate in period 3 through 4 after which it decrease a little bit in period 5 (although the difference is not statistically significant). When heart rate was normalized as the change from baseline, the CS rats had a significantly greater increase in heart rate when compared to the CNS rats, during periods 2, 3, and 4. (Figure 4B), and an overall greater increase in heart rate (treatment effect) than the CNS group.

The denervated groups showed no significant differences between or within groups in heart rate, (Figure 4A), nor any difference when heart rate was expressed as the change from baseline (Figure 4B). Furthermore, no differences were found between the denervated groups and the intact groups.

Data Exclusion

Rats were excluded from the data analysis for the following reasons

- Sudden death, upon postmortem the lungs shows a grey, flat to raised foci randomly distributed throughout the lungs (6 rats)
- Heat stress resulting in sudden rise in MAP (1)
- Death due to excess infusion of heparin-saline (1)
- Collected urine samples dried out in 1 ml syringe during storage (1)

- Hyper extension of the limbs and tremors caused by electrode exerting pressure on the spinal cord (1)
- Data discarded due to rats showing signs of dyspnea and hyperpnoea (2)
- Data discarded due to pressure going low below 100mmHg (1)

TABLE

Table 1: Baseline data (age, body weight, saline infusion, stimulation intensity, and surgery time) in four groups of male SHR rats

Group	Age (WK)	Body Weight (KG)	Saline Infusion Rate (ml/kg/hr)	Stimulation Intensity (V)	Surgery Time (min)
I	20 ± 0.9	0.3 ± 0.0	5.1 ± 0.1	0 ± 0	226.1 ± 6.6
II	20.1 ± 1.2	0.3 ± 0.0	5.0 ± 0.1	0.8 ± 0.1	221.7 ± 4.0
III	19.8 ± 0.6	0.3 ± 0.0	5.0 ± 0.2	0 ± 0	227.0 ± 6.7
IV	18.6 ± 0.5	0.3 ± 0.0	5.2 ± 0.1	0.7 ± 0.0	242.4 ± 7.1

Values are reported as mean ± standard error

Control Not-Stimulated (CNS): Intact Renal Nerve, Non-stimulated

Control Stimulated (CS): Intact Renal Nerve, Stimulated

Denervated Not-Stimulated (DNS): Dorsal Rhizotomy, Non-stimulated

Denervated Stimulated (DS): Dorsal Rhizotomy, Stimulated

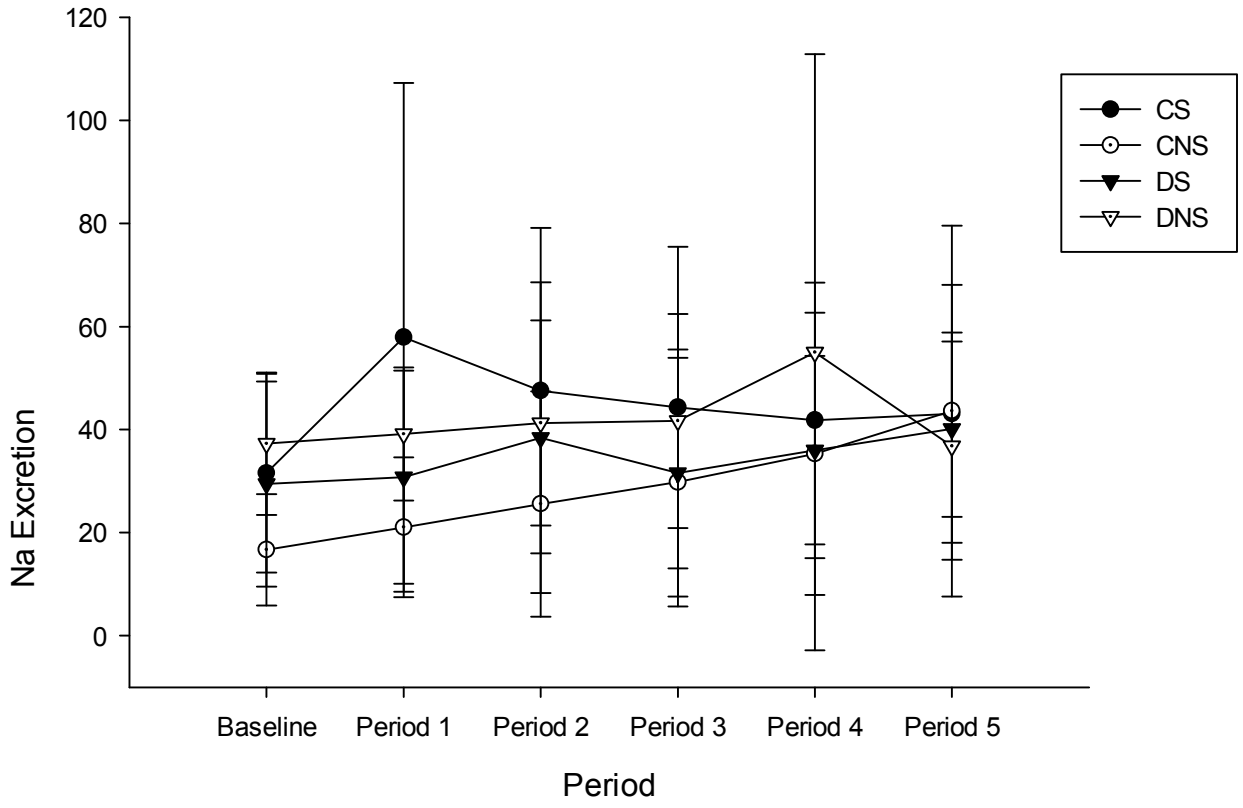

Figure 1A: Mean sodium Excretion during baseline, spinal stimulation (period 1), and post stimulation collection periods (periods 2-5) in rats subjected to no stimulation (CNS), stimulation during period 1 (CS), afferent renal denervation and no stimulation (DNS), and afferent renal denervation and stimulation during period 1 (DS).

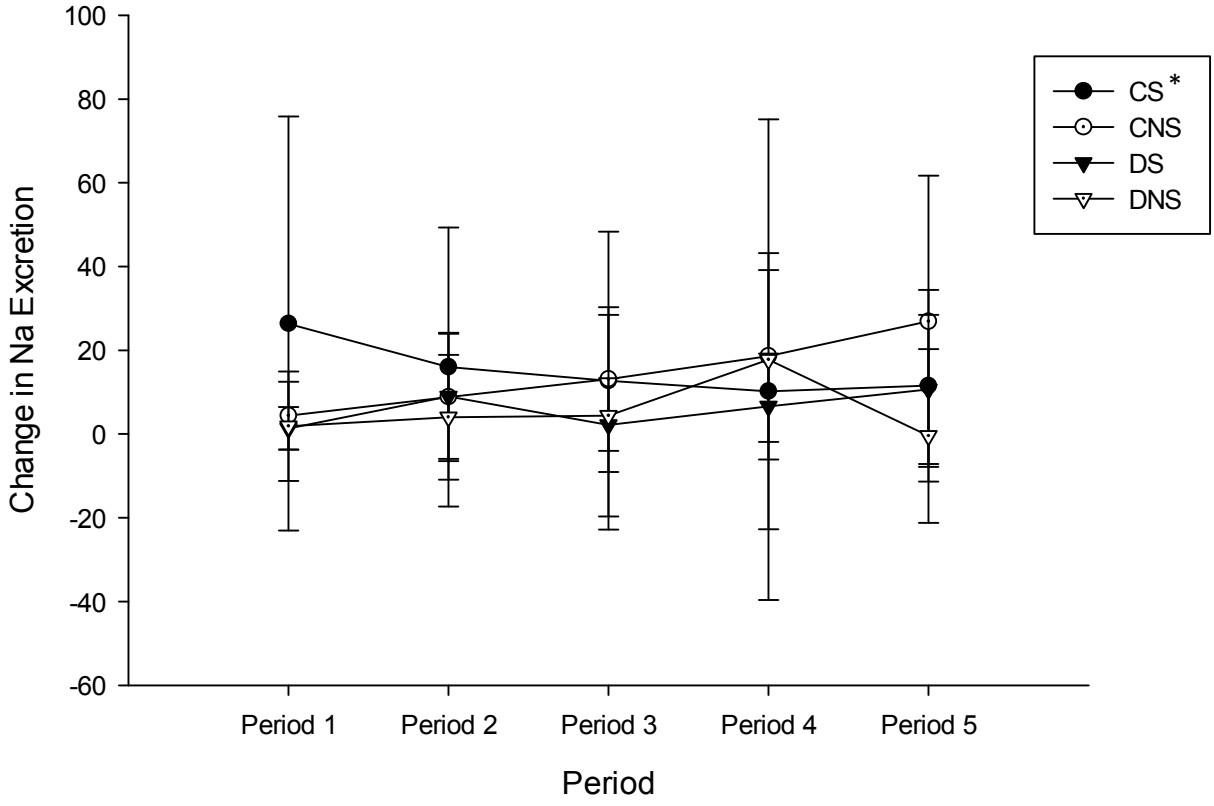

Figure 1B: Mean Change in Sodium from baseline, spinal stimulation (period 1), and post stimulation collection periods (periods 2-5) in rats subjected to no stimulation (CNS), stimulation during period 1 (CS), afferent renal denervation and no stimulation (DNS), and afferent renal denervation and stimulation during period 1 (DS).

* $P < 0.05$ when CS values over time (Treatment × time) are compared with CNS group with P=0.02

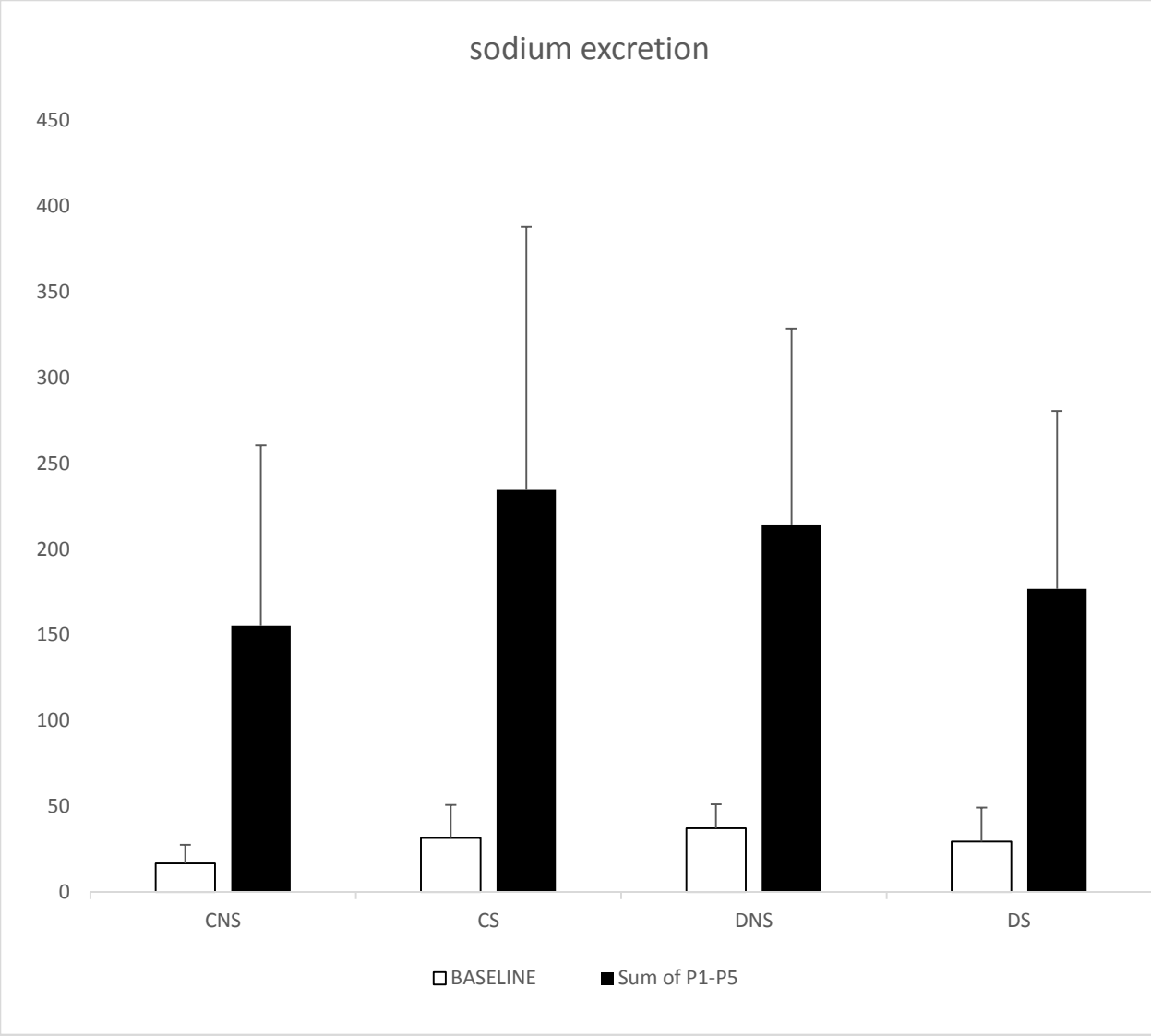

Figure 1C: Mean baseline and mean sum of sodium excretion for periods 1-5 in each of the 4 groups (CNS, CS, DNS, DS).

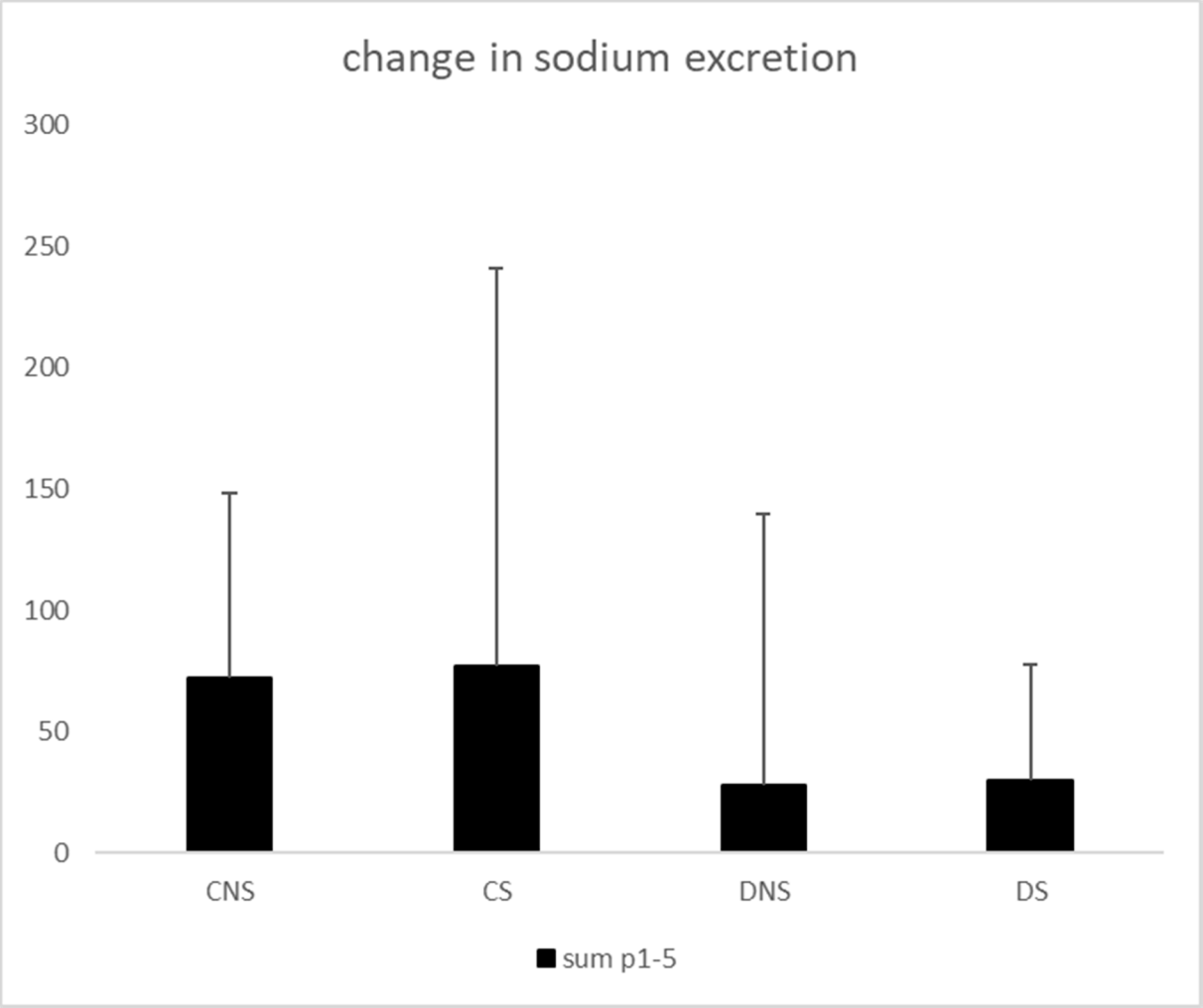

Figure 1D**:** Mean sum of the increase in sodium excretion over baseline for periods 1-5 (change in Na excretion) in each of the 4 groups (CNS, CS, DNS, DS)

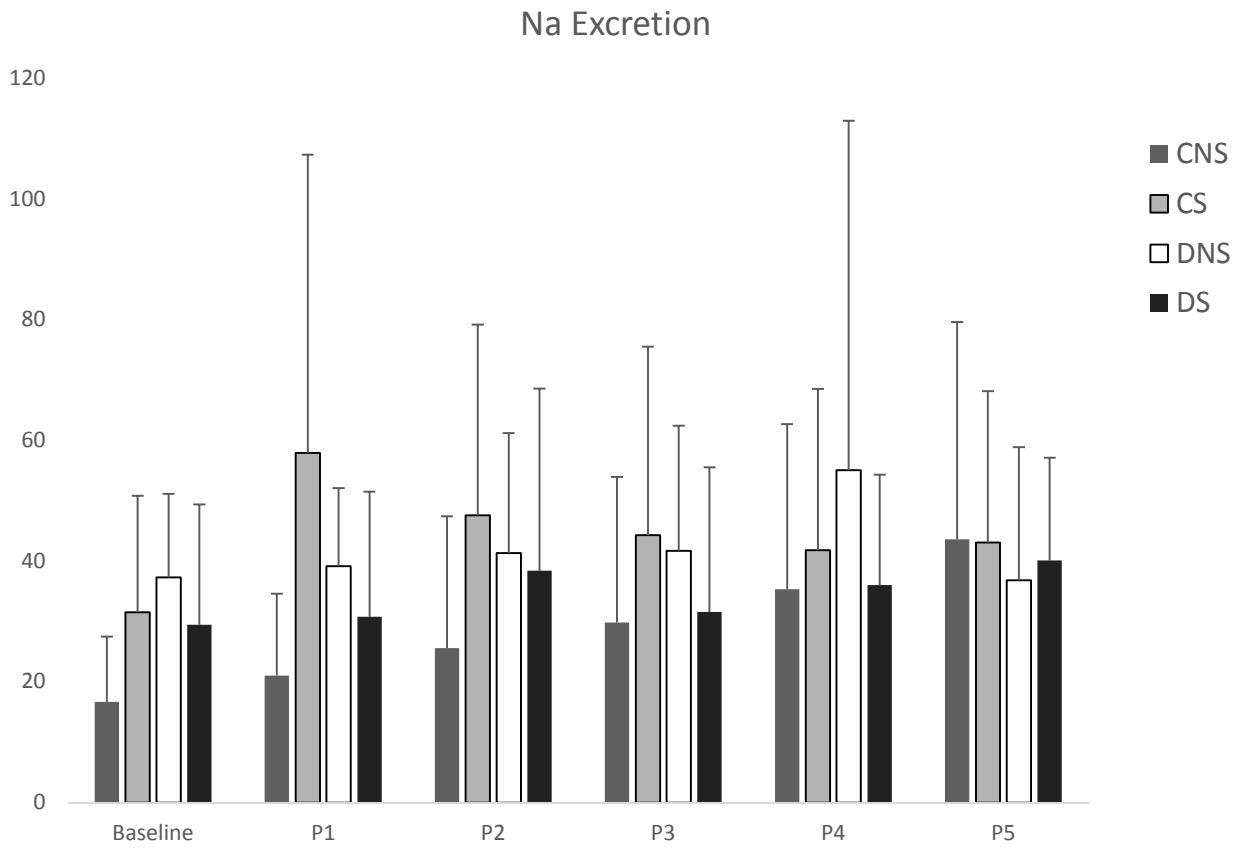

Figure 1E**:** Mean Na Excretion during baseline**,** spinal stimulation (period 1), and post stimulation collection periods (periods 2-5) in rats subjected to no stimulation (CNS), stimulation during period 1 (CS), afferent renal denervation and no stimulation (DNS), and afferent renal denervation and stimulation during period 1 (DS).

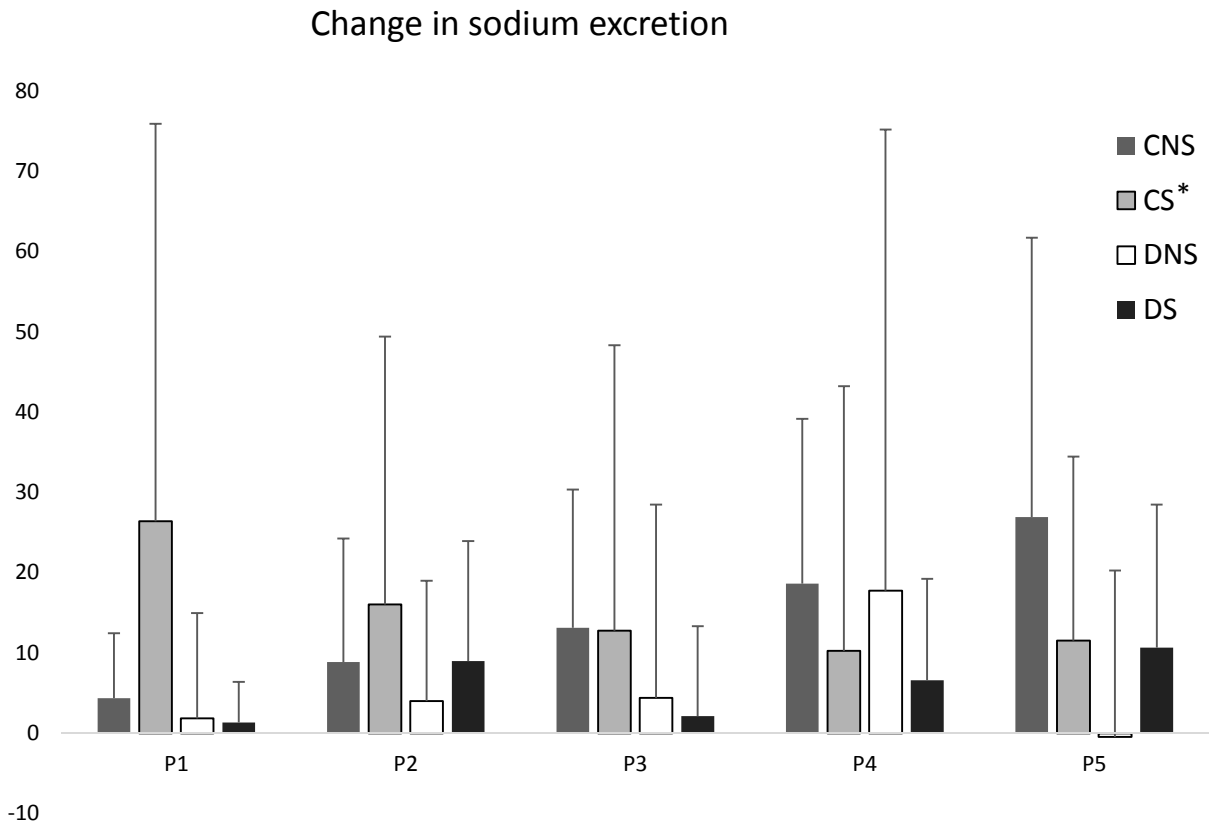

Figure 1F: Mean change in Na excretion from baseline, spinal stimulation (period 1), and post stimulation collection periods (periods 2-5) in rats subjected to no stimulation (CNS), stimulation during period 1 (CS), afferent renal denervation and no stimulation (DNS), and afferent renal denervation and stimulation during period 1 (DS).

* $P < 0.05$ when CS values over time (Treatment × time) are compared with CNS group with P=0.02

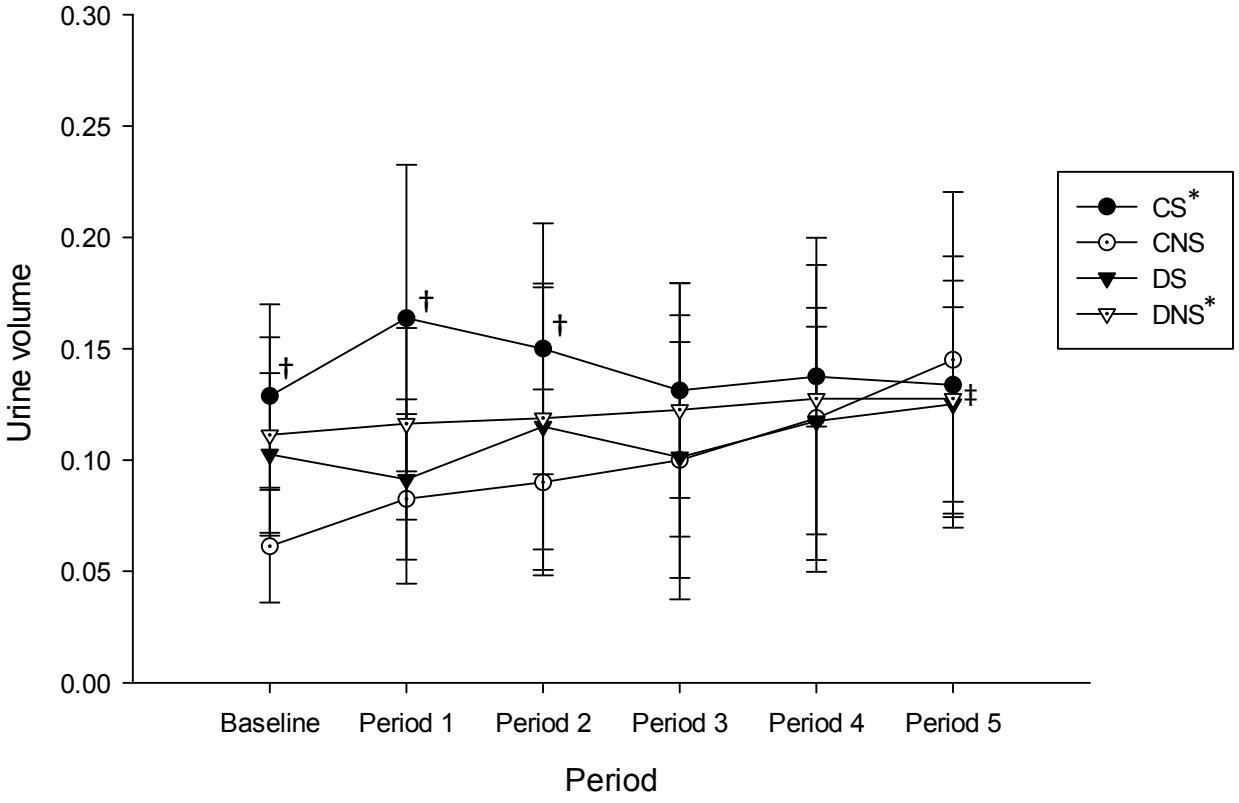

Figure 2A**:** Mean Urine Volume during baseline**,** spinal stimulation (period 1), and post stimulation collection periods (periods 2-5) in rats subjected to no stimulation (CNS), stimulation during period 1 (CS), afferent renal denervation and no stimulation (DNS), and afferent renal denervation and stimulation during period 1 (DS).

From SPSS, CNS is greater than baseline during periods 3, 4, and 5, DS during period 5

* P < 0.05 when CS values over time (Treatment × time) are compared to the CNS group

† P < 0.05 when value is compared to the same period in the CNS group

‡ P < 0.05 when value is compared to the baseline in the DNS group

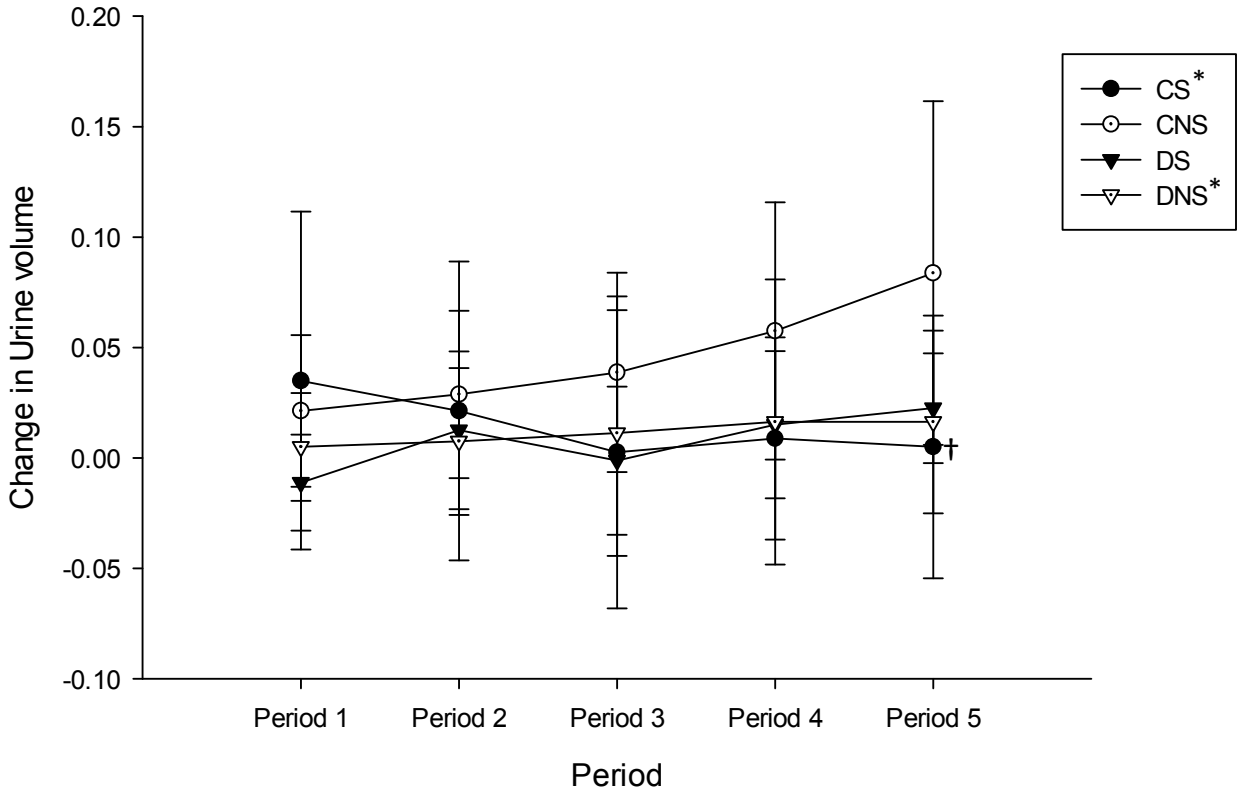

Figure 2B: Mean Change in urine volume during baseline, spinal stimulation (period 1), and post stimulation collection periods (periods 2-5) in rats subjected to no stimulation (CNS), stimulation during period 1 (CS), afferent renal denervation and no stimulation (DNS), and afferent renal denervation and stimulation during period 1 (DS).

* $P < 0.05$ when values over time (Treatment × time) are compared to CNS group

† $P < O.O5$ when CS values are compared to the same period in the CNS group

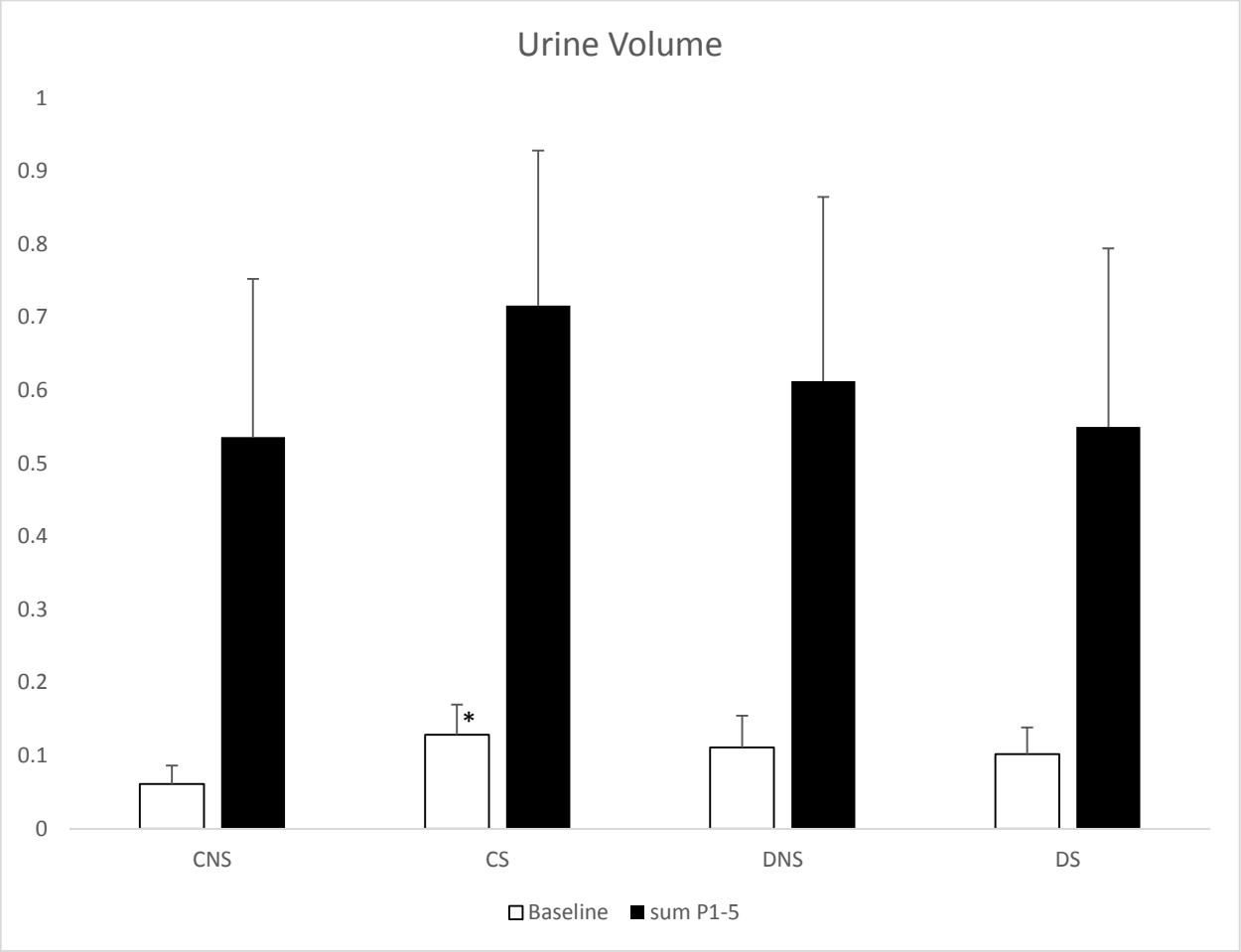

Figure 2C: Mean baseline and mean sum of urine volumes for periods 1-5 in each of the 4 groups (CNS, CS, DNS, DS).

* P<0.05 when values) are compared to CNS group within baseline

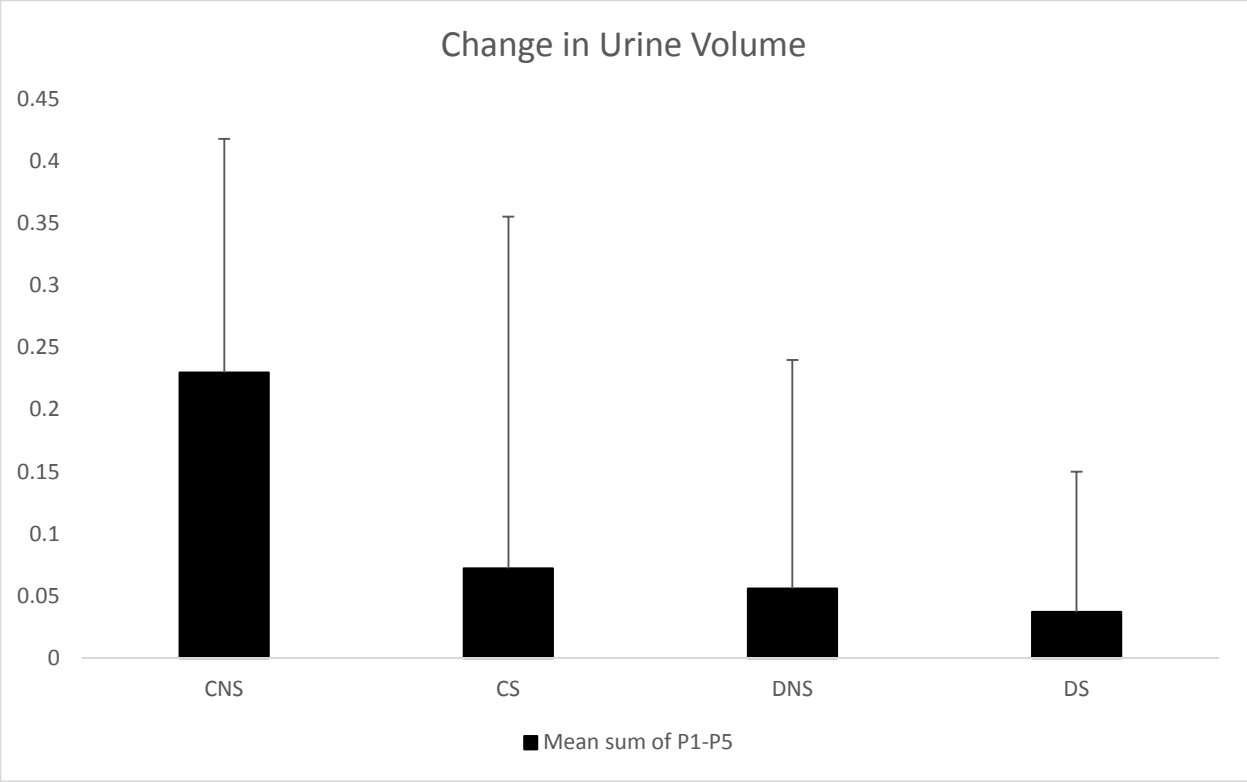

Figure 2D: Mean sum for change in urine volume from baseline for periods 1-5 in each of the 4 groups (CNS, CS, DNS, DS).

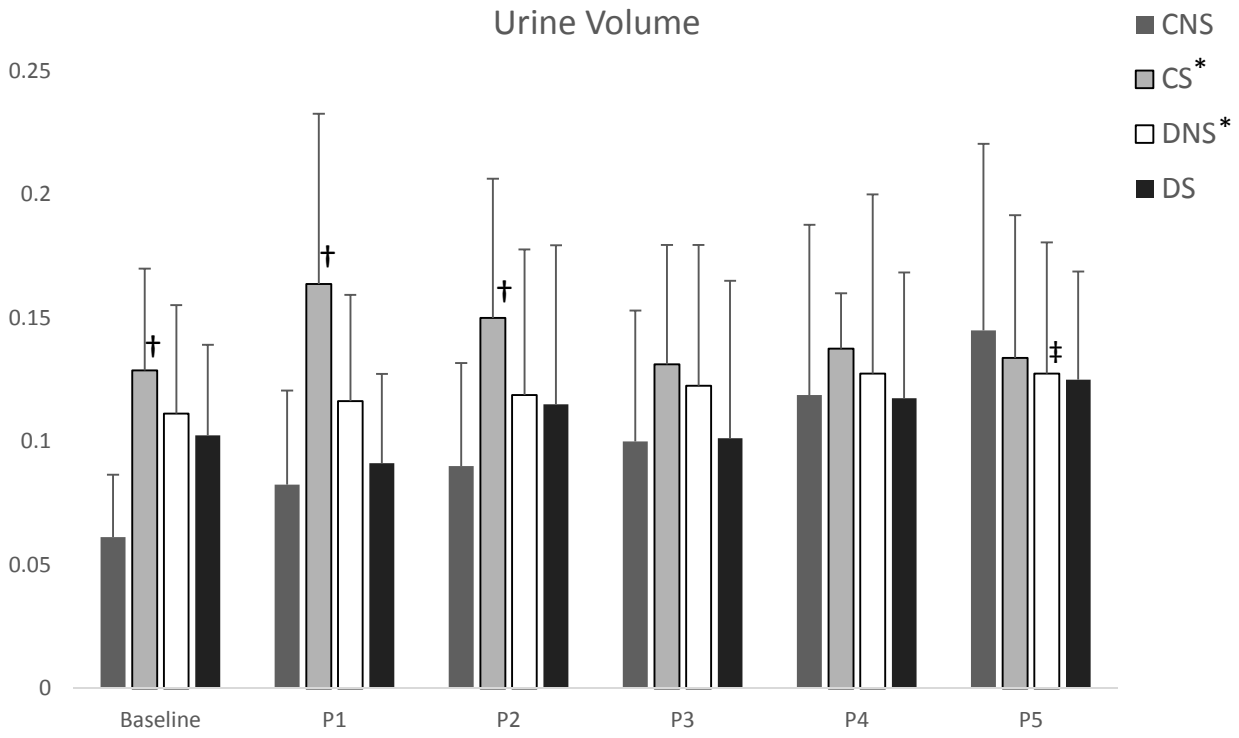

Figure 2E: Mean Urine Volume during baseline, spinal stimulation (period 1), and post stimulation collection periods (periods 2-5) in rats subjected to no stimulation (CNS), stimulation during period 1 (CS), afferent renal denervation and no stimulation (DNS), and afferent renal denervation and stimulation during period 1 (DS).

From SPSS, CNS is greater than baseline during periods 3, 4, and 5, DS during period 5

* $P < 0.05$ when values over time (Treatment × time) are compared to the CNS group

† $P < 0.05$ when value is compared to the same period in the CNS group

‡ $P < 0.05$ when value is compared to the baseline in the DNS group

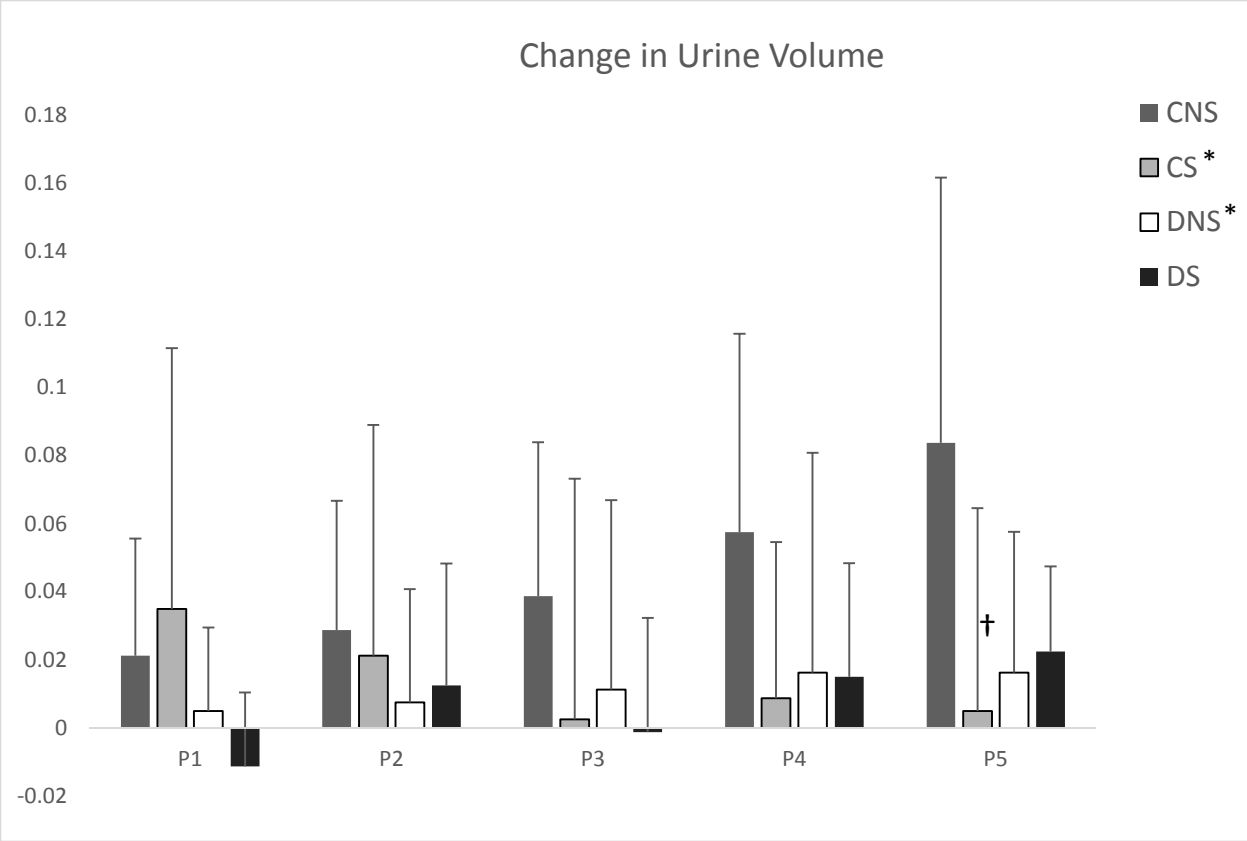

Figure 2F: Mean Change in urine volume during baseline, spinal stimulation (period 1), and post stimulation collection periods (periods 2-5) in rats subjected to no stimulation (CNS), stimulation during period 1 (CS), afferent renal denervation and no stimulation (DNS), and afferent renal denervation and stimulation during period 1 (DS).

* P < 0.05 when values over time (Treatment × time) are compared to CNS group

† P < O.O5 when CS values are compared to the same period in the CNS group

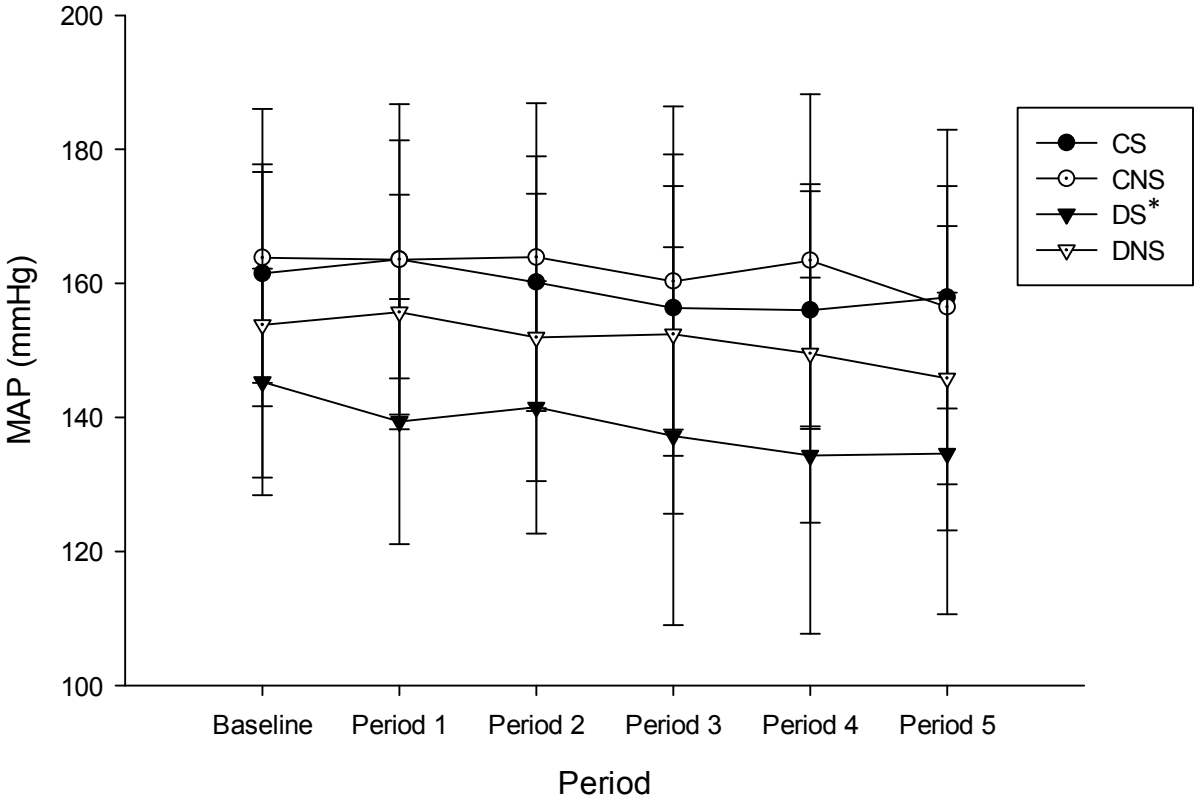

Figure 3A: Mean arterial pressure during baseline, spinal stimulation (period 1), and post stimulation collection periods (periods 2-5) in rats subjected to no stimulation (CNS), stimulation during period 1 (CS), afferent renal denervation and no stimulation (DNS), and afferent renal denervation and stimulation during period 1 (DS).

* $P < 0.05$ when DS (Treatment only) is compared to CS group

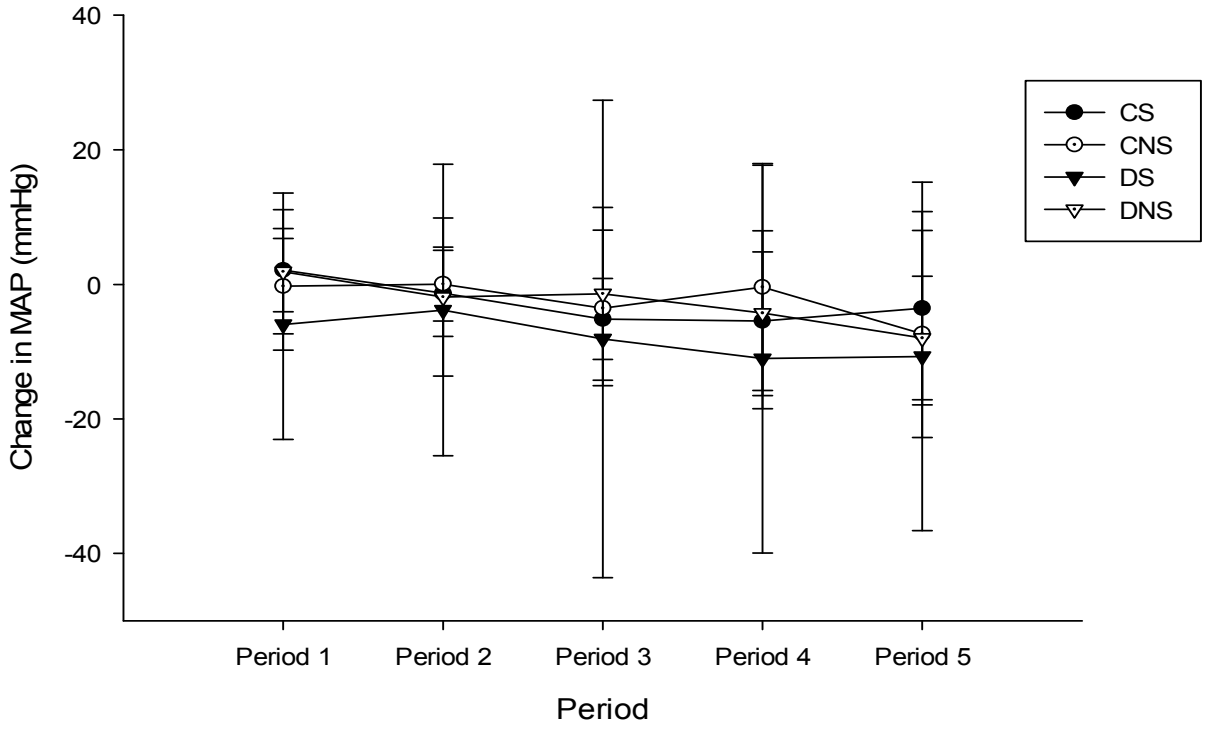

Figure 3B: Mean change in mean arterial pressure during baseline, spinal stimulation (period 1), and post stimulation collection periods (periods 2-5) in rats subjected to no stimulation (CNS), stimulation during period 1 (CS), afferent renal denervation and no stimulation (DNS), and afferent renal denervation and stimulation during period 1 (DS).

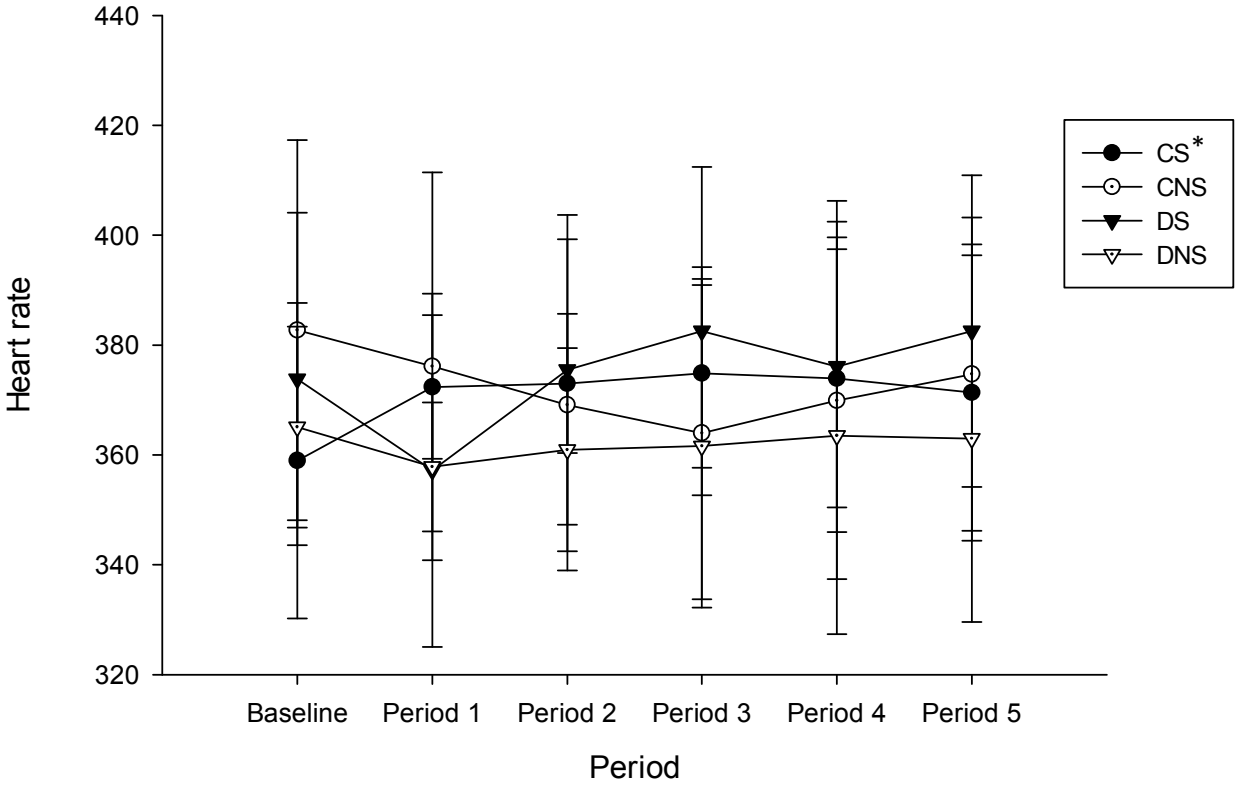

Figure 4A: Mean Heart rate during baseline, spinal stimulation (period 1), and post stimulation collection periods (periods 2-5) in rats subjected to no stimulation (CNS), stimulation during period 1 (CS), afferent renal denervation and no stimulation (DNS), and afferent renal denervation and stimulation during period 1 (DS).

* $P < 0.05$ when CS values over time (Treatment × time) are compared with CNS group.

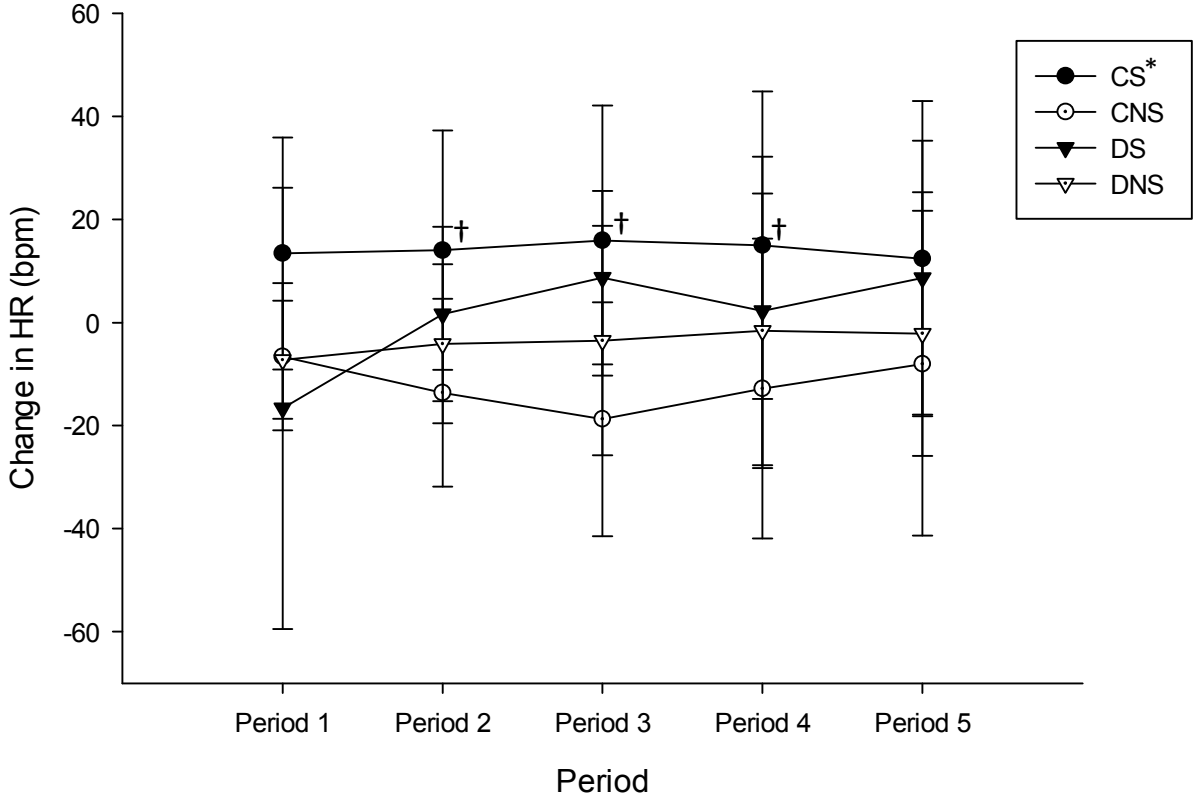

Figure 4B: Mean Change in heart rate during baseline, spinal stimulation (period 1), and post stimulation collection periods (periods 2-5) in rats subjected to no stimulation (CNS), stimulation during period 1 (CS), afferent renal denervation and no stimulation (DNS), and afferent renal denervation and stimulation during period 1 (DS).

* $P < 0.05$ when CS value (Treatment only) is compared to CNS group $P= 0.035$.

† $P < 0.05$ when CS values compared to the same period in the CNS group.

DISCUSSION

In previous studies in this lab, spinal cord stimulation (SCS) has been found to effectively increase urinary sodium excretion (UNa). Mager and Knoblich examined the effect of unilateral (left) dorsal column stimulation on urine sodium excretion in male SHR rats. These studies, demonstrated that stimulation at two-third of the motor threshold (67%) at the level of T_{11}-T_{12}, significantly increased the urinary sodium excretion during periods 4 and 5 (with stimulation occurring during period 2), when compared with the control group. Spinal cord stimulation at 90% of motor threshold did not increase urinary sodium excretion significantly in these studies (Mager and Knoblich, 2003).

In another study, Stearns and Knoblich applied unilateral dorsal column stimulation at T_{12}-T_{13} of male SHR and measured urine sodium excretion, mean arterial pressure, heart rate, and renal blood flow. The authors demonstrated that spinal cord stimulation did not change renal blood flow, mean arterial pressure or heart rate, but increased urine sodium excretion significantly in the treatment group during periods 3 and 4 (stimulation occurred during period 2), compared with the control group. Their study concluded that the natriuretic effect of spinal cord stimulation is independent of alterations in renal blood flow (Stearns and Knoblich, 2007).

Further studies conducted in the Knoblich laboratory in 2016 examined the effect of bilateral renal denervation on urinary sodium excretion following spinal cord stimulation (Knoblich and Mellati). These results showed that when stimulation was applied during period 2, urine sodium excretion increased over baseline values during period 2, 3, 4, and 5. When sodium excretion was expressed as the change from baseline, the increase from baseline sodium

excretion was greater in the stimulated rats than the control rats during periods 2, 3, and 4 (stimulation was applied during period 2). Complete renal denervation resulted in a greater baseline urinary sodium excretion and a lower mean arterial pressure. Complete renal denervation reversed the effect of SCS, resulting in a decline in urinary sodium excretion when compared to baseline. A similar pattern was observed in urine volume, increasing after SCS in the group with intact renal nerves, but decreasing in the denervated group. Although mean arterial pressure was not significantly affected by SCS in the intact rats, SCS in the denervated rats resulted in a sharp decline in MAP, providing a potential explanation for the decline in urinary sodium excretion.

Based on these prior studies, this research was designed to determine if spinal cord stimulation increases urinary sodium excretion in SHR by a mechanism involving the renal sensory nerves. For this purpose, four groups of rats were used. In the CNS group, no intervention was done, and this group was considered the control for CS group, which went through bilateral spinal cord stimulation. In the same way the DNS group (dorsal rhizotomy with no spinal cord stimulation) was designed as the control for the DS group (dorsal rhizotomy with spinal cord stimulation). Other group comparisons were done to determine the effect of denervation alone.

No significant difference between the groups were found in age, weight, surgery duration or saline infusion. Therefore, differences between groups cannot be attributed to differences in these variables.

Dorsal Column Spinal Stimulation in SHR with Intact Renal Nerves

In this study we observed variability in baseline urine sodium excretion among the rats in both groups. Similar variations in urine sodium excretion have been observed in other studies (Stearns and Knoblich, 2007, Marger and Knoblich, 2003, Mellati and Knoblich, 2016). This variation could be due to hypertension, or small variations in rat health and hydration, or in time and blood loss during the surgical setup. To account for the effect of high variability in sodium excretion during the baseline period, the change in urinary sodium excretion from baseline was calculated.

When data was normalized as change from baseline, bilateral dorsal column stimulation of the spinal cord at 67% of the motor threshold showed a significant difference in the pattern of urine sodium excretion (treatment × time) in the stimulated, intact rats (CS) when compared with the non-stimulated intact rats (CNS). In CS group, urine sodium excretion was increased during period 1 and decreased during periods 2, 3, 4, and 5. CNS group showed a gradual increase in urine sodium excretion as time progressed during the acute study. As reported in other studies in this lab, no significant difference was found in the raw sodium excretion during like time periods when the two groups where compared.

In neuropathic pain, spinal cord stimulation blocks the phenomenon of “wind-up of wider dynamic range neuronal response” and this may explain the effect of pain relief. During chronic noxious stimuli, spinal excitability increases. Consequently, due to central sensitization, pain gets much worse through the activity of local excitatory interneurons, resulting in even non-painful stimuli feeling painful (hyperalgesia). Spinal cord stimulation activates large diameter axons called Aβ fibers in the dorsal horn, which subsequently activate inhibitory interneurons to block

the local excitatory interneurons and transmission cells (WDR neurons). This blocks the wind-up process and prolongs analgesic effects (Guan, 2012). The same mechanism may activate the inhibitory interneurons to suppress afferent WDR renal sensory fibers and decrease the overall renal sympathetic nerve activity, interrupting the positive feedback loop between renal sensory input and efferent sympathetic outflow that has been described in hypertensive people and animal models. Since the sympathetic nerve effect is to decrease sodium excretion, reducing sympathetic outflow to the kidney would increase renal sodium excretion.

Urine volume increased during period 1 in the intact stimulated group (CS), then declined, producing a different pattern of excretion (treatment × time) than the intact non stimulated group (CNS) over the collection periods. Urine volumes in the CS group were significantly higher than in the CNS group during periods 1 and 2, but were also higher during baseline. When data was normalized as change from baseline, the CS group again had a significantly different pattern (treatment × time) of urine volume over time than the CNS group. It is likely that the increase in urine volume occurred as a result of the increase in urinary sodium excretion. This supports the hypothesis that spinal cord stimulation increases sodium excretion.

Stimulation increased heart rate in the CS rats, which then remained elevated, resulting in a significantly different (treatment × time) effect than the CNS rats. Furthermore, when data was normalized as change from baseline, the heart rate increases following stimulation were significantly greater than the non-stimulated rats during periods 2, 3, and 4. Mellati's study showed that heart rate declined in CS rats, becoming significantly lower than baseline during period 2. The CNS group in Mellati's study showed no significant changes in heart rate from baseline, and remained stable though out the study.

MAP gradually declined over the course of the acute study in the intact groups, and no differences were noted in the stimulated rats. This is similar to Mellati's results in which MAP declined through the course of the acute study.

Dorsal Column Stimulation in Renal Denervated SHR

Afferent renal nerve ablation (renal afferent denervation) did not alter baseline sodium excretion, urine volume, or heart rate. Denervation eliminated the effect of SCS on urinary sodium excretion and urine volume. This suggests that spinal cord stimulation is unable to increase urine sodium excretion in the absence of an afferent renal nerve. Bayliss (1901) demonstrated that dorsal root stimulation at high intensity induced peripheral vasodilation mediated by thin fibers. Hilton and Marshall (1980) studies confirmed Bayliss observation by demonstrating the antidromic effect of dorsal root stimulation on blood flow through the gastrocnemius muscle. The authors showed that dorsal root stimulation with a current pulse of 0.3-0.5msec duration for a period pf 15-20 seconds, produced a 50-60 %increase in muscle vascular conductance (Hilton and Marshall, 1980).

Foreman et al., (2003) characterized the fiber types responsible for spinal cord stimulation-induced vasodilation. The authors demonstrated that spinal cord stimulation at ≤60% of motor-threshold induced vasodilation via antidromic activation of myelinated fibers only. Spinal cord stimulation of ≥90% of motor-threshold induced vasodilation via antidromic activation of unmyelinated C-fibers (Foreman etal., 2003).

Wu et al. (2006), demonstrated that spinal cord stimulation activates TRPV-1 containing sensory fibers in the unmyelinated C-fibers or myelinated Aδ sensory fibers. Additionally the

authors showed that activation of TRPV-1 containing sensory fibers by SCS would result in the release of CGRP from sensory nerve terminals which would initiate the release of nitric oxide. Nitric oxide from the vascular endothelium binds to the vascular smooth muscle to induce vasodilation (Wu et al., 2006).

Simon et al. 1989, demonstrated that electric stimulation of the afferent renal nerve elicited a rise in vasopressin release 1 hour after stimulation which gradually returned to the control level by the third hour after stimulation. Thus, renal denervation may alter vasopressin release by interruption of the afferent renal nerves.

Denervation resulted in a lower mean arterial pressure than the intact groups, but this was only significant when combined with stimulation (DS vs CS). Mean arterial pressure gradually decreased in all groups. Stimulation had no clear effect on MAP in the denervated group when compared to the other groups. Mellati's study demonstrated that spinal cord stimulation in renal denervated SHR significantly decreased mean arterial pressure from an already reduced baseline, which persisted for the remainder of the acute study. Since this effect was not observed in our study, it appears that complete renal denervation is needed to produce a decrease in MAP with SCS.

The effect observed in this study that dorsal rhizotomy decreases baseline mean arterial pressure has been observed in other studies. Mellati's study demonstrated that complete renal denervation lowered MAP, which persisted during the acute study. Frederick et al. demonstrated a marked sudden decrease in mean arterial pressure after renal denervation (Frederic et al. 2003). Investigators have found that renal denervated animals have a lower plasma renin activity than intact animals (Collister and Osborn, 1998). This result indicates that the acute or sudden

decrease in arterial pressure observed after renal denervation may be the result of a decrease in renin release, which is not unexpected following elimination of the renal efferent sympathetic nerves, a stimulator of renin release. Another possibility is that renal vasomotor tone might be reduced in denervated rats, again secondary to loss of renal efferent sympathetic stimulation. The present study eliminated only the afferent renal nerves, and left the efferent nerves intact. Thus the smaller effect on MAP in this study may have been through mechanisms not related to the sympathetic efferent nerves. Janssen et al. 1989 subjected rats to renal denervation, selective dorsal rhizotomy or sham studies. Results showed that mean arterial pressures were significantly lowered in complete renal denervation compared with selective dorsal rhizotomy or sham. Previous studies have shown that selective deafferention by dorsal rhizotomy in SHR did not attenuate the subsequent increase in mean arterial pressure, but complete renal denervation did (Spripairojhikoon et al. 1989). The present study showed a somewhat smaller decrease in MAP in the denervated group than the intact group but this was only significant when combined with stimulation (DS vs CS).

Afferent renal denervation produced a different pattern of urine volume excretion between the non-stimulated groups (DNS vs CNS), both when raw data was analyzed and when data was expressed as change from baseline. Other studies have shown that there was no statistical difference in urine volume excretion when non-stimulated groups where compared (DNS vs CNS) (Mellati and Knoblich, 2016, Mager and Knoblich, 2003, Stearns and Knoblich, 2007). The mechanism by which this unique finding occurred remains unknown. The difference appears to be a more gradual increase in urine volume excretion in the DNS rats when compared to the stepper increase in the CNS rats. The higher baseline level in the denervated rats may be

responsible for the greater stability in urine volumes and could be an effect related to the elimination of the afferent renal nerve input, which can stimulate vasopressin as mentioned previously. Lower vasopressin at baseline may have resulted less water reabsorption in the kidney collecting ducts, resulting in a higher baseline urine volume.

Afferent renal denervation eliminated the effect of SCS on heart rate. No difference in heart rate in the denervated groups were observed between the SCS rats and the non-stimulated rats.

SUMMARY

In this experiment we have confirmed the effect of dorsal rhizotomy on urinary sodium excretion following dorsal spinal stimulation in SHR. We can conclude that dorsal column stimulation at the level of T11 – T12 at the voltage of 67% of motor threshold increased urinary sodium and water excretion from the kidney. This study demonstrated that denervation of the renal afferent nerve eliminated the response. In renal afferent denervated rats spinal cord stimulation was unable to increase renal sodium excretion and urine volume. This confirms spinal cord stimulation works through the renal afferent nerve via a retrograde transmission of electrical signal back into the kidneys. Furthermore we found that dorsal column stimulation significantly decreased MAP in the renal afferent denervated group when compared with the intact group. This is an exciting finding and could be the subject of further experiments to investigate the mechanism behind it.

Spinal cord stimulation could be a great alternative for the treatment of resistant hypertension, and less invasive than denervation. Further studies are needed to investigate the long-term effect of chronic or intermittent spinal cord stimulation on renal and cardiovascular physiology.

Evaluation of Hypotheses

As mentioned previously the purpose of this experiment is to understand the mechanism behind which dorsal spinal stimulation increases renal sodium and water excretion via renal

afferent nerve. For this purpose a couple of hypothesis were considered and all were accepted as below;

Hypothesis 1: the renal afferent nerve is responsible for transmitting a retrograde signal to the kidney, during dorsal spinal stimulation that affect renal sodium excretion,

According to the results of ΔU_{Na} in CS group compared to the CNS group, this hypothesis was accepted.

Hypothesis 2

Severance of the dorsal root of renal afferent nerve, just distal to the dorsal root ganglion, will eliminate the effect of dorsal spinal stimulation on renal sodium excretion.

The data analysis of U_{Na}, ΔU_{Na}, Uv and ΔUv showed that severance of the dorsal root eliminated the effect of SCS.

REFERENCES:

1. Aperia, A., Ibarra, F., Svensson, L. B., Klee, C., & Greengard, P. (1992). Calcineurin mediates alpha-adrenergic stimulation of Na+, K (+)-ATPase activity in renal tubule cells. *Proceedings of the National Academy of Sciences*, *89*(16), 7394-7397.
2. Barajas, L. (1964). THE INNERVATION OF THE JUXTAGLOMERULAR APPARATUS. AN ELECTRON MICROSCOPIC STUDY OF THE INNERVATION OF THE GLOMERULAR ARTERIOLES. *Laboratory investigation; a journal of technical methods and pathology*, *13*, 916-929.
3. Barajas, L., & Wang, P. (1979). Localization of tritiated norepinephrine in the renal arteriolar nerves. *The Anatomical Record*, *195*(3), 525-534.
4. Bayliss, W. M. (1901). On the origin from the spinal cord of the vaso-dilator fibres of the hind-limb, and on the nature of these fibres 1. *The Journal of physiology*, *26*(3-4), 173-209.
5. Bertog, S. C., Sobotka, P. A., & Sievert, H. (2012). Renal denervation for hypertension. *JACC: Cardiovascular Interventions*, *5*(3), 249-258.
6. Braunwald, N. S., Epstein, S. E., & Braunwald, E. (1970). Carotid sinus nerve stimulation for the treatment of intractable angina pectoris: surgical technic. *Annals of surgery*, *172*(5), 870.
7. Broseta, J., Barberá, J., De Vera, J. A., Barcia-Salorio, J. L., Garcia-March, G., González-Darder, J., ... & Joanes, V. (1986). Spinal cord stimulation in peripheral arterial disease: a cooperative study. *Journal of neurosurgery*, *64*(1), 71-80.

8. Brown, C. D., Higgins, M., Donato, K. A., Rohde, F. C., Garrison, R., Obarzanek, E., ... & Horan, M. (2000). Body mass index and the prevalence of hypertension and dyslipidemia. *Obesity research*, *8*(9), 605-619.

9. Burchiel, K. J., Anderson, V. C., Brown, F. D., Fessler, R. G., Friedman, W. A., Pelofsky, S., ... & Shatin, D. (1996). Prospective, multicenter study of spinal cord stimulation for relief of chronic back and extremity pain. *Spine*, *21*(23), 2786-2794.

10. Campese, V. M., & Kogosov, E. (1995). Renal afferent denervation prevents hypertension in rats with chronic renal failure. *Hypertension*, *25*(4), 878-882.

11. Chen, S. R., & Pan, H. L. (2003). Spinal GABAB receptors mediate antinociceptive actions of cholinergic agents in normal and diabetic rats. *Brain research*, *965*(1-2), 67-74.

12. Chobanian, A. V. (2009). The hypertension paradox—more uncontrolled disease despite improved therapy. *New England Journal of Medicine*, *361*(9), 878-887.

13. Ciriello, J., & Calaresu, F. R. (1983). Central projections of afferent renal fibers in the rat: an anterograde transport study of horseradish peroxidase. *Journal of the autonomic nervous system*, *8*(3), 273-285.

14. Ciriello, J., & de Oliveira, C. V. (2002). Renal afferents and hypertension. *Current hypertension reports*, *4*(2), 136-142.

15. Collister, J. P., & Osborn, J. W. (1998). Area postrema lesion attenuates the long-term hypotensive effects of losartan in salt-replete rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *274*(2), R357-R366.

16. Conrad CH, Brooks WW, Hayes JA, Sen S, Robinson KG, Bing OH. (1995). Myocardial fibrosis and stiffness with hypertrophy and heart failure in the spontaneously hypertensive rat. Circulation; 91(1):161-70.

17. Cui, J. G., T O'Connor, W., Ungerstedt, U., Linderoth, B., & Meyerson, B. A. (1997). Spinal cord stimulation attenuates augmented dorsal horn release of excitatory amino acids in mononeuropathy via a GABAergic mechanism. *Pain*, *73*(1), 87-95.

18. Dagmara Hering, Pawel J Winklewski. (2017). R1 Autonomic nervous system in acute kidney injury. Clinical and experimental pharmacology and physiology.vol 44,issue3,428.

19. Daniele, C. A., & MacDermott, A. B. (2009). Low-threshold primary afferent drive onto GABAergic interneurons in the superficial dorsal horn of the mouse. *Journal of Neuroscience*, *29*(3), 686-695.

20. DiBona GF, Kopp UC. (1997). Neural control of renal function. Physio Rev. 77:75-197.

21. Dibona GF. Sympathetic nervous system influences on the kidney (1989). Role in hypertension. Am J Hypertens. 2:1195-124.

22. Dibona, G. F. (2003). Neural control of the kidney: past, present, and future. *Hypertension*, *41*(3), 621-624.

23. Esler, M., Eikelis, N., Schlaich, M., Lambert, G., Alvarenga, M., Dawood, T., ... & Brenchley, C. (2008). Chronic mental stress is a cause of essential hypertension: presence of biological markers of stress. *Clinical and Experimental Pharmacology and Physiology*, *35*(4), 498-502.

24. Esler, M., Lambert, G., Vaz, M., Thompson, J., Kaye, D., Kalff, V., ... & Jennings, G. (1997). Central nervous system monoamine neurotransmitter turnover in primary and obesity-related human hypertension. *Clinical and experimental hypertension*, *19*(5-6), 577-590.

25. Esler, M., Lux, A., Jennings, G., Hastings, J., Socratous, F., & Lambert, G. (2004). Rilmenidine sympatholytic activity preserves mental stress, orthostatic sympathetic responses and adrenaline secretion. *Journal of hypertension*, *22*(8), 1529-1534.

26. Fisher, J. P., & Fadel, P. J. (2010). Therapeutic strategies for targeting excessive central sympathetic activation in human hypertension. *Experimental physiology*, *95*(5), 572-580.

27. Fisher, J. P., & Paton, J. F. R. (2012). The sympathetic nervous system and blood pressure in humans: implications for hypertension. *Journal of human hypertension*, *26*(8), 463.

28. Fisher, J. P., & Paton, J. F. R. (2012). The sympathetic nervous system and blood pressure in humans: implications for hypertension. *Journal of human hypertension*, *26*(8), 463.

29. Fletcher, E. C. (2001). Invited review: Physiological consequences of intermittent hypoxia: systemic blood pressure. *Journal of Applied Physiology*, *90*(4), 1600-1605.

30. Foss J. Fink G. Osborn J. (2016). Differential role of afferent and efferent renal nerves in the maintainance of early and late phase dahl S hypertension. American Journal of Physiology- Regulatory Integrative and comparative physiology. DOI:10.1152/ajpregu.00408.2015.

31. Foss, J. D., Wainford, R. D., Engeland, W. C., Fink, G. D., & Osborn, J. W. (2014). A novel method of selective ablation of afferent renal nerves by periaxonal application of capsaicin. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *308*(2), R112-R122.

32. Fu, Q., Zhang, R., Witkowski, S., Arbab-Zadeh, A., Prasad, A., Okazaki, K., & Levine, B. D. (2005). Persistent sympathetic activation during chronic antihypertensive therapy: a potential mechanism for long term morbidity?. *Hypertension*, *45*(4), 513-521.

33. Grassi, G. (2004). Sympathetic and baroreflex function in hypertension: implications for current and new drugs. *Current pharmaceutical design*, *10*(29), 3579-3589.

34. Grassi, G. (2009). Assessment of sympathetic cardiovascular drive in human hypertension: achievements and perspectives. *Hypertension*, *54*(4), 690-697.

35. Grassi, G., Seravalle, G., Dell'Oro, R., Turri, C., Bolla, G. B., & Mancia, G. (2000). Adrenergic and reflex abnormalities in obesity-related hypertension. *Hypertension*, *36*(4), 538-542.

36. Grassi, G., Turri, C., Dell'Oro, R., Stella, M. L., Bolla, G. B., & Mancia, G. (1998). Effect of chronic angiotensin converting enzyme inhibition on sympathetic nerve traffic and baroreflex control of the circulation in essential hypertension. *Journal of hypertension*, *16*(12), 1789-1796.

37. Guan, Y., Wacnik, P. W., Yang, F., Carteret, A. F., Chung, C. Y., Meyer, R. A., & Raja, S. N. (2010). Spinal cord stimulation-induced analgesiaelectrical stimulation of dorsal column and dorsal roots attenuates dorsal horn neuronal excitability in neuropathic rats. *Anesthesiology: The Journal of the American Society of Anesthesiologists*, *113*(6), 1392-1405.

38. Hausberg, M., Kosch, M., Harmelink, P., Barenbrock, M., Hohage, H., Kisters, K., ... & Rahn, K. H. (2002). Sympathetic nerve activity in end-stage renal disease. *Circulation*, *106*(15), 1974-1979.

39. Hill, M. A., Zou, H., Potocnik, S. J., Meininger, G. A., & Davis, M. J. (2001). Invited review: arteriolar smooth muscle mechanotransduction: Ca2+ signaling pathways underlying myogenic reactivity. *Journal of applied physiology*, *91*(2), 973-983.

40. Hilton, S. M., & Marshall, J. M. (1980). Dorsal root vasodilatation in cat skeletal muscle. *The Journal of physiology*, *299*(1), 277-288.

41. Jacob, F., Ariza, P., & Osborn, J. W. (2003). Renal denervation chronically lowers arterial pressure independent of dietary sodium intake in normal rats. *American Journal of Physiology-Heart and Circulatory Physiology*, *284*(6), H2302-H2310.

42. Janssen, S. P., Truin, M., Van Kleef, M., & Joosten, E. A. (2011). Differential GABAergic disinhibition during the development of painful peripheral neuropathy. *Neuroscience*, *184*, 183-194.

43. Jessurun, G. A. J., DeJongste, M. J. L., & Blanksma, P. K. (1996). Current views on neurostimulation in the treatment of cardiac ischemic syndromes. *PAIN®*, *66*(2-3), 109-116.

44. Julian P. Yaxley and Sam V. Thambar (2015). Resistant hypertension: an approach to management in primary care. Journal of Family Medicine and Primary Care, 4(2): 193-199.

45. Katholi, R. E., Carey, R. M., Ayers, C. R., Vaughan, J. E., Yancey, M. R., & Morton, C. L. (1977). Production of sustained hypertension by chronic intrarenal norepinephrine infusion in conscious dogs. *Circulation research*, *40*(5 Suppl 1), I118-26.

46. Katholi, R. E., Hageman, G. R., Whitlow, P. L., & Woods, W. T. (1983). Hemodynamic and afferent renal nerve responses to intrarenal adenosine in the dog. *Hypertension*, *5*(2_pt_2), I149.

47. Katholi, R. E., Rocha-Singh, K. J., Goswami, N. J., & Sobotka, P. A. (2010). Renal nerves in the maintenance of hypertension: a potential therapeutic target. *Current hypertension reports*, *12*(3), 196-204.

48. Katholi, R. E., Whitlow, P. L., Hageman, G. R., & Woods, W. T. (1984). Intrarenal adenosine produces hypertension by activating the sympathetic nervous system via the renal nerves in the dog. *Journal of hypertension*, *2*(4), 349-359.

49. Kawabe K, Watanabe TX, Shiono K, Sokabe H. (1978). Influence on blood pressure of renal isografts between spontaneously hypertensive and normotensive rats, utilizing the F1 hybrids. Japanese Heart Journal; 19(6): 886-94..

50. Koepke, J. P., & DiBona, G. F. (1985). Functions of the renal nerves. *Physiologist*, *28*(1), 47-52.

51. Koldas, L., Ayan, F., & Ikitimur, B. (2003). Short term effects of rilmenidine on left ventricular hypertrophy and systolic and diastolic function in patients with essential hypertension. *Japanese heart journal*, *44*(5), 693-704.

52. Kopp UC, Cicha MZ, Smith LA, Mulder J, Hokfelt T.(2007). Renal sympathetic nerve activity modulates afferent renal nerve activity by PGE_2-dependent activation of α_1- and α_2-adrenoceptors on renal sensory nerve fibers. Am J Physiol Regul Integr Comp Physiol 293: R1561–R1572.

53. Kopp UC, Cicha MZ, Smith LA. (2002). Endogenous angiotensin modulates PGE_2-mediated release of substance P from renal mechanosensory nerve fibers. Am J Physiol Regul Integr Comp Physiol 282: R19–R30.

54. Kopp, U. C., Cicha, M. Z., & Smith, L. A. (2003). Dietary sodium loading increases arterial pressure in afferent renal–denervated rats. *Hypertension*, *42*(5), 968-973.

55. Kopp, U. C., Jones, S. Y., & DiBona, G. F. (2008). Afferent renal denervation impairs baroreflex control of efferent renal sympathetic nerve activity. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *295*(6), R1882-R1890.

56. Kopp, U. C., Jones, S. Y., and DiBona, G. F. (2008). Afferent renal denervation impairs baroreflex control of efferent renal sympathetic nerve activity. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *295*(6), R1882–R1890.

57. Krum, H., Lambert, E., Windebank, E., Campbell, D. J., & Esler, M. (2006). Effect of angiotensin II receptor blockade on autonomic nervous system function in patients with essential hypertension. *American Journal of Physiology-Heart and Circulatory Physiology*, *290*(4), H1706-H1712.

58. Krum, H., Schlaich, M., Whitbourn, R., Sobotka, P. A., Sadowski, J., Bartus, K., ... & Abraham, W. T. (2009). Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. *The Lancet*, *373*(9671), 1275-1281.

59. Laterza, M. C., de Matos, L. D., Trombetta, I. C., Braga, A. M., Roveda, F., Alves, M. J., ... & Rondon, M. U. (2007). Exercise training restores baroreflex sensitivity in never-treated hypertensive patients. *Hypertension*, *49*(6), 1298-1306.

60. Lepori, M., Sartori, C., Trueb, L., Owlya, R., Nicod, P., & Scherrer, U. (1998). Haemodynamic and sympathetic effects of inhibition of nitric oxide synthase by systemic infusion of NG-monomethyl-L-arginine into humans are dose dependent. *Journal of hypertension*, *16*(4), 519-523.

61. Levy, M. N., & Pappano, A. J. (2007). Regulation of the heart beat. *Cardiovascular Physiology. 9th edn. Ed. W. Schmitt. Mosby Elsevier. p*, 104-105.

62. Lind, G., Schechtmann, G., Winter, J., Meyerson, B. A., & Linderoth, B. (2008). Baclofen-enhanced spinal cord stimulation and intrathecal baclofen alone for neuropathic pain: Long-term outcome of a pilot study. *European Journal of Pain*, *12*(1), 132-136.

63. Lindblom, U., & Meyerson, B. A. (1975). Influence on touch, vibration and cutaneous pain of dorsal column stimulation in man. *Pain*, *1*(3), 257-270.

64. Linderoth, B., & Foreman, R. D. (1999). Physiology of spinal cord stimulation: review and update. *Neuromudulation: Technology at the Neural Interface*, *2*(3), 150-164.

65. Linderoth, B., & Foreman, R. D. (1999). Physiology of spinal cord stimulation: review and update. *Neuromudulation: Technology at the Neural Interface*, *2*(3), 150-164.

66. Linderoth, B., & Meyerson, B. A. (1995, August). Dorsal column stimulation: modulation of somatosensory and autonomic function. In *Seminars in Neuroscience* (Vol. 7, No. 4, pp. 263-277). Academic Press.

67. Linderoth, B., & Meyerson, B. A. (2010). Spinal Cord StimulationExploration of the Physiological Basis of a Widely Used Therapy. *Anesthesiology: The Journal of the American Society of Anesthesiologists*, *113*(6), 1265-1267.

68. Linderoth, B., Fedoresak, I., & Meyerson, B. A. (1991). Peripheral vasodilatation after spinal cord stimulation: animal studies of putative effector mechanisms. *Neurosurgery*, *28*(2), 187-195.

69. Linderoth, B., Gazelius, B., Franck, J., & Brodin, E. (1992). Dorsal column stimulation induces release of serotonin and substance P in the cat dorsal horn. *Neurosurgery*, *31*(2), 289-297.

70. Liu, F. Y., Qu, X. X., Ding, X., Cai, J., Jiang, H., Wan, Y., ... & Xing, G. G. (2010). Decrease in the descending inhibitory 5-HT system in rats with spinal nerve ligation. *Brain research*, *1330*, 45-60.

71. Ljungqvist, A., & Wågermark, J. (1970). The adrenergic innervation of intrarenal glomerular and extra-glomerular circulatory routes. *Nephron*, *7*(3), 218-229.

72. M. Ezzati, A.D. Lopez, A. Rodgers, S. Vander Hoorn, C.J. Murray, Comparative Riask (2002). Assessment Collaborating Group. Selected major risk factors and global and regional burden of disease. Lancet, 360, PP. 1347-1360.

73. Mager C and Knoblich P (2003). Alteration of Renal Sodium Excretion during Dorsal column Stimulation, Thesis.

74. Mancia, G., Fagard, R., Narkiewicz, K., Redon, J., Zanchetti, A., Boehm, M., ... & Galderisi, M. (2013). 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of

Hypertension (ESH) and of the European Society of Cardiology (ESC). *Blood pressure*, *22*(4), 193-278.

75. Mannheimer, C., Eliasson, T., Augustinsson, L. E., Blomstrand, C., Emanuelsson, H., Larsson, S., ... & Hjalmarsson, A. (1998). Electrical stimulation versus coronary artery bypass surgery in severe angina pectoris: the ESBY study. *Circulation*, *97*(12), 1157-1163.

76. Meglio, M., Cioni, B., Dal Lago, A., De Santis, M., Pola, P., & Serricchio, M. (1981). Pain control and improvement of peripheral blood flow following epidural spinal cord stimulation: case report. *Journal of neurosurgery*, *54*(6), 821-823.

77. Mellati M & Knoblich P (2016). Renal denervation alters the urinary sodium excretion response to dorsal spinal stimulation, Thesis.

78. Melzack, R., & Wall, P. D. (1965). Pain mechanisms: a new theory. *Science*, *150*(3699), 971-979.

79. Meyerson, B. A., Herregodts, P., Linderoth, B., & Ren, B. (1994). An experimental animal model of spinal cord stimulation for pain. *Stereotactic and functional neurosurgery*, *62*(1-4), 256-262.

80. Miller, J. P., Eldabe, S., Buchser, E., Johanek, L. M., Guan, Y., & Linderoth, B. (2016). Parameters of spinal cord stimulation and their role in electrical charge delivery: a review. *Neuromodulation: Technology at the Neural Interface*, *19*(4), 373-384.

81. Mittal, B. V., & Singh, A. K. (2010). Hypertension in the developing world: challenges and opportunities. *American Journal of Kidney Diseases*, *55*(3), 590-598.

82. Mohrman DE and Heller L (2014). Chapter 9. Regulation of Arterial Pressure. In *Cardiovascular Physiology*, 8th edn, ed. Mohrman DE and Heller L. McGraw-Hill, New York, NY.

83. Müller, J., & Barajas, L. (1972). Electron microscopic and histochemical evidence for a tubular innervation in the renal cortex of the monkey. *Journal of ultrastructure research*, *41*(5-6), 533-549.

84. National Clinical Guideline Centre (UK). (2011). Hypertension: the clinical management of primary hypertension in adults: update of clinical guidelines 18 and 34. *National Institute for Health and Clinical Excellence: Guidance*.

85. North, R. B., Kidd, D. H., Zahurak, M., James, C. S., & Long, D. M. (1993). Spinal cord stimulation for chronic, intractable pain: experience over two decades. *Neurosurgery*, *32*(3), 384-395.

86. Oakley, J. C., & Prager, J. P. (2002). Spinal cord stimulation: mechanisms of action. *Spine*, *27*(22), 2574-2583.

87. Okamoto K, Aoki K. (1963). Development of a strain of spontaneously hypertensive rats. Japanese Circulation Journal. 27:282-93.

88. O'Callaghan, E. L., Choong, Y. T., Jancovski, N., & Allen, A. M. (2013). Central angiotensinergic mechanisms associated with hypertension. *Autonomic Neuroscience*, *175*(1-2), 85-92.

89. P.M Kearney, M. Whelton, K. Reynolds, P. Muntner, P.K. Whelton, J (2005). He Global burden of hypertension: analysis of worldwide data. Lancet, 365, pp. 217-223.

90. Pinto YM, Paul M, Ganten D. (1998). Lesson from rat models of hypertension: from Goldblatt to genetic engineering. Cardiovascular Research.39(1):77-88.

91. Papin E, Ambard L (1924). Resection of the nerves of the kidney for nephralgia and small hydronephrosis. *J Urol* ;**11**:337.

92. Radhakrishnan, R., & Sluka, K. A. (2003). Spinal muscarinic receptors are activated during low or high frequency TENS-induced antihyperalgesia in rats. *Neuropharmacology*, *45*(8), 1111-1119.

93. Rosas-Arellano, M. P., Solano-Flores, L. P., & Ciriello, J. (1999). c-Fos induction in spinal cord neurons after renal arterial or venous occlusion. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *276*(1), R120-R127.

94. Rosengren, A., Hawken, S., Ôunpuu, S., Sliwa, K., Zubaid, M., Almahmeed, W. A., ... & INTERHEART investigators. (2004). Association of psychosocial risk factors with risk of acute myocardial infarction in 11 119 cases and 13 648 controls from 52 countries (the INTERHEART study): case-control study. *The Lancet*, *364*(9438), 953-962.

95. Rozanski, A., Blumenthal, J. A., & Kaplan, J. (1999). Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. *Circulation*, *99*(16), 2192-2217.

96. Sander, M., Chavoshan, B., & Victor, R. G. (1999). A large blood pressure–raising effect of nitric oxide synthase inhibition in humans. *Hypertension*, *33*(4), 937-942.

97. Schade, C. M., Sasaki, J., Schultz, D. M., Tamayo, N., King, G., & Johanek, L. M. (2010). Assessment of patient preference for constant voltage and constant current spinal cord stimulation. *Neuromodulation: Technology at the Neural Interface*, *13*(3), 210-217.

98. Schechtmann, G., Song, Z., Ultenius, C., Meyerson, B. A., & Linderoth, B. (2008). Cholinergic mechanisms involved in the pain relieving effect of spinal cord stimulation in a model of neuropathy. *Pain*, *139*(1), 136-145.

99. Schechtmann, G., Song, Z., Ultenius, C., Meyerson, B. A., & Linderoth, B. (2008). Cholinergic mechanisms involved in the pain relieving effect of spinal cord stimulation in a model of neuropathy. *Pain*, *139*(1), 136-145.

100. Schlaich, M. P., Sobotka, P. A., Krum, H., Lambert, E., & Esler, M. D. (2009). Renal sympathetic-nerve ablation for uncontrolled hypertension. *New England Journal of Medicine*, *361*(9), 932-934.\

101. Schoffnegger, D., Heinke, B., Sommer, C., & Sandkühler, J. (2006). Physiological properties of spinal lamina II GABAergic neurons in mice following peripheral nerve injury. *The Journal of physiology*, *577*(3), 869-878.

102. Schultz, H. D., Li, Y. L., & Ding, Y. (2007). Arterial chemoreceptors and sympathetic nerve activity: implications for hypertension and heart failure. *Hypertension*, *50*(1), 6-13.

103. Shealy, C. N., Taslitz, N., Mortimer, J. T., & Becker, D. P. (1967). Electrical inhibition of pain: experimental evaluation. *Anesthesia & Analgesia*, *46*(3), 299-305.

104. Simpson, E. L., Duenas, A., Holmes, M. W., Papaioannou, D., & Chilcott, J. (2009). Spinal cord stimulation for chronic pain of neuropathic or ischaemic origin: systematic review

and economic evaluation. In *NIHR Health Technology Assessment programme: Executive Summaries*. NIHR Journals Library.

105. Simon, J. K., Zhang, T. X., & Ciriello, J. O. H. N. (1989). Renal denervation alters forebrain hexokinase activity in neurogenic hypertensive rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *256*(4), R930-R938.

106. Skøtt, O., & Jensen, B. L. (1993). Cellular and intrarenal control of renin secretion. *Clinical science*, *84*(1), 1-10.

107. Smithwick RH. (1951). Hypertensive cardiovascular disease; effect of thoracolumbar splanchnicectomy on mortality and survival rates. *J Am Med Assoc*.;**147**:1611–1615.

108. Sobotka P. Krum H, Francis D, Schlaich M. (2012). The role of renal denervation in the treatment of heart failure. Current cardiology reports. DOI: 10.1007/S118886-012-0258.

109. Song, Z., Meyerson, B. A., & Linderoth, B. (2008). Muscarinic receptor activation potentiates the effect of spinal cord stimulation on pain-related behavior in rats with mononeuropathy. *Neuroscience letters*, *436*(1), 7-12.

110. Song, Z., Meyerson, B. A., & Linderoth, B. (2011). Spinal 5-HT receptors that contribute to the pain-relieving effects of spinal cord stimulation in a rat model of neuropathy. *PAIN®*, *152*(7), 1666-1673.

111. Song, Z., Viisanen, H., Meyerson, B. A., Pertovaara, A., & Linderoth, B. (2014). Efficacy of kilohertz-frequency and conventional spinal cord stimulation in rat models of different pain conditions. *Neuromodulation: Technology at the Neural Interface*, *17*(3), 226-235.

112. Sripairojthikoon, W., Oparil, S., & Wyss, J. M. (1989). Renal nerve contribution to NaCl-exacerbated hypertension in spontaneously hypertensive rats. *Hypertension*, *14*(2), 184-190.

113. Stearns A and Knoblich P (2007). Electrical Stimulation of the Dorsal Spinal Column at T12-T13 increases Renal Sodium Excretion but not Renal Blood Flow, Thesis.

114. Stiller, C. O., Cui, J. G., O'Connor, W. T., Brodin, E., Meyerson, B. A., & Linderoth, B. (1996). Release of γ-aminobutyric acid in the dorsal horn and suppression of tactile allodynia by spinal cord stimulation in mononeuropathic rats. *Neurosurgery*, *39*(2), 367-375.

115. Stouffer, G. A., DiBona, G. F., Patel, A., Kaul, P., & Hinderliter, A. L. (2013). Catheter-based renal denervation in the treatment of resistant hypertension. *Journal of Molecular and Cellular Cardiology*, *62*, 18-23.

116. Straznicky, N. E., Lambert, E. A., Lambert, G. W., Masuo, K., Esler, M. D., & Nestel, P. J. (2005). Effects of dietary weight loss on sympathetic activity and cardiac risk factors associated with the metabolic syndrome. *The Journal of Clinical Endocrinology & Metabolism*, *90*(11), 5998-6005.

117. Suzuki, R., Rahman, W., Rygh, L. J., Webber, M., Hunt, S. P., & Dickenson, A. H. (2005). Spinal-supraspinal serotonergic circuits regulating neuropathic pain and its treatment with gabapentin. *Pain*, *117*(3), 292-303.

118. Tanaka, S., Barron, K. W., Chandler, M. J., Linderoth, B., & Foreman, R. D. (2003). Role of primary afferents in spinal cord stimulation-induced vasodilation: characterization of fiber types. *Brain research*, *959*(2), 191-198.

119. Thomas, Gail D., Weiguo Zhang, and Ronald G. Victor. "Nitric oxide deficiency as a cause of clinical hypertension: promising new drug targets for refractory hypertension." *Jama* 285.16 (2001): 2055-2057.

120. Ulett, G. A., Han, S., & Han, J. S. (1998). Electroacupuncture: mechanisms and clinical application. *Biological psychiatry*, *44*(2), 129-138.

121. Uppuluri, S. C., Storozynsky, E., & Bisognano, J. D. (2009). Baroreflex device therapy in the treatment of hypertension. *Current hypertension reports*, *11*(1), 69-75.

122. Wallin, B. G., Sundlöf, G., Strömgren, E. R. L. A. N. D., & Aberg, H. A. N. S. (1984). Sympathetic outflow to muscles during treatment of hypertension with metoprolol. *Hypertension*, *6*(4), 557-562.

123. Wang, Y. Y., Wu, S. X., Liu, X. Y., Wang, W., & Li, Y. Q. (2003). Effects of c-fos antisense oligodeoxynucleotide on 5-HT-induced upregulation of preprodynorphin, preproenkephalin, and glutamic acid decarboxylase mRNA expression in cultured rat spinal dorsal horn neurons. *Biochemical and biophysical research communications*, *309*(3), 631-636.

124. Weir, M. R., & Dzau, V. J. (1999). The renin-angiotensin-aldosterone system: a specific target for hypertension management. *American journal of hypertension*, *12*(S9), 205S-213S.

125. Wenzel, R. R., Spieker, L., Qui, S., Shaw, S., Lüscher, T. F., & Noll, G. (1998). I1-imidazoline agonist moxonidine decreases sympathetic nerve activity and blood pressure in hypertensives. *Hypertension*, *32*(6), 1022-1027.

126. Wolter, T. (2014). Spinal cord stimulation for neuropathic pain: current perspectives. *Journal of Pain Research*, *7*, 651–663.

127. Wu, M., Linderoth, B., & Foreman, R. D. (2008). Putative mechanisms behind effects of spinal cord stimulation on vascular diseases: a review of experimental studies. *Autonomic Neuroscience*, *138*(1-2), 9-23.

128. Wu, M., Komori, N., Qin, C., Farber, J. P., Linderoth, B., & Foreman, R. D. (2006). Sensory fibers containing vanilloid receptor-1 (VR-1) mediate spinal cord stimulation-induced vasodilation. *Brain research*, *1107*(1), 177-184.

129. Ye, S., Zhong, H., Yanamadala, V., & Campese, V. M. (2002). Renal injury caused by intrarenal injection of phenol increases afferent and efferent renal sympathetic nerve activity. *American journal of hypertension*, *15*(8), 717-724.

130. Young, C. N., Fisher, J. P., Gallagher, K. M., Whaley-Connell, A., Chaudhary, K., Victor, R. G., ... & Fadel, P. J. (2009). Inhibition of nitric oxide synthase evokes central sympatho-excitation in healthy humans. *The Journal of physiology*, *587*(20), 4977-4986.

131. Zhang, H. M., Chen, S. R., Cai, Y. Q., Richardson, T. E., Driver, L. C., Lopez-Berestein, G., & Pan, H. L. (2009). Signaling mechanisms mediating muscarinic enhancement of GABAergic synaptic transmission in the spinal cord. *Neuroscience*, *158*(4), 1577-1588.

132. Zucker, I. H., Patel, K. P., Schultz, H. D., Li, Y. F., Wang, W., & Pliquett, R. U. (2004). Exercise training and sympathetic regulation in experimental heart failure. *Exercise and sport sciences reviews*, *32*(3), 107-111.