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Characterization of Sympathetic Ganglion Sensitivity to Substance P in a Genetic  
and a Non-genetic Rat Model of Hypertension

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A dissertation  
presented to  
the faculty of the Department of Pharmacology  
East Tennessee State University

In partial fulfillment  
of the requirements for the degree  
Doctor of Philosophy in Biomedical Science

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by  
John Daniel Tompkins  
May 2003

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Keywords: hypertension, SHR, substance P, electrophysiology, sympathetic ganglia, DOCA

## ABSTRACT

### Characterization of Sympathetic Ganglion Sensitivity to Substance P in a Genetic and a Non-Genetic Rat Model of Hypertension

by

John Daniel Tompkins

Intravenous injection of substance P (SP) stimulates sympathetic ganglia to evoke a greater increase in renal sympathetic nerve activity, heart rate (HR) and blood pressure (BP) in hypertensive than normotensive rats due to upregulation of the NK<sub>1</sub> receptor. These experiments were designed to determine the cellular basis for the enhanced ganglionic responsiveness to NK<sub>1</sub> agonists in spontaneously hypertensive rats (SHR) in comparison to their normotensive counterparts, Wistar-Kyoto rats (WKY). Studies were also conducted to determine whether the increased ganglion responsiveness to SP in SHR is causally related to the increased BP or is a unique characteristic of this model of essential hypertension. Nerve recordings were made from the external carotid branch of the superior cervical ganglion (SCG) in pentobarbital anesthetized rats. Animals were treated with the ganglion blocking agent chlorisondamine (10.5 μmol/kg) and pre- and postganglionic SCG nerves were cut. SP (1.0 to 100 nmol/kg) evoked a greater increase in postganglionic nerve firing from the SCG of SHR vs. WKY. Intracellular microelectrode recordings were made from isolated SCG. Membrane properties were similar between strains. Picospritzer application of the NK<sub>1</sub> agonist GR-73632 (100 μM, 1 s) caused slow depolarization and increased neuron excitability. Depolarization amplitude and duration were similar between strains, however, a greater percentage of neurons were depolarized by the NK<sub>1</sub> agonist in SHR. To determine if the ganglion sensitivity to SP was correlated with blood pressure WKY were made hypertensive by unilateral nephrectomy and deoxycorticosterone acetate (DOCA)/salt treatment. Tail cuff BP was the same in treated WKY and untreated SHR. Increases in sympathetic nerve activity, HR and BP in response to SP (1.0 to 100 nmol/kg) were the same in treated and untreated WKY rats. In conclusion, SHR are more responsive to ganglion stimulation by NK<sub>1</sub> agonists due to a greater number of responsive cells within their SCG rather than an enhanced responsiveness of individual neurons. The increased sympathetic nerve responsiveness to SP is an inherent characteristic and not an adaptive response of sympathetic ganglion neurons to hypertension. This enhanced action of SP at sympathetic ganglia may contribute to the elevated sympathetic outflow observed in this model of hypertension.

## ACKNOWLEDGEMENTS

I wish to thank everyone in the Department of Pharmacology. I am privileged to have been part of such a wonderful group of people.

I must thank our chairman Dr. Dun for his leadership and exemplary scientific achievement.

Thank you David, Christina, Eugene, Catalin, Masa, Yoshi, Phoumy, Jess, and Alan for the good times and many fond memories.

Thank you Dr. Hoover, Dr. Ardell, Dr. Schoborg, and Dr. Williams for sharing your passion and enthusiasm for science with me.

Thank you Steve and Kim for the fishing trips and Friday-night dinners (good food and great companionship).

I am especially grateful for the leadership, support and encouragement provided by Dr. Hancock, mentor and good friend. Thanks for the long hikes, great meals and good conversation all the while.

Most importantly I wish to acknowledge the love and support of my family; Jay, Sherry, Mom, and Dad. You make it all possible.

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## CHAPTER 1

### INTRODUCTION

Increased sympathetic outflow plays an major role in the development and maintenance of high blood pressure in spontaneously hypertensive rats (SHRs) ( Bunag et al. 1975; Folkow 1975; Judy et al. 1976; Judy and Farrell 1979; Folkow 1982; Oparil 1986; Oparil et al. 1989; Ferrario and Averill 1991; Kumagai et al. 1992). Increased peripheral sympathetic output is also observed in humans with essential hypertension (Folkow 1975; Folkow 1982; Mark 1996). The increased sympathetic activity can raise blood pressure through stimulation of the heart, the peripheral vasculature, and the kidneys, causing increased cardiac output, increased vascular resistance, and fluid retention. Several mechanisms believed to underlie the increased nerve activity have been proposed but fail to accurately explain this condition in either scenario. Recent evidence suggests that abnormal neurotransmission through sympathetic ganglia may contribute to the elevated sympathetic tone in hypertensive rats (Magee and Schofield 1992; Magee and Schofield 1994). Multiple neuroactive substances function at the level of autonomic ganglia to modulate ganglion output to the periphery (Heym et al. 1991; Elfvin et al. 1993; Gibbins et al. 2000). Among these agents substance P, a sensory neuropeptide, has an excitatory effect on neurons of sympathetic ganglia (Dun and Karczmar 1979; Jiang et al. 1982; Konishi and Otsuka 1985). Substance P containing sensory nerve fibers innervate the peripheral vasculature (Malliani 1982; Dalsgaard et al. 1983; Holzer 1998; Holzer and Maggi 1998) and collaterals of these fibers innervate sympathetic ganglia (Oldfield and McLachlan 1978; Konishi et al. 1985; Cuello 1987; Quigg et al. 1990). This circuitry may thereby constitute a peripheral reflex mechanism for the regulation of cardiovascular function (Konishi et al. 1985; Mantyh

1991; Elfvin et al. 1993). Sympathetic ganglia of hypertensive rats show an enhanced responsiveness to substance P as compared to their normotensive counterparts (Hancock and Lindsay 1995; Hancock and Lindsay 2000; Schoborg et al. 2000). The increased sympathetic nerve activity in response to intravenous administration of substance P raises blood pressure in SHR (Schoborg et al. 2000). This evidence suggests substance P is involved in the increased sympathetic drive of hypertensive rats by enhancing ganglion outflow and may thus be involved in the elevation of blood pressure observed in this strain. This work investigated the cellular mechanism of the enhanced ganglion excitability to substance P and examined whether this phenomenon is correlated with the increased blood pressure or is a causative factor in the development of hypertension in SHR.

#### Hypertension and the SHR

Hypertension, the clinical term for high blood pressure, is the most common cardiovascular disease in the United States (American Heart Association 2001). Approximately 24% of all adults in the United States have high blood pressure. Patients are conventionally considered hypertensive when they have a diastolic blood pressure at or above 90 mm Hg, a systolic blood pressure at or above 140 mm Hg, or both after repeated determinations (American Heart Association 2001). This helps to characterize a group of individuals who are at risk of developing hypertension-related cardiovascular disease. While acute elevation of blood pressure is not associated with high mortality, long-term elevation of arterial pressure contributes to pathological changes in the vasculature and myocardium. These changes are associated with increased risk of stroke, disease of the coronary arteries, which can lead to myocardial infarction or sudden cardiac death, and can be major contributors to cardiac failure, renal insufficiency and dissecting aneurysm of the aorta (Oates 1996).

Hypertension can be categorized as primary or secondary dependant on the cause. With secondary hypertension an elevated blood pressure develops due to a known underlying pathological condition. Pheochromocytoma is an example of secondary hypertension in which a chromaffin cell carcinoma secretes excess catecholamines, evoking sympathetic hyperactivity. Primary or essential hypertension is by far the most common form of hypertension in humans, accounting for more than 95% of all observed cases (Oparil 2000). In contrast to secondary hypertension, the underlying cause of essential hypertension is unknown. Essential hypertension is known to cluster in families and most likely represents a collection of genetically based diseases or syndromes with a number of resultant inherited biochemical abnormalities (Oparil 2000). Many pathophysiological factors have been implicated in the genesis of essential hypertension, including increased sympathetic nervous system activity, over-production of sodium-retaining hormones and vasoconstrictors, increased activity of vascular growth factors, and altered cellular ion transport (Oparil 2000). Environmental factors (i.e. smoking, stress, poor diet) contribute significantly to the progression of the disease, and primary hypertension ultimately manifests in structural changes of the heart and vasculature that contribute to the maintenance of the elevated arterial pressure (Oparil 2000).

The SHR is the most widely studied animal model of hypertension and is considered an acceptable model for human essential hypertension given their genetic predisposition to hypertension and the similar pathophysiological development of the disease (Yamori and Okamoto 1974; Lovenberg 1986; Mulvany 1992; Pinto et al. 1998). As in human patients with essential hypertension, SHRs also exhibit a polygenic predisposition to the development of hypertension (Louis et al. 1969), environmental factors contribute to the progression of the disease and a structural upward resetting of the heart and vessels occurs in response to the

elevated arterial pressure and sustains the elevated blood pressure (Yamori et al. 1979; Yamori 1989; Yamori 1991; Folkow 1993).

SHRs were developed in 1963 at the Kyoto Institute from outbreed Wistar (Okamoto 1969; Okamoto et al. 1973). Rats that displayed an elevated arterial blood pressure were cross breed and offspring from these pairs were selected for “spontaneous” development of hypertension. Subsequent interbreeding has resulted in a strain of rats with phenotypic homology that provide standardized, handy and time-compressed caricatures for the investigation of essential hypertension.

#### Autonomic Control of Blood Pressure

The cardiovascular system is regulated by a complex hierarchy of feedback-regulated control systems with considerable overlap in the regulation of vascular pressure. The autonomic nervous system plays a central role in the regulation of blood pressure on a short-term basis with its ability to rapidly and profoundly modify cardiovascular function. The excitatory effects of sympathetic innervation are balanced by the inhibitory parasympathetic inputs at multiple tissues. At rest the cardiovascular system is a closed-loop pressure-regulated system. Basal vascular tone is maintained by sympathetic innervation. Increases in sympathetic drive to the heart and periphery increase the rate and force of myocardial contraction and increase peripheral vascular resistance. Blood pressure, the product of cardiac output and peripheral vascular resistance, is subsequently elevated. The increased vascular pressure activates pressure sensitive cells or baroreceptors that “feedback” to the central nervous system to attenuate sympathetic drive. In this way basal vascular tone is maintained near normal physiological values on a beat-by-beat basis. These nervous control mechanisms are ideally suited for short-term circulatory control while renal excretory functions are believed to be the primary regulators of blood

pressure in the long term (Guyton 1980). In addition to neuronal connections to autonomic effector tissues, neuroendocrine control mechanisms also modulate blood pressure but operate more diffusely and generally have a slower time course. The renin-angiotensin-aldosterone system participates in short- as well as long-term blood pressure control by influencing renal sodium absorption and by direct and possibly centrally mediated effects on blood vessels (Baum 1990).

### Sympathetic Neuroanatomy

The general components of the sympathetic innervation of the heart and vasculature are well understood. Autonomic outflow from the CNS originates from “centers” (i.e., nuclei or more diffusely arranged groups of cells) in the brainstem and hypothalamus. These regions are closely related and are subject to excitatory and inhibitory input from afferent fibers and from higher brain structures. Neural tracts originating from medulla and hypothalamus descend the spinal cord and innervate, directly or via interneurons, cell bodies of the majority of preganglionic sympathetic neurons located in the intermediolateral column of the thoracolumbar spinal cord (Baum 1990; Randall 1994).

The efferent division of the sympathetic part of the autonomic nervous system is structurally organized as a two-neuron pathway from the central nervous system to the peripheral effector organ (Fig. 1). Preganglionic sympathetic neurons targeting the heart are located in the lower cervical and upper thoracic segments of the spinal cord. Nerve fibers from these cells project to the ganglion cell bodies of the upper thoracic ganglia and form axodentric synapses with the postganglionic cell membrane. Unmyelinated postganglionic nerve fibers then project from the ganglion cell bodies to multiple tissues (Randall 1994). Stimulation of sympathetic nerves innervating nodal tissue, conduction tissue and the myocardium increase heart rate, conduction

velocity and myocardial contractility. These effects are mediated by a release of norepinephrine at the target site.

A large portion of the sympathetic nervous system is concerned with distributing electrical signals that initiate and maintain the degree of constriction of blood vessels throughout the body (McLachlan 1995). Most blood vessels are richly innervated by sympathetic fibers. However, the density of innervation of individual arteries and veins varies considerably. Postganglionic nerve fibers from sympathetic ganglia form a network of anastomosing filaments that course the periphery of vessels. Arteries typically receive a greater density of innervation than veins however this density can vary considerably. Stimulation of sympathetic nerves or intraarterial injection of norepinephrine results in vasoconstriction and an increase in vascular resistance.

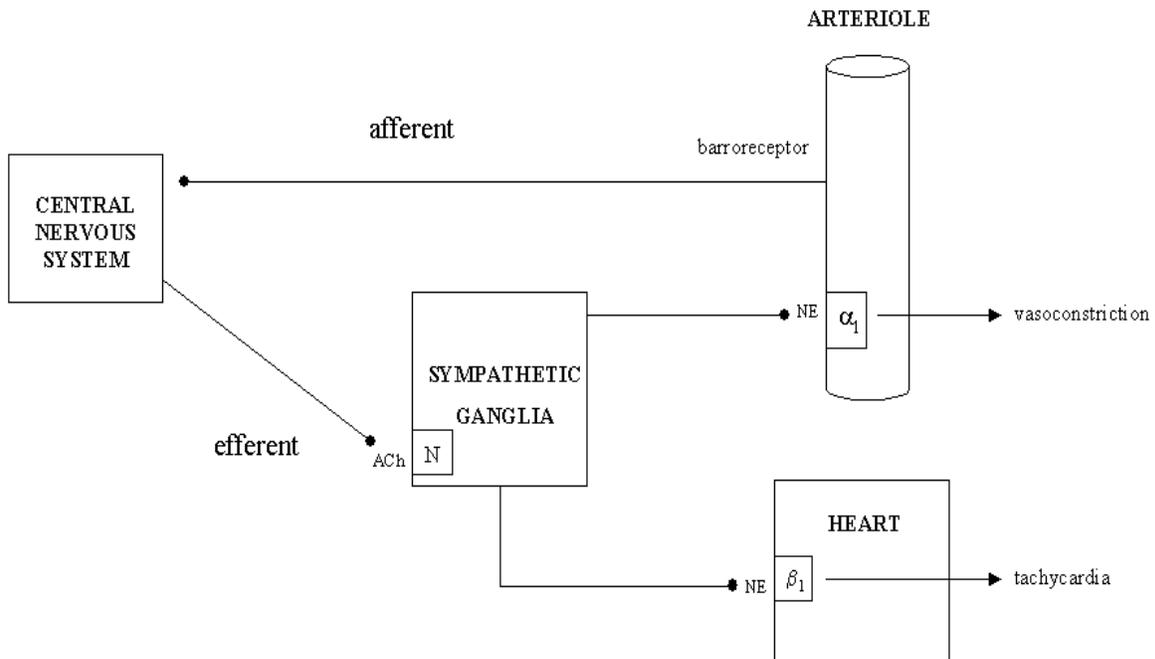


FIG 1. Schematic illustration of sympathetic innervation of the heart and vasculature. Preganglionic efferent sympathetic neurons converge on postganglionic neurons at sympathetic ganglia. Preganglionic neurotransmission is cholinergically mediated via nicotinic receptors. Postganglionic adrenergic neurons release norepinephrine at peripheral arterioles and heart to mediate vasoconstriction and tachycardia respectively. Stretch activated baroreceptors within carotid sinus and aortic arch attenuate central sympathetic neurochemical drive.

## Ganglionic Neurotransmission

The intervening ganglia within this two-neuron pathway have classically been viewed as simple relay stations; however, it is currently understood that autonomic ganglia represent important peripheral control centers whereby central neurochemical input can be modulated to more effectively meet the demands of the target tissue. Here a heterogeneous population of neuroactive elements function to modulate ganglionic output to the periphery (Lundberg and Hökfelt 1986; Elfvin et al. 1993; Gibbins and Morris 2000; Gibbins et al. 2000). In addition to the classic innervation by acetylcholine containing preganglionic nerve fibers, paravertebral sympathetic ganglia receive a diverse input from peptidergic, noradrenergic, aminergic and nitric oxide containing nerve fibers (Hökfelt et al. 1975; Hökfelt et al. 1977; Dalsgaard et al. 1982; Mantyh et al. 1992; Elfvin et al. 1993; Gibbins and Morris 2000). The noradrenergic innervation may arise from interganglionic neurons or postganglionic neurons in adjacent ganglia (Baum 1990). The peptidergic innervation originates from preganglionic fibers originating in the intermediolateral column of the spinal cord (Jimenez et al. 2002) and from peptide containing axon collaterals of dorsal root primary afferents (Oldfield and McLachlan 1978; Konishi et al. 1985; Cuello 1987; Quigg et al. 1990).

Neurotransmission at the ganglia has long been realized to be more complex than that described by a single neurotransmitter-receptor system. Intracellular recordings from ganglion neurons reveal at least four different types of synaptic events evoked by stimulation of preganglionic nerve fibers (Volle and Hancock 1970; Dun and Karczmar 1979; Weight et al. 1979; Dun 1980). The primary event is a fast excitatory postsynaptic potential (EPSP) that involves a rapid depolarization of the postsynaptic membrane by acetylcholine. This response is mediated by ganglion nicotinic receptors. The secondary events are believed to modulate this

primary event. The secondary events are insensitive to hexamethonium or other nicotinic antagonists. They include the slow EPSP, late slow EPSP, and an inhibitory postsynaptic potential (IPSP). The slow EPSP is mediated by agonists at muscarinic receptors and is blocked by antagonists that are selective for M<sub>1</sub> muscarinic receptors (Libet 1970). The IPSP is believed to be mediated by the release of catecholamines from interneurons by acetylcholine (Dun 1980; McAfee et al. 1980). The late slow EPSP can last for several minutes and is initiated by the action of peptides, including substance P, released within the ganglia (Dun and Karczmar 1979; Dun and Jiang 1982; Konishi and Otsuka 1985; Szucs et al. 1999).

#### Enhanced Sympathetic Nerve Activity in Hypertension

Multiple lines of evidence support a role of the sympathetic nervous system in the pathogenesis of hypertension in hypertensive rats (Okamoto et al. 1967; Okamoto 1969; Folkow 1975; Judy et al. 1976; Schramm and Chornoboy 1982; Howes 1984; McCarty 1986; Jubelin and Kannan 1990; Mark 1996). Both indirect and direct assays of sympathetic nerve activity demonstrate increased sympathetic outflow. Norepinephrine turnover is increased in systemic vascular tissues (Yamori et al. 1972) and plasma catecholamine and dopamine-beta-hydroxylase activity are elevated (Yamori et al. 1972; Roizen et al. 1975). Microneurography, a technique using microelectrodes to directly measure peripheral or muscle sympathetic nerve activity, shows elevated sympathetic nerve activity in anesthetized and conscious SHR (Okamoto et al. 1967; Judy et al. 1976; Schramm and Chornoboy 1982). In addition to increased basal sympathetic outflow, SHR also display a sympathetic hyperresponsiveness to stressful stimuli (Yamori et al. 1969; Koepke et al. 1987) and hypothalamic stimulation (Bunag and Takeda 1979). Early interference with sympathetic cardiovascular regulation prevents the development of the

hypertension in the SHR (Clark 1971; Weiss et al. 1974; Schramm and Chornoboy 1982; Kushinsky and Bell 1983; Lee et al. 1987) and arterial blood pressure is lowered to similar levels in anesthetized WKY, Wistar and SHRs following blockade of preganglionic neurotransmission (Hancock and Lindsay 1995; Hancock and Lindsay 2000).

The mechanism underlying the increased sympathetic tone has not been clearly defined, nor is it understood at what level sympathetic tone is augmented. One suggestion is that baroreceptor modulation of sympathetic control is either defective (Judy and Farrell 1979) or inactivated by aberrant central neurochemical drive (Haeusler 1975). A lack of central inhibitory input would enable tonically active peripheral sympathetic outflow as the systems errs to maintain elevated arterial pressure. The hypothalamic centers where sympathetic outflow originates have also been shown to be tonically active in SHRs (Yamori and Okamoto 1969). Interestingly, abnormalities in the function of the peripheral autonomic ganglia may also be involved in amplifying sympathetic output to the periphery. Transmission of neuronal activity is elevated in sympathetic ganglia from hypertensive animals (Magee and Schofield 1992). Cyclic nucleotide levels are also elevated in sympathetic ganglia suggestive of increased second messenger activity (Ariano and Kenny 1987). It is uncertain whether the increased ganglionic neurotransmission is due to a hyperexcitable postganglionic membrane, facilitation of preganglionic neurotransmitter release or other factors modulating ganglion activity. An increase in the stimulation induced release of, and hyperresponsiveness to, norepinephrine has been shown at peripheral postganglionic nerve endings (Ekas and Lokhandwala 1981; Westfall and Meldrum 1985). SHR ganglion neurons have also been shown to have hyperexcitable membranes (Yarowsky and Weinreich 1985; Jubelin and Kannan 1990), a decreased concentration of the inhibitory transmitter dopamine and an increased production of

catecholamines in response to cholinergic stimulation (Lutold et al. 1979; Ariano and Kenny 1987; Debinski and Kuchel 1989). The postsynaptic effects of ACh are similar between SHR and WKY rat postganglionic neurons (Magee and Schofield 1995). One goal of this study was to use basic intracellular microelectrode recording techniques to investigate the active and passive membrane properties of superior cervical ganglion (SCG) neurons from hypertensive and normotensive animals. Recent studies have also suggested that endogenous substance P may play a role at sympathetic ganglia to contribute to the heightened postganglionic sympathetic nerve activity in SHRs (Gurusinghe and Bell 1989a; Gurusinghe and Bell 1989b; Hancock and Lindsay 2000; Schoborg et al. 2000).

### Substance P

The neuropeptide Substance P was first isolated from the equine gut and characterized based on its ability to promote vasodilation in anesthetized rabbits (von Euler and Gaddum 1931). Lembeck first proposed the idea that substance P may function as a neurotransmitter of primary sensory neurons (Lembeck 1953). Isolation and sequencing of the peptide's structure by Leeman and colleagues helped clarify the role of this peptide in sensory neurotransmission (Chang et al. 1971). Substance P belongs to a family of structurally similar, fast-acting peptides termed tachykinins. There are three major mammalian tachykinins; substance P, neurokinin A and neurokinin B. Substance P and neurokinin A are processed from the same gene. A separate gene encodes the neurokinin B peptide (Bonner et al. 1987; Nakanishi 1987; Krause et al. 1989). There are three tachykinin receptor subtypes termed NK<sub>1</sub>, NK<sub>2</sub> and NK<sub>3</sub>. NK<sub>1</sub> receptors are expressed in a wide variety of tissues including central nervous system neurons and vascular

endothelial cells (Maggio 1988; Dam et al. 1988; Helke et al. 1990). Substance P has a 1000 fold higher affinity for NK<sub>1</sub> receptors than neurokinin A or neurokinin B.

#### Substance P's Involvement in Local Regulation of Blood Pressure

Substance P containing sensory nerve fibers project from dorsal root ganglia to the peripheral vasculature as well as to the dorsal horn of the spinal cord whereby peripheral sensory information is relayed to the central nervous system (Hökfelt et al. 1975; Dalsgaard et al. 1983). Collaterals of the peripherally directed fibers also project to prevertebral (Hökfelt et al. 1977; Dalsgaard et al. 1982; Cuello 1987; Matthews et al. 1987; Elfvin et al. 1993) or paravertebral sympathetic ganglia (Gurusinghe and Bell 1989b; Quigg et al. 1990; Elfvin et al. 1993). Stimulation of sensory collaterals to these ganglia or application of exogenous substance P elicits slow depolarization of the postganglionic sympathetic neurons (Dun and Karczmar 1979; Tsunoo et al. 1982); tachykinin receptor antagonists block the membrane response in both experimental conditions (Konishi and Otsuka 1985). Accordingly, sensory afferents may release substance P at sympathetic ganglia to modulate ganglion function (Dun and Jiang 1982; Konishi et al. 1985; Cuello 1987). Substance P containing sensory afferents may likewise constitute a peripheral reflex mechanism involved in the local control of blood pressure (Fig. 2).

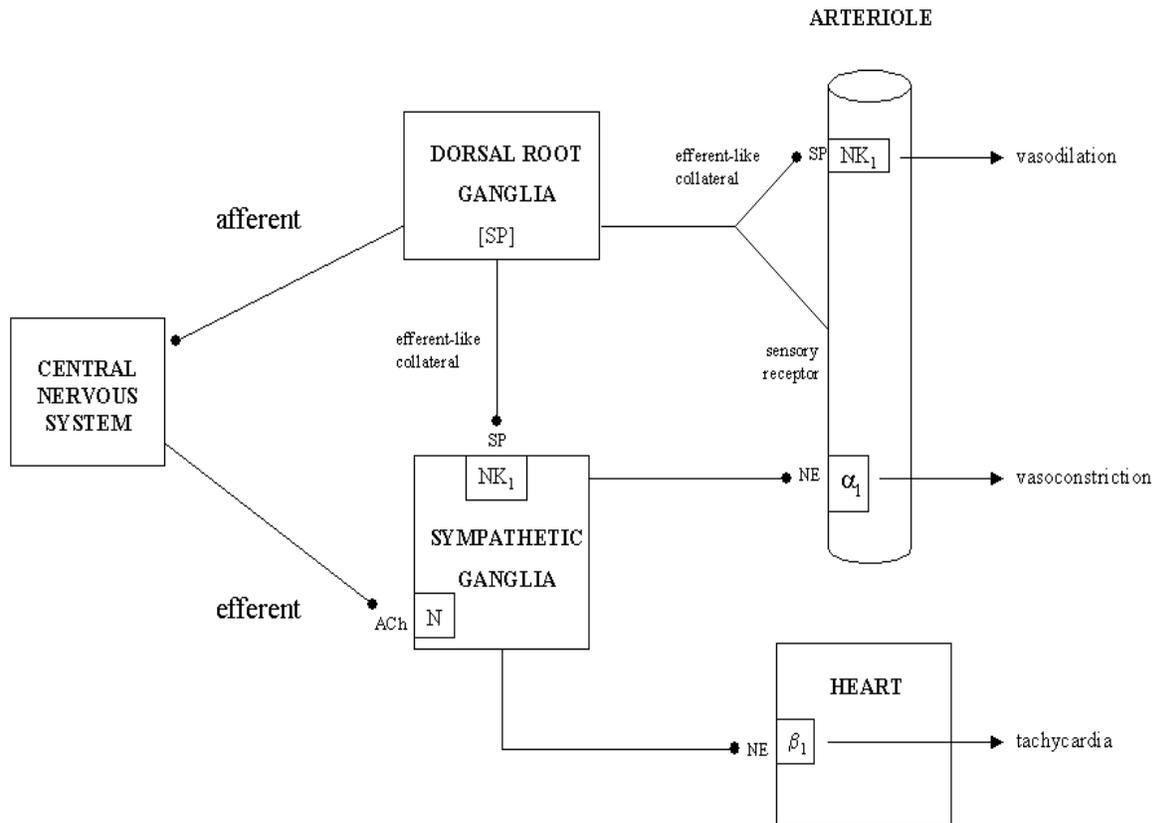


FIG 2. Hypothetical model illustrating short-loop reflex modulation of peripheral sympathetic outflow by substance P containing sensory afferents. Sensory afferent fibers, originating from dorsal root ganglia, innervate peripheral vasculature and central nervous system to relay sensory information from the periphery to the CNS (Hökfelt et al. 1975; Dalsgaard et al. 1983). Efferent-like collaterals to these fibers innervate peripheral arterioles and sympathetic ganglia (Hökfelt et al. 1977; Dalsgaard et al. 1982; Malliani 1982; Dalsgaard et al. 1983; Cuello 1987; Matthews et al. 1987; Elfvin et al. 1993; Holzer 1998; Holzer and Maggi 1998). Release of substance P from these peripheral processes modulates ganglion neurotransmission and evokes peripheral vasodilation (Dun and Karczmar 1979; Tsunoo et al. 1982; Holzer 1998; Holzer and Maggi 1998). This circuitry may hereby constitute short-loop reflex arcs involved in the peripheral modulation of cardiovascular function.

### Effect of Substance P *In Vivo*

Intravenous injection of substance P increases blood pressure and heart rate in rats (Hancock and Lindsay 1995; Hancock and Lindsay 2000; Schoborg et al. 2000). These responses are due to stimulation of tachykinin receptors at sympathetic ganglia as they are blocked by adrenergic receptor antagonists and unaffected by ganglion blockade or destruction of the brain stem and

spinal cord (Hancock and Lindsay 1995; Hancock and Lindsay 2000; Schoborg et al. 2000). Substance P can stimulate NK<sub>1</sub>, NK<sub>2</sub>, or NK<sub>3</sub> receptors but has greatest potency on NK<sub>1</sub> receptors. Blockade of this receptor inhibits the pressor response to the ganglionic action of substance P (Schoborg et al. 2000). These observations suggest that substance P modulates sympathetic nerve activity and blood pressure and that the effect is mediated by NK<sub>1</sub> receptors. Also supporting the role of tachykinins in the control of blood pressure is the observation that afferent fiber denervation by chronic exposure to the sensory nerve toxin capsaicin reduces sympathetic drive to the heart and peripheral vasculature (Virus et al. 1981; Lembeck and Skofitsch 1982).

In addition to its action on sympathetic ganglia, substance P causes vasodilation (von Euler and Gaddum 1931) by a direct action on endothelial NK<sub>1</sub> receptors (Maggio 1988) to release endothelium-derived relaxant factor (Furchgott et al. 1984). The ganglionic stimulation caused by substance P, however, appears to be able to override the vasodilator response with the result that substance P increases blood pressure (Hancock and Lindsay 1995). Depletion of catecholamines from peripheral nerves by reserpine blocks the pressor response and unmasks the vasodilator response further demonstrating that the pressor response is due to the ganglionic action of substance P (Hancock and Lindsay 1995).

#### Substance P's Involvement in Hypertension of SHR

Several lines of evidence suggest that there is a selective increase in the action of substance P on sympathetic ganglia of hypertensive rats and that the responsiveness of the ganglia to substance P is correlated with blood pressure. SCG of genetically hypertensive rats are more densely innervated by substance P-immunoreactive fibers than are ganglia from normotensive

rats (Gurusinghe and Bell 1989a; Gurusinghe and Bell 1989b). Evidence from our laboratory showed a three-fold increase in the expression of the NK<sub>1</sub> receptor message and NK<sub>1</sub> receptor protein in SCG of SHR (Schoborg et al. 2000). Furthermore, we showed that intravenous (i.v.) injection of substance P or an NK<sub>1</sub> selective agonist elicits enhanced renal sympathetic nerve activity, tachycardia and a pressor response in SHR. Conversely, in WKY rats substance P causes vasodilatation and has minimal effects on sympathetic nerve activity (Schoborg et al. 2000).

While the sympathetic ganglia supplying the renal innervation in SHR have an enhanced sensitivity to i.v. administration of substance P (Hancock and Lindsay 1995; Hancock and Lindsay 2000; Schoborg et al. 2000), it is unknown whether this is a unique feature of renal sympathetic ganglia or a common occurrence of sympathetic ganglia in hypertensive animals. Evidence of increased innervation of SCG by substance P containing nerve fibers, combined with the observations of increased NK<sub>1</sub> receptor protein and mRNA would suggest that these ganglia also display an increased sensitivity to substance P. To test this hypothesis we made extracellular recordings from a branch of the external carotid nerve to measure the increase in SCG outflow to close arterial injection of substance P in hypertensive and normotensive rats. These ganglia were selected because of the existing evidence from our lab showing an increased expression of NK<sub>1</sub> receptors and evidence from other labs showing an enhanced innervation by substance P containing nerve fibers (Gurusinghe and Bell 1989b; Schoborg et al. 2000). These ganglia are also easily accessible, easily isolated and easily removed for intracellular recording experiments.

Intracellular recordings were made from SCG neurons to investigate the cellular mechanism of the increased excitability of the ganglia to substance P. Intravenous injections of substance P

evoke increased ganglion activity (Hancock and Lindsay 2000; Schoborg et al. 2000) and substance P has been shown to depolarized sympathetic ganglion neurons (Dun and Karczmar 1979; Tsunoo et al. 1982). The cause of the enhanced ganglion excitability to substance P is unknown. Tachykinin NK<sub>1</sub> receptors are upregulated in sympathetic ganglia of hypertensive rats (Schoborg et al. 2000). How this upregulation of receptors manifests as enhanced ganglion stimulation to intravenous administration of substance P is unknown. It is possible that an upregulation of NK<sub>1</sub> receptors on individual neurons would elicit an augmented depolarization of ganglion neurons in the presence of substance P. Or this may only increase the probability of response to substance P and not affect the magnitude of the depolarization. In addition to investigating the resting membrane properties of these cells to determine whether increased membrane excitability contributes to the increased sympathetic outflow in hypertensive rats, we also investigated the magnitude of the membrane depolarization in response to picospritzer application of substance P and the NK<sub>1</sub> selective agonist GR73632. These studies should elucidate the cellular mechanism for the enhanced ganglion excitability to substance P.

Sympathetic afferent fibers in blood vessels respond to a variety of stimuli, including pressor drugs, arterial occlusion, changes in venous return and mechanical distension, by increasing their firing (Malliani 1982). As previously mentioned, fibers from dorsal root ganglion cells pass centrally to the dorsal horns of the spinal column and peripherally to tissues innervated by sympathetic nerves. Collaterals of peripherally directed afferent fibers also pass to autonomic ganglia (Hökfelt et al. 1977; Dalsgaard et al. 1982; Cuello 1987; Matthews et al. 1987; Gurusinghe and Bell 1989b; Quigg et al. 1990; Elfvin et al. 1993). The release of sensory peptides from peripheral sensory nerve terminals has been shown for a variety of pharmacological and physiopathological stimuli, and the concept that sensory fibers release

transmitters at all terminals is well accepted (Cuello 1987). This pathway from peripheral sensory endings to dorsal root sensory neurons and ganglia again raises the possibility that sensory peptides can modulate ganglion function by short reflex loops not involving the central nervous system (Fig. 2). This raises the possibility that the increased sensory innervation and increased sensitivity of sympathetic ganglia to the stimulant action of substance P are adaptive responses initiated by stimuli originating in the vasculature as a result of the elevated blood pressure and/or other factors associated with hypertension. Alternatively, the changes associated with ganglion substance P could be an inherent characteristic of SHR. These two alternatives will be systematically evaluated in this study using the deoxycorticosterone acetate (DOCA)/salt treated rat as a model for hypertension of nongenetic origin and SHR as a model for hypertension of genetic origin. If the increased ganglion neuronal responsiveness to substance P is an inherent trait of SHRs then it would not be expected to occur in rats with a nongenetic form of hypertension. This reasoning is based on the working hypothesis that if the increased responsiveness in SHR is secondary to the development of hypertension then it would be found in rats with hypertension of non-genetic as well as genetic origin. Knowing this is fundamental to knowing whether substance P is a causative factor in the development of hypertension or a consequence of the hypertension.

In, summary, several lines of evidence converge to indicate that feedback modulation of cardiovascular function is mediated by substance P at sympathetic ganglia and inherent differences in this modulation may be important in the development or maintenance of hypertension. Our working hypothesis is that the increased responsiveness of SHRs to tachykinins is due to an upregulation of tachykinin receptors at sympathetic ganglia and that this is an inherent characteristic of SHRs. The following goals will be addressed;

## Goals of this Study

I. Investigate the excitatory effect of substance P on postganglionic output from the superior cervical ganglia in SHR and WKY rats. Extracellular recordings were made from a postganglionic nerve of the SCG to determine effects of i.v. substance P on postganglionic firing.

II. Compare the effects of a selective NK<sub>1</sub> agonist on the active and passive membrane properties of individual superior cervical ganglion neurons from SHR and WKY rats.

Intracellular microelectrode recordings were used to determine if there was a greater response of individual neurons in the SCG to the selective NK<sub>1</sub> agonist GR-73632. This was done to determine the basis for the enhanced responsiveness of sympathetic ganglia in SHRs to substance P. Passive and active membrane properties of individual SCG neurons were also investigated between hypertensive and normotensive animals.

III. Determine if making WKY rats hypertensive by DOCA-salt treatment results in an up-regulation of NK<sub>1</sub> responses compared to untreated age-matched SHRs. These experiments were conducted to test the postulate that the increased sensitivity of sympathetic ganglia to the stimulant action of substance P is an inherent characteristic of SHRs and not an adaptive response to the hypertension.

## CHAPTER 2

### MATERIALS AND METHODS

#### Source of Animals

The first series of extracellular nerve recordings and all intracellular experiments were performed with male Harlan (Indianapolis, IN), SHR and WKY rats (12 to 14 weeks old). These rats were purchased at 10 weeks of age and allowed to acclimatize 1-2 weeks prior to each experiment. Male 4-week-old SHR and WKY rats were purchased from Charles River laboratories (Wilmington, MA) for the experiments involving DOCA/salt treated animals and age matched SHRs.

All animals were housed individually at 24°C on a 12 hr light/dark cycle in the university's animal care facility. Animals, except those treated with DOCA/salt, received normal tap water and rat chow *ad libitum*. All experiments were conducted in accordance with NIH Guidelines for the Care and Use of Laboratory Animals. Experimental protocols were reviewed and approved by the University Animal Care Committee.

#### In Vivo Studies

##### General Methods

Twelve- to 14-week-old animals were anesthetized with 32 mg/kg sodium/pentobarbital and 50 mg/kg ketamine by intraperitoneal (i.p.) injection. Anesthesia was supplemented as needed by intravenous (i.v.) injection of 1.5 mg/kg sodium/pentobarbital. The trachea was cannulated to facilitate respiration. The left femoral vein was cannulated for i.v. delivery of drugs. Blood pressure was recorded from the femoral artery via a cannula connected to a Gould Statham

pressure transducer and a Gould Brush 2400S polygraph (Cleveland, OH). Heart rate was derived from the pressure pulse signal by a Gould ECG/Biotach amplifier. Body temperature was monitored rectally with a thermistor probe and maintained at 37° C with a thermostatically controlled heating pad. The left internal and external carotid arteries were ligated to direct carotid blood flow to the superior cervical ganglia. The pre- and postganglionic sympathetic nerves were cut reducing the possibility that nerve responses to substance P were mediated by stimulation of the central nervous system or preganglionic nerve fibers. A branch of the external carotid nerve was isolated, stripped of connective tissue and placed over bipolar, silver chloride electrodes. The preparation was sealed with Quick-Cast (World Precision Instruments; Sarasota, FL).

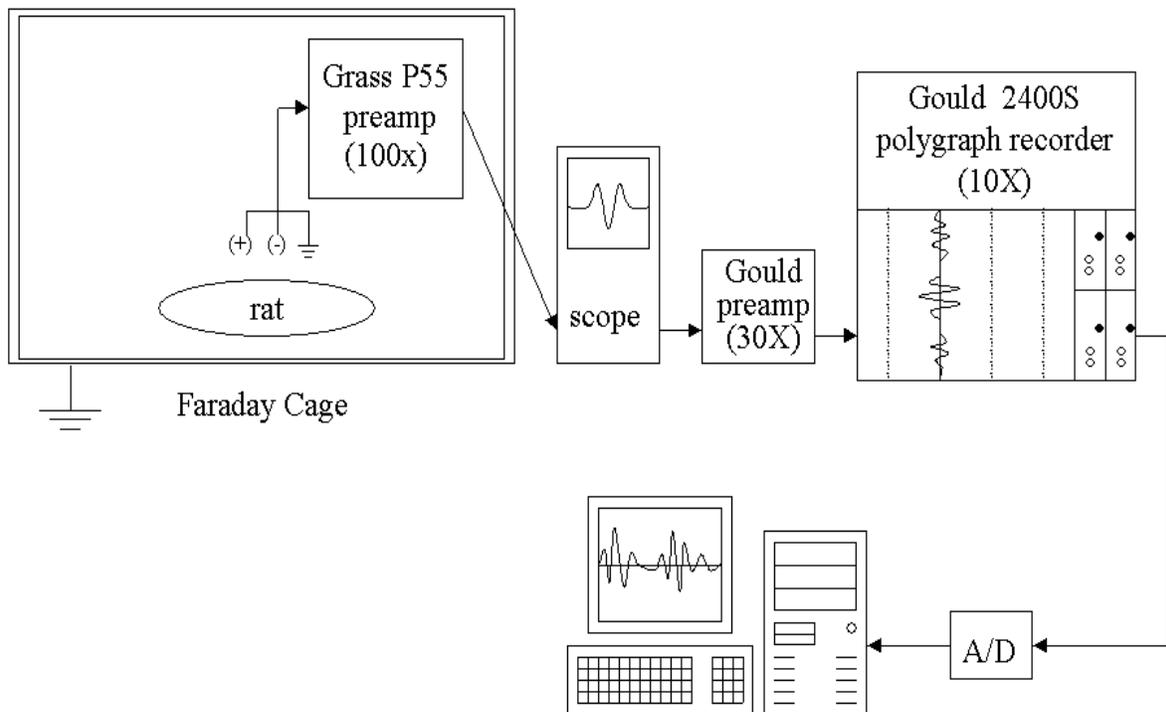


FIG. 3. Schematic illustration of the equipment used for recording extracellular nerve activity. Raw nerve activity is recorded in a grounded Faraday cage providing isolation from electromagnetic interference. First stage amplification is performed the cage near the signal source. The amplified signal is serially connected to an oscilloscope for visualization, to a polygraph recorder for second stage amplification and graphic translation and to a computer for data acquisition and analysis.

## Extracellular Nerve Recording

Nerve activity was recorded using a bipolar silver electrode ( $\text{Ø} = 0.008''$ ). The electrode was connected to a Grass model P55 pre-amplifier using a shielded input cable. At the amplifier raw nerve activity was bandpass filtered (30 Hz - 3 KHz) and amplified (gain = 100). This amplifier provides signal conditioning and amplification near the signal source reducing the problem of noise and cable capacity effects on the high impedance signal source. The amplifier signal output was viewed on a Tektronix oscilloscope (model 5110) and relayed to a Gould Isolated Preamp (model 18-5407-58, gain = 30) and a Gould Universal Amplifier (model 13-4615-58, gain = 10) for secondary amplification. Total signal gain was 30,000x. A permanent record of nerve activity was made on paper by a Gould 2400S polygraph recorder. The serial connections made and the equipment used are illustrated in figure 3.

The amplified nerve signal was further relayed to an analog-to-digital converter for digital data acquisition and analysis using a Pentium-1(200 MHz) based IBM compatible personal computer. The analog data stream was sampled at 2 KHz with 14-bit resolution using Strawberry Tree's Flash-12 data acquisition board and the Workbench for Windows software. Data from the sampled waveform were stored directly to the computer hard disk for future analysis.

## Data Analysis

Recorded data were replayed and analyzed with a program we designed using the Workbench for Windows software. The percent change in nerve firing was calculated after intraarterial injection of drugs. To calculate the percent change in nerve activity, the sampled signal must first be broken into discrete blocks of time and the power of each segment was then

calculated. Power analysis was done using the Fast Fourier Transform (FFT). This method has previously been reported in the literature as an acceptable means to quantify changes in nerve activity (Hancock and Lindsay 2000; Schoborg et al. 2000). The FFT is based on Fourier's theorem that every periodic signal can be decomposed into a series of harmonic functions, or more simply, any shape wave can be represented by a sum of many harmonic waves. The FFT is an adaptation of Fourier's theorem useful for rapid computation of discrete (time limited) waveform frequency components. Power spectrum output from the FFT graphs the "power", which is proportional to the square of the amplitude, versus the frequency for each sinusoidal component in the sampled waveform. Thus the signal is expressed graphically with frequency represented on the x-axis versus voltage on the y-axis. This representation of a neurogram or nerve recording is termed the autospectrum.

For FFT analysis, data were organized into sequential blocks of time containing 2048 samples. At a sampling frequency of 2 KHz this corresponds to a sample time of 1.024 seconds. It is essential that this bin or block size be a power of two for the FFT. These blocks of data were then analyzed by FFT analysis. The resulting power spectrum for a sample bin is shown in figure 4. The total power for each block is summated to give the total area under the curve by integration (Fig. 5).

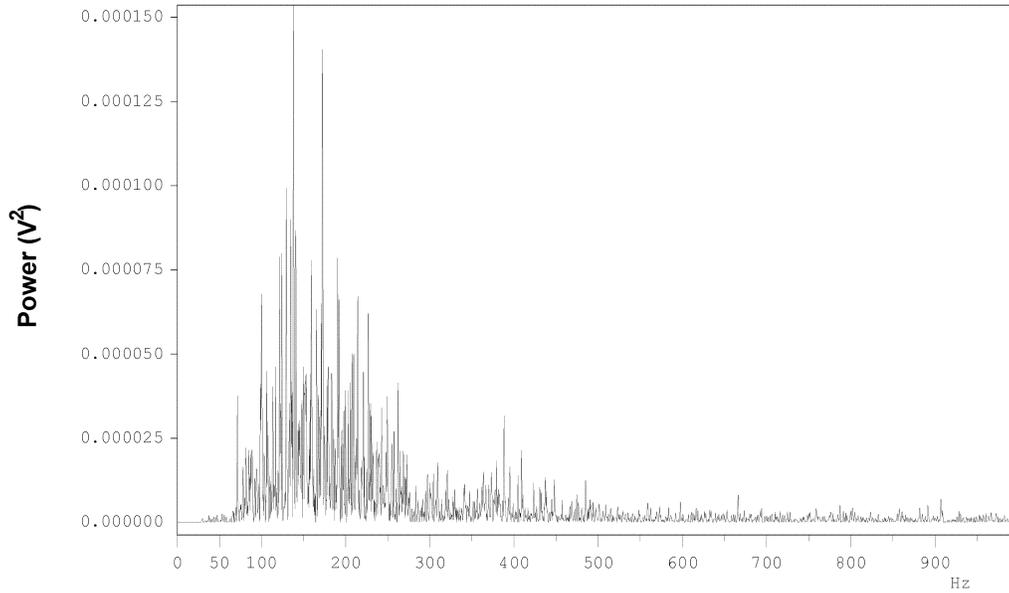


FIG. 4. Power spectrum output from Fast Fourier Transform (FFT). One-second bins of digitized data from the sampled waveform (sampling rate = 2 KHz) were analyzed by FFT. Output from the FFT graphically depicts waveform power (Volts<sup>2</sup>) as a function of waveform frequency. This spectrum is an analysis of nerve activity recorded from the external carotid nerve in a spontaneously hypertensive rat. This bin depicts the peak nerve response to 32 nmol/Kg substance P.

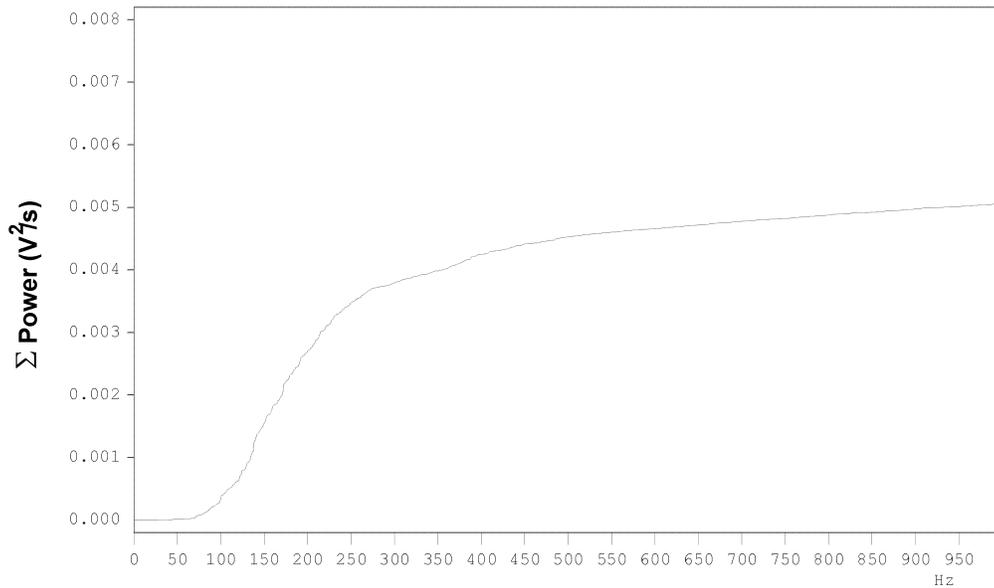


FIG. 5. Integral of the power spectrum from figure 4. Power across all frequencies from the output of the Fast Fourier Transform was summated by integration. This figure shows the magnitude of the waveform power summated from low to high frequency for one bin of data ( $t = 1.024$  s).

Total power for each block or time segment is then plotted continuously on the digital chart recorder beneath the nerve signal as shown in figure 6. The percent increase in total power is calculated as the percent increase of peak power from baseline. The baseline measurement is taken as the stable power of nerve activity prior to administration of drugs ( $Y_1$  in Fig. 6). The peak measurement is taken at the plateau of the increased power after drug administration ( $Y_2$  in Fig. 6). These values are reported for the changes in nerve firing evoked by intraarterial injection of drugs.

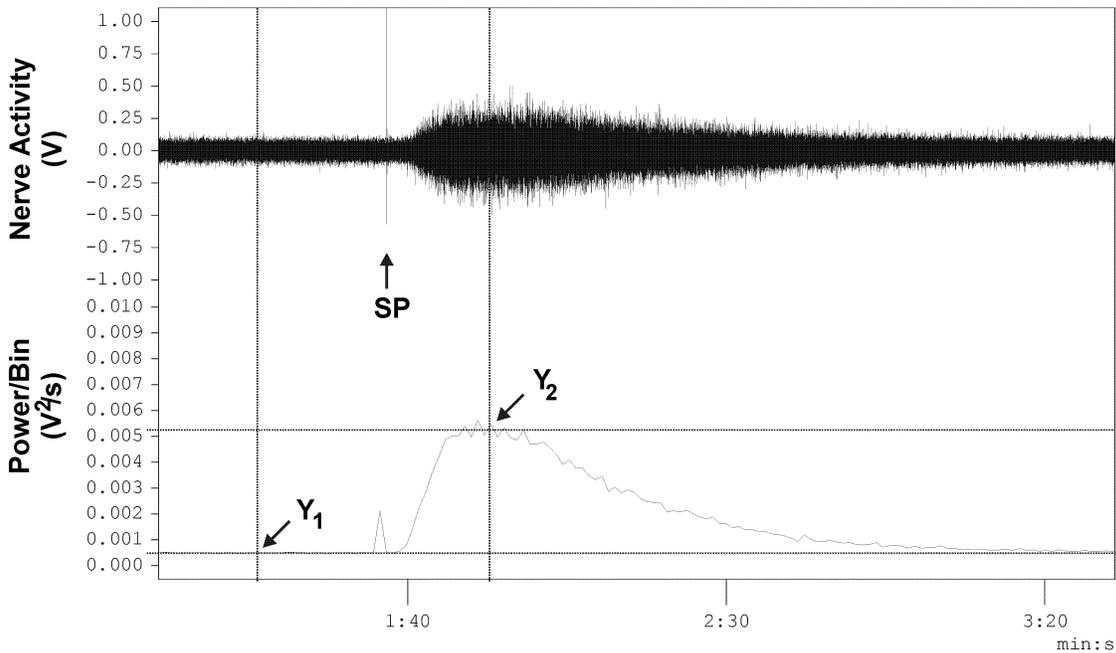


FIG. 6. Calculation of the percent change in nerve activity. Upper tracing is representative of the digitized waveform acquired with bipolar silver electrodes from the external carotid nerve of a spontaneously hypertensive rat. This tracing depicts the nerve response to intraarterial injection of substance P (SP). Upper arrow marks injection artifact. The lower tracing plots the maximum power for each sequential bin of sampled data. Vertical dashed lines indicate the time of the baseline power measurement ( $Y_1$ ) and the peak power measurement ( $Y_2$ ). Horizontal dashed lines indicate the autospectral power at each point ( $Y_1$  &  $Y_2$ ). The percent change in nerve activity is calculated as the percent increase in power from baseline ( $Y_1$ ) to peak power ( $Y_2$ ). This response illustrates a 1000% increase in nerve firing.

### Measurement of SCG Responsiveness to Substance P

Experiments were begun approximately one hour after completion of the surgery. Ganglion nicotinic receptors were blocked by i.v. administration of chlorisondamine (10.5  $\mu\text{mol/kg}$ ) reducing the possibility that the effects of substance P on blood pressure and heart rate were due to actions involving preganglionic sympathetic fibers or the central nervous system and to prevent reflex modulation of the cardiovascular response. Slow administration of chlorisondamine (45 min) produces a complete blockade of the blood pressure and heart rate increases to 1.0  $\mu\text{mol/kg}$  of 1,1-dimethyl-4-phenylpiperazinium (DMPP) without causing an acute autonomic crisis. Nicotinic receptor blockade by chlorisondamine persists for the duration of the experiment as shown by the blockade of the ganglion response to DMPP immediately following chlorisondamine administration and at the end of the experiment. The effects of 1.0, 3.2, 10, 32 and 100 nmol/kg doses of substance P on blood pressure, heart rate and external carotid nerve firing are recorded and the resulting dose response curves are generated. Injections of substance P began 30 minutes after administration of chlorisondamine, and the different doses were given in random order at thirty-minute intervals. The 100 nmol/kg dose of substance P was always given last. We have previously shown that tachyphylaxis to substance P does not occur with this regimen (Hancock and Lindsay 1995; Hancock and Lindsay 2000; Schoborg et al. 2000).

### DOCA/salt Hypertension

Unilateral Nephrectomy. Six-week-old WKY rats were randomly assigned to one of two treatment groups; DOCA or SHAM. DOCA animals were unilaterally nephrectomized. Surgical procedures were performed in a room dedicated to small animal survival surgery where aseptic technique was utilized. Animals were anesthetized by combined i.p. injection of ketamine (45

mg/kg) and xylazine (10 mg/kg). A 2 cm dorsal midline incision was made with its cranial terminus at the level of the 13<sup>th</sup> rib. The abdominal wall was opened 1.5 to 2 cm lateral to the spine. With blunt forceps the left kidney was seized and retracted through each incision. Care was exercised to minimize disturbance of the adrenal gland. The left renal artery, vein and ureter were ligated with 2-0 absorbable Vicryl suture and cut distal to the ligature to remove the kidney. The abdominal muscle was approximated with 4-0 absorbable Vicryl suture (Ethicon, Somerville, NJ) and the skin was closed with 2-0 absorbable Vicryl suture. Post-operatively, animals were put in clean cages with fresh bedding and the cages were placed on a heating pad for eight hours. Sham nephrectomies were performed identically in all respects, with the exception that no ligation took place and the kidney was replaced intact.

DOCA/salt Treatment. DOCA animals were made hypertensive by chronic treatment with deoxycorticosterone-acetate (DOCA) and high salt diet. Three days after surgery DOCA rats began to receive weekly subcutaneous (s.c.) injections of DOCA (30 mg/kg) suspended in sesame oil (Sigma, ST. Louis, MO) and were switched to a 1 % NaCl solution in place of normal tap water. SHAM rats received weekly s.c. injections of sesame oil alone and were given normal tap water to drink.

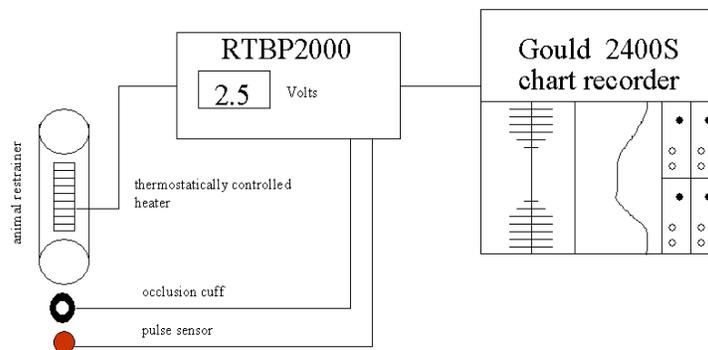


FIG. 7. Schematic illustration of the RTBP2000 system and Gould 2400S chart recorder for non-invasive heart rate and blood pressure measurement.

Non-Invasive Tailcuff Plethysmography. Blood pressure, heart rate and weight were recorded weekly from all animals. Heart rate and blood pressure were measured non-invasively from conscious animals using the RTBP2000 system from Kent Scientific (Torrington, CT) (Figs. 7 and 8). Animals were restrained in a cylindrical rodent restrainer equipped with a thermostatically controlled heater (Kent Scientific). Animals were warmed to promote vasodilatation in the tail. An occlusion cuff and piezoelectric pulse sensor were placed around the base of the tail. Gain and positioning of the pulse sensor were adjusted until a pulse was detectable. The occlusion cuff was inflated and the increased pressure occluded blood flow to the tail ablating the pulse signal. The occlusion pressure was then gradually reduced to restore perfusion of the tail. Systolic pulse pressure was read as the occlusion cuff pressure at which the pulse signal returned (Fig. 8). Heart rate was derived directly from the pulse signal. Animals were conditioned to the restrainer daily for two weeks before measurements were made. Three measurements were made from each animal and the mean value was recorded. This technique for chronically measuring systolic blood pressure from conscious animals is routinely cited in the literature and has been shown to be an accurate means for following the development of hypertension in DOCA/salt treated animals (Lamprecht et al. 1977; Basso et al. 1985; Anderson et al. 1988; Kirchner et al. 1993; Kubo et al. 2000).

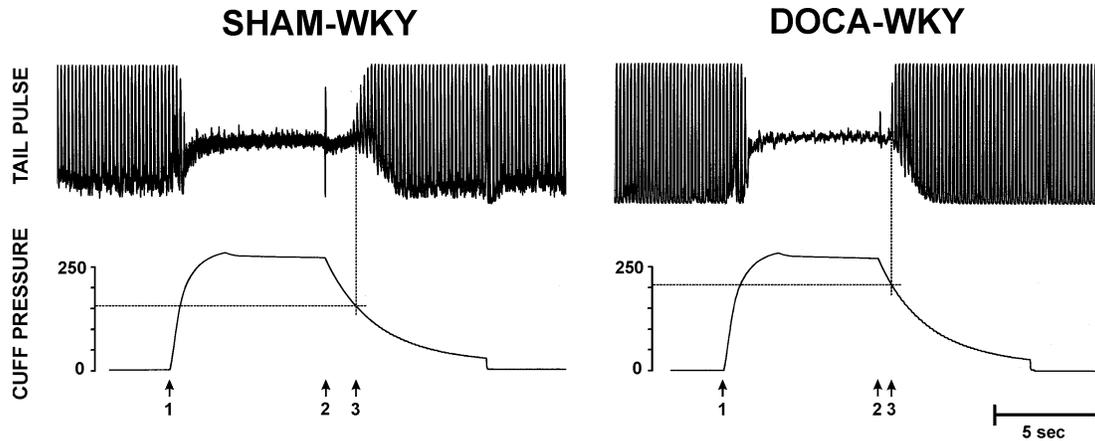


FIG. 8. Tail cuff measurement of heart rate and blood pressure. Heart rate and blood pressure are measured weekly from conscious DOCA, SHAM and SHR by non-invasive tail cuff plethysmography. Upper tracings are of the pulse signal and bottom tracings are occlusion cuff pressure (mmHg). Occlusion cuff pressure is increased (1) to occlude the pulse signal. As the pressure in the cuff is reduced (2), systolic blood pressure is read at the point the pulse signal returns (3). Tracings on the left are from a SHAM rat. Systolic blood pressure for this animal was 158 mmHg and heart rate was 330 beats/min. On the right, this DOCA rat (4 weeks after DOCA/salt treatment) had a systolic blood pressure of 208 mmHg and heart rate of 340 beats/min.

#### Measurement of SCG Responsiveness to Substance P in DOCA/salt Treated Animals

Extracellular nerve recordings as well as intra-arterial blood pressure and heart rate recordings were made from SHR, DOCA and SHAM rats at 12 to 14 weeks of age. The left external carotid artery was cannulated with 20 gauge polyethylene tubing for close arterial injection of DMPP and substance P to the superior cervical ganglia. Substance P was applied by close arterial injection to minimize systemic effects of the peptide. Microinjection of 50  $\mu$ l substance P into the common carotid artery yields a similar ganglion response as i.v. administration with a quicker onset.

#### In Vitro Studies

##### Intracellular Recording

Rats were anesthetized via i.p. injection of 50 mg/kg sodium/pentobarbital. The carotid artery with attached SCG was removed from the anesthetized animals, placed in a custom

recording chamber and continuously superfused with a modified Krebs solution (pH 7.38 – 7.40) of the following composition (in mM): 120 NaCl, 25 NaHCO<sub>3</sub>, 1.1 NaH<sub>2</sub>PO<sub>4</sub>, 5 KCl, 11 Dextrose, 2 MgCl<sub>2</sub>, 2.5 CaCl<sub>2</sub>. Buffer was delivered to the chamber by gravity flow at the rate of 2 ml/min and continuously gassed with 95% O<sub>2</sub>/5% CO<sub>2</sub>. The temperature of the superfusate was maintained at 34°C by a thermostatically controlled heater (Warner Instruments, Hamden, CT). The SCG was separated from the carotid artery and pinned to the Sylgard (Dow Corning, Midland, MI) floor of the chamber. Overlying connective tissue was teased away and pinned around the ganglia for support. After one hour of equilibration, intracellular recordings were made from superficial SCG neurons using standard microelectrode recording techniques. Recordings were made with high impedance (60-80 MΩ) borosilicate glass micropipettes (Sutter Instrument Co., Novato, CA) filled with filtered 3 M KCl. Transmembrane potentials were recorded in a current clamp configuration using A-M Systems Neuroprobe amplifier (model 1600, Everett, WA). Before cells were impaled the reference potential was adjusted to zero, the electrode resistance was nullified and the transmural capacity was compensated. Cells were allowed to stabilize for about three minutes after impalement before membrane properties were measured. Intracellular depolarizing and hyperpolarizing current pulses, triggered by a Grass S88 stimulator (Quincy, MA) were given through the recording electrode. Intracellular current and voltage signals were visualized on a Tektronix analog oscilloscope and on the monitor of a Dell Dimension 200 MHz Pentium computer. The computer was equipped with Strawberry Tree's "Flash 12" analog to digital converter and Workbench software suite for data acquisition and analysis (Kent Scientific, Litchfield, CT). GR-73632 ([100 μM], RBI, Natick, MA) was applied in 1-second pulses through a 5 μm bore diameter micropipette by local pressure ejection using a General Valve Picospritzer II (Fairfield, NJ).

## Data Analysis

Only neurons with stable resting membrane potentials more negative than -40 mV were included in the analysis. Cell input resistance was estimated from the slope of the line plotting membrane potential displacements from resting membrane potential against the amplitude of a series of hyperpolarizing pulses. Action potential duration was measured at two-thirds peak amplitude. Afterhyperpolarization (AHP) duration was measured from the point at which the repolarizing potential crossed the level of the resting membrane potential to the point at which the potential had returned to one-half the peak amplitude of the AHP (Edwards et al. 1995; Smith 1999; Zhang et al. 2001). Only depolarizations evoked by GR-73632 that were equal to or greater than 2 mV are to be included in the analysis. Depolarization durations were measured from the onset of the depolarization to the point at which the potential returned to the resting membrane potential or to the point at which the potential stabilized.

## Source of Drugs

All drugs were diluted with saline. Rapid IV injection of 50  $\mu$ l saline did not affect nerve firing, blood pressure or heart rate. The drugs used and their sources were as follows: sodium/pentobarbital (Abbott Labs, North Chicago, IL), ketamine (Fort Dodge, Fort Dodge, IA), GR-73632 and substance P (RBI, Natick, MA), DMPP (Sigma, St. Louis, MO), chlorisondamine was a gift from Novartis Pharmaceuticals Inc (East Hanover, NJ).

## Statistical Analysis

Data values are expressed as mean  $\pm$  SEM. GraphPad Prism software for windows (GraphPad Software v.3.0, San Diego, CA) was used to perform one-way ANOVA and

Newman-Keuls posttests on the means of the non-invasive measurements of heart rate and blood pressure as well as weight among DOCA, WKY and SHR animals. Baseline values for heart rate and mean arterial pressure in anesthetized animals were also compared this way.

Comparisons of mean arterial pressure (MAP) before and after ganglion blockade were made with a students t-test (one-tail). Nerve, heart rate, and blood pressure responses to administration of substance P were compared among treatment groups by GLM ANOVA and Fishers LSD post tests using NCSS 97 (NCSS Statistical Software, Keysville, UT). Increases in heart rate, blood pressure, and nerve firing were plotted as dose-response curves using GraphPad Prism version 3.00 software. P values <0.05 were considered significant.

For the intracellular studies, neuron membrane properties, action potential characteristics and GR-73632 evoked depolarization amplitudes and durations were compared using an unpaired t-test. Proportions of cells responding to GR-73632 within the SCG of hypertensive and normotensive rats were analyzed with Chi-square analysis. Dependency of the AHP amplitude with the cell input resistance was examined by correlation analysis. R squared ( $r^2$ ) is given as the square of the correlation coefficient. P values <0.05 were considered significant.

## CHAPTER 3

### RESULTS

#### *In Vivo* Studies

##### Measurement of SCG responsiveness to Substance P

Basal blood pressure and heart rate values were greater in SHR than in WKY rats; however, nerve firing was similar between strains. Prior to ganglion blockade, mean blood pressure was  $101 \pm 9$  mmHg in WKY rats and  $192 \pm 10$  mmHg in SHRs ( $p < 0.01$ ), heart rate was  $240 \pm 17$  beats/min in WKY rats and  $305 \pm 15$  beats/min in SHRs ( $p < 0.01$ ) and external carotid nerve firing was  $872 \pm 209 \mu\text{V}^2/\text{s}$  in WKY rats and  $1,237 \pm 472 \mu\text{V}^2/\text{s}$  in SHRs. After treatment with  $10.5 \mu\text{mol/kg}$  of chlorisondamine, mean blood pressure was  $73 \pm 8$  mmHg in WKY rats and  $88 \pm 5$  mmHg in SHRs, heart rate was  $143 \pm 6$  beats/min in WKY rats and  $158 \pm 6$  beats/min in SHRs ( $p < 0.05$ ) and external carotid nerve firing was  $464 \pm 82 \mu\text{V}^2/\text{s}$  in WKY rats and  $287 \pm 22 \mu\text{V}^2/\text{s}$  in SHRs ( $p < 0.05$ ).

Intravenous injection of substance P elicited dose-dependent increases in external carotid nerve activity in SHRs and WKY rats (Figs. 9 and 10). Figure 9 shows increases in nerve firing evoked by  $32 \text{ nmol/kg}$  substance P in a SHR and a WKY rat. The responses were significantly greater in SHRs at 10, 32 and 100 nmol/kg doses (Fig. 10).

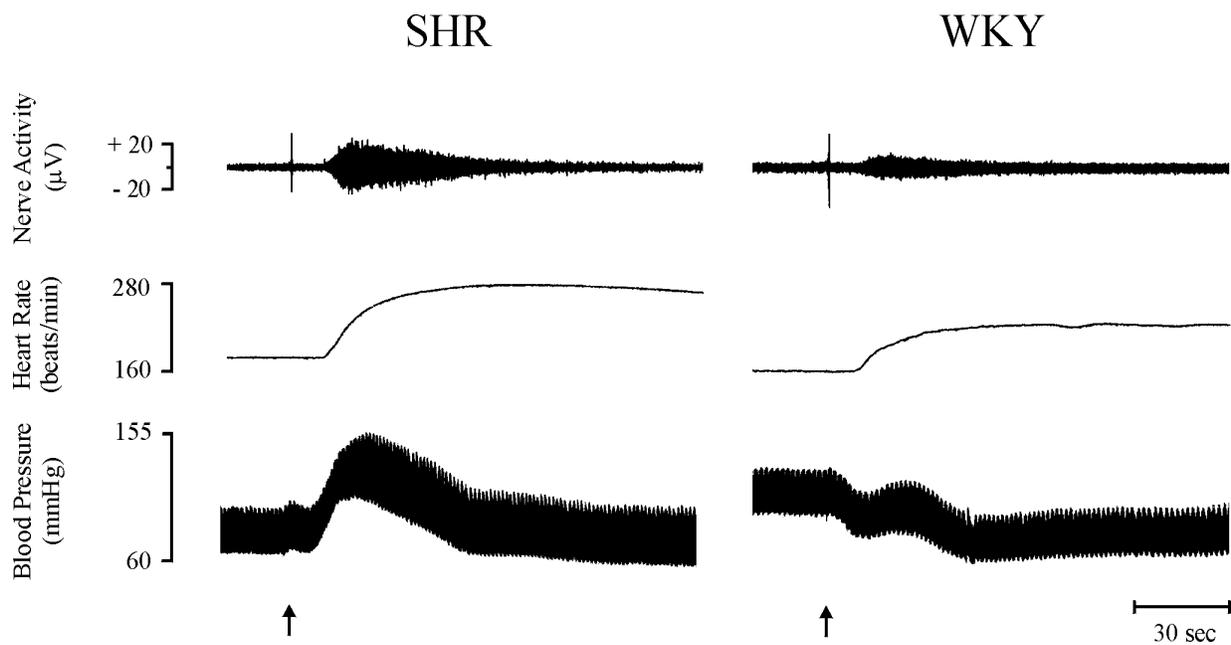


FIG. 9. External carotid nerve, heart rate and blood pressure responses to i.v. substance P (32 nmol/kg) in a SHR and a WKY rat. Arrows indicate time of injection.

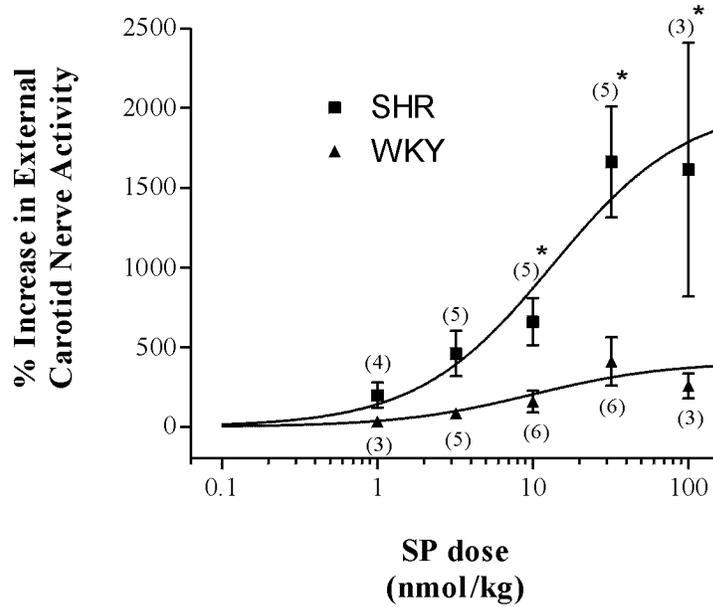


FIG. 10. Increased external carotid nerve firing in response to i.v. substance P (SP) in SHR and WKY rats. There was a significant difference in the response between strains, ( $F(4,45) = 3.1, p < 0.05$ ). \* Significant difference between responses at the same dose. Number of rats is in parentheses.

TABLE 1. Effect of Substance P (SP) on Blood Pressure and Heart Rate in SHR and WKY Rats<sup>a</sup>

Dose SP (nmol/kg)	Blood Pressure Change (mmHg)		Heart Rate Change (bpm)	
	SHR	WKY	SHR	WKY
100	48 ± 5* (4)	10 ± 2 (3)	50 ± 3* (4)	16 ± 2 (3)
32	41 ± 5* (6)	- 1 ± 3 (6)	83 ± 7* (6)	52 ± 5 (6)
10	18 ± 2* (6)	- 6 ± 3 (6)	62 ± 5* (6)	24 ± 8 (6)
3.2	11 ± 2* (6)	- 7 ± 3 (5)	53 ± 6* (6)	15 ± 7 (5)
1.0	1 ± 2* (5)	- 7 ± 2 (3)	31 ± 6* (5)	2 ± 4 (3)

<sup>a</sup> Values are maximum changes in mean arterial pressure or heart rate ± SEM after i.v. injection of substance P. Number of animals contributing to means in parentheses.

\* Significantly different from WKY at same dose.

Blood pressure and heart rate responses to Substance P are reported as the maximum change in heart rate or mean arterial pressure from baseline values. Substance P evoked dose-dependent increases in heart rate and blood pressure in SHR (Table 1). WKY rats respond with a dose-dependent increase in heart rate but blood pressure was lowered. Responses to 32 nmol/kg substance P are shown in figure 9. Heart rate and blood pressure changes were significantly greater at all doses in SHR.

### DOCA/salt Hypertension

Non-invasive Tailcuff Plethysmography. Systolic blood pressure was significantly elevated in 6-week-old SHR compared to age matched DOCA and SHAM rats ( $153 \pm 9$  mmHg in SHR (n = 12) vs.  $123 \pm 8$  mmHg in DOCA (n = 12) and  $116 \pm 8$  mmHg in SHAM (n = 14)). Blood pressure gradually increased in DOCA animals over 6 weeks of DOCA/salt treatment (Fig. 11). At 12 weeks of age, DOCA and SHR had a similar degree of hypertension as indicated by systolic blood pressure values measured by non-invasive tailcuff plethysmography ( $233 \pm 10$  mmHg in DOCA vs.  $219 \pm 9$  mmHg in SHR). These values were significantly greater than those from sham-treated animals at 8, 9, 10, 11, and 12 weeks of age. Heart rate was greater in DOCA ( $389 \pm 16$  beats/min) and SHR ( $409 \pm 14$  beats/min) in comparison to SHAM rats ( $349 \pm 14$  beats/min) at the end of treatment (Fig. 11). Weight gain was similar in DOCA and SHAM rats from 6 to 9 weeks of age (Fig. 12). SHR were significantly smaller than the other groups up to 10 weeks. At 12 weeks of age DOCA animals weighed significantly less ( $252 \pm 6$  g) than SHAM ( $292 \pm 5$  g) or SHR ( $286 \pm 6$  g).

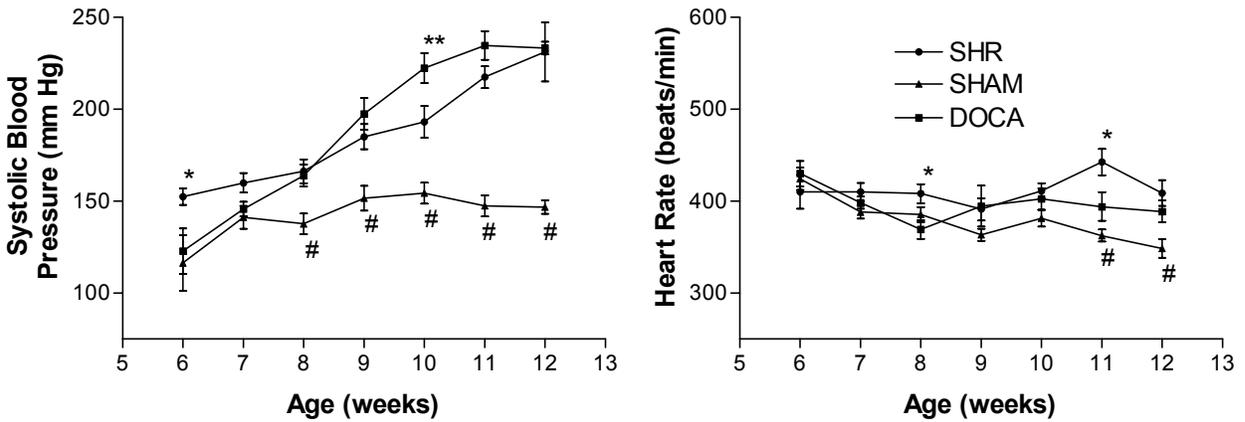


FIG. 11. Heart rate and blood pressure values recorded from SHAM, DOCA and SHRs. Systolic blood pressure was recorded from conscious animals by non-invasive tail cuff plethysmography. Comparisons were made by GLM ANOVA and post hoc analysis by Newman-Keuls test of means. Statistical significance at  $p < 0.05$ . Legend at right used for both figures. \* different from DOCA and SHAM, # different from DOCA and SHR, \*\* different from SHR and SHAM.

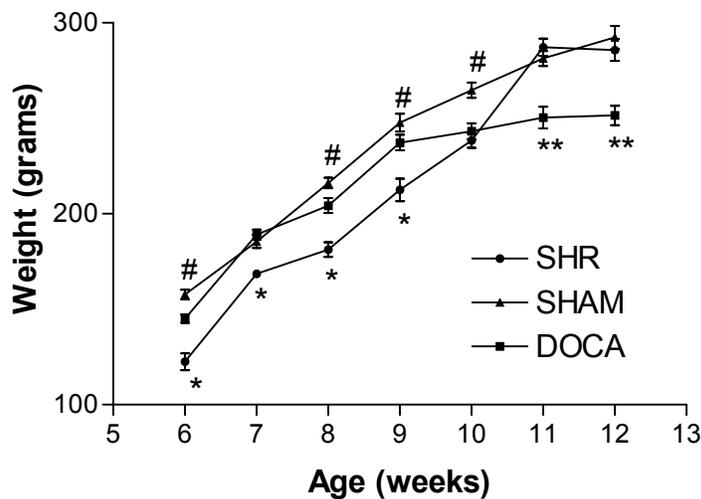


FIG. 12. Recorded weekly weight for SHR, DOCA and SHAM animals. Comparisons were made by GLM ANOVA and post hoc analysis by Newman-Keuls test of means. Statistical significance at  $p < 0.05$ . \* different from DOCA and SHAM, # different from SHR and DOCA, \*\* different from SHR and SHAM.

### Measurement of SCG Responsiveness to Substance P in DOCA/salt Treated Animals

Rats were anesthetized with i.p. injections of sodium pentobarbital (32 mg/kg) and ketamine (50 mg/kg) for experiments involving extracellular nerve recording. Mean arterial blood pressure (MAP) was significantly greater in SHR<sub>s</sub> ( $172 \pm 7$  mmHg,  $n = 12$ ) than in DOCA ( $100 \pm 7$  mmHg,  $n = 12$ ;  $p < 0.01$ ) and SHAM rats ( $112 \pm 15$  mmHg,  $n = 14$ ;  $p < 0.01$ ) after anesthesia (baseline, Fig. 13). Heart rate was also significantly greater in this strain ( $315 \pm 11$  beats/min,  $n = 12$ ) in comparison to DOCA ( $193 \pm 11$  beats/min,  $n = 12$ ;  $p < 0.01$ ) and SHAM rats ( $236 \pm 20$  beats/min,  $n = 14$ ;  $p < 0.01$ ).

The selective ganglion nicotinic receptor agonist DMPP was administered prior to ganglion blockade with chlorisondamine to test nerve activity. There was not a significant difference between the percent increase in nerve activity between DOCA ( $6024 \pm 1708$  %), SHAM ( $6715 \pm 1456$  %) or SHR<sub>s</sub> ( $2195 \pm 672$  %) ( $F(2,24)=2.446$ ,  $p > 0.05$ ).

Chlorisondamine significantly lowered blood pressure in all three groups (Fig. 13). Blood pressure was lowered to  $64 \pm 2$  mmHg ( $n = 11$ ,  $p < 0.01$ ) in SHAM rats,  $77 \pm 5$  mmHg ( $n = 11$ ,  $p = 0.03$ ) in DOCA rats and to  $74 \pm 3$  mmHg ( $n = 11$ ,  $p < 0.01$ ) in SHR<sub>s</sub>. Heart rate was also significantly lowered from  $247 \pm 17$  to  $197 \pm 22$  beats/min in SHAMs ( $n = 11$ ,  $p = 0.04$ ) and from  $283 \pm 13$  to  $174 \pm 5$  beats/min in SHR<sub>s</sub> ( $n = 11$ ,  $p < 0.01$ ). There was not a significant change in the DOCA rat heart rate ( $193 \pm 11$  beats/min before,  $191 \pm 15$  beats/min after chlorisondamine;  $n = 11$ ,  $p = 0.29$ ).



FIG. 13. *Blood pressure before and after chlorisondamine. Anesthetized animals were treated with the ganglion blocking agent chlorisondamine to prevent baro-reflex modulation of heart rate and blood pressure. Mean arterial blood pressure (MAP) was significantly greater in SHR before chlorisondamine treatment. Chlorisondamine significantly lowered blood pressure in all three treatment groups. After treatment, MAP was significantly less in SHAM animals than either DOCA or SHR. Comparisons were made using repeated measures ANOVA. Statistical significance was observed at  $p < 0.05$ . \* different from DOCA and SHAM at baseline, # different from DOCA and SHR after chlorisondamine.*

Substance P increased blood pressure in SHRs (Fig. 14). DOCA and SHAM animals did not show a pressor response (Fig. 14). The dose dependency of the blood pressure response is shown in figure 15. The three dose response curves were significantly different ( $F(2,4)=6.14$ ,  $p < 0.05$ ). The magnitude of the pressor response was greater in SHRs at all doses tested (Fig. 15). DOCA and SHAM animals responded similarly. In these animals substance P only elicited a depressor response.

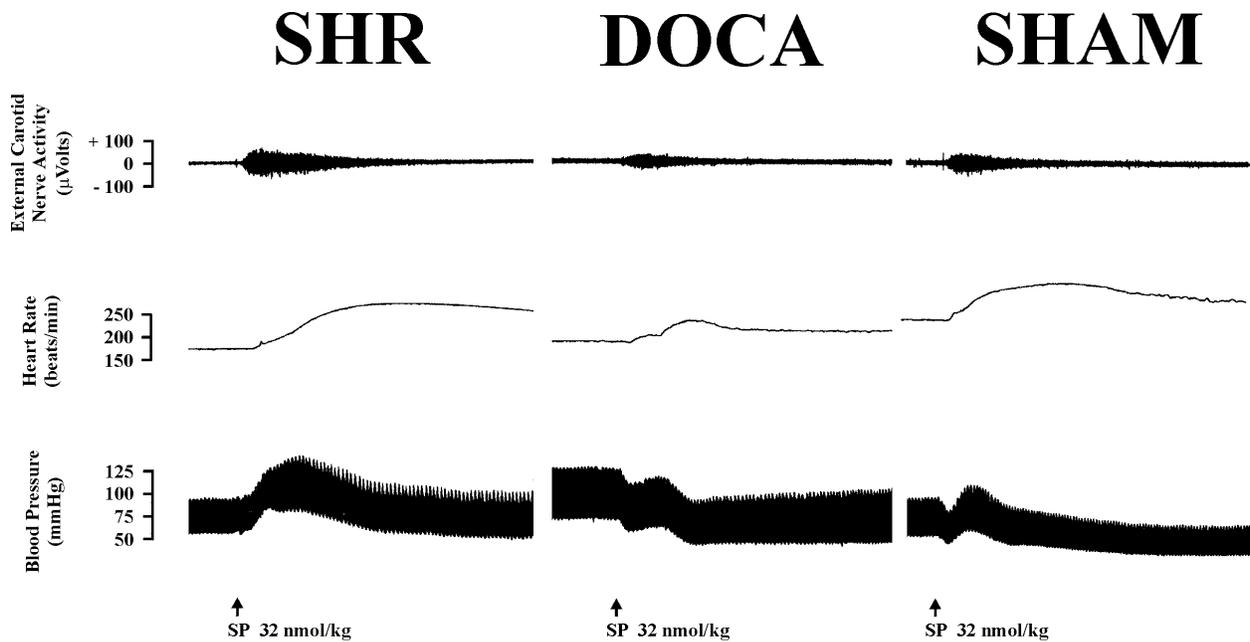


FIG. 14. Polygraph tracings of the nerve, heart rate and blood pressure responses to intra-arterial injection of substance P (SP) in an anesthetized SHAM, DOCA and SHRs. Rats were treated with chlorisondamine (10.5  $\mu$ mol/kg) to block nicotinic receptors in autonomic ganglia and the pre- and postganglionic nerves were cut. Nerve responses are representative of mean response for each group. Arrows indicate drug injection.

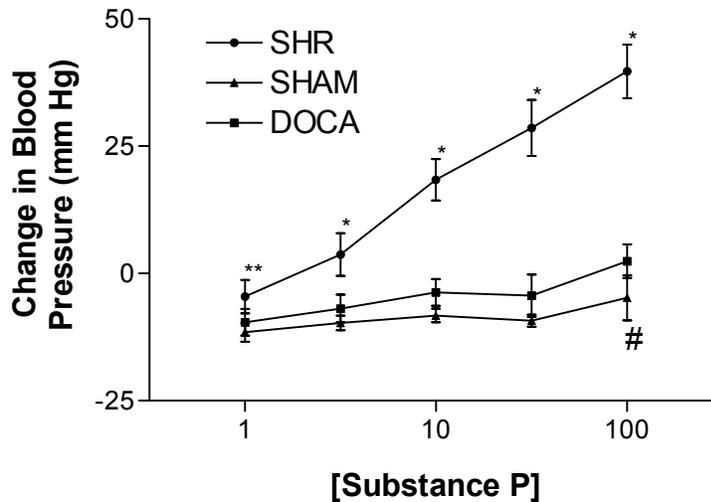


FIG. 15. Change in arterial blood pressure to close arterial injection of Substance P. Arterial blood pressure was recorded via cannulation of the femoral artery. Comparisons were made by GLM ANOVA and post hoc analysis by Newman-Keuls analysis of means. Statistical significance at  $p < 0.05$ . \*\* different from SHAM, \* different from DOCA and SHAM, # different from DOCA and SHR.

Close arterial injection of substance P elicited a dose dependant tachycardia in all animals (Figs. 14 and 16). The three dose response curves were significantly different ( $F(2,4)=4.0$ ,  $p < 0.05$ ). The change in heart rate was less in DOCA animals than SHRs at 3.2, 10, 32 and 100 nmol/kg doses of substance P. The magnitude of the tachycardia was greater in SHAM compared to DOCA rats at 10 and 32 nmol/kg doses of substance P. The 32 nmol/kg dose of substance P elicited the greatest change in heart rate in all animals (Fig. 16). At this dose heart rate was increased in SHAM rats from  $197 \pm 22$  to  $243 \pm 24$  beats/min ( $n = 11$ ), in DOCA rats from  $191 \pm 15$  to  $217 \pm 17$  beats/min ( $n = 11$ ) and in SHR from  $170 \pm 4$  to  $249 \pm 11$  beats/min ( $n = 10$ ).

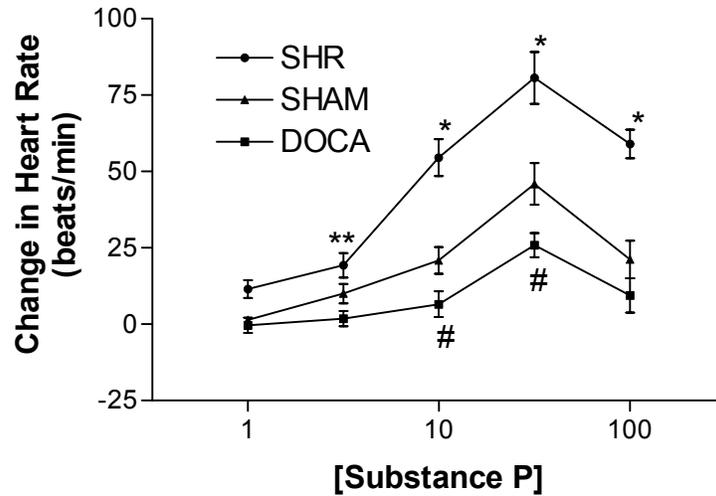


FIG. 16. Change in heart rate in response to close arterial injection of substance P. Heart rate was derived from the pressure pulse signal. Comparisons were made by GLM ANOVA and post hoc analysis by Bonferroni test of means. Statistical significance at  $p < 0.05$ . \*\* different from DOCA, \* different from SHAM and DOCA, # different from SHR and SHAM.

Substance P elicited dose-dependant increases in external carotid nerve activity in each of the treatment groups studied (Fig. 17). Figure 14 shows the increase in nerve firing evoked by 32 nmol/kg of substance P in a DOCA, SHAM and SHR. Nerve responses to substance P were significantly greater in SHRs at 1, 10, 32, and 100 nmol/kg doses (Fig. 17). SHAM animals responded greater than DOCAs to 32 nmol/kg substance P.

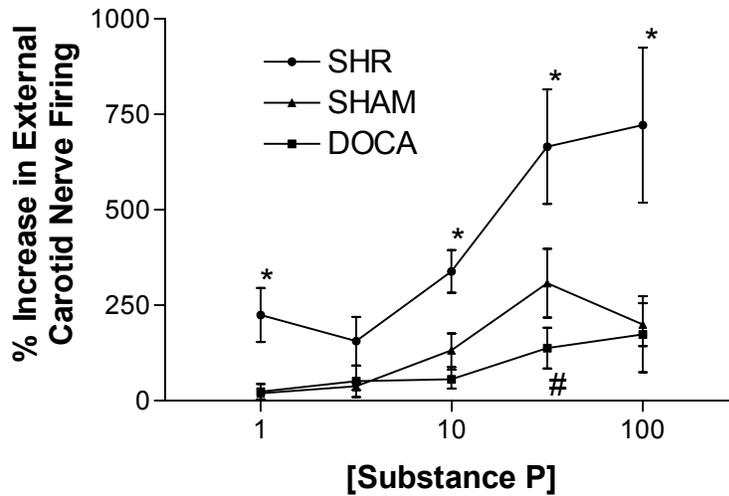


FIG. 17. Increases in carotid nerve activity in response to close arterial injection of substance P. Comparisons were made by GLM ANOVA and post hoc analysis by Bonferroni test of means. Statistical significance at  $p < 0.05$ . \* different from DOCA and SHAM, # different from SHAM and SHR.

### In Vitro Studies

#### Intracellular Recording

Membrane Properties of SCG neurons in SHR and WKY rats. Passive and active membrane properties of SCG neurons are shown in Table 2. A total of 70 cells (28 animals) were sampled from SHRs and 42 cells (16 animals) from WKY rats. With the exception of the amplitude of the action potentials evoked by intracellular stimulation, which were slightly greater in WKY rats than in SHRs, the membrane properties of these neurons were similar in the 2 strains.

TABLE 2. *Membrane Properties and Action Potential Characteristics of SHR and WKY Superior Cervical Ganglion Neurons<sup>a</sup>*

	SHR	WKY
Resting Membrane Potential (mV)	- 49 ± 0.8 (70)*	- 50 ± 1 (42)
Cell Input Resistance (MΩ)	63 ± 4 (68)	63 ± 6 (40)
Intracellularly Stimulated Action Potentials		
Threshold (mV)	- 39 ± 0.6 (70)	- 39 ± 0.8 (42)
Amplitude (mV)	50 ± 0.7* (70)	53 ± 1 (42)
Overshoot (mV)	1.2 ± 0.6 (70)	2.5 ± 0.8 (42)
Duration (ms)	1.7 ± 0.03 (70)	1.6 ± 0.05 (42)
AHP Amplitude (mV)	16 ± 0.4 (69)	15 ± 0.5 (42)
AHP Duration (ms)	102 ± 7 (69)	110 ± 8 (42)

<sup>a</sup> Action potentials were evoked by intracellular depolarizing currents delivered through the recording electrode. Values are expressed as mean ± SEM. Numbers of measurements contributing to means are in parentheses.

\* Significantly different from WKY.

Neurons were characterized as phasic or tonic based on the firing pattern evoked by intracellular depolarizing current pulses of 0.1-0.5 nA with a 1 second duration (Fig. 18). Cells firing 2 or fewer action potentials were classified “phasic”. Cells that fired multiple action potentials were classified “tonic”. There was no significant difference in the percentage of tonic cells between hypertensive and normotensive animals. Twenty percent (14/70) of SHR SCG neurons were tonic versus 17% (7/41) of WKY rat neurons. The cell input resistance ( $58 \pm 3.6 \text{ M}\Omega$  (n = 88) vs  $86 \pm 7.5 \text{ M}\Omega$  (n = 18);  $p < 0.01$ ) and the AHP amplitude ( $15 \pm 0.3 \text{ mV}$  (n = 89) vs  $18 \pm 0.6 \text{ mV}$  (n = 20);  $p < 0.01$ ) were greater in tonic neurons than in phasic neurons from both strains. There was no correlation between input resistance and AHP magnitude in tonic cells ( $r^2 = 0$ ). Action potential amplitude and duration were similar in the 2 types of cells.

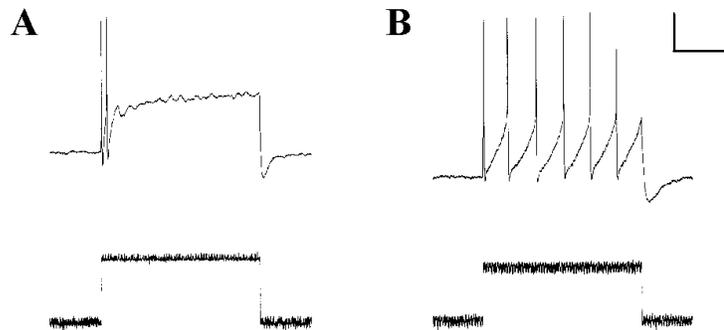


FIG. 18. *Characterization of SCG neurons. A-B: top traces, transmembrane potential, bottom traces, stimulus current. A: Phasic neuron; 1 second duration current pulse (0.53 nA) evoked 2 action potentials (APs). Resting membrane potential - 45 mV. B: Tonic neuron; 1 second duration current pulse (0.48 nA) evoked multiple APs. Resting membrane potential was - 54 mV. Vertical calibration bar: 15 mV. Horizontal calibration bar: 20 msec.*

Membrane responses to GR-73632. Pressure application of the  $\text{NK}_1$ -selective agonist GR-73632 evoked a similar long lasting depolarization of SCG neurons in SHRs (Figs. 19 and 20) and WKY rats. In SHRs, GR-73632 elicited a mean depolarization of  $4.7 \pm 0.5 \text{ mV}$ . There was

no significant difference in the percentage of tonic and phasic neurons that responded to GR-73632. The duration of the depolarization was  $49 \pm 7$  seconds ( $n = 22$ ). In WKY rats, GR-73632 elicited a depolarization of  $4.5 \pm 0.8$  mV. The duration of the depolarization was  $49 \pm 6$  seconds ( $n = 6$ ). A long-lasting increase in membrane excitability accompanied the depolarization in both strains. This is evidenced by the firing of anodal break action potentials at the offset of the hyperpolarizing current pulses following GR-73632 application (Fig. 19). Only a few cells (7/90) fired spontaneous action potentials in response to GR-73632 (Fig. 20). The number of cells where GR-73632 caused firing was similar in SHRs and WKY rats. Substance P had similar effects to those of GR-73632 on SCG neurons of SHRs and WKYs (data not shown).

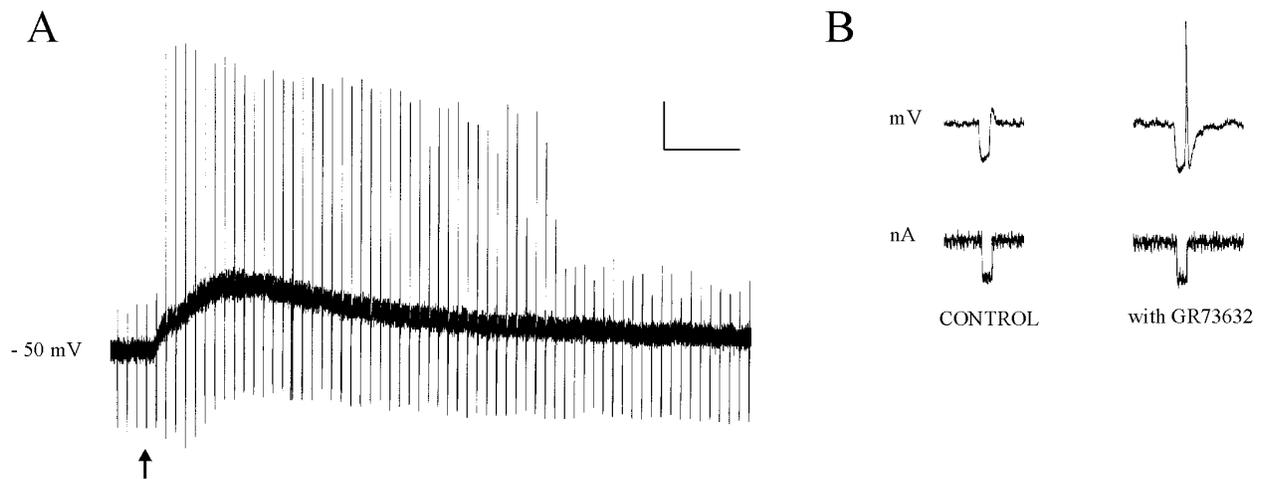


FIG. 19. GR-73632 induced depolarization and increased membrane excitability of SCG neurons. *A*: Transmembrane potential tracing from a SHR SCG neuron. GR-73632 ( $100 \mu\text{M}$ , 1 second pulse; indicated by arrow) elicited a 7 mV depolarization. Hyperpolarizing current pulses applied at 1 Hz were used to elicit hyperpolarizing electrotonic potentials (downward deflections). Vertical calibration bar: 5 mV. Horizontal calibration bar: 10 second. Resting membrane potential was - 50 mV. *B*: Anodal break action potentials evoked by the offset of the hyperpolarizing current pulse (0.2 nA; lower tracings) illustrate increase in membrane excitability that lasted 50 second after GR-73632 application. Calibration bar from *A*: Vertical scale: 10 mV. Horizontal scale: 35 msec.

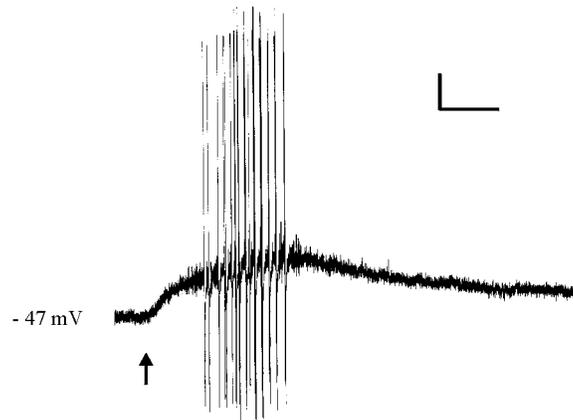


FIG. 20. *Transmembrane potential response of a phasic WKY rat SCG neuron to GR-73632 (100 $\mu$ M, 1 second pulse). Twenty-five spontaneous action potentials occurred at the plateau of the membrane depolarization (8 mV maximum amplitude, 67 second duration). Vertical calibration bar: 5 mV. Horizontal calibration bar: 7.5 msec.*

There was a significant trend for more SCG neurons to respond to GR-73632 in SHRs than in WKY rats. Thirty-seven percent (22/59) of SCG neurons in SHRs were depolarized by GR-73632 versus 19% (6/31) of WKY rats ( $p < 0.05$ ; Fig. 21).

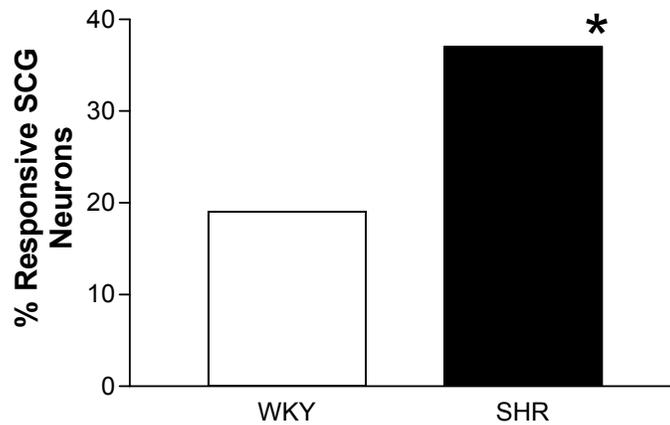


FIG. 21. *Percentage of WKY and SHR SCG neurons responsive to GR-73632. A significantly greater percentage of SCG neurons were depolarized by the NK<sub>1</sub> agonist GR-73632 in SHR vs. WKY rats ( $p < 0.05$ ).*

## CHAPTER 4

### DISCUSSION

This study showed that stimulation of SCG tachykinin receptors by i.v. injection of substance P causes a prominent increase in postganglionic nerve firing in SHRs. Blood pressure and heart rate were also elevated. In contrast, substance P decreased blood pressure and only slightly increased external carotid nerve firing and heart rate in WKY rats. Intracellular electrodes were used to determine the cellular basis for the enhanced effect in hypertensive rats. Local application of the NK<sub>1</sub> receptor agonist GR-73632 evoked slow depolarization and increased excitability of SCG neurons. These effects were similar in the two strains; however, there was a significant trend for more neurons from SHRs to respond to the tachykinin agonist. These observations suggest that the greater sympathetic nerve, blood pressure and heart rate responses observed in response to ganglion stimulation of NK<sub>1</sub> receptors in SHRs are due to a greater number of sympathetic ganglion neurons expressing functional NK<sub>1</sub> receptors rather than differences in the effect of tachykinins on the membrane properties of sympathetic neurons. This study also showed that changes in sympathetic nerve activity, heart rate and blood pressure in response to substance P were similar between WKY made hypertensive by DOCA/salt treatment and normotensive WKY. These results suggest that the increased sympathetic ganglion responsiveness to substance P observed in SHR is not correlated with elevated blood pressure.

#### Characterization of Sympathetic Ganglion Responsiveness to Substance P in SHR and WKY

Increases in renal nerve firing, blood pressure and heart rate caused by substance P in SHRs is due to stimulation of sympathetic ganglia (Hancock and Lindsay 2000). Those experiments

utilized ganglion blockade to reduce the possibility that the effects of the tachykinins were due to actions involving the central nervous system, and reserpine to eliminate the possibility that responses were due to actions on peripheral tissues. In the present study, in addition to using ganglion blockade, the ganglia were decentralized by sectioning preganglionic nerves. Thus, increases in nerve firing recorded from the external carotid nerve were due to direct ganglion stimulation. These observations also confirm the results from renal nerve recordings and suggest that up-regulation of NK<sub>1</sub> receptors is a characteristic of paravertebral ganglia in SHR<sub>s</sub>.

The predominant tachykinin receptor in sympathetic ganglia appears to be the NK<sub>1</sub> type (Seabrook et al. 1992; Zhao et al. 1993; Hawcock et al. 1995; Schoborg et al. 2000) and we have shown that this receptor mediates the pressor response to substance P (Schoborg et al. 2000). Other tachykinin receptor types may be present (Couture et al. 1989; Seabrook et al. 1992) but do not appear to be necessary for the pressor response. Our preliminary studies showed that the responses of individual neurons to GR-73632 and substance P produce similar effects on firing from the SCG and on blood pressure indicating that the effects of substance P were due to stimulation of NK<sub>1</sub> receptors. A NK<sub>1</sub> selective agonist was, therefore, used for intracellular recording studies to increase the likelihood that its effects were on cardiovascular neurons. The precise targets for specific neurons however were not determined.

The present study showed that more neurons responded to the tachykinins in SHR<sub>s</sub> than in WKY rats. For the neurons that responded, the effects of substance P (data not shown) and GR-73632 on the membrane potential and action potential characteristics were the same in the 2 strains. Although action potential amplitudes were slightly greater in ganglion neurons of WKY rats than in those of SHR<sub>s</sub>, the biological significance of the differences is not known. Other properties of the action potentials were similar in the 2 strains. Given these data, the increases in

blood pressure and postganglionic sympathetic nerve firing likely reflect a greater number of neurons in SCG excited by tachykinins in SHR.

The effects of substance P to increase firing from the SCG are consistent with studies that showed that substance P stimulated paravertebral ganglia to increase renal nerve firing and that this effect was greater in ganglia of SHR than those of WKY rats. In equimolar doses, the renal nerve response to GR-73632 was less than the response to substance P in SHR. The reason for this difference was not clear but may be due to stimulation of NK<sub>2</sub> and NK<sub>3</sub> receptors by substance P. This conclusion was supported by the observation that selective NK<sub>2</sub> and NK<sub>3</sub> receptor agonists have effects on renal nerve firing similar to those of substance P but of much smaller magnitude (Hancock et al. 1998; Hancock et al. 1999). Responses to NK<sub>2</sub> and NK<sub>3</sub> receptor agonists were the same in SHR and WKY rats. Thus, the effect of substance P to increase firing from the SCG probably represents activation of all three tachykinin receptors. The contribution of stimulating NK<sub>2</sub> and NK<sub>3</sub> receptors however is the same in SHR and WKY rats and they don't appear to be involved in blood pressure regulation (Schoborg et al. 2000).

Active and passive membrane properties control AP generation and may regulate neurotransmission (Burke 1987). Previous investigators have shown that SCG neurons from SHR lack spike accommodation and suggested that this may be responsible for the enhanced basal sympathetic tone associated with the hypertension in this strain (Yarowsky and Weinreich 1985; Jubelin and Kannan 1990). No other differences in resting membrane properties were observed between hypertensive and normotensive animals in those studies. In the present study, we used similar methods to characterize neurons in the SCG of SHR and WKY rats. Application of a depolarizing current pulse to tonic cells elicited multiple action potentials at a constant frequency. These cells lack any spike accommodation. We did not find a difference in

either the basic membrane properties or the percentage of tonic cells between hypertensive and normotensive rats. Accordingly, our results suggest that differences in the active and passive neuronal membrane properties of the SHR alone do not account for the increased sympathetic nerve activity observed in this strain. This conclusion is supported by experiments demonstrating a disproportional pairing of synaptic input and output at high frequencies of extracellular stimulation of the cervical sympathetic trunk in SHRs (Magee and Schofield 1992). Compound action potential magnitudes, recorded from the internal carotid nerve, were significantly greater in SHRs at low frequencies (1-80 Hz) of nerve stimulation whereas WKY rats responded greater at high frequencies (80-100 Hz). If enhanced excitability of the postganglionic cell membrane played a significant role in facilitating presynaptic transmission then one would assume that synaptic transmission would be greater in SHRs across all frequencies of nerve stimulation (Magee and Schofield 1992).

Studies have shown that sympathetic ganglia of SHRs are larger (Kondo et al. 1990) and contain more sympathetic neurons (Messina et al. 1996) than ganglia of normotensive rats. This raises the possibility that the enhanced ganglionic response to substance P in SHRs is due to a greater number of neurons in ganglia of hypertensive rats. Our previous studies, however, showed that the enhanced response to substance P does not seem to be due to a generalized increase in sensitivity or cell number because renal nerve, blood pressure and heart rate responses to DMPP were the same in SHRs and WKY rats (Hancock and Lindsay 1995).

Substance P and its receptors appear to be differentially regulated in various tissues of hypertensive rats in a manner that is consistent with their involvement in hypertension. Kopp and her associates (Kopp et al. 1998) have shown an impaired afferent renal nerve response to increased pelvic pressure in SHRs due to either an impaired release of substance P or an

impaired activation of substance P receptors. This impaired activation of renal sensory neurons is believed to contribute to hypertension by increasing water and sodium retention. Decreased circulating levels of substance P in SHR and in humans with essential hypertension have also been reported in the literature (Rathsack et al. 1983; Faulhaber et al. 1987). Since substance P causes vasodilatation, the decreased circulating levels could contribute to the elevation of peripheral vascular resistance. There is also an impairment of the peripheral vasodilator response to substance P and other endothelium-dependant vasodilators which might tend to enhance the effect of decreased circulating substance P (Pompei et al. 1993; Egashira et al. 1995). We have shown that substance P can increase sympathetic nerve activity and increase blood pressure by an action on sympathetic ganglia (Hancock and Lindsay 1995; Hancock and Lindsay 2000; Schoborg et al. 2000). Chen and coworkers have reported increased substance P-like immunoreactivity in the hypothalamus, in the rostral ventral surface of the medulla oblongata and in the intermediolateral cell column of the thoracic spinal cord of SHR (Chen et al. 1990). These observations suggest the involvement of substance P in the central regulation of vasomotor tone as another potential site of action for this peptide contributing to the hypertension of the SHR. In context with the present observations, it is likely that substance P and its receptor are involved in the pathogenesis of hypertension in SHR.

Characterization of Sympathetic Ganglion Responsiveness to Substance P  
in a Nongenetic Form of Hypertension

It is unknown whether the increased ganglion responsiveness to substance P observed in SHR is a unique characteristic of this model of hypertension or whether it is induced by the elevated arterial pressure. Substance P containing sensory afferents innervate the peripheral

vasculature and spinal cord and are known to relay peripheral sensory information to the central nervous system. Collaterals to these fibers modulate ganglion function (Fig. 2). The afferent fibers in blood vessels respond to a variety of stimuli, including pressor drugs, arterial occlusion, changes in venous return and mechanical distension, by increasing their firing (Malliani 1982). The increased sensory innervation and increased sensitivity of sympathetic ganglia to the stimulant action of substance P observed in SHR's could, therefore, be an adaptive response initiated by stimuli originating in the vasculature as a result of the elevated blood pressure.

DOCA/salt treated animals were used to investigate the effect of an induced increase in arterial pressure on the ganglion responsiveness to substance P. Chronic DOCA/salt administration evoked an elevated arterial pressure in WKY rats over the course of six weeks with a similar time course of development as the hypertension observed in SHR's. Weekly non-invasive measurements of systolic blood pressure were made to monitor the progression of hypertension in DOCA and SHR's. At 12 weeks of age, DOCA hypertensive rats did not show increased ganglion responsiveness to substance P. Ganglion nerve activity, heart rate and blood pressure responses to intraarterial injection of substance P were similar in DOCA treated and control WKY animals. These results suggest that the observed increase in ganglion sensitivity to substance P in SHR's is a unique trait of this genetic model of hypertension and is not causally related to the increased arterial pressure.

Tail cuff monitoring of blood pressure is routinely cited in the literature as an accurate and reliable means of non-invasive measurement of arterial pressure (Lamprecht et al. 1977; Basso et al. 1985; Anderson et al. 1988; Kirchner et al. 1993; Kubo et al. 2000). Results from this procedure demonstrated that arterial pressure of DOCA animals was significantly elevated over controls from 8 through 12 weeks of age. However, after anesthesia with the barbiturate sodium

pentobarbital, intraarterial blood pressure values were similar in DOCA/salt and sham treated WKY rats and both were significantly lower than in SHRs. Further treatment with the ganglion blocking agent chlorisondamine lowered blood pressure to similar values in SHR and DOCA rats that were both significantly elevated from controls.

Why the conscious state mean arterial pressure was significantly higher in DOCA than control rats before but not after pentobarbital anesthesia is unclear. Such sensitivity to anesthetics in this kind of experimental hypertension has, however, previously been reported (Iriuchijima et al. 1975; Iriuchijima et al. 1976). Pentobarbital has been shown to cause hypotension, particularly at high doses (Wixson et al. 1987). This effect is partially mediated by actions on the sympathetic nervous system. Pentobarbital suppresses central and peripheral noradrenergic nerve activity (Takemoto 1992) and is associated with a significant reduction of norepinephrine spillover and metabolic clearance (Maignan et al. 2000). As in the pathogenesis of hypertension in the SHR, the importance of the sympathetic nervous system in DOCA/salt hypertension is well documented (de Champlain et al. 1969; Iriuchijima et al. 1975; Takeda and Bunag 1980; de Champlain et al. 1987; de Champlain et al. 1989). Central depletion of catecholamines has been shown to prevent or reverse the hypertension in this model (Lamprecht et al. 1977). Direct recordings from visceral sympathetic fibers also illustrate increased sympathetic fiber activity (Takeda et al. 1988). Plasma catecholamines are also elevated (Bouvier and de Champlain 1986). These findings suggest an important role of neural mechanisms in maintaining DOCA hypertension. Why blood pressure wasn't lowered in SHRs to a similar degree is unknown but probably reflects differences in the pathways or mechanisms for the hypertensive state between the 2 models.

Chronic administration of DOCA induces sodium retention and, in the presence of a high salt intake, produces a volume-dependant type of hypertension (de Gracia et al. 2000). The pathogenesis of DOCA/salt hypertension is not fully understood but a number of consistent features have been observed allowing for a generalized concept to be formed. Administration of DOCA changes hormonal and neural pressor mechanisms. Relevant prehypertensive mechanisms include increased activation of the brain renin-angiotensin system, increased sympathetic nerve activity and a blunted baro-reflex response (Schenk and McNeill 1992). In addition DOCA/salt hypertension induces characteristic changes in vascular structure and reactivity that contribute to the overall pathology (Walker and Boyd 1983).

Intraarterial injection of substance P elicits a biphasic blood pressure response in rats (Hancock and Lindsay 1995; Hancock and Lindsay 2000; Schoborg et al. 2000). A transient pressor response precedes a long-lasting depressor response. The increase in pressure is mediated by stimulation of sympathetic ganglia while the depressor response is mediated by stimulation of endothelial NK<sub>1</sub> receptors. Kohlmann and colleagues have previously shown that a selective NK<sub>1</sub> antagonist raises blood pressure in DOCA/salt treated rats (Kohlmann, Jr. et al. 1995b; Kohlmann, Jr. et al. 1997). From these results they speculate that substance P expression is elevated in DOCA/salt treated WKY rats as a counter regulatory mechanism to the elevated arterial pressure (Kohlmann, Jr. et al. 1997). These results demonstrate that DOCA/salt treatment may affect tachykinin expression. In the present experiments, intraarterial injection of substance P lowered blood pressure in SHAM and DOCA rats to a similar extent over the range of doses tested. Kohlmann suggests that the participation of substance P in the pathogenesis of elevated BP could vary according to the phase of the hypertensive state and/or the experimental model (Kohlmann, Jr. et al. 1995b). In their experiments they found that while substance P

expression appeared to be upregulated during the benign or early phase of hypertension in DOCA/salt treated rats the selective NK<sub>1</sub> antagonist had no effect on blood pressure during later phases (Kohlmann, Jr. et al. 1995a). Since the vascular responses were similar in treated and untreated WKY, our results suggest we are looking at what they termed the malignant or more advanced phase of DOCA/salt induced hypertension.

In conclusion, these studies showed that stimulation of NK<sub>1</sub> receptors causes a greater increase in postganglionic sympathetic nerve firing, blood pressure and heart rate in SHR than in WKY rats. This enhanced sympathetic ganglion responsiveness in SHRs appears to be due to a greater number of neurons expressing NK<sub>1</sub> receptors. This conclusion is based on results from our studies with intracellular microelectrode recordings that showed that more neurons in the SCG respond to NK<sub>1</sub> receptor stimulation in hypertensive rats than in normotensive rats. Further, the enhancement of the response does not appear to be due to a greater responsiveness of individual neurons since NK<sub>1</sub> receptor stimulation had similar effects on the membrane properties of SCG neurons and in evoking firing from the neurons in the two strains. These studies also showed that the postganglionic sympathetic nerve, heart rate and blood pressure responses to substance P are similar in hypertensive and normotensive WKY rats. This suggests that the enhanced ganglion responsiveness to substance P observed in SHRs is not correlated with the elevated blood pressure. The cause of the upregulation of tachykinergic systems in ganglia of SHRs is unknown and may be due to factors unrelated to hypertension. The question of whether the heightened ganglion responsiveness to tachykinins contributes to the elevation of sympathetic nerve activity and blood pressure in SHRs has not been determined. The results of this and other studies (Dun and Karczmar 1979; Virus et al. 1981; Tsunoo et al. 1982; Lembeck and Skofitsch 1982; Gurusinge and Bell 1989a; Gurusinge and Bell 1989b; Hancock and

Lindsay 1995; Hancock and Lindsay 2000; Schoborg et al. 2000) show, however, that substance P has actions that could contribute to the increased sympathetic nerve activity and possibly the elevated blood pressure in SHR.

#### Note in Proof

In support of our conclusions from the aforementioned experiments, we have preliminary data that shows that preventing the development of hypertension in SHR does not affect SCG sensitivity to substance P (Tompkins and Hancock 2003). SHR were prevented from developing hypertension by chronic treatment with the angiotensin converting enzyme inhibitor captopril. These animals (n = 3) were maintained from 6 weeks of age on drinking water containing captopril at a concentration giving a dose of 60 mg/kg/day. Tail cuff and invasive blood pressure values were the same as those in untreated WKY at the end of the 12 week treatment period. External carotid nerve activity, blood pressure and heart rate responses to substance P (1.0 to 100 nmol/kg) were similar in captopril-treated SHR and untreated SHR. The observations that responses to substance P in SHR kept from becoming hypertensive are the same as in hypertensive SHR supports the idea that the increased sympathetic nerve response to substance P is an inherent characteristic of SHR and not an adaptive response of sympathetic ganglion neurons to hypertension.

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